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Mitotic Functions of Kinesin-5

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Abstract

In all eukaryotic cells, molecular motor proteins play essential roles in spindle assembly and function. The homotetrameric kinesin-5 motors in particular generate outward forces that establish and maintain spindle bipolarity and contribute to microtubule flux. Cell cycle dependent phosphorylation of kinesin-5 motors regulates their localization to the mitotic spindle. Analysis of live cells further shows that kinesin-5 motors are highly dynamic in the spindle. Understanding the interactions of kinesin-5 motors with microtubules and other spindle proteins is likely to broaden the documented roles of kinesin-5 motors during cell division.

Keywords

Eg5; mitosis; spindle; kinesin; dynein

Introduction

One of the first mitotic motors to be identified was the kinesin, BimC (Enos and Morris, 1990). This kinesin was identified in a temperature-sensitive fungal library screen in search of strains that were defective in cellular division at the restrictive temperature (Morris, 1976). A similar screen carried out in fission yeast identified a related kinesin, Cut7 (Hagan and Yanagida, 1990). Mutations in either of these motor proteins blocked spindle pole body separation and thus prevented the successful completion of mitosis (Enos and Morris, 1990; Hagan and Yanagida, 1990). Subsequent work has identified BimC/Cut7 orthologs in *Xenopus* (Eg5), *S. cerevisiae* (Cin8p and Kip1p), *Drosophila* (KLP61F), human (hsEg5), *C. elegans* (BMK-1), and *Arabidopsis* (AtKRP125a,b,c and AtF16L2); with the exception of *C. elegans*, the gene product plays a critical role in mitosis (Bannigan et al., 2007; Bishop et al., 2005; Blangy et al., 1995; Heck et al., 1993; Hoyt et al., 1992; Le Guellec et al., 1991; Reddy and Day, 2001; Roof et al., 1992; Sawin et al., 1992). This group of related kinesins, subsequently classified as the kinesin-5 family (Lawrence et al., 2004), localizes to spindle microtubules and structures present at spindle poles.

Structurally the kinesin-5 polypeptide consists of an N-terminal head domain, which contains the motor; an internal stalk domain, capable of forming coiled coils; and a C-terminal tail domain (Le Guellec et al., 1991). Four of these ~125kDa monomers associate to form a homotetrameric complex with motor domains positioned at each end of the tetramer's long

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axis (Blangy et al., 1995; Cole et al., 1994; Kashina et al., 1996). Such an arrangement allows kinesin-5 motors to crosslink and slide apart antiparallel microtubules, a behavior that has been directly observed *in vitro* (Kapitein et al., 2005). Eg5 is a relatively slow motor, moving at ~2-3µm/min, and has been shown to be moderately processive (Cole et al., 1994; Kwok et al., 2006; Sawin et al., 1992). This is in contrast to other mitotic motors, notably dynein, which is capable of rapid minus end-directed microtubule-based transport *in vitro* (~75µm/min) (Paschal et al., 1987), although dynein-dependent transport within the spindle is considerably slower (~6µm/min) (Heald et al., 1996; Rusan et al., 2002). The biophysical properties of Eg5 *in vitro* (Kapitein et al., 2008; Kapitein et al., 2005; Valentine and Gilbert, 2007) will not be reviewed here.

Contribution of Kinesin-5 motors to the establishment and maintenance of bipolar spindles

In every model system analyzed, with the exception of *C. elegans* (Bishop et al., 2005), spindle assembly requires kinesin-5 activity (Bannigan et al., 2007; Blangy et al., 1995; Heck et al., 1993; Hoyt et al., 1992; Le Guellec et al., 1991; Reddy and Day, 2001; Roof et al., 1992; Sawin et al., 1992). In fungi, kinesin-5 motor activity is additionally required for maintenance of a bipolar spindle prior to anaphase and elongation of the spindle during anaphase B (Hoyt, 1994). In *S. cerevisiae*, for example, previously separated spindle pole bodies collapse in response to kinesin-5 inhibition (Saunders and Hoyt, 1992).

