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Extra Views

How Do Kinetochores CLASP Dynamic Microtubules?

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ABSTRACT

Maintenance of genetic stability during cell division requires binding of chromosomes to the mitotic spindle, a process that involves attachment of spindle microtubules to kinetochores. This enables chromosomes to move to the metaphase plate, to satisfy the spindle checkpoint and finally to segregate during anaphase. Recent studies on the function MAST in *Drosophila* and its human homologue CLASP1, have revealed that these microtubule-associated proteins play an essential role for the kinetochore-microtubule interaction. CLASP1 localizesto the plus ends of growing microtubules and to the most external kinetochore domain. Depletion of CLASP1 causes abnormal chromosome congression, collapse of the mitotic spindle and attachment of kinetochores to very short microtubules that do not show dynamic behavior. These results suggest that CLASP1 is required at kinetochores to regulate the dynamic behavior of attached microtubules.

The correct segregation of the genetic material during cell division requires the interaction between spindle microtubules and chromosomes. In this process microtubules play an essential role through their interaction with a specialized structure at the centromeres called the kinetochore (for reviews see refs. 1, 2). The importance of proper microtubule-kinetochore attachment is such that eukaryotic cells have evolved a checkpoint that specifically monitors several aspects of this process, and blocks mitotic progression when kinetochore attachment is incomplete (reviewed in ref. 3).

Microtubules grow by incorporation of subunits in the form of an α- and β-tubulin heterodimer. Each monomer harbours energy in the state of GTP. However, while the GTP on α-tubulin is buried and unchangeable, the GTP on β-tubulin is exposed (reviewed in ref. 4). Hydrolysis of the exchangeable GTP bound to β-tubulin is thought to destabilize the microtubule. As a result it has been hypothesized that microtubule growth (polymerisation) is driven by a putative stabilising GTP-cap at the microtubule plus-ends. Complete hydrolysis of the GTP-cap, together with a decreasing pool of soluble tubulin subunits, is thought to trigger microtubule depolymerization. Structurally, the ends of growing microtubules consist of two-dimensional open sheets that close into a tube. By contrast the protofilaments at the ends of shrinking microtubules are bent, suggesting that there is also a structural transition associated with the switch between microtubule polymerization and depolymerization (reviewed in ref. 5).

During mitosis, the dynamic cycle of microtubule polymerization and depolymerization is thought to be the major force for searching and ultimately catching a kinetochore. Accordingly, microtubule plus ends continuously probe the cytoplasmic space until they contact a kinetochore and become stabilized, while those that do not soon depolymerize.[6,7] However, the molecular mechanism that allows kinetochores to bind and stabilize spindle microtubules remains a fundamental question in mitosis research.[8]

When compared with other microtubule populations in the dividing cell, the regulation of microtubule dynamics at the kinetochore is likely to involve additional controls. This is evident after cooling cells in metaphase, which induces astral and interpolar, but not kinetochore microtubules to disassemble.[9] Interestingly, the turnover rate of kinetochore microtubules is much slower than astral microtubules, although they can still incorporate tubulin subunits at the kinetochore in vivo.[10,11] In addition, microtubule plus ends captured by the kinetochore can also depolymerize at the kinetochore while remaining attached.[12-15] From these observations, one can conclude that a captured microtubule is stabilized at the kinetochore not because depolymerization is prevented, or because it does not incorporate new tubulin subunits, but because kinetochores contain molecules that stabilize microtubule plus ends. Importantly, whilst attached to the kinetochore, the 15–20 microtubules that form a kinetochore fibre grow and shrink in a coordinated fashion as a result of microtubule polymerisation and depolymerization events.[16] However, the question of what coordinates the dynamic behaviour of individual microtubules, so that they function as a bundle with apparently identical parameters of dynamic instability remains

