

The role of centromere-binding factor 3 (CBF3) in spindle stability, cytokinesis, and kinetochore attachment¹

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Abstract: The spindle midzone is critical for spindle stability and cytokinesis. Chromosomal passenger proteins relocalize from chromosomes to the spindle midzone after anaphase onset. The recent localization of the inner-kinetochore, centromere-binding factor 3 (CBF3) complex to the spindle midzone in budding yeast has led to the discovery of novel functions for this complex in addition to its essential role at kinetochores. In G1/S cells, CBF3 components are detected along dynamic microtubules, where they can “search-and-capture” newly replicated centromeres. During anaphase, CBF3 is transported to the microtubule plus-ends of the spindle midzone. Consistent with this localization, cells containing a mutation in the CBF3 subunit Ndc10p show defects in spindle stability during anaphase. In addition, *ndc10-1* cells show defects during cytokinesis, resulting in a defect in cell abscission. These results highlight the importance of midzone-targeted proteins in coordinating mitosis with cell division. Here we discuss these findings and explore the significance of CBF3 transport to microtubule plus-ends at the spindle midzone.

Key words: spindle midzone, passenger protein, inner centromere protein (INCENP), microtubule plus-end.

Résumé : Les composantes du milieu du fuseau mitotique sont essentielles à la stabilité du fuseau et à la cytokinèse. Les protéines chromosomiques passagères se re-localisent à partir des chromosomes vers le milieu du fuseau après le début de l’anaphase. La localisation récente de facteur de liaison centromérique 3 (CBF3), un complexe du kinétochore interne liant le centromère, au milieu du fuseau chez la levure à bourgeonnement a conduit à la découverte de nouvelles fonctions pour ce complexe, en plus de son rôle essentiel dans le kinétochore. Chez les cellules en phase G1/S, les composantes de CBF3 sont détectées le long des microtubules dynamiques où elles peuvent « chercher et capturer » les centromères nouvellement répliqués. Durant l’anaphase, CBF3 est transporté à l’extrémité positive des microtubules au milieu du fuseau. Conséquemment, les cellules comportant des mutations de la sous-unité Ndc10p de CBF3 présentent des défauts dans la stabilité du fuseau lors de l’anaphase. De plus, les cellules *ndc10-1* présentent des défauts lors de la cytokinèse, résultant en un déficit de l’abscission cellulaire. Ces résultats mettent en évidence l’importance des protéines ciblées au milieu du fuseau dans la coordination de la mitose avec la division cellulaire. Nous discutons ici de ces résultats et explorons le rôle du transport de CBF3 à l’extrémité positive des microtubules au milieu du fuseau.

Mots clés : milieu du fuseau, protéine passagère, protéines centromériques internes (INCENP), extrémité positive des microtubules.

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Introduction

Successful cell division requires the coordination of chromosome segregation and cytokinesis. In many higher eukaryotes, this coordination is manifested in the determination of the cytokinetic furrow via mitotic spindle position

(Rappaport 1996). The proteins that localize to the spindle midzone in these cells contribute to furrow positioning and later to cytokinesis. By contrast, in many plants and fungi, in which the plane of division is determined before spindle formation, the spindle must align itself perpendicular to the division plane to ensure that equal complements of the ge-

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nome will be segregated to opposite sides of the division plane (Pearson and Bloom 2004). In these cells, proteins directed to the spindle midzone may function as a signal for the initiation of cytokinesis, thus preventing cell division until the mitotic spindle has elongated along the proper axis. Regardless of whether the spindle defines the division plane or the cell shape defines the orientation for the spindle, the coordination of chromosome segregation and cytokinesis remains essential to the viability of the resulting daughter cells.

