REVIEW ARTICLE
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The Molecular Basis for Kinesin Functional Specificity During Mitosis

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Microtubule-based motor proteins play key roles during mitosis to assemble the bipolar spindle, define the cell division axis, and align and segregate the chromosomes. The majority of mitotic motors are members of the kinesin superfamily. Despite sharing a conserved catalytic core, each kinesin has distinct functions and localization, and is uniquely regulated in time and space. These distinct behaviors and functional specificity are generated by variations in the enzymatic domain as well as the non-conserved regions outside of the kinesin motor domain and the stalk. These flanking regions can directly modulate the properties of the kinesin motor through dimerization or self-interactions, and can associate with extrinsic factors, such as microtubule or DNA binding proteins, to provide additional functional properties. This review discusses the recently identified molecular mechanisms that explain how the control and functional specification of mitotic kinesins is achieved. © 2013 Wiley Periodicals, Inc.

Key Words: mitosis; kinesin; microtubules; motors; regulation

Introduction

Microtubules are essential players in mitosis [Mitchison and Kirschner, 1984; Goshima and Scholey, 2010]. However, the dynamic properties and polarity of microtubules are not sufficient to organize microtubules into a spindle or align the chromosomes. Multiple microtubule-based motors, as well as non-motor microtubule-associated proteins, are key to mediating these processes. Most eukaryotic cells contain a single minus-end directed cytoplasmic dynein motor and multiple kinesin motor proteins that perform distinct microtubule-based processes, apart from higher plants that lack dynein. Kinesins have a conserved ATPase domain, but each kinesin family member utilizes the energy from ATP hydrolysis to perform different functions, relying on both its motor domain and its non-motor regions to create functional specificity. Although the motor domains of kinesins have been extensively studied, this domain represents only one part of the kinesin. Regions flanking the catalytic domain have divergent primary sequences and are essential for creating functional diversity. These divergent regions provide a molecular basis for unique interactions with cargo molecules, protein partners, and self-interaction that can also be uniquely regulated by post-translational modifications. Many of the early studies that revealed the molecular mechanism underlying kinesin diversity were conducted on kinesins involved in vesicular transport. Recently, there have been several reports revealing that mitotic kinesins require additional partners to target to the correct subcellular location. These regulatory domains and associated partners of the kinesin motors are thus key to understanding how mitotic kinesins target to and function at the correct place within the cell. Functional accuracy and specificity during mitosis is key to achieve correct chromosome segregation. Here, I review the intrinsic and extrinsic factors that provide specificity to mitotic kinesins and the underlying mechanisms for their correct targeting and function, focusing particularly on the kinesin proteins that play a role during mitosis.

Additional Supporting Information may be found in the online version of this article.

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Identification and Structural Characterization of the Kinesins

The first kinesin, Kinesin-1, was identified from squid giant axons [Vale et al., 1985] as a microtubule-based motor that was distinct from the minus end-directed cytoplasmic dynein motor. Subsequent work demonstrated that Kinesin-1 is conserved across species [Brady, 1985; Scholey et al., 1985; Neighbors et al., 1988; Saxton et al., 1988] and shares a common ancestor with the actin-based motor

myosin [Kull et al., 1996]. Since the original discovery of the first kinesins, many additional kinesins have been identified due to the high degree of conservation of the kinesin motor domain that possesses the catalytic ATPase activity. In the early 1990s, it became apparent that a superfamily of kinesin motors existed with divergent motor-flanking regions and unique functions [Vale and Goldstein, 1990].

Biochemical and structural characterization of the Kinesin-1 established the tripartite architecture of this motor [Hirokawa et al., 1989; Yang et al., 1989]. The motor region containing the ATPase domain forms a large globular structure, also termed the kinesin "head." Most kinesins have a coiled-coil region, the "stalk," that flanks the motor domain and is often important for dimerization. Stalk length and flexibility can vary greatly between kinesins with the stalk in Kinesin-1 of 80 nm, but CENP-E's coiled-coil region being 230 nm long [Hirokawa et al., 1989; Kim et al., 2008]. The hinge region linking the motor domain to the stalk is termed the "neck." At the other end of the stalk, the "tail" region is highly divergent, allowing kinesin specification. The kinesin superfamily can be subdivided into three groups depending on the position on the motor region within the polypeptide chain. The kinesin head is at the N-terminus in Kinesin-1 to -12 and at the C-terminus of Kinesin-14. Kinesin-13's are atypical with the motor domain flanked by additional regions on each side [Hirokawa, 1998]. In total, the kinesin superfamily shares common structural features such as the neck region and the presence or absence of a dimerization domain but the organization and position of these domains with respect to the ATPase region create functional diversity.

Phylogenetic Organization and Functional Diversity of Kinesins

After the initial discovery of Kinesin-1 in diverse species, additional kinesin-like molecules were identified in various organisms [Brady, 1985; Scholey et al., 1985; Vale et al., 1985; Yang et al., 1988]. The minus bend directed motor Kar3 and the Eg5 homologues Cut7 and BimC were identified in fungi [Enos and Morris, 1990; Hagan and Yanagida, 1990; Meluh and Rose, 1990]. This marked the birth of the kinesin superfamily [Vale and Goldstein, 1990]. The sequence of the *Drosophila Khc* gene, encoding the heavy chain of Kinesin-1, allowed the identification of multiple kinesin genes, which were initially termed kinesin-like proteins (KLP) based on sequence homology of the ATPase domain [Yang et al., 1989; McDonald and Goldstein, 1990; McDonald et al., 1990]. A phylogeny of the kinesin superfamily was established to classify the kinesins according to their sequences [Moore and Endow, 1996; Hirokawa, 1998]. Kinesins are classified into 14 families according to their sequence and function [Lawrence et al., 2004]. Large-scale genome sequencing further refined this kinesin phylogeny and helped identify new kinesins [Dagenbach and Endow, 2004; Miki et al., 2005; Wickstead and Gull, 2006]. The large scale sequence data on eukaryotic kinesins however challenged the proposed classification and stated that Kinesin-4 and -10 should be united into one class, while Kinesin-12 members Kif12 and Kif15 should be in separate classes [Wickstead and Gull, 2006]. In this review, we follow the classification nomenclature proposed by Lawrence et al. [2004]. The Kinesin-1 to -12 families are plus-end directed motors that move towards the rapidly growing end of microtubules, whereas Kinesin-13 are microtubule depolymerases. There is only one minus end directed family, Kinesin-14, most likely due to dynein acting as a main contributor to minus-end directed transport along microtubules. In species lacking dynein, motors may adopt bidirectional properties. For example, budding yeast Cin8 single motors are minus-end directed on individual microtubules. However, teams of Cin8 become plus-end directed motors [Roostalu et al., 2011]. All eukaryotes contain multiple kinesin motors with conserved function, suggesting the last common ancestor also possessed multiple kinesins [Wickstead et al., 2010]. The number of kinesins within a species can vary widely due to gene loss (e.g., loss of Kinesin-14 in Apicomplexa), gene duplications and subsequent divergence, generating diversity, and versatility within each kinesin motor family [Vale, 2003; Wickstead and Gull, 2006]. Budding yeast contain six different kinesins belonging to five different subfamilies, Cin8 and Kip1 being partially redundant, whereas human cells encode 45 different kinesin family members [Hirokawa et al., 2009bb], most of which are plus end directed motors. Flowering plants such as *Arabidopsis* have at least 61 kinesins, of which 21 are minus-end directed motors perhaps to compensate for the lack of dynein in plants [Lawrence et al., 2001; Reddy and Day, 2001; Lee and Liu, 2004]. Overall, each kinesin fulfills functions distinct from its related family members and has unique properties. Here, I will focus on the diverse kinesin proteins that play essential roles during mitosis to organize the spindle and direct chromosome alignment and segregation.

