RESEARCH PAPER

BioArchitecture 3:3, 69–76; May/June 2013; © 2013 Landes Bioscience

Insights from an erroneous kinetochore-microtubule attachment state

Stuart Cane,[1,2,†] Philip T. McGilvray[1,†] and Thomas J. Maresca[1,2,*]

[1]Biology Department; University of Massachusetts; Amherst, MA USA; [2]Molecular and Cellular Biology Graduate Program; University of Massachusetts; Amherst, MA USA

[†]These authors contributed equally to this work.

Keywords: cell division, kinetochore, microtubule, syntelic attachment, error correction, spindle assembly checkpoint, aurora B kinase, Mad1, BubR1

Abbreviations: ABK, aurora B kinase; BubR1, budding uninhibited by benzimidazoles related 1; HEC1, highly expressed in cancer protein 1; kt-MT, kinetochore-microtubule; Mad1, mitotic arrest-deficient 1; Mad2, mitotic arrest-deficient 2; Ndc80, nuclear division cycle 80; NOD, no-distributive disjunction; PEF, polar ejection force; pN, piconewton; SAC, spindle assembly checkpoint

Faithful distribution of the genome requires that sister kinetochores, which assemble on each chromatid during cell division, interact with dynamic microtubules from opposite spindle poles in a configuration called chromosome biorientation. Biorientation produces tension that increases the affinity of kinetochores for microtubules via ill-defined mechanisms. Non-bioriented kinetochore-microtubule (kt-MT) interactions are prevalent but short-lived due to an error correction pathway that reduces the affinity of kinetochores for microtubules. Interestingly, incorrect kt-MT interactions can be stabilized by experimentally applying force to misoriented chromosomes. Here, a live-cell force assay is utilized to characterize the molecular composition of a common type of improper kt-MT attachment. Our force-related studies are also discussed in the context of current models for tension-dependent stabilization of kt-MT interactions.

Whenever a cell divides it is faced with the incredibly complex challenge of evenly distributing its entire complement of replicated chromosomes into two daughter cells. Amazingly, healthy cells accurately divvy up their genomes 99 out of every 100 times that they divide.[1] So how do cells pull off such a herculean task with such high fidelity? The solution is provided, in large part, by two critical and convergent cellular networks:[2] the error correction pathway, which destabilizes improper interactions between chromosomes and spindle microtubules, and the spindle assembly checkpoint (SAC) pathway, which delays anaphase onset until every chromosome interacts properly with spindle microtubules.

"Proper interaction," in this context, refers to chromosome biorientation—the geometric configuration that best ensures sister chromatids will end up in different daughter cells. During the initial phases of cell division, a complex called the kinetochore, consisting of multiple copies of more than 100 different proteins, assembles on the centromeres of sister chromatids, which remain held together through DNA catenation and protein-based cohesion. The kinetochore consists of domains with distinct molecular compositions. The inner kinetochore contains DNA-binding components and, consequently, interfaces with the centromeric chromatin whereas the microtubule-attachment factors reside in the outer kinetochore.[3,4] The core kt-MT attachment complex consists of 8–9 proteins (depending on the organism) and is referred to as the KMN (KNL1/Blinkin, Mis12 complex, Ndc80 complex) network.[5-7] Thus, the kinetochore connects the chromosomes to spindle microtubules. The kinetochore also serves as the physical locale from which the SAC signal originates. Checkpoint proteins including Mad1 and BubR1 accumulate at improperly attached kinetochores[8] and delay anaphase onset by catalyzing the assembly of a soluble inhibitor of the anaphase promoting complex/cyclosome (APC/C).[9-11] Not surprisingly, the error correction and SAC pathways converge at kinetochores to promote segregation of bioriented chromosomes.

Chromosomes become bioriented when their sister kinetochores attach to dynamic microtubules emanating from opposite spindle poles. Chromosome biorientation generates tension across sister kinetochores because kinetochore-microtubules (kt-MTs) pull the physically linked chromatids in opposite directions. In turn, tension increases the affinity of kinetochore-associated microtubule-binding factors for microtubules.[12-15] Thus, bioriented attachments are selectively stabilized. While bioriented attachments may be the most stable kt-MT interaction, erroneous, non-bioriented kt-MT attachments form very frequently. In fact, a recent analysis of meiosis I kinetochores in mouse oocytes revealed that nearly 90% of kinetochores established improper kt-MT interactions at least once before becoming bioriented.[16] However, erroneous kt-MT attachments are transient because they are destabilized by a centromere enriched kinase called aurora B kinase (ABK),[17,18] which reduces the affinity of improperly attached kinetochores for microtubules via phosphorylation of KMN components including the microtubule-binding factor Ndc80.[5,6]