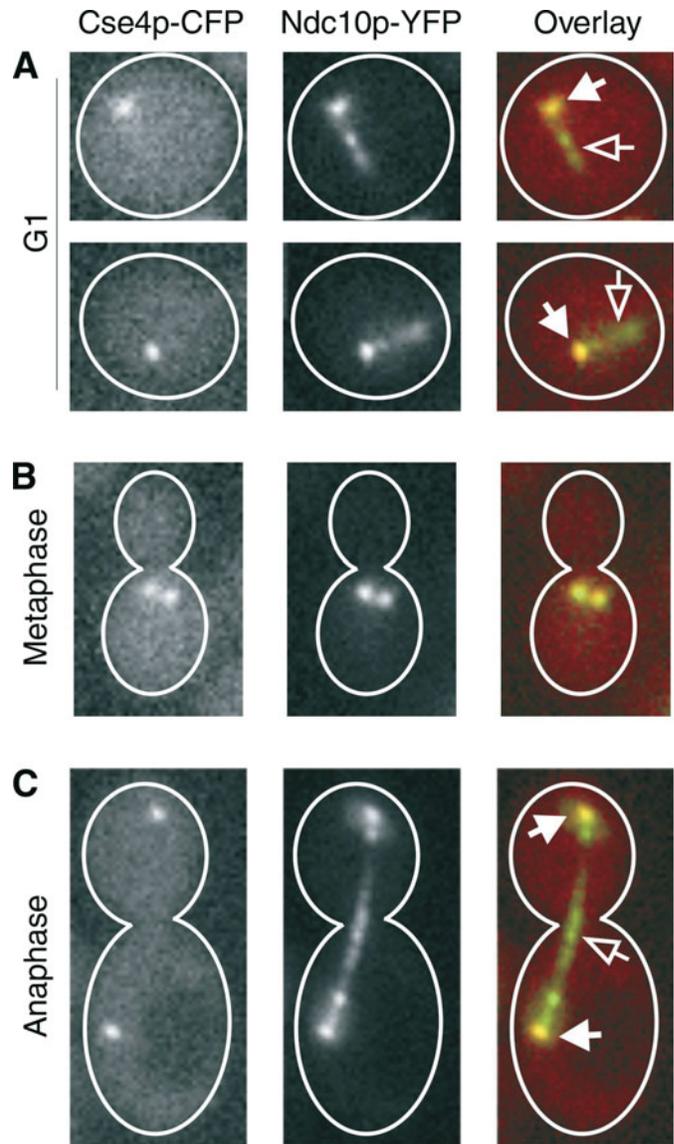
Among the proteins likely to couple chromosome segregation with cytokinesis are those that localize first to centromeres and later to the midzone of the anaphase spindle. The spindle midzone comprises overlapping microtubule plus-ends and is the future site of cytokinesis. First identified in 1987, chromosomal passenger proteins undergo programmed relocalization from chromosomes to the spindle midzone during anaphase (Cooke et al. 1987). This spatial-temporal regulation reflects the changing functions of these proteins during mitosis. Passenger proteins, including aurora B kinase, inner centromere protein (INCENP), survivin, TD-60, Dasra B / Borealin, and Dasra A / Borealin-2, first localize to chromosomes, where they function in chromatin compaction, and subsequently relocalize to the spindle midzone after anaphase onset (Adams et al. 2000; Andreassen et al. 1991; Gassmann et al. 2004; Kaitna et al. 2000; Kang et al. 2001; Leverson et al. 2002; Sampath et al. 2004; Wheatley et al. 2001). Consistent with their localization to the midzone, passenger proteins contribute to both spindle stability and cytokinesis (Andrews et al. 2003; Carmena and Earnshaw 2003).

Homologs of the passenger proteins aurora B, survivin, and INCENP have been identified in both *Saccharomyces cerevisiae* (Ipl1p, Bir1p, and Sli15p) and *Schizosaccharomyces pombe* (Ark1, Bir1/Cut17, and Pic1). In addition to sequence conservation across eukaryotes, the functions of these proteins are well conserved. As in tissue cells, these proteins have been shown to function during chromosome segregation, spindle regulation, and cytokinesis (Biggins et al. 1999; Buvelot et al. 2003; Kang et al. 2001; Kim et al. 1999; Leverson et al. 2002; Morishita et al. 2001; Petersen et al. 2001; Uren et al. 1999; Yoon and Carbon 1999).

In addition to forming the scaffold for Ipl1p, Bir1p, and Sli15p localization, the yeast anaphase spindle midzone hosts a number of other proteins involved in mitosis, including the spindle structural proteins Ase1p and Slk19p (Pellman et al. 1995; Schuyler et al. 2003; Sullivan et al. 2001; Zeng et al. 1999). We have recently found that the inner kinetochore complex centromere-binding factor 3 (CBF3) (consisting of Ndc10p, Ctf13p, Cep3p, and Skp1p) is transported to the spindle midzone during anaphase, where it localizes to the plus-ends of microtubules (Bouck and Bloom 2005). CBF3 remains associated with microtubule plus-ends during spindle disassembly and is detected along dynamic projections emanating from the spindle pole body in G1 (Fig. 1A).

The localization of CBF3 to growing and shortening microtubule plus-ends and the spindle midzone is the first demonstration of an inner-kinetochore, centromere-binding complex moving to the midzone (Bouck and Bloom 2005). This finding highlights the midzone as a key site within the cell where proteins may interact as part of the signaling pro-

Fig. 1. Non-centromeric localization of Ndc10p-YFP along microtubules. Asynchronously growing cells expressing Cse4p-CFP (centromere-specific histone H3 variant) and Ndc10p-YFP (pseudocolored as red and green, respectively) were imaged at various cell cycle stages by fluorescence microscopy. (A) In unbudded cell, Cse4p appears as a single spot, representing clustered centromeres (solid arrow), while Ndc10p is also detected, along a projection extending from the cluster (hollow arrow). (B) During metaphase, Cse4p and Ndc10p colocalize to 2 clusters representing bioriented sister chromatids. (C) In anaphase, Ndc10p localizes to the interpolar microtubules and spindle midzone (hollow arrow), while Cse4p appears as 2 discrete clusters.



cess essential for coordinating cellular functions. Here we discuss 3 possible roles for CBF3 in addition to its previously defined role in chromosome segregation: (i) midzone CBF3 could contribute to anaphase spindle stability; (ii) midzone CBF3 could act as part of the pathway necessary to ensure that cytokinesis follows chromosome segregation; and (iii) CBF3 associated with microtubule plus-ends could form a “pre-kinetochore” structure that matures into a com-

plete kinetochore in the subsequent cell cycle. These novel CBF3 roles are not mutually exclusive of each other and represent a means by which CBF3 may function at different points throughout the cell cycle.