Similar to fungi, kinesin-5 orthologs in *Xenopus* and *Drosophila* contribute to both spindle assembly and maintenance. Addition of monastrol to *Xenopus* bipolar spindles induces rapid collapse of the bipolar array into a monopole. In this system, poles move together at a rate of ~1µm/min although spindles that are 'trapped' between the coverslip and slide shortened more slowly (Kapoor et al., 2000). In *Drosophila* embryos, the kinesin-5, KLP61F, is required to maintain metaphase spindle length and to drive anaphase B spindle elongation (Sharp et al., 2000) (Sharp et al., 1999). In this system, bipolar spindles in cells injected with anti-KLP61F antibodies collapsed into monopoles at a rate of 5.7µm/min (Sharp et al., 1999). However, in *Drosophila* embryos, centrosome movement to opposite sides of the nuclear envelope (prior to NEB) proceeds even in the presence of anti-KLP61F antibodies (Sharp et al., 1999). In these prophase cells, initial centrosome separation requires cytoplasmic dynein (Robinson et al., 1999). Mechanistically, cortical dynein pulling on astral microtubules is thought to generate outward forces that drive centrosome separation (Sharp et al., 1999); similar processes may operate in mammalian cells as well (Vaisberg et al., 1993). In the *C. elegans* embryo, inhibition of BMK-1, the sole kinesin-5 in this organism, does not block mitosis. In these cells, BMK-1 functions to modulate strong cortical pulling forces, acting as a brake, thus restricting centrosome separation (Saunders et al., 2007) (Grill et al., 2003).

In cultured mammalian cells, kinesin-5 activity is required for the establishment of a bipolar spindle, but the contribution of Eg5 to maintenance of bipolar spindle morphology is somewhat less clear. In BS-C1 and HeLa cells containing bipolar spindles, addition of monastrol (Mayer et al., 1999) or anti-Eg5 antibodies, respectively, did not result in spindle collapse (Blangy et al., 1995; Kapoor et al., 2000). In LLC-Pk1 epithelial cells expressing GFP-tubulin (Rusan et al., 2001), however, addition of monastrol to metaphase spindles results in spindle shortening though not complete collapse to a monopolar phenotype (Ferenz et al., 2009). Although it is possible that different cell lines respond differentially to inhibition of Eg5, an alternative possibility is that the modest spindle shortening (~30%) apparent in LLC-Pk1 cells was overlooked in the earlier studies. Taken together, these results show that Eg5 is required to establish and maintain spindle bipolarity, but in mammalian cells additional plus-end directed motor proteins likely aid in such maintenance. These results paired with the localization of Eg5

to antiparallel microtubules also imply that kinesin-5 contributes to bipolar spindle assembly and maintenance by generating an outward force.

Contribution of kinesin-5 motors to the balance of forces in the mitotic spindle

The pioneering studies of kinesin-5 motors in fungi further demonstrated that these motors can be counteracted by opposing forces; in other words, kinesin-5 motors were capable of engaging in antagonistic relationships. The first example of such antagonistic activity was the observation that mutations in the *S. cerevisiae* minus-end directed kinesin Kar3 could partially suppress the collapsed spindle phenotype resulting from loss of Cin8p and Kip1p (Saunders and Hoyt, 1992). Similar situations have been demonstrated in additional fungal and animal systems (Mountain et al., 1999; O'Connell et al., 1993; Pidoux et al., 1996; Sharp et al., 1999). Importantly, motor proteins capable of antagonizing kinesin-5 proteins are not limited to the Kar3 type. For example, the loss or inhibition of Eg5 can be rescued through loss or inhibition of dynein (Ferenz et al., 2009; Gaglio et al., 1996; Mitchison et al., 2005; Tanenbaum et al., 2008).

Although the existence of a balance of forces in the metaphase spindle is now well established, the site, or sites, where forces are generated has not been examined, in part due to the difficulties in determining where motors are active within the live cell. To address this issue, we recently used a spindle assembly assay (Tulu et al., 2006) to test the hypothesis that antagonistic motors generate force at overlapping antiparallel microtubules. In these experiments, the initial distance between centrosomes, and hence the degree of overlap between antiparallel microtubules, is highly variable and can therefore be used to test the effects of motor protein inhibition on the outcome of spindle assembly. We predicted, and confirmed, that Eg5 inhibited cells formed bipolar not monopolar arrays when spindle assembly initiated with well-separated (>5.5 μm) centrosomes. The results of our live cell studies, paired with *in silico* modeling, support our hypothesis that Eg5-based antagonistic motor activity requires overlapping antiparallel microtubules in the mammalian mitotic spindle (Ferenz et al., 2009). Additional experiments in other model systems are needed to ascertain if this model is generally applicable.

