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A Bir1-Sli15 Complex Connects Centromeres to Microtubules and Is Required to Sense Kinetochore Tension

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SUMMARY

Proper connections between centromeres and spindle microtubules are of critical importance in ensuring accurate segregation of the genome during cell division. Using an in vitro approach based on the sequence-specific budding yeast centromere, we identified a complex of the chromosomal passenger proteins Bir1 and Sli15 (Survivin and INCENP) that links centromeres to microtubules. This linkage does not require Ipl1/Aurora B kinase, whose targeting and activation are controlled by Bir1 and Sli15. Ipl1 is the tension-dependent regulator of centromere-microtubule interactions that ensures chromosome biorientation on the spindle. Elimination of the linkage between centromeres and microtubules mediated by Bir1-Sli15 phenocopies mutations that selectively cripple Ipl1 kinase activation. These findings lead us to propose that the Bir1-Sli15-mediated linkage, which bridges centromeres and microtubules and includes the Aurora kinase-activating domain of INCENP family proteins, is the tension sensor that relays the mechanical state of centromere-microtubule attachments into local control of Ipl1 kinase activity.

INTRODUCTION

During mitosis, kinetochores assemble on the centromeric regions of each sister chromatid to act as the primary chromosomal attachment sites for spindle microtubules (Cleveland et al., 2003; Kline-Smith et al., 2005; Maiato et al., 2004). Kinetochores exhibit both end-on and lateral interactions with microtubules. End-on connections between the outer kinetochore and microtubule plus ends couple chromosome motility to changes in the polymerization and depolymerization of bound microtubules (Inoue and Salmon, 1995). Lateral interactions between kinetochores and spindle microtubules are associated with poleward as well as equatorial chromosome movements that facilitate the establishment of stable end-on connections (Kapoor et al., 2006; Rieder and Alexander, 1990; Tanaka et al., 2005).

In addition to their mechanical role in segregation, kinetochores serve as signaling hubs that inhibit anaphase onset until every chromosome in the cell is properly connected. The kinetochore-based mitotic checkpoint pathway relays the presence of any unattached kinetochores into inhibition of the ubiquitin protein ligase that triggers sister chromatid separation and mitotic exit (Cleveland et al., 2003; Nasmyth, 2005; Pinsky and Biggins, 2005). Classic micromanipulation studies in insect spermatocytes as well as recent work using chromosome engineering in budding yeast have highlighted the importance of tension in selectively stabilizing correctly bioriented chromatid pairs (Dewar et al., 2004; Li and Nicklas, 1995; Nicklas and Koch, 1969; Stern and Murray, 2001). Biorientation places kinetochore-microtubule connections under tension, whereas incorrect syntelic attachments, where the kinetochores on both sisters are connected to the same spindle pole, do not. The conserved Aurora B kinase is required to eliminate syntelic attachments, facilitating new connection attempts until the correct configuration is achieved (Biggins and Murray, 2001; Dewar et al., 2004; Ditchfield et al., 2003; Hauf et al., 2003; Kallio et al., 2002; Lampson et al., 2004; Pinsky et al., 2003; Tanaka et al., 2002). The action of Aurora B generates unoccupied kinetochores that in turn signal via the mitotic checkpoint pathway to prevent anaphase onset (Pinsky et al., 2006). Thus, the choreography of chromosome segregation is comprised of an intimate feedback between the mechanics of kinetochore-microtubule connections and localized signaling pathways.

Efforts to reconstitute the mechanical and regulatory functions of kinetochores in vitro have been limited by the complexity of the underlying centromeric DNA (Cleveland et al., 2003). An exception to this complexity is budding yeast, where centromeres consist of a well-defined ~125 base-pair region (Clarke, 1998; McAinsh et al., 2003). The biochemical identification of CBF3, the protein complex that directly binds the key *cis*-acting CDEIII domain (Lechner and Carbon, 1991), provided further impetus for analyzing kinetochore-microtubule interactions in vitro. Previous studies have demonstrated that budding yeast centromeric (CEN) DNA will bind to microtubules following incubation in a cell extract (Hyman et al., 1992; Kingsbury and Koshland, 1991; Severin et al., 1997; Sorger et al., 1994). This interaction requires the CBF3 complex and is subject to regulation by Ipl1, the budding yeast Aurora B kinase, and the counteracting phosphatase PP1/Glc7 (Biggins et al., 1999; Sassoon et al., 1999). However, CBF3 is not sufficient, indicating that other factor or factors are necessary to link CBF3-CEN DNA to microtubules (Sorger et al., 1994). Here, we extend this in vitro approach to biochemically identify the missing factor(s). Our results reveal that a complex of two chromosomal passenger proteins, Bir1/Survivin and Sli15/INCENP, connects CBF3-CEN DNA to microtubules in vitro. This connection is independent of Ipl1, whose activation and targeting are controlled by Bir1 and Sli15. In vivo analysis of Sli15 mutants that eliminate the in vitro activity leads us to propose that the Bir1-Sli15-mediated linkage between CBF3-CEN DNA and microtubules acts as a tension sensor that activates Ipl1 in the vicinity of incorrect syntelic attachments.