Ndc10p function in spindle stability

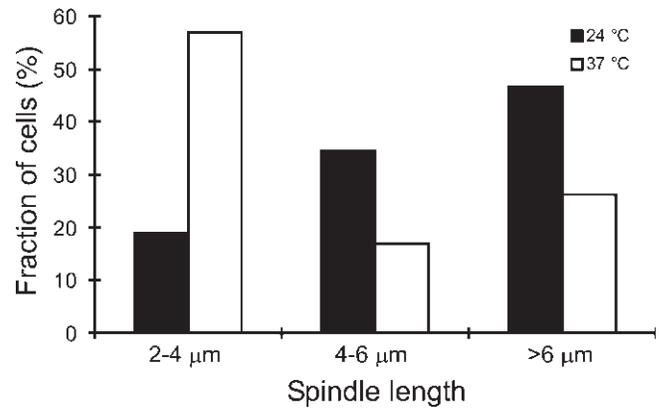
The spindle midzone comprises overlapping microtubule plus-ends. In budding yeast, these microtubules are arranged with defined spacing and organization (Winey et al. 1995). As the spindle elongates during anaphase B, antiparallel microtubules slide against each other as they polymerize, producing the force necessary to separate the spindle pole bodies. Microtubule sliding is presumably a result of plus-end-directed motor proteins crosslinking antiparallel microtubules. Failure to promote microtubule polymerization or to crosslink and slide microtubules would result in short or fragile anaphase spindles.

The spindle midzone is populated by the dynamic plus-ends of microtubules. In budding yeast, microtubule minus-ends (embedded in the spindle pole bodies) do not contribute to microtubule dynamics (Maddox et al. 2000; Tanaka et al. 2005). Growth and shortening of microtubules appear to occur exclusively at plus-ends. At the spindle midzone, factors promoting microtubule polymerization must act to promote the microtubule growth necessary for spindle elongation.

Among the proteins at the midzone, Slk19p and Sli15p have been identified as contributing to spindle stability (Pellman et al. 1995; Schuyler et al. 2003; Sullivan et al. 2001; Zeng et al. 1999). Additionally, Ipl1p plays a role in regulating the timing of spindle disassembly, as *ipl1-321* mutants spend an additional 6 min in anaphase before spindle disassembly (Buvelot et al. 2003). The mechanism by which Ipl1p regulates the timing of spindle disassembly has not been determined, but 2 likely possibilities exist. Upon full spindle elongation, Ipl1p could activate a microtubule “depolymerase” at microtubule plus-ends to initiate spindle disassembly. One possibility for this microtubule depolymerase is Kar3p, which shows microtubule depolymerase activity in vitro, localizes weakly to spindle microtubules, and contains 4 putative Ipl1p phosphorylation sites based on the described consensus sequence (Cheeseman et al. 2002; Endow et al. 1994; Page et al. 1994). Alternatively, Ipl1p might regulate the timing of spindle disassembly through the destabilization or delocalization of microtubule plus-end stabilizing proteins. Consistent with this hypothesis, levels of Ase1p and Slk19p at the midzone decrease during anaphase (Bouck and Bloom 2005; Schuyler et al. 2003). Observation of the localization and stability of these proteins at the midzone in *ipl1* mutants would address this possibility.

CBF3 also contributes to spindle stability. Using *ndc10-1*, a temperature-sensitive allele of *NDC10* known to disrupt kinetochore structure and function and to bypass the spindle checkpoint, we examined spindle length and morphology in cells expressing GFP-Tub1p (Fraschini et al. 2001; Gardner et al. 2001; Goh and Kilmartin 1993). Under nonpermissive conditions, there is enrichment in 2–4 μm spindles in asynchronously growing cells lacking functional Ndc10p (Fig. 2). Time-lapse microscopy revealed the cause of this enrichment: spindles repeat cycles of failed elongation during

Fig. 2. Spindle elongation defect in cells lacking functional Ndc10p. Asynchronously growing *ndc10-1* cells were shifted to nonpermissive conditions for 90 min, and then spindle length was measured using GFP-Tub1p. *ndc10-1* cells showed an enrichment of shorter spindles, indicating a defect in spindle elongation.



anaphase. Spindles in *ndc10-1* cells partially elongate (usually to about 5–6 μm), collapse, re-form, and then begin to elongate again (Bouck and Bloom 2005). This phenotype was attributed to lost Ndc10p function rather than lost kinetochore function, since cells lacking Nuf2p (another essential kinetochore protein) and Mad2p (allowing the cells to bypass the spindle checkpoint) showed no defects in spindle elongation (Bouck and Bloom 2005). Thus, in addition to functioning at the kinetochore, Ndc10p appears to play a role in stabilizing the spindle during anaphase elongation.