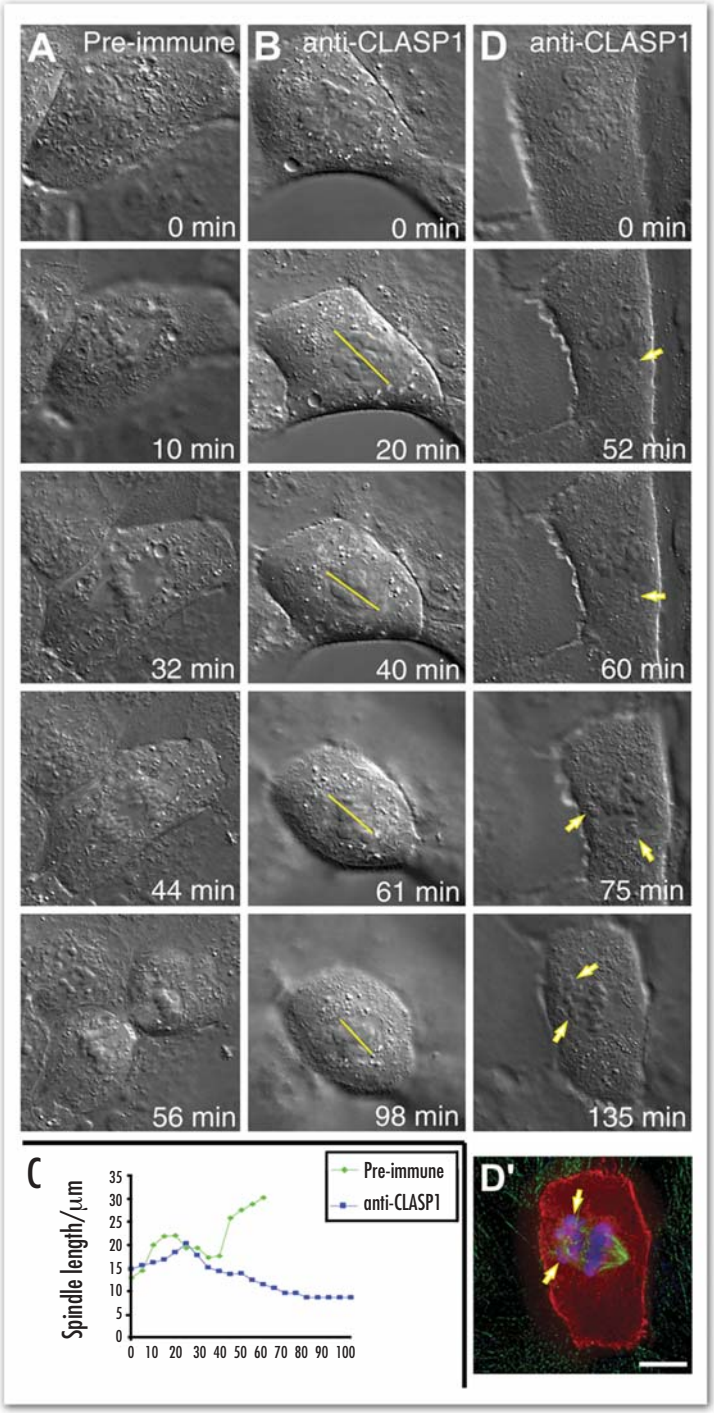

Figure 1. Mitotic progression of cells injected with control or anti-CLASP1 antibodies. (A) Time-lapse series of a CF-PAC cell immediately after injection with pre-immune or (B, D) anti-CLASP1 antibodies analysed by DIC microscopy. (B) Time-lapse series of a CF-PAC cell injected with anti-CLASP1 antibodies showing the progressive collapse of the spindle and chromosomes statically clustered. The position and size of the spindle is marked with a yellow scale bar. (C) Measurements of correspondent spindle length after injection with preimmune and anti-CLASP1 antibodies. (D) Time-lapse series of a CF-PAC cell injected with anti-CLASP1 antibodies showing deficient chromosome congression (arrows). (D') Same cell as in D that was fixed and processed for immunofluorescence immediately after time lapse imaging, showing the spindle (green) and the chromosomes (blue). The cell was identified using anti-rabbit antibodies (red). Bar is 10 μm.