Bioriented kinetochores under tension are not subjected to error correction nor do they contribute to production of an

*Correspondence to: Thomas J. Maresca; Email: tmaresca@bio.umass.edu
Submitted: 06/28/13; Revised: 07/11/13; Accepted: 07/11/13
http://dx.doi.org/10.4161/bioa.25734

inhibitory SAC signal.[13,19] Does production of tension merely correlate with the cessation of error correction and SAC signaling or does it actively contribute to their suppression? We and others have demonstrated that experimentally applying tension to misoriented kinetochores in living cells overrides ABK-mediated error correction and production of the SAC signal.[13,14,20] The data reveal that tension plays an active role in opposing the error correction and SAC pathways although the means by which it does so are not entirely clear. For example, it has been hotly debated as to whether kinetochore tension is capable of providing a direct input to production of a SAC signal or if the soluble inhibitory signal is only generated by unattached kinetochores.[21] While it is our opinion that existing data are insufficient to definitively rule out a direct contribution of tension to SAC signaling, the fact that tension stabilizes kt-MT interactions places tension upstream of attachment in the SAC pathway; therefore, we believe tension must be considered an important regulator of SAC signaling.

We recently developed a live-cell polar ejection force (PEF) assay in *Drosophila melanogaster* S2 cells to more closely examine the effects of force and the tension it produces at kinetochores.[20] The PEF assay takes advantage of an intrinsic force-producing cellular component, specifically the chromokinesin NOD (Drosophila kinesin-10),[22-24] to experimentally elevate the force, known as the polar ejection force,[25,26] that pushes chromosome arms away from spindle poles. In the PEF assay, inducible NOD overexpression results in a dose-dependent increase in the percentage of a specific type of erroneous kt-MT interaction called a syntelic attachment, in which both sister kinetochores are attached to the same spindle pole. Syntelic attachments are normally short-lived due to the activity of ABK. We postulate that syntelic attachments, despite being misoriented, are stabilized in the PEF assay through a tension-dependent mechanism in which the poleward pulling force generated by kt-MTs[27,28] is opposed by the away-from-the-pole pushing force produced by NOD.[23] In turn, elevated tension at the syntelic kinetochores overwhelms ABK-mediated error correction and the SAC is satisfied in the presence of erroneous yet artificially stabilized kt-MT attachments. The ability to experimentally produce high numbers of stable syntelic attachments in the PEF assay offers a valuable opportunity to study the nature of these transient kt-MT interactions and how they contribute to production of a SAC signal.

In order to evaluate the checkpoint response to the syntelic attachments produced in NOD-overexpressing cells, a cell line was generated expressing both NOD-mCherry and an EYFP-tagged version of the checkpoint protein Mad1. A sharp reduction of Mad1 levels at the kinetochore, due in part to dynein-mediated "stripping" and poleward transport along kt-MTs, accompanies SAC satisfaction and precedes anaphase onset.[29-31] In accordance with the observation that NOD-expressing cells enter anaphase in the presence of syntelic attachments, we found that Mad1-EYFP levels were reduced at both bioriented and syntelic attachments in cells arrested in metaphase with the proteasome inhibitor MG132.[20]

While NOD-expressing S2 cells clearly satisfied the SAC in the presence of stable syntelic attachments, they did so more slowly than wild type cells. Furthermore, the duration of mitosis increased with the number of syntelic attachments (unpublished observation). To better understand why mitotic progression was slower in the presence of stable syntelic attachments, we examined kinetochore Mad1-EYFP dynamics as cells progressed through cell division in the absence of MG132. Interestingly, Mad1 depletion often occurred more rapidly at bioriented kinetochores than at syntelic attachments (**Fig. 1A**, **Movie S1**). In support of our previous observation that stable syntelic attachments established robust kinetochore fibers,[20] Mad1-EYFP particles could be seen streaming poleward along kt-MTs (**Fig. 1**, **Movies S1 and S2**). Thus, dynein actively depletes Mad1 from NOD-stabilized syntelic attachments.