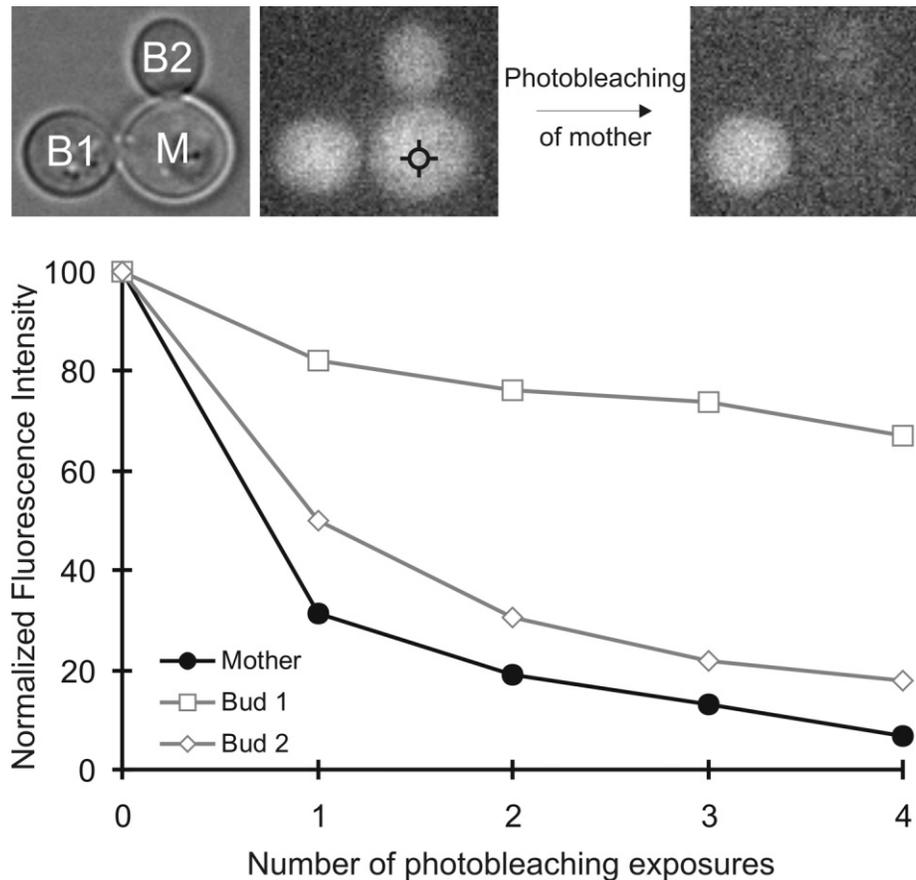
Since CBF3 has neither microtubule-binding nor motor properties, it seems unlikely that CBF3 itself plays a direct role in crosslinking or stabilizing antiparallel microtubules at the spindle midzone (Sorger et al. 1994). As part of the kinetochore, CBF3 serves as a foundation or scaffold for assembly of the remainder of the kinetochore (McAinsh et al. 2003). Likewise, at the spindle midzone, CBF3 might aid the assembly of microtubule-associated proteins directly responsible for spindle stability. The organization and dynamics of these proteins (Ase1p, Slk19p, and Sli15p) in *ndc10-1* cells could shed light on this possibility.

Coordination of chromosome segregation with cell division

In most eukaryotes, nuclear envelope breakdown and chromosome condensation mark entry into mitosis. Dissolution of the nuclear envelope also enables interaction between the mitotic machinery and the cell cortex, thus allowing the orchestration of chromosome segregation and cytokinesis.

For many years, the formation of a midbody complex appeared to be essential for the positioning of the cytokinetic furrow and completion of cytokinesis, but more recently it has been shown that cells lacking a bipolar spindle (and thus a spindle midzone–midbody) are able to furrow and complete cytokinesis following an induced anaphase (Canman et al. 2003). In these cells, the stable attachments of microtubules to cortical regions may allow for the delivery of factors necessary for completion of cell division. This model allows for

Fig. 3. Multicell clusters in *ndc10-1* mutants caused by cell separation defect. *ndc10-1* cells expressing cytoplasmic GFP were shifted to nonpermissive temperature for 3 h. GFP within the mother was specifically photobleached with 4 laser (488 nm) exposures. Fluorescence intensity measurement of the mother and both buds showed partitioning between the mother and Bud 1, indicating that cytokinesis was complete, but cell separation was defective.



the coordination of the mitotic and cytokinetic machineries through spatial regulation and delivery of proteins. Thus, the delivery of certain components to microtubule plus-ends, rather than delivery of these components to the midbody, might be the critical signaling event for cytokinesis.

In budding yeast the nucleus remains intact during mitosis. Wild-type budding yeast forms binucleates and anucleates (a result of undergoing cytokinesis prior to either proper spindle alignment or spindle elongation) extremely rarely, and even mutants affecting spindle positioning show <5% binucleate cells (Li et al. 1993). This suggests that budding yeast has a mechanism by which the mitotic spindle signals to the bud neck for initiation of cytokinesis.