Most kinesins appear to have non-overlapping functions, but these can be masked by the existence of multiple parallel pathways. For example, RNAi-based screens in *Drosophila* S2 cells have defined groups of kinesin motors that play synergistic roles in chromosome congression or bipolar assembly [Goshima and Vale, 2003b; Goshima et al., 2005b]. In metazoans, kinesin motors with distinct molecular functions act synergistically in parallel pathways to ensure the robustness of bipolar spindle assembly [Mountain et al., 1999; Sharp et al., 1999; Tanenbaum et al. 2008, 2009]. In general, the kinesins that participate in related functional processes do not belong to the same family, and have different domain structures such that they act together through distinct molecular mechanisms. For example, the Kinesin-12 Kif15 and the Kinesin-13 MCAK are important for maintaining a bipolar spindle when the activity of the Kinesin-5 Eg5 is compromised [Tanenbaum

et al., 2009]. Overall, mitotic kinesins have distinct cellular functions that allow fine-tuning and robustness of mitotic microtubule-based processes.

Although the kinesin motors domains all bind to microtubules and hydrolyze ATP, the way in which this enzymatic activity is harnessed to direct intracellular functions varies dramatically. The unique roles that kinesins play in a given process arise primarily from the functional contributions of the divergent non-motor regions, through both their intrinsic features and extrinsic associated factors. Here, I will discuss the primary molecular mechanisms that appear to create this kinesin functional specification:

1. Functional diversity of the kinesin motor domain.
2. Oligomerization and self-interactions.
3. Non-conserved regions that modify the properties or targeting of the motor domain, or alter motor activity.
4. Divergent regions that interact with microtubules, thereby providing a second microtubule binding site to the kinesin.
5. Interactions with proteins that target kinesins to a particular sub-cellular location, for example by providing a microtubule or DNA binding activity.
6. Post-translational modifications of kinesins.

Functional Diversity of the Motor and Neck Domains

A significant focus of work on kinesin motor proteins has involved the analysis of the molecular and catalytic mechanisms underlying the ATP hydrolysis cycle of the motor and how this chemical energy is coupled to produce mechanical force [Cross, 2004; Carter and Cross, 2006; Block, 2007]. Molecular snapshots of kinesin motor domains in distinct nucleotide-bound states by X-ray crystallography or kinesins bound to microtubules visualized using cryo-electron microscopy have provided important insights into the catalytic mechanism by which kinesins utilize ATP to produce a conformational change that can be converted into a mechanical force [Vale et al., 2000; Marx et al., 2009]. Depending on the unique features of the kinesin motor domain, this force manifests in different ways. For processive kinesins, this force can be converted into movement and stepping along the microtubule lattice using a hand-over-hand mechanism [Asbury et al., 2003; Kaseda et al., 2003; Yildiz et al., 2004]. In contrast, non-processive kinesins such as Ncd, adopt a microtubule hold-and-release mechanism that does not involve stepping. Finally, microtubule depolymerases utilize force to induce or stabilize a curved conformation of single protofilaments thereby causing microtubule catastrophe and disassembly [Niederstrasser et al., 2002; Ogawa et al., 2004; Varga et al., 2006]. Some kinesins can act both as processive motors and as microtubule depolymerases. For example, Kar3/Cik1 is a *S. Cerevisiae* Kinesin-14 that has minus end directed activity and microtubule directed plus end depolymerase activity [Endow et al., 1994; Sproul et al., 2005]. It is under debate whether the Kinesin-8 members also act both as depolymerases and processive motors. The yeast Kinesin-8 Kip3 is a microtubule depolymerase, proposed to depolymerize microtubules in an age-dependent manner [Varga et al., 2006; Gardner et al., 2011; Su et al., 2011]. Yet, mammalian Kinesin-8 members do not show intrinsic microtubule depolymerase activity in vitro, but rather causes pausing in microtubule dynamics [Stumpff et al., 2008; Peters et al., 2010; Stumpff et al., 2012]. Kif18a depletion results in longer spindles which could correlate with microtubule depolymerase activity [Mayr et al., 2007]. However, spindle length also increases in absence of kinetochore proteins mediated-pulling forces [DeLuca et al., 2002; Toso et al., 2009]. It is possible that Kinesin-8 motors, while processive in most species, have acquired new additional depolymerase properties to compensate for the lack of Kinesin-13 in *S. cerevisiae*.

High structural conservation of the motor domain is necessary to preserve the ATPase properties and microtubule binding properties of the kinesin. Overall, a layer of β-strands sandwiched by α-helices form the highly conserved core of the kinesin motor domain, which also contains the microtubule binding region [Hirokawa et al., 1998a]. However, comparative analyses of known kinesin structures have identified unique features within the ATPase domain that create functional specificity of the motor. Variations in the primary and tertiary sequences of kinesins are mapped primarily to the loop regions within the motor domain [Kozielski et al., 1999; Ogawa et al., 2004; Neumann et al., 2006; Cochran et al., 2009; Peters et al., 2010]. These structural variations impact the affinity of the motor for the microtubule, its processivity, the nucleotide-gated switch to modulate the ATP cycle, and its kinetic parameters. Kinesins can be classified according to these kinetic parameters and properties [Friel and Howard, 2012]. The adjacent neck region is also conserved across kinesin families, although less so than the motor domain. The neck is essential for specifying the directionality of kinesin movement along the microtubule and gives rise to unique properties within kinesin families [Case et al., 1997; Henningsen and Schliwa, 1997; Endow and Waligora, 1998; Sablin et al., 1998; Endow, 1999]. In addition, comparative structural analysis of kinesin motors in different nucleotide-bound states and conformations allows the evaluation of individual mitotic kinesins as cancer drug targets [Rath and Kozielski, 2012]. In total, while being conserved, the motor domains of kinesins show structural and biophysical differences translate into unique functional properties.

Oligomerization and Self-interactions

In many cases, kinesins homo-multimerize through a coiled-coil region called the stalk, which allows them to