Why, then, do the syntelic attachments often lose Mad1 more slowly than bioriented attachments? Ultimately, depletion of kinetochore-associated Mad1 depends on its off-rate, which is regulated by dynein-mediated stripping, being greater than its on-rate. While Mad1 is clearly being stripped from the stable syntelic kinetochores, the relatively slow pace of its reduction suggests that it is also being replenished and, moreover, that the Mad1 on-rate is higher at these sites than at bioriented attachments. This difference warrants further investigation especially because (1) NOD-mediated syntelic attachments were found to be as stable as bioriented attachments[20] and (2) Mad1 levels are generally believed to reflect the degree of kt-MT attachment stability.

Anaphase onset normally occurs ~10 min after the Mad1 binding partner Mad2 is completely lost from the kinetochores of the last chromosome to align on the metaphase plate.[29] However, we observed that Mad1 was detectable at some syntelically attached kinetochores at anaphase onset, albeit at significantly lower levels than at the start of imaging (**Fig. 1A and B** and **Movies S1 and S2**). While this discrepancy could reflect a difference in the dynamics of Mad1 vs. Mad2 depletion from kinetochores, we presume that low levels of Mad2 are also present at the stable syntelic attachments where Mad1 persists. Thus, we favor the idea that Mad1/Mad2 at stable syntelic attachments contributes to production of a wait-anaphase signal, but that once kinetochore-associated levels of Mad1/Mad2 drop below a critical, but still observable, threshold the signal is no longer strong enough to block anaphase onset. A logical extension of this hypothesis is that the SAC is mediated by a titratable inhibitory signal derived from the sum total of the signal inputs from each kinetochore. This would result in the production of wait-anaphase signals of variable potencies, reflected in varying degrees of mitotic delay, rather than an "all-or-none" signal. Such a mechanism would explain why the duration of mitosis increases with the number of stable syntelic attachments. Not only does the model predict that a cell with a greater number of stable syntelics will spend a longer time in mitosis than a cell with fewer; it also predicts that the more syntelically attached kinetochores with detectable Mad1 are found in a given cell, the greater the Mad1 reduction that must occur at each such kinetochore before anaphase onset can occur.

It is also notable that not all stable syntelic attachments behave in the same manner. For example, often Mad1-EYFP was absent from some syntelic attachments yet detectable at other syntelics