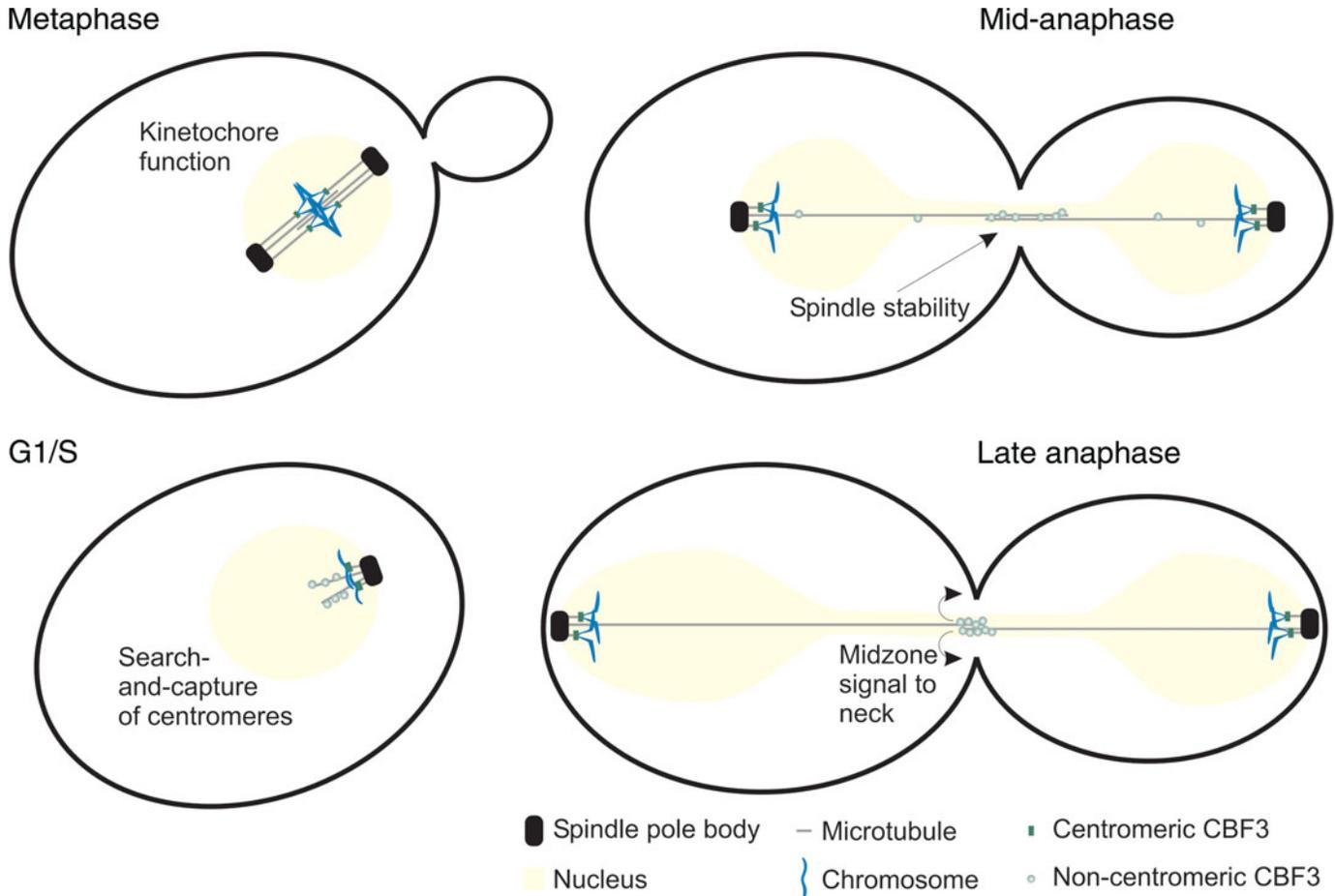
We have found that a previously unidentified phenotype of *ndc10-1* cells is the formation of multicell clusters (Fig. 3). This morphology can be a result of either failure to complete cytokinesis or failure in cell abscission following cytokinesis. FLIP (fluorescence loss in photobleaching) analysis of cytoplasmic green fluorescent protein (GFP) expressed in *ndc10-1* multibudded cells showed that these cells successfully complete cytokinesis but fail to separate from each other afterwards (Fig. 3). When the mother cell was specifically photobleached, fluorescence was lost in only 1 of the 2 attached daughters. This suggests that the bud that retained fluorescence had completed cytokinesis, whereas the other

bud still shared a continuous cytoplasm with the mother cell. Cell wall digestion of fixed, multicell clusters also showed that these cells had completed cytokinesis but had failed to separate from each other (Bouck and Bloom 2005).

To better understand the basis of this failure in cell separation, the localization and organization of septins were examined in *ndc10-1* cells. In wild-type cells, septins form a ring at the bud neck, which splits into 2 rings around the time of cytokinesis (Lippincott et al. 2001). Septins have been proposed to recruit or retain factors to the bud neck essential for cytokinesis and cell separation (Dobbelaere and Barral 2004). In *ndc10-1* cells, the septin ring fails to split into 2 rings (Bouck and Bloom 2005). This defect likely causes the disorganization or delocalization of components needed at the neck during cytokinesis and cell separation.

How might the kinetochore communicate with the cytokinetic machinery? We can imagine 2 plausible means by which the kinetochore signals to the mother–bud neck in a closed mitosis. The first is signaling through the spindle pole body. Spindle pole bodies nucleate microtubules to both the nucleus and cytoplasm. These microtubules may act as signaling conduits emanating from a central hub, the spindle pole. During anaphase, centromere–kinetochore complexes are drawn close to the nuclear side of the spindle pole body, and during spindle disassembly many passenger proteins

Fig. 4. Model of the changing roles of CBF3. During G1/S, CBF3 may be involved in a search-and-capture mechanism for the capture of centromeres. Once kinetochores are formed CBF3 is essential for kinetochore function. During anaphase, CBF3 localizes to the midzone, where it might play a role in stabilizing the anaphase spindle. Once the spindle has fully elongated, CBF3 is enriched at the midzone, where it could signal to the cytokinesis machinery at the mother–bud neck.