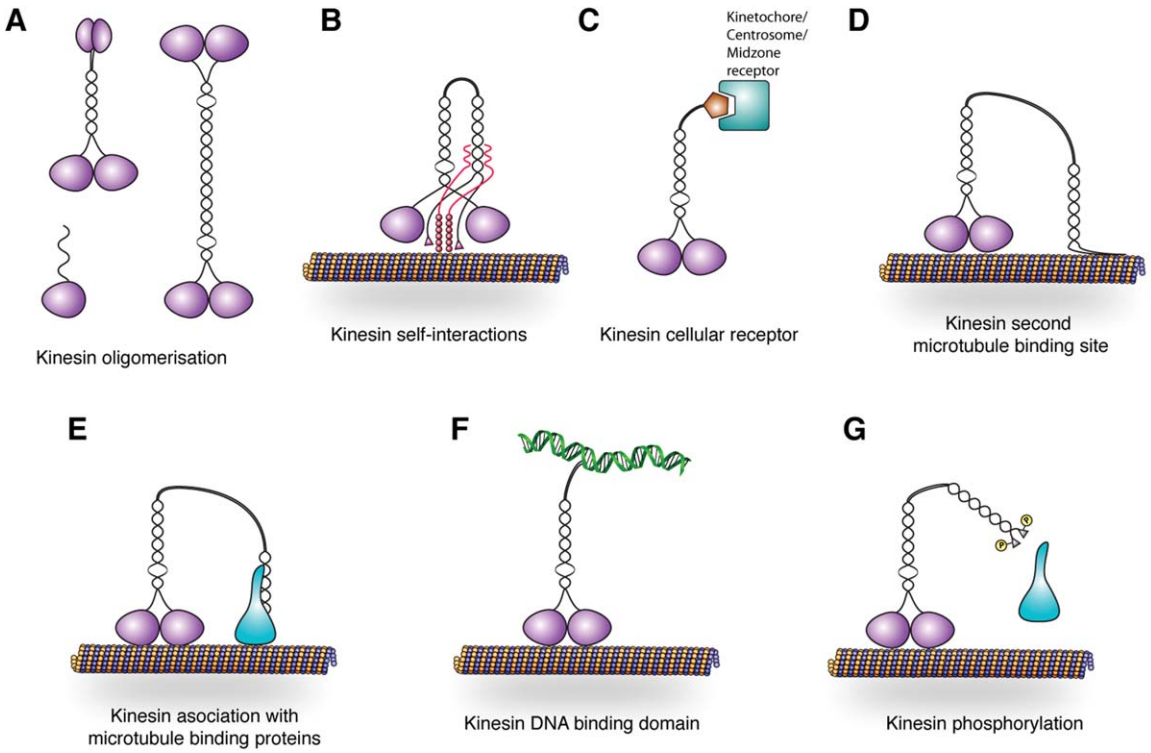

Fig. 1. Schematic diagram showing how the non-motor regions of kinesins may acquire properties that would specify unique function to the catalytic motor domain. (**A**) The non-motor domains can support the monomeric or oligomeric state of kinesins. (**B**) The non-motor regions can self interact or interact with the ATPase domain of kinesins, most of the time to inhibit kinesin function. (**C**) The non-motor regions can direct the targeting of the kinesin to a specific localization that acts as a kinesin receptor. (**D**) Non-motor regions may have a microtubule binding domain or (**E**) may associate with a microtubule binding protein. (**F**) Chromokinesins have a DNA binding region. (**G**) All of these interactions can be regulated by post-translational modifications to add an additional layer of regulation to kinesin function. The schematic diagram of kinesins was adapted from the review Verhey and Hammond [2009].

step along the length of the microtubule in a hand-over-hand manner with one head remaining associated with the microtubule at all times (Fig. 1A). The Kinesin-5 family forms tetramers and functions to slide antiparallel microtubules away from each other, especially during formation of a bipolar spindle [Cole et al., 1994; Kashina et al. 1996a, 1996b; Kapitein et al., 2005; van den Wildenberg et al., 2008]. This tetramerization property is essential to allow each pair of motor heads to crosslink and slide along the length of proximally-associated antiparallel microtubules. One long coiled-coil adjacent to the N-terminal motor domain forms the central stalk and dimerization interface of the motor pair. Three additional downstream coiled-coils mediate the tetramerization of the Kinesin-5 complex [Weinger et al., 2011]. While many kinesins multimerize to allow them to walk along microtubules, some kinesins can even display highly processive motility acting as monomers. The first kinesins to be identified as monomeric are Kif1a and Kif1b, both of which are involved in axonal transport of synaptic vesicles and mitochondria in neurons [Nangaku et al., 1994; Okada et al., 1995]. Kif1a and Kif1b lack a dimerization domain and form a globular compact structure, yet are highly processive in vitro [Hirokawa and Noda, 2008; Hirokawa et al., 2009a]. However, Kif1a is also found in a dimeric state in vivo that is essential for its motility [Tomishige et al., 2002; Lee et al., 2004; Hammond et al., 2009]. The controlled transition of a kinesin monomeric to dimeric state might regulate the activity of such kinesins in cells. Subsequently, the Kinesin-10 chromokinesins have also been shown to be functionally monomeric [Matthies et al., 2001; Shiroguchi et al., 2003]. Kid displays plus-end directed motility [Yajima et al., 2003; Stumpff et al., 2012], whereas its meiotic counterpart NOD in *Drosophila* has been proposed to lack motility [Matthies et al., 2001]. Recent work suggests that Nod may be able to track the plus ends of microtubules [Cane et al., 2013]. For non-canonical kinesins that do not display motility, oligomerization can also be important. Dimerization of the Kinesin-13 MCAK/Kif2c increases the efficiency of the microtubule depolymerase activity relative to a monomeric depolymerase domain of MCAK in vitro [Maney et al., 2001; Hertzer et al., 2006]. Overall, kinesins can form higher order structures through coiled-coil regions, such as dimers or tetramers that further define their properties.

Kinesins can also use self-interactions to regulate their activity and prevent non-productive ATP hydrolysis. Binding of non-motor regions to the kinesin ATPase

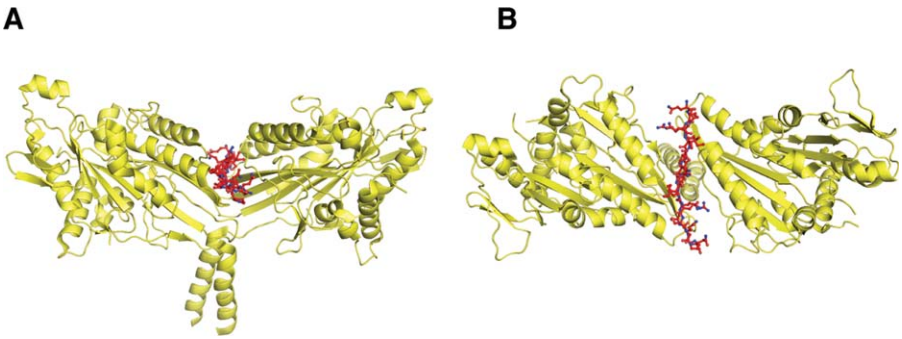

Fig. 2. Structure of Kinesin-1 bound to its tail. (**A**) Side and (**B**) top view of *Drosophila melanogaster* kinesin motor domain dimer complexed with tail domain. This is the only structure to date to show interactions of a kinesin motor domain with other regions (based on the structure published in Kaan et al. [2011], PDB: 2Y65).

domain traps the ATPase domain in an auto-inhibited state (Fig. 1B). Auto-inhibition is particularly well-documented with motors involved in the transport of cargos [Verhey and Hammond, 2009]. The most abundant kinesin, Kinesin-1, has been used as a paradigm for studies on auto-inhibition of kinesins. Kinesin-1 transports cargos throughout the cell. To avoid the unnecessary use of ATP, Kinesin-1 remains in an autoinhibited state until cargo binding occurs, which activates the motor [Cai et al., 2007]. Early electron microscopy and biophysical studies revealed that kinesin-1 adopts a folded conformation where the motor domain is in close proximity with the tail region [Hackney et al., 1991, 1992]. Full-length Kinesin-1 has only limited ATPase activity due to the tail region binding to and inhibiting the ATPase motor domains, with one tail binding to the two heads [Coy et al., 1999; Friedman and Vale, 1999; Stock et al., 1999; Hackney et al., 2009]. The inhibitory tail binds at the interface of a motor domain dimer and makes a second point of attachment between the two motors, thereby preventing movement of one motor with respect to the other (Fig. 2) [Kaan et al., 2011]. Similarly, an auto-inhibitory mechanism via the C-terminal tail of the Kinesin-7, CENP-E, and the Kinesin-2, Kif17 has been proposed to regulate their motility [Espeut et al., 2008; Hammond et al., 2010]. The inhibition of CENP-E is relieved by CDK1 or Mps1-dependent phosphorylation in the tail region. Finally, the extreme C-terminal region of MCAK is thought to influence its catalytic activity and affinity of MCAK for microtubules [Moore and Wordeman, 2004]. This auto-inhibition mechanism does not appear to be conserved across species, as the removal of the C-terminal tail of *Xenopus* MCAK results in a decrease in depolymerase activity [Hertzer et al., 2006]. Typically, the self-interaction of a non-motor region with the motor domain of the kinesin results in inhibition that is relieved by an active mechanism. This auto-inhibition mechanism represents an interesting paradigm for the temporal or spatial control of a kinesin.