Contribution of Kinesin-5 motors to spindle flux

Following spindle formation, microtubule marking experiments have revealed the presence of a unique form of microtubule motion, called spindle flux, that results from the coordinated addition and loss of tubulin subunits from opposite ends of spindle microtubules (Mitchison, 1989). With the exception of yeast (Maddox and Salmon, 2000), flux has been observed in all eukaryotic systems examined to date (at rates between 0.5 and 3.0 $\mu\text{m}/\text{min}$) during both metaphase and anaphase (Desai et al., 1998; Dhonukshe et al., 2006; LaFountain et al., 2004; Maddox et al., 2002; Mitchison, 1989; Mitchison and Salmon, 1992; Sawin and Mitchison, 1991; Zhai et al., 1995). Motion that is consistent with flux is also apparent in mammalian prophase cells (Ferenz and Wadsworth, 2007). Though inhibition of flux in human tissue culture cells does not prevent mitotic progression, it does drastically increase the number of lagging anaphase chromosomes (Ganem et al., 2005). This is consistent with the known relationship between poleward flux and anaphase chromosome-to-pole motion (Rogers et al., 2005; Zhai et al., 1995) and more recent data indicating that flux is responsible for the temporal synchrony of chromosome segregation (Matos et al., 2009). In human cells, flux may additionally make a contribution to centrosome separation (Toso et al., 2009).

Given the ability of kinesin-5 motors to crosslink and slide antiparallel microtubules, the presence of these motors at the spindle midzone ideally positions them to contribute to spindle flux. In favor of this possibility, inhibition of Eg5 eliminates flux in metaphase spindles

assembled in *Xenopus* egg extracts (Miyamoto et al., 2004; Shirasu-Hiza et al., 2004). In the same system, displacement of the flux depolymerase from spindle poles results in spindle elongation at rates consistent with Eg5-mediated microtubule-microtubule sliding (Gaetz and Kapoor, 2004). In contrast to these results, however, inhibition of Eg5 with monastrol in metaphase Ptk or LLC-Pk1 cells results in only a modest reduction in flux (Cameron et al., 2006; Ferenz and Wadsworth, 2007). Furthermore, flux remains operational in mammalian monopolar spindles that lack antiparallel microtubules (Cameron et al., 2006; Ferenz and Wadsworth, 2007) implying that the mechanistic contribution kinesin-5 makes to flux may vary by model system. Because centrosomes can be the site of force generation in mammalian cells (Waters et al., 1996), flux has been modeled as a feeder/chipper in which Eg5 (located at spindle poles) feeds microtubules to a depolymerase, also localized at spindle poles (Cassimeris, 2004). The differential activity of Eg5 regarding flux in *Xenopus* and mammalian systems may relate to the high proportion of antiparallel microtubules found in *Xenopus* spindles.

Unexpectedly, inhibition of Eg5 did not reduce the rate of flux in prophase cells. Instead, the frequency of poleward (P) vs away-from-the-pole (AP) motion was altered following inhibition of Eg5 (from ~54% P motion in control to ~12% in monastrol treated prophase cells) (Ferenz and Wadsworth, 2007). These results support the possibility that Eg5 additionally functions to cross-link and tether spindle microtubules (Kapitein et al., 2005). If Eg5 is the dominant cross-linker in prophase spindles, then treatment with monastrol could result in microtubules becoming untethered, leading to the increase in AP motions of microtubules driven by the action of antagonistic motors (Ferenz and Wadsworth, 2007). In contrast, other proteins may function redundantly with Eg5 to tether microtubules in metaphase cells, so loss of Eg5 function would not result in an increase in the frequency of AP motion (Ferenz and Wadsworth, 2007).

Mitotic localization and regulation of kinesin-5 motors

Kinesin-5 proteins localize to spindle microtubules, with an enrichment at centrosomes or spindle pole bodies, but are not detectable on astral microtubules (Figure 1). Although the spindle localization is consistent with a mitotic function, the concentration of the protein at spindle poles rather than at regions of microtubule overlap is somewhat unexpected. However, this observation is consistent with the possibility that kinesin-5 functions on both parallel, and antiparallel microtubules (Kapitein et al., 2005; van den Wildenberg et al., 2008). This possibility is supported by observations showing that inhibition of kinesin-5 leads to disruption of centrosomes and spindle poles (Groen et al., 2008).