(including Ipl1p, Sli15, Ndc10p, Cep3p) reel back to the spindle pole bodies on depolymerizing microtubule plus-ends (Bouck and Bloom 2005; Buvelot et al. 2003). Either of these events could act as a signal transduced from the nuclear face to the cytoplasmic face of the spindle pole body. This signal could then be carried to the neck by cytoplasmic microtubules or diffusion of signaling molecules from the cytoplasmic face of the spindle pole body.

Alternatively, the delivery of proteins to microtubule plus-ends at the midzone during late anaphase could be the initiating step in signaling to the neck that chromosomes have segregated. The spindle midzone is located close to the mother–bud neck. Formation of the midzone complex could cause a signal to the neck through steric interaction of the nuclear envelope with the neck cortex, or through signaling through nuclear pores located near the midzone. Interestingly, many spindle checkpoint proteins have been localized to the nuclear pore complexes, and spindle checkpoint mutants also form multicell clusters when treated with microtubule depolymerizing agents (Hoyt et al. 1991; Iouk et al. 2002; Li and Murray 1991). Together, these data suggest that failure to form a midzone complex (whether caused by spindle fragility in *ndc10-1* cells or microtubule poisons) leads to a failure to complete cell division.

Building a kinetochore

One of the greatest questions remaining in the field of mitosis research is how a centromere–kinetochore–microtubule attachment is formed. Three possibilities exist: first, a kinetochore can be built on a centromere and wait for a dynamic microtubule to find it. Second, a kinetochore could form on a microtubule and search for a centromere. Third, part of the kinetochore could form on the centromere and part on a microtubule; when these complementary complexes find each other a kinetochore is formed.

Discoveries of the last few years suggest that the kinetochore might be divided up into microtubule-associated and centromere-associated complexes. The outer kinetochore Dam1 complex has recently been shown to form rings around microtubules in vitro (Miranda et al. 2005; Westermann et al. 2005). This structure, if present in vivo, would likely remain associated with microtubules despite the presence or absence of centromeric DNA. In fact, the interface between the Dam1 ring complex and the Ndc80 complex appears to be the site of detachment–reattachment as mediated by the Ipl1p kinase (Shang et al. 2003). Since other kinetochore proteins and complexes, including the CBF3 complex, Cbf1p, and Cse4p, associate directly with centromeric DNA, it seems likely that

the kinetochore is made up of complexes associated more directly with either the centromere or microtubules.

While increased understanding of kinetochore structure yields insights into how the subcomplexes of the kinetochore are arranged with respect to each other, it fails to directly address the question of how the centromere–kinetochore–microtubule structure is formed. The recent localization of CBF3 components to the plus-ends of growing and shrinking microtubules following spindle disassembly suggests that this complex may function in the next cell cycle in a “search-and-capture” mechanism to establish new kinetochore attachments (Bouck and Bloom 2005; Tanaka et al. 2005). Since the CBF3 complex specifically binds centromeric DNA and has now been localized with dynamic microtubules, this “pre-kinetochore” complex is capable of tethering centromere-bound chromosomes to microtubules (Fig. 1A). Upon CBF3 binding of a centromere, additional kinetochore proteins and complexes could be recruited to this structure in a process of kinetochore maturation. The centromere–kinetochore–microtubule attachment has to be strong enough to withstand forces exerted during metaphase, and the quality of attachments has to be monitored by the spindle checkpoint. The addition of these complexes might offer increased stability and checkpoint function to the kinetochore.

Conclusion

While microtubules have long been appreciated for their roles in nuclear positioning, nuclear orientation, and chromosome segregation, the formation of the spindle midzone and the delivery of proteins to microtubule plus-ends appear to be additional essential microtubule functions. Proteins associated with the spindle midzone are optimally positioned to act in the coordination of chromosome segregation and cytokinesis as a result of their dissociation from chromosomes after anaphase onset and their localization at the plane of division. We have found that the inner kinetochore complex CBF3 is targeted to the spindle midzone during anaphase and that it remains associated with dynamic nuclear microtubules in G1/S cells. CBF3 association with microtubule plus-ends could facilitate search-and-capture of centromeres following centromere replication in early S phase (Fig. 4). Once kinetochores are formed, CBF3 is essential to kinetochore function (i.e., tethering chromosomes to dynamic microtubule plus-ends). During anaphase, CBF3 is found along the inter-polar microtubules and may function to stabilize the elongating spindle. CBF3 is enriched at the spindle midzone in late anaphase, where it might function in signaling the completion of chromosome segregation. These functions show that CBF3 may contribute to kinetochore assembly, spindle stability, and the coordination of chromosome segregation with cytokinesis.