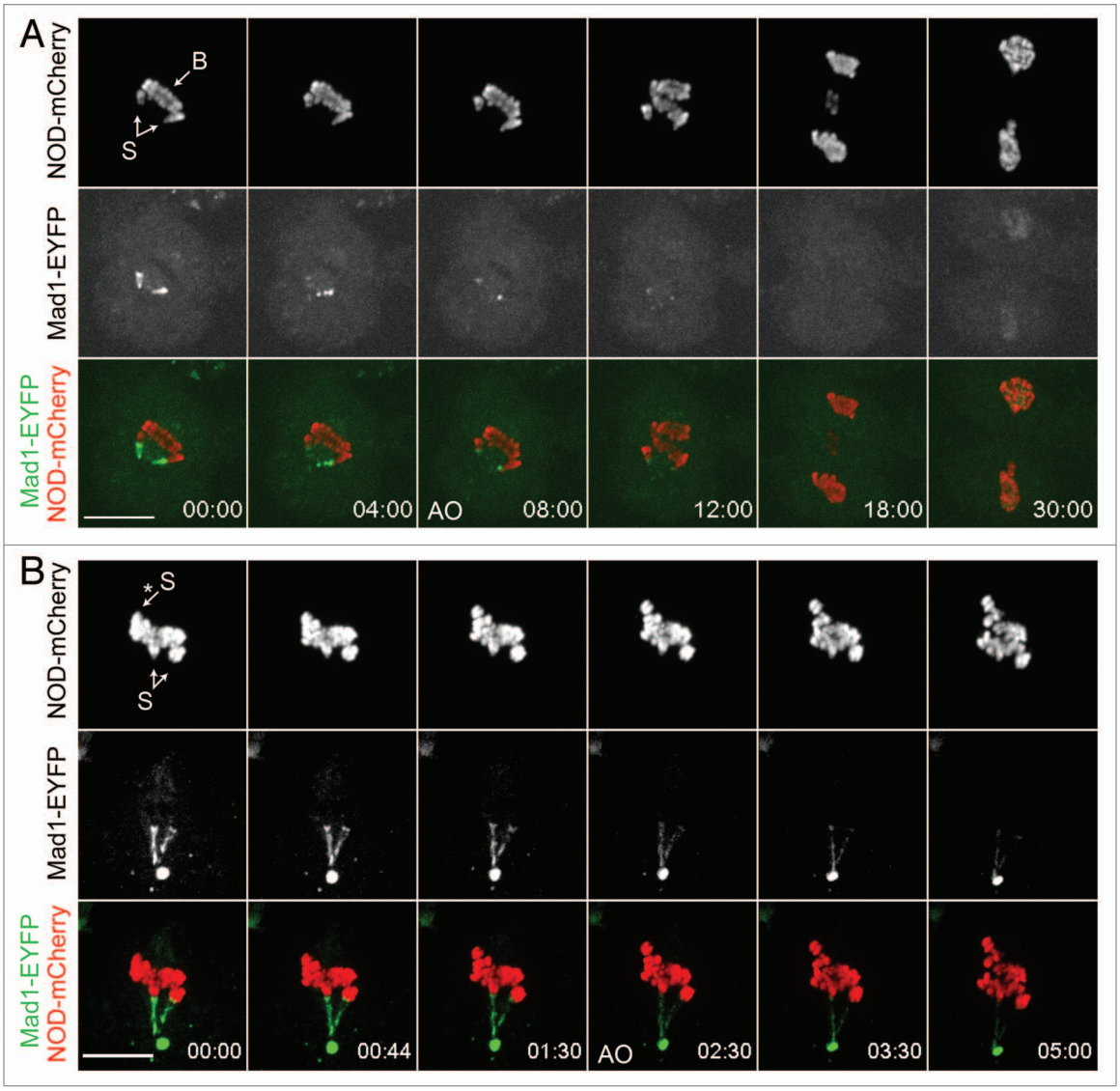

Figure 1. Mad1 depletion from syntelic attachments is often gradual and incomplete before anaphase onset. (**A**) Still images of time-lapse microscopy (**Movie S1**) showing a cell with a mixture of syntelic and bioriented kinetochores. Mad1-EYFP persists at the syntelic kinetochores after it has been depleted from bioriented kinetochores. (**B**) Still images of time-lapse microscopy (**Movie S2**) showing a different cell with syntelic and bioriented attachments. One syntelic (arrow with asterisk) lacks detectable Mad1 but Mad1 persists at two other syntelics (arrows). In both **A and B**, the cells enter anaphase (AO) despite having detectable (though reduced) levels of Mad1 at two syntelic attachments. In each case, Mad1 can also be seen streaming away from the syntelic attachments along kinetochore fibers. In merged images NOD-mCherry is red and Mad1-EYFP is green. Scale bars are 10 μm. Materials and Methods: Drosophila S2 cells expressing kinesin 1-NOD-mCherry and Mad1-EYFP[20] were cultured in the Schneider media (Life Technologies) supplemented with 10% heat inactivated fetal bovine serum (Life Technologies) and 0.5× antibiotic-antimycotic cocktail (Life Technologies). The cells were seeded onto concanavalin A (Sigma) treated acid-washed coverslips (Corning) for 1 h after an overnight treatment with 500 μM $CuSO_4$ to induce NOD expression. The coverslips were next assembled into rose chambers containing the Schneider media and imaged at room temperature on a Nikon TiE inverted microscope with a CSU-X1 spinning disk confocal head (Yokogawa) and an iXON EMCCD camera (Andor Technology) using a Nikon 100× 1.4 NA Plan Apo violet corrected (VC) series DIC objective. Confocal images of the EYFP and mCherry channels were acquired every 2 min (**Fig. 1A**) or every 15 sec (**Fig. 1B**).

in the same cell (**Fig. 1B** and **Movie S2**). We do not currently understand what causes this stochasticity; however, the fact that Mad1 is depleted from syntelic attachments to the same extent as from bioriented chromosomes following a two-hour MG132 treatment suggests that the non-uniform behavior seen in untreated cells may be related to the age of the syntelic attachment. We propose that Mad1 is depleted gradually from stable syntelics because the dynein-driven off-rate of Mad1 is only slightly greater than its on-rate. The observation of anaphase onset with detectable levels of kinetochore-associated Mad1 and active Mad1 streaming has additional implications for checkpoint regulation. First, it suggests that the kinetochore fiber-associated Mad1-containing particles do not produce a sufficiently potent wait-anaphase signal to maintain a mitotic delay. Since Mad1 was not replenished at kinetochores following anaphase onset (**Fig. 1A and B**) key regulators of the Mad1 on-rate must change at the metaphase to anaphase transition.