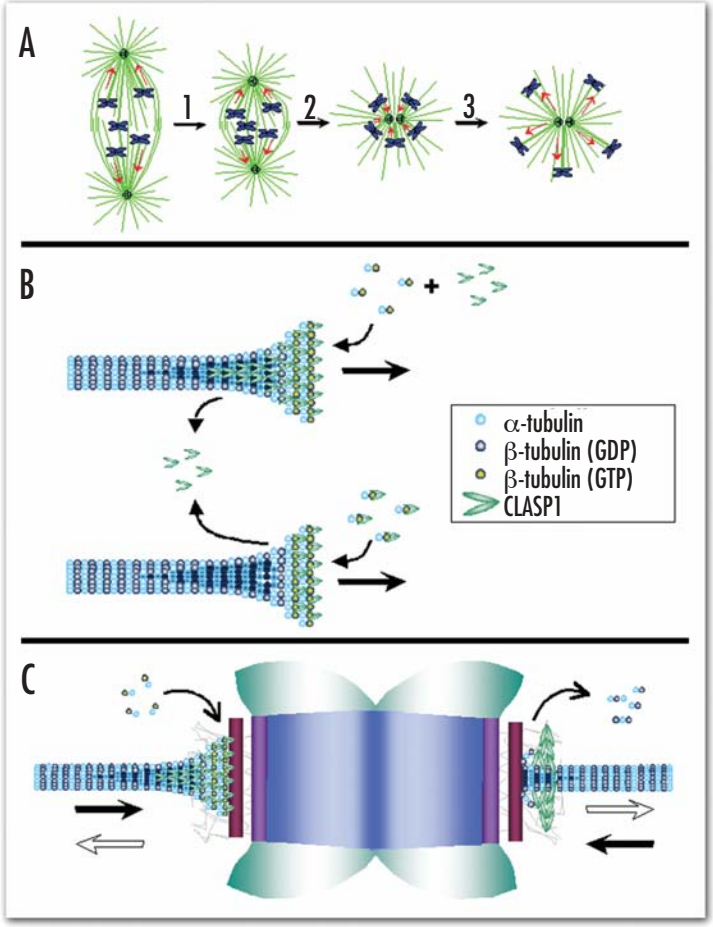

Figure 2. Schematic illustration of the possible role of CLASP1 in the regulation of spindle microtubule dynamics. (A) Interference with CLASP1 function leads to deficient chromosome congression due to abnormal stabilization of the kinetochore fiber that favors microtubule catastrophe. The inability of kinetochore microtubules to rescue from shrinking to growth imbalances the forces exerted on the spindle during metaphase-anaphase transition leading to the collapse of the spindle (1). The resulting monopolar spindles show the chromosomes buried deeper within the aster, with most kinetochores attached to short microtubules that are unable to grow (2). The addition of low doses of nocodazole or taxol that promote microtubule stabilization, rescues kinetochore microtubule growth and chromosomes are normally expelled to the periphery of the mono-aster (3). (B) CLASP1 may target to growing microtubule plus-ends by recognition of the open sheets which expose the luminal side of the microtubule or by co-polymerization with the tubulin heterodimer due to direct interaction with β-tubulin bound to GTP. The later would implicate that CLASP1 and maybe other +TIPs, define the putative GTP-cap at the microtubule-plus ends, whose true extent is not known in vivo. (C) At the kinetochore, CLASP1 may prevent depolymerization of attached microtubules resulting in the promotion of growth and allowing the free addition and lost of tubulin subunits.

open.

To date, no kinetochore component has been shown to regulate microtubule dynamics by promoting microtubule stabilization. Based on in vitro motility assays, CENP-E, a component of the fibrous corona and outer plate of mammalian kinetochores from prometaphase through anaphase,[17,18] was proposed to be one molecule responsible for attachment of kinetochores to depolymerizing microtubules.[19,20] However, CENP-E itself does not seem to play a direct role in regulating kinetochore microtubule dynamics since disrupting CENP-E function in vivo does not affect kinetochore velocity towards or away from the pole, or even the capacity of most chromosomes to become attached and align at the metaphase plate.[21,22]

Therefore, in the absence of CENP-E, the initial attachment of a single kinetochore, as well as its ability to hold onto shortening microtubule ends is not compromised in vivo. On the other hand, MCAK/XKCM1 has recently been shown to be required for chromosome congression.[25] However, this protein only promotes microtubule depolymerization[26] and does not localise at the kinetochore outer plate, where most microtubule plus ends terminate. Instead, it is found in the centromeric heterochromatin beneath the kinetochore.[27,28]