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References

Adams, R.R., Wheatley, S.P., Gouldsworthy, A.M., Kandels-Lewis,

- S.E., Carmena, M., Smythe, C. et al. 2000. INCENP binds the Aurora-related kinase AIRK2 and is required to target it to chromosomes, the central spindle and cleavage furrow. *Curr. Biol.* **10**: 1075–1078.
- Andreassen, P.R., Palmer, D.K., Wener, M.H., and Margolis, R.L. 1991. Telophase disc: a new mammalian mitotic organelle that bisects telophase cells with a possible function in cytokinesis. *J. Cell Sci.* **99**(3): 523–534.
- Andrews, P.D., Knatko, E., Moore, W.J., and Swedlow, J.R. 2003. Mitotic mechanics: the auroras come into view. *Curr. Opin. Cell Biol.* **15**: 672–683.
- Biggins, S., Severin, F.F., Bhalla, N., Sassoan, I., Hyman, A.A., and Murray, A.W. 1999. The conserved protein kinase Ipl1 regulates microtubule binding to kinetochores in budding yeast. *Genes Dev.* **13**: 532–544.
- Bouck, D.C., and Bloom, K.S. 2005. The kinetochore protein Ndc10p is required for spindle stability and cytokinesis in yeast. *Proc. Natl. Acad. Sci. U.S.A.* **102**: 5408–5413.
- Buvelot, S., Tatsutani, S.Y., Vermaak, D., and Biggins, S. 2003. The budding yeast Ipl1/Aurora protein kinase regulates mitotic spindle disassembly. *J. Cell Biol.* **160**: 329–339.
- Canman, J.C., Cameron, L.A., Maddox, P.S., Straight, A., Tirnauer, J.S., Mitchison, T.J. et al. 2003. Determining the position of the cell division plane. *Nature (London)*, **424**: 1074–1078.
- Carmena, M., and Earnshaw, W.C. 2003. The cellular geography of aurora kinases. *Nat. Rev. Mol. Cell Biol.* **4**: 842–854.
- Cheeseman, I.M., Anderson, S., Jwa, M., Green, E.M., Kang, J., Yates, J.R., III. et al. 2002. Phospho-regulation of kinetochore-microtubule attachments by the Aurora kinase Ipl1p. *Cell*, **111**: 163–172.
- Cooke, C.A., Heck, M.M., and Earnshaw, W.C. 1987. The inner centromere protein (INCENP) antigens: movement from inner centromere to midbody during mitosis. *J. Cell Biol.* **105**: 2053–2067.
- Dobbelaere, J., and Barral, Y. 2004. Spatial coordination of cytokinetic events by compartmentalization of the cell cortex. *Science (Washington, D.C.)*, **305**: 393–396.
- Endow, S.A., Kang, S.J., Satterwhite, L.L., Rose, M.D., Skeen, V.P., and Salmon, E.D. 1994. Yeast Kar3 is a minus-end microtubule motor protein that destabilizes microtubules preferentially at the minus ends. *EMBO J.* **13**: 2708–2713.
- Fraschini, R., Beretta, A., Lucchini, G., and Piatti, S. 2001. Role of the kinetochore protein Ndc10 in mitotic checkpoint activation in *Saccharomyces cerevisiae*. *Mol. Genet Genomics*, **266**: 115–125.
- Gardner, R.D., Poddar, A., Yellman, C., Tavormina, P.A., Monteagudo, M.C., and Burke, D.J. 2001. The spindle checkpoint of the yeast *Saccharomyces cerevisiae* requires kinetochore function and maps to the CBF3 domain. *Genetics*, **157**: 1493–1502.
- Gassmann, R., Carvalho, A., Henzing, A.J., Ruchaud, S., Hudson, D.F., Honda, R. et al. 2004. Borealin: a novel chromosomal passenger required for stability of the bipolar mitotic spindle. *J. Cell Biol.* **166**: 179–191.
- Goh, P.Y., and Kilmartin, J.V. 1993. NDC10: a gene involved in chromosome segregation in *Saccharomyces cerevisiae*. *J. Cell Biol.* **121**: 503–512.
- Hoyt, M.A., Totis, L., and Roberts, B.T. 1991. *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. *Cell*, **66**: 507–517.
- Iouk, T., Kerscher, O., Scott, R.J., Basrai, M.A., and Wozniak, R.W. 2002. The yeast nuclear pore complex functionally interacts with components of the spindle assembly checkpoint. *J. Cell Biol.* **159**: 807–819.
- Kaitna, S., Mendoza, M., Jantsch-Plunger, V., and Glotzer, M. 2000. Incenp and an aurora-like kinase form a complex essential for