Specification of Kinesin Function by Non-motor Regions

Kinesins are large molecules and contain multiple divergent domains flanking the motor ATPase domain. These regions may associate with distinct kinesin "receptors" within a cell, thereby creating specificity that targets a given kinesin to a defined subcellular location (Fig. 1C). The role of non-motor regions in specific targeting was proposed once the extent of the functional diversity within the kinesin superfamily was realized [Vale and Goldstein, 1990]. Extensive work on kinesins involved in trafficking, particularly in neurons, has provided information on how kinesin diversity of the non-motor regions is essential for targeting to specific subcellular locations [Goldstein and Yang, 2000; Hirokawa and Noda, 2008; Hirokawa et al., 2009b].

The non-motor regions of mitotic kinesins also direct the targeting of the motor to the correct subcellular location. The highly conserved microtubule cross-linking protein PRC1 (Ase1p in yeast and MAP65 in plants) is important for specifying the spindle mid-zone, a region of overlapping microtubules that determine the cleavage plane in anaphase and telophase [Jiang et al., 1998; Smertenko et al., 2000; Mollinari et al., 2002; Schuyler et al., 2003; Verbrugghe and White, 2004; Verni et al., 2004]. In addition, PRC1 crosslinks antiparallel microtubules through a spectrin-fold domain and bundles microtubules in vitro [Subramanian et al., 2010]. In cells, PRC1 acts as a receptor for multiple mitotic kinesins in the spindle mid-zone during anaphase. The N-terminus of Kif14 flanking the motor region associates with PRC1 and localizes to the mid-zone, independently of the motor domain [Gruneberg et al., 2006]. PRC1 also associates with the C-terminal region of the chromokinesin Kif4a to control the length of the mid-zone microtubules [Kurasawa et al., 2004; Bieling et al., 2010b; Subramanian et al., 2010]. The Kif4a/PRC1 complex accumulates at microtubule ends in a microtubule-length dependent manner [Subramanian et al., 2013]. Phosphorylation of PRC1 by Cdk1/cyclinB prevents its association

with Kif4a until anaphase. Regulation of the PRC1-Kif4a interaction thus provides a spatial and temporal context to restrict this interaction until after the metaphase-anaphase transition [Zhu and Jiang, 2005; Zhu et al., 2006]. PRC1 also associates with additional kinesins such as MKLP1/Kif23 and MKLP2/Kif20 to target them to the central spindle and mid-zone during the late stages of mitosis [Verni et al. 2004; Gruneberg et al., 2006; Bassi et al., 2013]. Disruption of PRC1 localization leads to altered localization of these kinesins and multiple defects in cytokinesis. Therefore PRC1 acts as a receptor to recruit mitotic kinesins to the mid-zone and allow correct cytokinesis.

Within a kinesin family, the divergent N-termini can provide diversity in targeting the conserved catalytic activity. When expressed alone, the motor domains of the kinesins-13 family proteins localize uniformly throughout the mitotic spindle [Welburn and Cheeseman, 2012]. However, in the context of the full-length protein, each of the three human family members (Kif2a, Kif2b, and Kif2c/MCAK) displays a distinct localization within the cell where they play different functions (Figs. 3A and 3B). The divergent non-motor regions provide targeting specificity. The N-terminus of MCAK/Kif2c directs the kinesin to centromeres [Maney et al., 1998; Walczak et al., 2002]. At kinetochores, MCAK associates with Sgo2 in an Aurora B dependent manner to promote kinetochore alignment [Tanno et al., 2010; Rivera et al., 2012]. The N-terminus of Kif2a is also responsible for targeting the Kif2a microtubule depolymerase activity to the centrosome, but its interaction partners that target Kif2a to centrosomes are unknown [Welburn and Cheeseman, 2012]. Interestingly, the Kinesin-13 member Kif24 utilizes its motor domain to target to the centrosome [Kobayashi et al., 2011]. Thus comparative analysis highlights the differences between closely related proteins within a kinesin family and identifies both intrinsic and extrinsic factors that contribute to creating kinesin specificity and targeting.

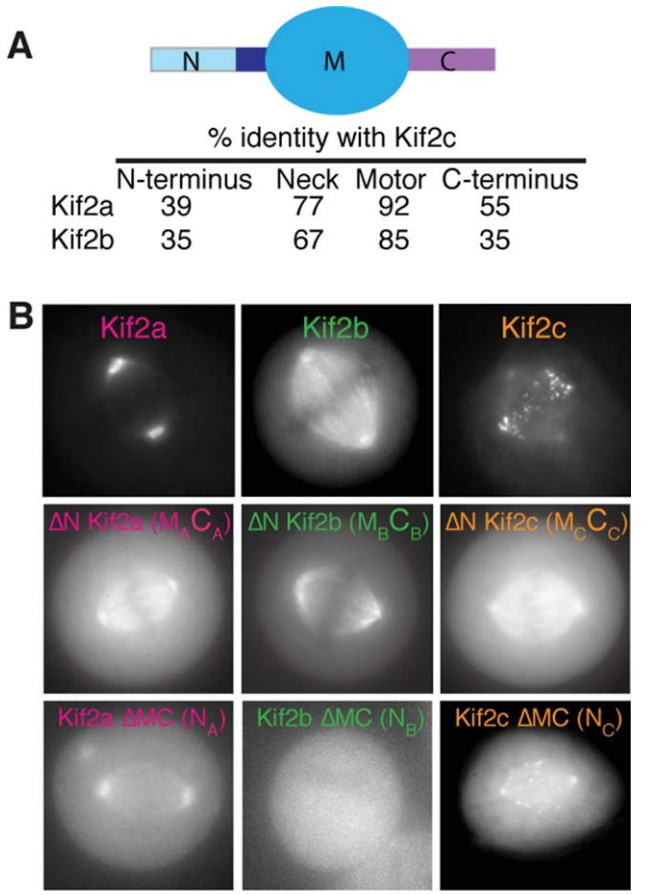

Fig. 3. The N-terminus of Kinesin-13 is a primary determinant of kinesin localization. (**A**) Schematic diagram showing the Kinesin-13 domains and their percentage similarity with respect to Kif2c/MCAK. (**B**) Images of HeLa cells transiently expressing GFP fusions to full length and domains of Kif2a, Kif2b, and Kif2c. The ΔN and ΔMC domains represent GFP-fusions lacking the N-terminus or the motor and C-terminal domains of the kinesins, respectively. Adapted from Welburn and Cheeseman [2012].

Microtubule Interactions with Kinesin Non-motor Regions

Since the discovery of kinesins, multiple studies have identified a second microtubule binding site in kinesins in addition to the canonical motor domain that is essential for the correct function and targeting of the kinesin (Fig. 1D). The yeast Kar3 was the first motor proposed to have an N-terminus that can bind independently to microtubules, based on localization studies [Meluh and Rose, 1990]. The presence of a second nucleotide-insensitive microtubule domain has also been identified in Kinesin-1, -7, -8, -10, and -14 [Navone et al., 1992; Yen et al., 1992; Karabay and Walker, 1999; Shiroguchi et al., 2003; Wendt et al., 2003; Seeger and Rice, 2010; Moua et al., 2011]. The microtubule binding properties of the non-motor regions differ from those of the motor domains such that they are likely to provide a second low affinity binding site for the kinesin to increase its affinity for microtubules. I will focus here on the second microtubule binding domains of three families: (1) Kinesin-14, which are minus end directed motors and crosslink parallel microtubules, (2) Kinesin-5, which crosslinks anti-parallel microtubules, and (3) Kinesin-8, which depolymerizes and/or regulates microtubule plus ends.