During interphase, Eg5 is diffusely cytoplasmic. What is the cue that transitions Eg5 to its mitotic location? Kinesin-5 family members were originally referred to as BimC motors because sequence comparisons showed similarity between BimC and its orthologs in the motor region and in a conserved region in the C-terminal tail, referred to as the 'BimC box', which contains a consensus site for phosphorylation by Cdk1 (Blangy et al., 1995). Mutation of a conserved threonine to alanine within the BimC box prevents motor localization to the spindle, demonstrating that the association of Eg5 with spindle microtubules is regulated by cell-cycle dependent phosphorylation (Blangy et al., 1995; Cahu et al., 2008; Sawin and Mitchison, 1995). In addition to Cdk1, recent work shows that the NIMA-family kinase, Nek6, phosphorylates a small fraction of Eg5 and rescue experiments show that this phosphorylation is important for the mitotic function of Eg5 (Rapley et al 2008). Finally, in *C. elegans*, the Eg5 ortholog BMK-1 (which lacks the conserved threonine in the BimC box) interacts with and is phosphorylated by the aurora B kinase, AIR-2, in the C-terminal tail domain and this interaction is important for the localization of BMK-1 to spindle microtubules (Bishop et al., 2005).

Dynamics of kinesin-5 motors

Although genetic and biochemical approaches have provided important information about the mitotic functions of molecular motors, an ultimate goal is to visualize the dynamic behavior and interactions of spindle components in live cells. Several recent studies have provided such information for kinesin-5 motors in live cells. In yeast, which have an intranuclear spindle composed of relatively few microtubules, Cin8p contributes to chromosome congression by stimulating catastrophe transitions at microtubule plus ends (Gardner et al., 2008). In *Xenopus*, photoactivation studies show that Eg5 is static in the spindle midzone, consistent with a role for the motor in sliding antiparallel microtubules (Uteng et al., 2008). However, in the half-spindle, Eg5 was observed to move poleward in a dynein-dependent manner (Uteng et al., 2008). In *Drosophila* embryos, fluorescence recovery after photobleaching and fluorescence speckle microscopy have shown that KLP61F-GFP turns-over very rapidly, both near the equator and poles, and can undergo short poleward runs in the half-spindle (Cheerambathur et al., 2008). The dynamic behavior of KLP61F is similar to the rapid turnover of microtubules in these cells and support a model in which kinesin-5 motors can both crosslink and slide antiparallel microtubules (Cheerambathur et al., 2008).

The dynamic behavior of kinesin-5 motors is likely regulated by interactions with microtubules and with other spindle components. Eg5 has been shown to bind to the p150 subunit of dynactin, to the spindle assembly factor TPX2 and is a component of the HURP complex (Blangy et al., 1997; Eckerdt et al., 2008; Koffa et al., 2006). How might these interactions contribute to Eg5 function? One possibility is that binding of Eg5 to two antiparallel microtubules in the spindle midzone may restrict interactions with other spindle components. Conversely, an interaction of Eg5 with the p150 subunit of dynactin in the half spindle could promote poleward transport of Eg5 that was not actively generating outward forces (Uteng et al., 2008). Similarly, an interaction between Eg5 and TPX2 (which is enriched toward spindle poles) may promote the interaction of Eg5 with parallel microtubules (Figure 2). The phosphorylation state of Eg5, and its binding partners, are likely to contribute to the regulation of these interactions. Additional experiments in live cells throughout mitosis are necessary to understand the regulation of Eg5 in mitotic cells.

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Figure1.

Localization and activity of Eg5 in mammalian cells. (A) Indirect immunofluorescence of an LLC-Pk1 cell stained with antibodies to tubulin (MTs) and Eg5. Notice that Eg5 is enriched near spindle poles and excluded from astral microtubules. (B) Image of a living LLC-Pk1 cell expressing Eg5-EGFP. Bars = 10 μ m. (C) Model showing Eg5 activity in the mitotic spindle. Static Eg5 at the spindle equator (yellow) crosslinks antiparallel microtubules and generates a force that opposes dynein/dynactin (blue). In the half spindle, interactions with TPX2 (purple) may target Eg5 (yellow) to parallel microtubules and interactions with dynein may promote poleward motion.