We reasoned that factors that regulate microtubule dynamics at the kinetochores might be highly conserved. Previously we have shown that members of the widely conserved MAST/Orbit family of microtubule-associated proteins localize at the kinetochores and are essential for spindle assembly in *Drosophila*.[29-31] From an analysis of living embryos and RNAi in *Drosophila* tissue culture cell lines we found that MAST/Orbit is required for chromosome congression and also for the maintenance of spindle bipolarity. This suggested that the protein might play a role in microtubule dynamics and/or microtubule-kinetochore attachment.[32] More recently,[33] we addressed this issue by studying one of the human homologues of MAST, known as CLASP1, which is part of a growing class of proteins known as plus-end tracking proteins or +TIPs.[34,35] We found that CLASP1 localises preferentially near the plus-ends of growing microtubules during spindle assembly. More interesting, independently of its localization to the microtubule plus-ends, CLASP1 was also found in a very external region of the human kinetochore, which we termed the outer corona. Except for the CLASP-binding partner CLIP-170, which also plays a role in the early stages of microtubule-kinetochore attachment,[36] this localization pattern is unique among the microtubule plus-end tracking proteins, most of which require microtubules for kinetochore targeting. However, unlike CLIP-170, CLASP1 remains at the kinetochores attached to a full complement of microtubules, i.e., upon the completion of chromosome congression and throughout anaphase, suggesting that it may modulate the dynamics of attached microtubules throughout the duration of mitosis.

Functional analyses of CLASP1, either by overexpression of a dominant-negative construct that lacks the kinetochore-binding domain or by antibody microinjection, indicated that it directly modulates spindle function. An intriguing phenotype was produced from overexpressing the dominant-negative construct:

1. collapse of the spindle;
2. impressive microtubule bundling; and
3. abnormal behaviour of kinetochore-attached microtubules, which are depleted of endogenous CLASP1.

Most interesting, despite the absence of CLASP1 at kinetochores, CLIP-170 still strongly accumulated at the unattached outward-facing kinetochore of monooriented chromosomes within the monoaster. This not only demonstrated that CLIP-170 targets the kinetochore independently of CLASP1, but it also supported previous ultrastructural findings that inward-facing kinetochores of monooriented chromosomes are indeed attached to very short microtubules close to the centrosomes. These observations suggested that CLASP1 is not required for the attachment process per se but that it instead has a role in regulating microtubule dynamics at the kinetochore, namely in the promotion of microtubule growth.

Consistently, microinjection of CLASP1-specific antibodies suppressed microtubule dynamics at the kinetochore which, in turn, induced the chromosomes to localize deep within a the monoasters that resulted from spindle collapse (Fig. 1B, compare movie 1 and 2 in supplementary data and Fig. 2A). Curiously, the length of the spindle in cells injected with anti-CLASP1 antibodies was very similar to that in control injected cells, however, by the time of transition to anaphase, the spindle collapsed (Fig. 1C).

One interesting possibility is that in the absence of CLASP1 the mitotic spindle cannot support the forces required for chromosome segregation when anaphase is triggered. Consequently, the chromosomes are consistently associated with shorter kinetochore fibres and therefore are positioned in close proximity with the center of the monoaster (Fig. 2A). This phenotype is highly unusual, even for monopolar spindles, since polar ejection forces (also known as the polar wind) are thought to exert a pushing force on chromosome arms that act cooperatively with chromosome-bound kinesin motor proteins to expel the chromosomes from the interior of the asters.[37,38] Furthermore, a significant percentage of injected cells showed defects in chromosome congression (Fig. 1D-D', movie 3 in supplementary data and Fig. 2A). Overall, CLASP1 function can be largely understood in terms of regulating microtubule dynamics at the kinetochore. Remarkably, if cells microinjected with anti-CLASP1 antibodies were briefly exposed to taxol or nocodazole, kinetochore-attached microtubules elongated and chromosomes relocated to the periphery of the aster (Fig. 2A). This finding suggests that CLASP1 might promote microtubule rescue at the kinetochores, i.e., the transition from shrinkage to growth of attached microtubules.