Early biochemical work on the Kinesin-14 Ncd showed that this minus end directed motor had a second microtubule-binding site, independent of its C-terminal motor domain. The N-terminal domain can bind to and bundle microtubules in vitro [Chandra et al., 1993]. In fact, the N-terminal 200 amino acid tail contains two separate microtubule binding sites that are ATP-insensitive [Karabay and Walker, 1999; Wendt et al., 2003]. The mammalian, *S. cerevisiae,* and *S. pombe* Kinesin-14 counterparts HSET, Kar3, and Klp2 also have N-terminal microtubule binding domains in addition to the C-terminal motor

domain [Meluh and Rose, 1990; Ando et al., 1994; Kuriyama et al., 1995; Braun et al., 2009]. Kinesin-14 family members possess two microtubule-binding sites at the N- and C-terminus of the polypeptide, which function in focusing microtubules at the spindle poles. The current working model for Kinesin-14 function is that the two microtubule binding sites enable parallel crosslinking of microtubules and pole focusing [Mountain et al., 1999; Goshima and Vale, 2003; Goshima et al., 2005a]. Indeed, recent work in vitro demonstrated that *S. pombe* Klp2 and Drosophila Ncd are sufficient to crosslink parallel microtubules [Braun et al., 2009; Fink et al., 2009]. It remains to be determined how Kinesin-14 functions in reconstituted assays in presence of Eg5 and other microtubule crosslinkers, to examine the contribution of Kinesin-14 to spindle formation, and define the mechanistic basis for their ability to preferentially crosslink parallel microtubules rather than microtubules of mixed polarity.

The function of a second microtubule-binding region in *Xenopus* Kinesin-5 (the functional homologue of Eg5 in humans) has also been well characterized. Kinesin-5 possesses a second microtubule-binding region downstream of the motor domain that is essential for Kinesin-5 microtubule crosslinking function and kinesin processivity. This activity is required even though Kinesin-5 forms tetrameric assemblies that already contain multiple microtubule binding sites [Weinger et al., 2011]. The second binding site does not alter the motility of the Kinesin-5 assembly, but instead increases its processivity. Collectively, the non-motor regions of Kinesin-5 associate with microtubules to maintain kinesin association with both antiparallel microtubules, despite stochastic dissociation of the Kinesin-5 motor heads from microtubules. This ensures robust sliding of antiparallel microtubules with respect to each other.

The Kinesin-8 family also uses a non ATP-sensitive microtubule-binding site for its function. In humans, Kif18a has been implicated in chromosome alignment [Mayr et al., 2007; Stumpff et al., 2008; Savoian and Glover, 2010], whereas Kif18b regulates the dynamics of astral microtubules, particularly during prometaphase [Stout et al., 2011; Tanenbaum et al., 2011]. The motor domains of Kif18b and Kif18a are highly conserved, whereas their C-terminal regions are more divergent. Kinesin-8 function is conserved across eukaryotes including yeast (Kip3, Klp5/6) and *Drosophila* (Klp67), despite some species-specific differences. The C-terminus of Kif18a homologues is essential for kinesin targeting across species [Savoian and Glover, 2010]. Recent studies have shown that the C-terminal region of Kif18a and Kip3 contains a microtubule-binding domain that is distinct from the motor domain [Mayr et al., 2011; Stumpff et al., 2011; Su et al., 2011; Weaver et al., 2011]. This microtubule-binding site diffuses along the microtubule lattice and increases the dwell time of the motor on microtubules, which thereby increases the processivity of the kinesin. This allows Kinesin-8 to target to the end of microtubules. In addition, the Kinesin-8 C-terminal tail modulates its catalytic activity. In yeast, Kip3 can act as both a microtubule polymerase and a depolymerase, while the human Kif18a motor domain acts as a capping factor in vitro at the microtubule plus ends to stabilize microtubules [Du et al., 2010; Stumpff et al., 2011; Su et al., 2011; Weaver et al., 2011]. Interestingly, in yeast this non-motor microtubule-binding domain interacts both with both tubulin dimers and the microtubule lattice. Thus, to stabilize the plus ends of microtubules, Kip3 may act on tubulin conformations found at plus ends of microtubules to straighten the protofilaments by cross-linking adjacent tubulin dimers [Su et al., 2011]. Kip3 may also locally increase the free tubulin concentration, thereby promoting microtubule polymerization, similarly to plus end binding proteins such as TOG and CLASP [Al-Bassam and Chang, 2011]. This second microtubule-binding site modulates the functional properties of Kinesin-8 motors, which results in differential localization and function. In total, it appears that a second microtubule binding region that is distinct from the motor domain of a kinesin contributes a pivotal role to the functional properties of a motor without interfering with its processivity and is widely used by kinesins.

Kinesin-Associated Proteins

In addition to the intrinsic properties and interactions of kinesin proteins, extrinsic associations with interacting proteins are critical for kinesin function. Recent targeted and broad-scale proteomic studies have analyzed the interactions of kinesin proteins to identify associated factors [Hutchins et al., 2010; Maliga et al., 2013]. Although many of these interactions remain to be fully characterized, it appears that such kinesin interactors play diverse roles in modulating kinesin function and localization. Here, I will discuss how kinesin associated proteins have defined properties to generate kinesin protein complexes with unique functions (Supporting Information Table S1).

Kinesins and Microtubule Associated Proteins

Although kinesin motors have been extensively studied in isolation, multiple kinesins associate with additional proteins for correct function (Fig. 1E). In some cases, these extrinsic associated factors display their own microtubule binding activity, which then modify the function of the kinesin.

A subset of microtubule-associated proteins enhances the targeting of multiple kinesins to microtubules. The spindle assembly factor Tpx2 associates with microtubules as the spindle assembles [Wittmann et al., 2000]. Tpx2 was first identified as a Targeting protein for Xklp2 in *Xenopus* [Wittmann et al., 1998]. Kif15, the human counterpart of Xklp2 essential for spindle bipolar maintenance, also localizes to the spindle in a Tpx2 manner [Tanenbaum et al.,

2009; Vanneste et al., 2009]. In addition, the C-terminus of Tpx2 interacts with Eg5 to promote spindle bipolarity and K-fiber assembly [Eckerdt et al., 2008; Ma et al., 2010]. Interestingly, despite the microtubule cross-linking properties of the Eg5 tetramer, Tpx2 is required to localize Eg5 to the spindle microtubules and this interaction is essential for correct spindle formation [Ma et al., 2011]. Tpx2 is a large protein that acts as a platform to recruit Eg5 and Kif15 through distinct binding sites. This potentially allows Tpx2 to recruit them simultaneously to ensure robust spindle elongation and maintenance.

In the Kinesin-10 family, the chromokinesin Kid localizes to chromosomes and contributes to chromosome alignment. Kid shows microtubule plus end directed motility and contributes to polar ejection forces [Yajima et al., 2003; Stumpff et al., 2012; Wandke et al., 2012]. However, to localize to the spindle, Kid requires its interacting partner CHICA [Santamaria et al., 2008]. CHICA displays microtubule binding activity in vitro and localizes to spindles independently of Kid. In absence of CHICA, Kid does not target to spindles, but to date, this does not impair spindle dynamics and mitotic progression.

Other kinesins also associate with proteins that possess their own microtubule-binding domain to target to microtubules in different ways. For example, within the Kinesin-13 family of microtubule depolymerases, Kif2b and Kif2c/MCAK both associate with distinct microtubule-associated proteins. MCAK/Kif2c associates with the "end binding" (EB) family of proteins, which bind specifically to the plus ends of growing microtubules [Mennella et al., 2005; Lee et al., 2008]. MCAK binds to EB1 through a SXIP binding motif [Honnappa et al., 2009], and plus end binding is further enhanced through interactions with Tip150 and Kif18b [Jiang et al., 2009; Stout et al., 2011; Tanenbaum et al., 2011]. Both the motor domain and the MCAK-interacting domains of Kif18b are required for robust depolymerization, suggesting that Kif18b enhances MCAK activity at microtubule plus ends, rather than have intrinsic depolymerase activity [Tanenbaum et al., 2011]. In vitro MCAK enrichment at the plus ends does not prevent microtubule growth under these conditions, but increases the number of catastrophes, making microtubules more dynamic [Montenegro Gouveia et al., 2010]. It is possible that the in vitro system is lacking an MCAK activation partner such as Kif18b to promote depolymerization. In total, this plus end targeting mechanism provides MCAK with unique functional properties to control microtubule dynamics.