- chromosome segregation and efficient completion of cytokinesis. *Curr. Biol.* **10**: 1172–1181.
- Kang, J., Cheeseman, I.M., Kallstrom, G., Velmurugan, S., Barnes, G., and Chan, C.S. 2001. Functional cooperation of Dam1, Ipl1, and the inner centromere protein (INCENP)-related protein Sli15 during chromosome segregation. *J. Cell Biol.* **155**: 763–774.
- Kim, J.H., Kang, J.S., and Chan, C.S. 1999. Sli15 associates with the ipl1 protein kinase to promote proper chromosome segregation in *Saccharomyces cerevisiae*. *J. Cell Biol.* **145**: 1381–1394.
- Leverson, J.D., Huang, H.K., Forsburg, S.L., and Hunter, T. 2002. The *Schizosaccharomyces pombe* aurora-related kinase Ark1 interacts with the inner centromere protein Pic1 and mediates chromosome segregation and cytokinesis. *Mol. Biol. Cell*, **13**: 1132–1143.
- Li, R., and Murray, A.W. 1991. Feedback control of mitosis in budding yeast. *Cell*, **66**: 519–531.
- Li, Y.Y., Yeh, E., Hays, T., and Bloom, K. 1993. Disruption of mitotic spindle orientation in a yeast dynein mutant. *Proc. Natl. Acad. Sci. U.S.A.* **90**: 10096–10100.
- Lippincott, J., Shannon, K.B., Shou, W., Deshaies, R.J., and Li, R. 2001. The Tem1 small GTPase controls actomyosin and septin dynamics during cytokinesis. *J. Cell Sci.* **114**: 1379–1386.
- Maddox, P.S., Bloom, K.S., and Salmon, E.D. 2000. The polarity and dynamics of microtubule assembly in the budding yeast *Saccharomyces cerevisiae*. *Nat. Cell Biol.* **2**: 36–41.
- McAinsh, A.D., Tytell, J.D., and Sorger, P.K. 2003. Structure, function, and regulation of budding yeast kinetochores. *Annu. Rev. Cell Dev. Biol.* **19**: 519–539.
- Miranda, J.J., De Wulf, P., Sorger, P.K., and Harrison, S.C. 2005. The yeast DASH complex forms closed rings on microtubules. *Nat. Struct. Mol. Biol.* **12**: 138–143.
- Morishita, J., Matsusaka, T., Goshima, G., Nakamura, T., Tatebe, H., and Yanagida, M. 2001. Bir1/Cut17 moving from chromosome to spindle upon the loss of cohesion is required for condensation, spindle elongation and repair. *Genes Cells*, **6**: 743–763.
- Page, B.D., Satterwhite, L.L., Rose, M.D., and Snyder, M. 1994. Localization of the Kar3 kinesin heavy chain-related protein requires the Cik1 interacting protein. *J. Cell Biol.* **124**: 507–519.
- Pearson, C.G., and Bloom, K. 2004. Dynamic microtubules lead the way for spindle positioning. *Nat. Rev. Mol. Cell Biol.* **5**: 481–492.
- Pellman, D., Bagget, M., Tu, Y.H., Fink, G.R., and Tu, H. 1995. Two microtubule-associated proteins required for anaphase spindle movement in *Saccharomyces cerevisiae*. *J. Cell Biol.* **130**: 1373–1385.
- Petersen, J., Paris, J., Willer, M., Philippe, M., and Hagan, I.M. 2001. The *S. pombe* aurora-related kinase Ark1 associates with mitotic structures in a stage dependent manner and is required for chromosome segregation. *J. Cell Sci.* **114**: 4371–4384.
- Rappaport, R. 1996. Cytokinesis in animal cells. Cambridge University Press.
- Sampath, S.C., Ohi, R., Leismann, O., Salic, A., Pozniakovski, A., and Funabiki, H. 2004. The chromosomal passenger complex is required for chromatin-induced microtubule stabilization and spindle assembly. *Cell*, **118**: 187–202.
- Schuyler, S.C., Liu, J.Y., and Pellman, D. 2003. The molecular function of Ase1p: evidence for a MAP-dependent midzone-specific spindle matrix. Microtubule-associated proteins. *J. Cell Biol.* **160**: 517–528.
- Shang, C., Hazbun, T.R., Cheeseman, I.M., Aranda, J., Fields, S., Drubin, D.G., and Barnes, G. 2003. Kinetochores protein interactions and their regulation by the Aurora kinase Ipl1p. *Mol. Biol. Cell*, **14**: 3342–3355.
- Sorger, P.K., Severin, F.F., and Hyman, A.A. 1994. Factors required for the binding of reassembled yeast kinetochores to microtubules in vitro. *J. Cell Biol.* **127**: 995–1008.
- Sullivan, M., Lehane, C., and Uhlmann, F. 2001. Orchestrating anaphase and mitotic exit: separase cleavage and localization of Slk19. *Nat. Cell Biol.* **3**: 771–777.
- Tanaka, K., Mukae, N., Dewar, H., van Breugel, M., James, E.K., Prescott, A.R. et al. 2005. Molecular mechanisms of kinetochores capture by spindle microtubules. *Nature (London)*, **434**: 987–994.
- Uren, A.G., Beilharz, T., O’Connell, M.J., Bugg, S.J., van Driel, R., Vaux, D.L., and Lithgow, T. 1999. Role for yeast inhibitor of apoptosis (IAP)-like proteins in cell division. *Proc. Natl. Acad. Sci. U.S.A.* **96**: 10170–10175.
- Westermann, S., Avila-Sakar, A., Wang, H.W., Niederstrasser, H., Wong, J., Drubin, D.G. et al. 2005. Formation of a dynamic kinetochores-microtubule interface through assembly of the Dam1 ring complex. *Mol. Cell*, **17**: 277–290.
- Wheatley, S.P., Carvalho, A., Vagnarelli, P., and Earnshaw, W.C. 2001. INCENP is required for proper targeting of Survivin to the centromeres and the anaphase spindle during mitosis. *Curr. Biol.* **11**: 886–890.
- Winey, M., Mamay, C.L., O’Toole, E.T., Mastronarde, D.N., Giddings, T.H., Jr., McDonald, K.L., and McIntosh, J.R. 1995. Three-dimensional ultrastructural analysis of the *Saccharomyces cerevisiae* mitotic spindle. *J. Cell Biol.* **129**: 1601–1615.
- Yoon, H.J., and Carbon, J. 1999. Participation of Bir1p, a member of the inhibitor of apoptosis family, in yeast chromosome segregation events. *Proc. Natl. Acad. Sci. U.S.A.* **96**: 13208–13213.
- Zeng, X., Kahana, J.A., Silver, P.A., Morphew, M.K., McIntosh, J.R., Fitch, I.T. et al. 1999. Slk19p is a centromere protein that functions to stabilize mitotic spindles. *J. Cell Biol.* **146**: 415–425.