Since taxol treatment appears to partially rescue CLASP1 function, the mechanism by which taxol suppresses microtubule dynamics may help to elucidate how CLASP1 regulates microtubule dynamics at the kinetochore. Structurally, the taxol-binding site is situated on the luminal side of the microtubule wall, in the middle of β-tubulin subunit.[39] Curiously, we have found that in the presence of taxol, CLASP1 binding to microtubules is dramatically affected causing it to become more widely distributed throughout the spindle while remaining apparently unchanged at the kinetochore. This suggests that CLASP1 accumulation at the microtubule plus-ends may involve the recognition of a particular structural feature on the microtubule lattice that is being mimicked by taxol. Taxol is known to promote microtubule stabilization by mimicking the GTP state of tubulin.[40] Furthermore, MAST/Orbit binds tubulin in a GTP-dependent manner[30] and Stu1, the single orthologue of CLASPs in *S. cerevisiae*, specifically associates with β-tubulin,[41] the microtubule subunit where GTP hydrolysis takes place during polymerisation. In this context, it is tempting to speculate that this family of proteins may regulate some aspect of microtubule dynamics at or near the stabilizing GTP-cap by specific recognizing the open sheets of growing microtubules (which expose the luminal side of the microtubule during growth) or have a preferential binding to the GTP state of the lattice (Fig. 2B).

It is also significant that CLASP1 accumulates at the outer kinetochore corona independently of its binding to the microtubule plus-ends. This puts CLASP1 in a favourable position within the kinetochore to promote microtubule growth while allowing attached microtubules to keep their ends free for incorporating tubulin subunits (Fig. 2C). Through a direct influence on how the microtubule neck is organized, CLASP1 at the kinetochores may also prevent full microtubule depolymerization by promoting the transition to a growth phase (Fig. 2C). This interpretation would also readily explain how interference with the function of MAST/CLASPs leads to the collapse of the spindle, attributing to the kinetochore essential spindle stabilization properties that have been previously neglected from experiments of spindle assembly in vitro.[42,43] In support of our model, recent experiments on how spindles form in cultured vertebrate cells lacking chromosomes and kinetochores reveals that the two interacting arrays of astral microtubules are not able to sustain the normal spindle architecture.[44] Moreover, it has been previously demonstrated that normal spindles form around kinetochores that have been induced

to detach from the chromosomes,[45] but that chromosome fragments lacking kinetochores do not stabilize microtubules within their vicinity.[6] Thus, kinetochores do not appear to be required for spindle morphogenesis during mitosis in animal cells, but they may be required to stabilize its structure.

It will be interesting in the future to determine how the proposed role for CLASP1 co-relates with the function of other +TIPs at the kinetochore interface and dissect the mechanism by which CLASPs and other +TIPs recognize and influence microtubule dynamics at the plus-end. Furthermore, it would be of major importance to investigate how the machinery that regulates microtubule dynamics at the kinetochore communicates with the spindle checkpoint that monitors microtubule attachment and controls progression through mitosis.

Please visit the following link to download the following supplemental movies:

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Movie 1.Time-lapse video microscopy analysis by DIC of a human CF-PAC cell injected with pre-immune antibodies during late prophase. Chromosomes can be seen to fully congress to the metaphase plate within the first 15 min upon NEB. Metaphase lasts for ~25 min and the cell enters anaphase. During telophase/cytokinesis chromosomes in the two daughter cells start to decondense after ~1 hour from NEB.

Movie 2. Time-lapse video microscopy analysis by DIC of another human CF-PAC cell injected with anti-CLASP1 antibodies during late prophase. Chromosome oscillations are abnormal and the chromosomes accumulate as a disorganized mass in the center of the cell. The spindle can be seen to progressively collapse.

Movie 3. Time-lapse video microscopy analysis by DIC of a human CF-PAC cell injected with anti-CLASP1 antibodies during late prophase. During prometaphase full congression of all chromosomes is not accomplished even after more than 2 h 30 m. At this time, the cell was fixed and processed for immunofluorescence to reveal the details of the spindle and chromosomes (see Figure 1D').

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