Despite the presence of a conserved catalytic core, the Kinesin-13 family member Kif2b, is regulated differently. Kif2b associates with the microtubule binding protein Cep170 and a related protein Cep170R. The Cep170 microtubule binding activity provides a second high-affinity microtubule-binding site for Kif2b and facilitates Kif2b targeting to the mitotic spindle [Welburn and Cheeseman, 2012]. Interestingly, Cep170 also interacts with Kifc3 and facilitates Kifc3 targeting to microtubules. Cep170 may also interact with Kif2a and Kif2c [Maliga et al., 2013], but the nature of these interactions is unclear. Overall, Cep170 may act as a general kinesin interactor, similarly to Tpx2, to recruit and coordinate several motor activities on the spindle [Hutchins et al., 2010; Welburn and Cheeseman, 2012; Maliga et al., 2013]. Kif2c/MCAK and Kif2a also associate with the microtubule-associated protein ICIS in *Xenopus* extracts, which stimulates their activity at the inner centromere [Ohi et al., 2003; Knowlton et al., 2009]. Thus, extrinsic factors that control Kinesin-13 specificity are unique to each kinesin, thereby creating functional diversity within a highly conserved kinesin family.

Although the proteins described above direct the localization of kinesins to microtubule structures throughout mitosis, some microtubule-associated proteins are important in recruiting kinesins to substructures, such as kinetochores, centrosomes, or the spindle mid-zone. At kinetochores, Clasp1 recruits multiple kinesins such as CENP-E and Kif2b during prometaphase [Hannak and Heald, 2006; Maffini et al., 2009; Manning et al., 2010]. The microtubule binding protein PRC1 recruits Kif14, MKLP1/Kif23/Centralspindlin, MKLP2/Kif20 and Kif14 at the central spindle and the mid-zone during the late stages of mitosis, as described previously [Kurasawa et al., 2004; Gruneberg et al., 2006; Bassi et al., 2013]. Such kinesin-interacting proteins therefore create a temporal and spatial switch for kinesin recruitment and activity to a particular structure.

Interestingly, the yeast minus-end directed motor Kar3 complex can form a heterodimer with two alternative partners Cik1 or Vik1 that have a motor-like domain, but not nucleotide-binding properties [Manning et al., 1999; Allingham et al., 2007]. The presence of the second non-catalytic microtubule-binding site increases the affinity of Kar3 for microtubules and binding becomes cooperative. Association of Kar3 with either Cik1 or Vik1 creates distinct kinesin complexes with different biochemical properties, thereby increasing functional diversity of the Kar3 motor complex [Page et al., 1994; Manning et al., 1999; Barrett et al., 2000]. Overall, in vitro and in vivo approaches have provided a molecular understanding for how extrinsic kinesin associated proteins can facilitate timely kinesin targeting to specific locations and modify the properties of the kinesin motor to enhance its affinity for microtubules.

Kinesin Interactions with Chromatin and Regulation by Nuclear Import

Chromokinesin Interactions with DNA

Chromokinesins associate with both chromosomal DNA and spindle microtubules during mitosis and are implicated in various functions during mitosis (Fig. 1F). In metazoans,

there are three families of chromokinesins (Kinesin-4, -10, and -12) that are important for bipolar spindle formation, chromosome condensation, chromosome alignment, and chromosome segregation [Mazumdar and Misteli, 2005; Vanneste et al., 2011]. The Kinesin-12 family chromokinesin Kif15 is important for maintaining bipolar spindle assembly, particularly in absence of Eg5 [Tanenbaum et al., 2009; Vanneste et al., 2009]. The *Xenopus* homologue Xklp2 is also important for spindle maintenance, but it localizes to centrosomes, rather than chromosomes [Boleti et al., 1996]. Within the Kinesin-10 family, the *Drosophila* NOD plays a role in meiosis [Afshar et al., 1995; Matthies et al., 2001; Cochran et al., 2009]. Kid (Kinesin-like DNA binding protein) is present in mitotic cells, where it contributes to chromosome alignment [Tokai et al., 1996; Levesque and Compton 2001; Levesque et al., 2003]. Its *Xenopus* counterpart xKid (also termed Kif22) plays a role in chromosome alignment during mitosis [Antonio et al., 2000; Funabiki and Murray, 2000]. Both Kinesin-4 and the Kinesin-10 have been proposed to cooperate positively to align chromosomes [Brouhard and Hunt, 2005; Bieling et al., 2010a]. In *Drosophila*, Klp3 and NOD also synergize their activities to align chromosomes [Goshima and Vale, 2003]. Kinesin-10/Kid is the major contributor to polar ejection force for chromosomes [Brouhard and Hunt, 2005; Bieling et al., 2010a; Wandke et al., 2012; Cane et al., 2013]. Kinesin-4 inhibits microtubule dynamics and promotes pausing of the plus ends by altering the structure of the microtubule lattice [Bringmann et al., 2004; Stumpff et al., 2012]. However, recent studies in cells have challenged the view that the chromokinesins Kid and Kif4a work cooperatively, and have instead suggested that Kif4a and Kid play antagonistic roles for chromosome oscillations in human cells [Stumpff et al., 2012]. Kif4a has also been implicated in chromosome condensation and cytokinesis [Kurasawa et al., 2004; Mazumdar et al., 2004]. Its role in mitotic chromosome structure is dependent on the presence of the Kif4a motor domain [Samejima et al., 2012], although this function also likely depends on its chromatin-binding properties. Kid is also important for chromosome compaction during the later stages of mitosis [Ohsugi et al., 2008]. Taken together, each of the chromokinesin families performs unique functions in mitosis.

Despite the fact that the loading of each chromokinesin family onto chromosomes occurs at the onset of mitosis, each chromokinesin family has distinct DNA-targeting domains that create molecular specificity. Based on sequence analysis, NOD and Kid have a DNA binding helix–hairpin–helix (HhH) domain that binds to chromatin [Antonio et al., 2000; Yajima et al., 2003]. The Kinesin-4 family member Kif4 was first proposed to regulate vesicular trafficking in mice [Sekine et al., 1994]. Human Kif4a was then observed on chromosomes and proposed to be a chromokinesin [Lee et al., 2001]. Kif4a requires a basic zipper motif and a Cysteine-rich motif at its C-terminus to target to chromatin [Wu and Chen, 2008]. In addition, Kif4a associates with the chromosome condensation factors Condensin I and II to target to chromosomes [Samejima et al., 2012]. The Kif4a chromosome targeting mechanism therefore relies on additional chromatin-associated proteins and is tightly regulated to allow Kif4a relocalization to the midzone during the later stages of mitosis [Zhu and Jiang, 2005; Zhu et al., 2006]. Overall, chromokinesins utilize distinct DNA binding properties and unique protein associations that enhance their timely targeting to chromosomes.