RESULTS

A Quantitative In Vitro Assay for the Interaction of CBF3-Bound CEN DNA with Microtubules

In the presence of a yeast cell extract, fluorescent beads coupled to CEN DNA will bind to immobilized microtubules adsorbed to a coverslip surface. Although required, CBF3 is not sufficient for binding, indicating the presence of additional factor or factors in the extract that connect CBF3-bound CEN DNA to microtubules (Sorger et al., 1994). To identify these factor(s), we adapted the bead assay to enable rapid, reproducible testing of column fractions (Figure 1A). As expected, beads coated with CEN DNA exhibited robust microtubule binding in the presence of wild-type extract, whereas beads coated with a mutant CEN DNA that renders it nonfunctional in vivo failed to bind (Figure 1B).

To generate a source of CBF3 for in vitro complementation assays, we optimized a partial purification using CEN DNA band shift to monitor CBF3 activity (Figure 1C). This procedure yielded ~50-fold partially purified (PP)CBF3 that exhibited a robust band shift (Figure 1C) but did not support binding of CEN DNA beads to microtubules on its own (Figure 1D). Extracts prepared from strains harboring mutations in CBF3 lacked CEN DNA band shift but complemented (PP)CBF3 in the bead-microtubule-binding assay (data not shown, Sorger et al., 1994). Trypsin treatment indicated that the complementing activity is protease sensitive (see Figure S1 in the Supplemental Data available with this article online). Taken together, these results confirmed the existence of an unknown factor that connects CBF3-bound CEN DNA to microtubules in vitro and established a robust assay that could be used for its identification.

A Conventional Purification Strategy Identifies Bir1 as a Candidate for the Activity that Links CBF3-Bound CEN DNA to Microtubules

To identify the protein or proteins that connect CBF3-bound CEN DNA to microtubules, we utilized two strategies. First, we tested CBF3-complementing activity in extracts either prepared from mutant strains or immunodepleted of candidate kinetochore-localized or microtubule-binding proteins. At the budding yeast kinetochore, the Dam1 ring complex plays an important role in bioriented microtubule attachments and the Mis12, Ctf19, and Ndc80 complexes are suggested to direct assembly of the microtubule-binding interface (reviewed in De Wulf et al. [2003], McAinsh et al. [2003], and Tanaka et al. [2005]). However, neither mutations in nor immunodepletions of these complexes perturbed the in vitro linkage between CEN DNA and microtubules (Table S1). Other candidates, including motor and nonmotor microtubule-binding proteins, were similarly excluded. These results indicated the presence of a CBF3-dependent linkage between CEN DNA and microtubules that did not involve any of the obvious candidates suggested by prior studies.

Parallel to the candidate analysis, we pursued an unbiased conventional purification, using the quantitative in vitro assay (Figure 2A). Complementing activity was measured relative to a standard curve generated by serially diluting the starting material for the purification step into a constant amount of (PP)CBF3 (Figures 2B and 2C). Negative controls in which mutant CEN DNA beads were used, or (PP)CBF3 was omitted, verified the specificity of binding. By combining three purification steps in series, we enriched the complementing activity ~400-fold (Figure 2D). However, attempts at additional purification resulted in a significant loss of specific activity that did not appear to be due to separation of different components.

Since the purification did not achieve sufficient enrichment to directly correlate complementing activity with copurifying proteins, we identified candidates that could be functionally tested by performing mass spectrometry (Washburn et al., 2001) on the highest specific activity material—the Mono S cation-exchange pool (Figures 2E and 2F). This approach identified 247 polypeptides with greater than 10% sequence coverage in the Mono S pool (Table S2). Database functional annotations identified a single protein within this large set previously implicated in centromere function (Figure 2G; Table S2). This protein was Bir1, the budding yeast homolog of the Survivin subunit of the chromosomal passenger complex. This