Kinesin Interactions with Nuclear Import Machinery

The loading of chromokinesins onto chromosomes is also dependent upon chromosome-derived Ran-GTP signals, highlighting that the association of chromokinesins with DNA is controlled by both intrinsic and extrinsic factors [Levesque and Compton, 2001; Mazumdar et al., 2004; Vanneste et al., 2009]. Ran-GTP present in the vicinity of chromosomes disrupts the Kid-Importin α/β interaction, allowing Kid loading onto chromosomes [Tahara et al., 2008]. The Kinesin-12 member Kif15 associates with the chromatin factor KI-67 to load onto chromatin [Vanneste et al., 2009], and relies on Tpx2 (Targeting Protein for XKpl2) for its spindle targeting [Tanenbaum et al., 2009]. Interestingly, Tpx2 is itself tightly regulated by the Ran-GTP gradient and is critical for spindle assembly [Wittmann et al., 1998, 2000; Gruss et al., 2001]. Other kinesins may also be influenced by the chromosome-derived Ran-GTP gradient. The Kinesin-8 Kif18a has been reported recently to interact with HURP, a K-fiber associated protein important for spindle assembly [Ye et al., 2011]. HURP localization to the spindle is Ran-dependent [Koffa et al., 2006; Sillje et al., 2006]. It remains to be determined whether disruption of the Ran-GTP gradient would alter Kif18a localization through HURP mislocalization. Although the Kinesin-8 family has been reported to walk along microtubules and accumulates at the plus end, Kif18a selectively targets to K-fiber microtubules within the spindle microtubules. The Ran-dependent HURP association with Kif18a could explain this targeting specificity. However, in taxol-treated cells, Kif18a targets non-specifically to all microtubules. Thus, Kif18a's specific localization to K-fibers is at least partly due to their stability relative to non-kinetochore microtubules [Masuda et al., 2011; Stumpff et al., 2011]. In conclusion, the Ran gradient plays a role supporting spindle formation and chromosome alignment. The Ran pathway regulates a number of mitotic kinesins to achieve correct chromosome biorientation and spindle dynamics.

Interactions of Kinesins with Other Proteins to Modulate their Activity

Kinesins can associate with microtubule associated proteins or DNA, as described above. In addition, there are

examples of kinesins associating with proteins that have catalytic properties to provide additional and new functions to kinesin complex. A well-studied example is the centralspindlin complex, which has two key enzymatic activities. Centralspindlin is composed of the Kinesin-6 family member MKLP1 and a kinesin-associated protein that has a RhoGTPase activating protein domain (RacGAP1). The *C. elegans* centralspindlin counterpart ZEN-4-CYK4 is particularly well studied biochemically and structurally, revealing that the formation of a heterodimeric stable complex is essential for its function [Jantsch-Plunger et al., 2000; Mishima et al., 2002; Pavicic-Kaltenbrunner et al., 2007]. As a result, the centralspindlin complex has multiple activities conferred by its different properties. Centralspindlin positions the spindle division plane, clusters microtubules in the mid-zone, regulates the mid-zone, and contributes to abscission by controlling Rho family GTPases [White and Glotzer, 2012]. Thus, association of a kinesin with a protein bearing enzymatic activity provides the kinesin complex with new catalytic functions to perform accurate mitosis.

Mitotic Kinesins as Recruitment Hubs

Molecular motors can generate force to assemble and maintain the mitotic spindle and align the chromosomes. However, a subset of kinesins also plays a role in recruiting molecules to cellular structures such as kinetochores in mitosis. For example, kinesins have been reported to interact and recruit signaling and checkpoint proteins [Hutchins et al., 2010; Kim et al., 2010; Meadows et al., 2011]. Mitotic motors including the Kinesin-7 CENP-E and Kinesin-8 Klp5/6 have a PP1 phosphatase binding motifs. Current models propose that these motors act as platforms at the kinetochore to recruit the checkpoint silencing phosphatase PP1, which is essential for bi-orientation and stabilization of kinetochore-microtubule attachments [Kim et al., 2010; Meadows et al., 2011]. Both Kinesin-8 and CENP-E localize to kinetochores during prometaphase. CENP-E leaves the kinetochore as chromosomes become biorientated [Yen et al., 1991, 1992]. CENP-E and Kinesin-8 could therefore recruit PP1 to kinetochores as microtubule attachments are being stabilized. At stable end-on attachments, KNL-1, the primary PP1 receptor at kinetochores is spatially separated from Aurora B at the inner centromere and dephosphorylated, thereby creating additional PP1 binding sites [Liu et al., 2010]. In fission yeast, both Klp5/6 and Spc7/Knl-1 are necessary to silence the checkpoint [Meadows et al., 2011]. In addition, the recruitment of a phosphatase such as PP1 to kinetochore-targeted motors can fine-tune the activity of these motors by opposing Aurora B and Plk1 activity as tension is established [Liu et al., 2010]. At kinetochores, CENP-E has also been proposed to act as a "cyclin" for BubR1 kinase activity, [Mao et al., 2003; Weaver et al., 2003; Mao et al., 2005] although whether BubR1 has kinase activity that is required for mitosis is debated [Suijkerbuijk et al., 2012; Elowe, 2011]. In total, processive kinesins may use their unique non-motor domains to act as an interaction platform and recruit molecules to a particular structure to drive mitotic progression.

Regulation of Interactions by Post-translational Modifications

In addition to associating with other proteins, kinesin activity is controlled by post-translational modifications (Fig. 1G). The most extensively studied post-translational modification that affects mitotic motors is phosphorylation. A large subset of kinesins only associate with microtubules in mitosis. These microtubule-kinesin associations coincide with a dramatic increase in protein phosphorylation that occurs upon mitotic entry [Dephoure et al., 2008]. In some cases, mitotic kinesins are retained in the nucleus in interphase by their nuclear localization signal preventing their association with the cytoplasmic microtubule cytoskeleton, such as Kid and Kif18a [Tokai et al., 1996; Du et al., 2010]. However, other kinesins that play roles in mitosis are present in the cytoplasm during interphase and do not bind to microtubules [Houliston et al., 1994; Vanneste et al., 2009]. Often, phosphorylation directly controls the ability of motors to associate with microtubules [Syred et al., 2013]. Based on large-scale phospho-proteomic analyses, the motor domain of kinesins is rarely post-translationally modified. In contrast, the non-motor regions are often phosphorylated, which is key for differentially regulating the members of a kinesin family. Recent work has provided molecular insights into the role of phosphorylation on mitotic motor function and regulation for a small number of kinesins as a rapidly reversible way to fine-tune molecular motors.

Phosphorylation May Regulate Kinesin Affinity for Microtubules

Phosphorylation may dramatically modify the electrostatic surface properties of the kinesin to increase its overall negative charge. As a result, phosphorylation can reduce the affinity of a kinesin for microtubules through electrostatic interference with the acidic, negatively charged tubulin lattice. Both motile and depolymerizing kinesins use electrostatic complementarity to facilitate directional and processive movement on the lattice [Thorn et al., 2000]. Kinesins have a positively charged neck region that interacts with the microtubule to promote kinesin diffusion on the lattice through electrostatic interactions [Thorn et al., 2000; Niederstrasser et al., 2002; Ovechkina et al., 2002]. Addition of negative charges in the kinesin neck reduces the run length of conventional kinesin [Thorn et al., 2000]. The positively charged neck region of the Kinesin-13

MCAK/Kif2c is critical for depolymerase activity [Cooper et al., 2010]. Phosphorylation of the neck region (Ser192 MCAK in humans, S196 in *Xenopus*) by Aurora B reduces its depolymerase activity [Andrews et al., 2004; Lan et al., 2004; Ohi et al., 2004]. The negative charge may neutralize the positively charged neck region to ultimately reduce kinesin binding to the microtubule and reduce depolymerase activity. Thus, regulation by phosphorylation of the neck region modulates the activity of kinesins dramatically.

CENP-E phosphorylation of a region close to the neck region on T422 is critical for mitosis [Kim et al., 2010]. Both Aurora A and B phosphorylate this residue to reduce the affinity of the motor for microtubules and its processivity. Aurora A-phosphorylated CENP-E can rapidly search and capture of chromosomes close to the poles, due to its lower affinity for microtubules which allows for greater sampling. As CENP-E moves the chromosomes along existing K-fibers to the equator of the cell, CENP-E is dephosphorylated by its binding partner PP1, which increases the processivity of the motor and allows chromosome alignment. Thus, CENP-E phosphorylation and binding to a phosphatase allows the fine-tuning the activity of the motor during chromosome congression and alignment. Thus phosphorylation of regions that interact with microtubules may also directly affect the processive properties of the kinesin.

Surprisingly, phosphorylation of a kinesin in a non-motor region can also increase its affinity for microtubules, through a yet unknown mechanism. For example, phosphorylation of the Kinesin-5 Eg5 by Cdk1 in its C-terminal region (T927 in humans, T937 in *Xenopus*) increases its affinity for microtubules in vitro [Cahu et al., 2008]. This phosphorylation site is present in a highly conserved region known as the BimC box and is necessary for timely targeting of Eg5 to the spindle [Heck et al., 1993; Blangy et al., 1995; Sawin and Mitchison, 1995] to ensure that spindle bipolar assembly occurs robustly at the onset of mitosis. However, this regulatory site is not functionally conserved throughout species. Mutation of the BimC region and this phosphorylation site in the fission yeast Eg5 homologue Cut7 does not impair spindle formation and mitosis [Drummond and Hagan, 1998]. Interestingly, this region also contains the second microtubule binding site in Eg5 and is essential for its correct function [Weinger et al., 2011]. Thus phosphorylation provides a controlled way to increase the activity and targeting of Eg5 specifically at the onset of mitosis. How phosphorylation contributes mechanistically to increasing the affinity of Eg5 is still not well understood.

Phosphorylation May Disrupt Protein–Protein Interactions

The addition of a phosphate group to an acceptor site dramatically changes the properties of that amino acid. Phosphorylation can disrupt protein–protein interactions through electrostatic repulsion or steric hindrance following the addition of a phosphate group. In this way, the targeting of Kif2c/MCAK to microtubule plus ends can be selectively abolished by phosphorylation of its N-terminal targeting domain. An SXIP motif in the N-terminal region of MCAK supports its interaction with EBs [Honnappa et al., 2009], and targets MCAK to the plus ends of microtubules. The region close to this EB-binding motif is phosphorylated by Aurora B [Andrews et al., 2004; Lan et al., 2004], which disrupts the MCAK-EB interaction [Honnappa et al., 2009]. Consequently, phosphorylation prevents MCAK localization to microtubule plus ends in the vicinity of chromosome-bound Aurora B, spatially restricting MCAK depolymerase activity [Moore et al., 2005; Tanenbaum and Medema 2011]. Aurora B phosphorylation of MCAK also weakens the MCAK–Kif18b interaction, although it is not clear if Kif18b associates with MCAK or a MCAK–EB1 complex to target to the plus ends [Tanenbaum et al., 2011]. Overall, Aurora B phosphorylation of MCAK reduces the presence of both MCAK and Kif18b destabilizing enzymes at the plus ends of microtubules by abolishing protein–protein interactions.

Phosphorylation Can Inhibit Intramolecular Interactions

To control the activity of kinesins in a rapid and reversible manner, phosphorylation can act to control kinesin conformation. For example, the mitotic Kinesin-7 CENP-E adopts a self-inhibitory conformation with the C-terminal tail folding onto the motor region to inhibit its ATPase activity. The C-terminal tail binds to the motor region to abolish motility. This inhibition is alleviated by CDK and Mps1-dependent phosphorylation [Espeut et al., 2008]. Phosphorylation of the non-motor regions of MCAK might also regulate intramolecular interactions [Zhang et al., 2011].

Phosphorylation to Create Protein–Protein Interactions

Phosphorylation can create binding motifs for phospho-binding proteins such as FHA domains and 14-3-3 proteins. The phospho-binding domain can then promote kinesin targeting and dimerization or sequester the phospho-protein to alter its function. For example, Kif23/MKLP1 activity is controlled by its phosphorylation and association with 14-3-3 [Mishima et al., 2004; Douglas et al., 2010]. MKLP1/Kif23 (part of the Centralspindlin complex) plays a role at the spindle mid-zone to complete cytokinesis. Phosphorylation in the C-terminus of MKLP1/Kif23 creates a binding site for 14-3-3. 14-3-3 then sequesters Centralspindlin and prevents its aberrant clustering, but also reduces the affinity of Centralspindlin for microtubules. Importantly, this interaction is further regulated by the spatially restricted Aurora B kinase, which phosphorylates Kif23/MKLP1 on the neighboring serine at

P-2 to abolish the Kif23/MKLP1-14-3-3 interaction and to allow centralspindlin clustering at the mid-zone where Aurora B activity is present [Douglas et al., 2010]. This phospho-dependent 14-3-3-Kif23/MKLP1 interaction is also antagonized by ARF6 to further control the activity of the Centralspindlin complex [Joseph et al., 2012]. 14-3-3 proteins have also been reported to associate with other motors, such as KLC and Kif1c, but not in the context of mitosis [Dorner et al., 1999; Johnson et al., 2011].

Phosphorylation to Regulate Kinesin Localization

Kinesin phosphorylation may affect its localization or targeting properties. ZEN-4/MKLP1 targeting to the spindle is inhibited by CDK1/cyclinB phosphorylation [Mishima et al., 2004]. Phosphorylation on T9 and T450 reduces the affinity of the motor for microtubules. The counteracting Cdc14 phosphatase, found at the central spindle dephosphorylates MKLP1/ZEN-4 to localize MKLP1/ZEN-4 to the mid-zone. Mutations of CDK consensus sites in *Drosophila* Pav (MKLP1/ZEN4 homologue) also regulate the timely targeting of Pav to the central spindle [Goshima and Vale, 2005]. MCAK localization is also regulated both by Aurora A and Aurora B through yet unknown mechanisms. Aurora A phosphorylates MCAK in the C-terminus (S719 in *Xenopus*, equivalent to S715 in humans) and Kif2a to regulate their targeting to the spindle pole, without affecting their depolymerase activity [De Luca et al., 2006; Zhang et al., 2008; Jang et al., 2009]. In *Xenopus* egg extracts, Aurora B phosphorylation of the N-terminus through a combinatorial phosphorylation controls its targeting. For example, phosphorylation of T95 downregulates targeting to chromatin targeting while S110 phosphorylation promotes MCAK recruitment to the chromosome arms [Zhang et al., 2007]. *Xenopus* MCAK requires Aurora B phosphorylation to target to the centromere [Lan et al. 2004; Ohi et al. 2004]. MCAK is particularly recruited to merotelically attached kinetochores in an Aurora B dependent fashion, with an increase in active MCAK at kinetochores [Knowlton et al., 2006]. This would enhance the destabilization of erroneous kinetochore-microtubule attachments.

This is an exciting time for dissecting how motors are regulated, given the number of post-translational modifications for kinesins that have been identified and are unique to each member, but have not yet been characterized. Although cell biological approaches provide insights to the physiological significance of these phosphorylation events, defining mechanistically the role of phosphorylation on kinesin function requires recombinant proteins as well as biochemical and biophysical tools.

Conclusions

Unlike cytoplasmic dynein, which uses multiple accessory proteins to create functional specificity and targeting, there are many functionally distinct kinesins that co-exist within each species and work non-redundantly during mitosis. Mitotic kinesins are specific to cell division and represent strong anti-cancer targets [Rath and Kozielski, 2012]. However, redundant pathways for spindle assembly and function that exist during mitosis may allow resistance to anti-mitotic drugs [Raaijmakers et al., 2012]. Defining how kinesins work together in parallel pathways and their unique functional properties would allow researchers to evaluate what combination of drug targets would be required to kill cancer cells. Unlike kinases, the ATP binding site of kinesins does not form a closed pocket, which makes designing inhibitors to target the conserved catalytic core of kinesins a challenge. Understanding how the non-motor regions regulate kinesin activity could uncover protein–protein interactions as well as protein–ATP interactions that could be evaluated as new anti-mitotic drug targets [Welburn and Endicott, 2005].

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