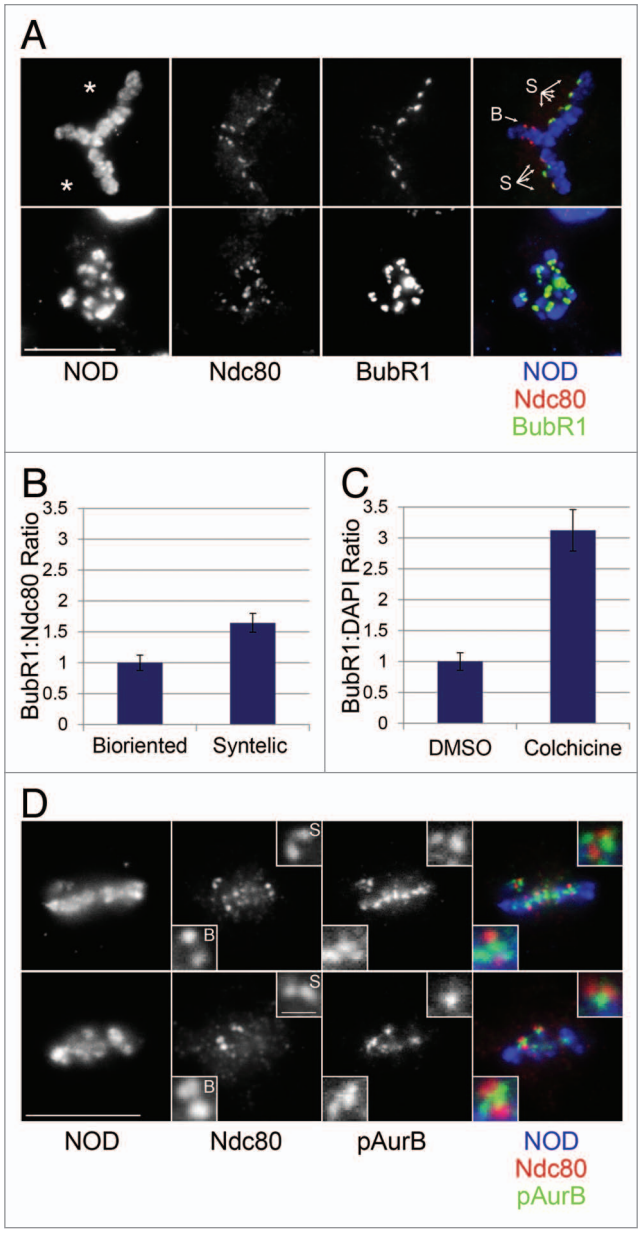

Figure 2. BubR1 levels at syntelic attachments are intermediate between levels at unattached and bioriented kinetochores. (**A-B**) BubR1 levels at syntelic attachments (S) are measured by quantitative immunofluorescence to be ~1.7 fold higher at syntelics relative to bioriented (**B**) attachments. In merged images NOD is blue, Ndc80 is red and BubR1 is green. (**C**) Unattached kinetochores in colchicine-treated cells exhibit a 3.2 fold increase of BubR1 levels relative to bioriented attachments in DMSO-treated controls. (**D**) ABK is properly localized and active (phosphorylated) at syntelic attachments, but the erroneous attachments are not corrected. The insets show 4× zooms of representative attachments (bioriented [B] in the lower left and syntelics [S] in the upper right). In merged images NOD is blue, Ndc80 is red and phospho-Aurora B (pAurB) is green. Scale bars are 10 μm and 1 μm in insets. Error bars represent the SEM. Materials and Methods: S2 cells expressing BubR1-GFP and NOD-mCherry (**A, B, C**) or only NOD-EGFP (**D**), after overnight induction with 25 μM $CuSO_4$ and a two hour treatment with 10 μM MG132 (Sigma) (**A, B and C**) followed by a 1 h treatment with either 0.1% DMSO or 25 μM colchicine (**A and C only**) were adhered to concanavalin A-coated coverslips before being rinsed in BRB80 and fixed in 10% paraformaldehyde. Cells were then permeabilized for 10 min in 1× PBS + 1% Triton X-100, washed 3× for 5 min in 1× PBS + 0.1% Triton, and blocked in Boiled Donkey Serum for 30–60 min. The cells were stained overnight at 4°C with either chicken anti-Ndc80 (Maresca lab) diluted 1:100 or rabbit anti-phospho-AuroraA/B/C (Cell Signaling Technology) at 1:1000 in Boiled Donkey Serum. Coverslips were washed 3× for 5 min in 1× PBS + 0.1% Triton and then incubated at room temperature for 45 min with appropriate secondary antibodies (Jackson ImmunoResearch) at 1:200 and DAPI (1:100) diluted in Boiled Donkey Serum. Coverslips were washed 3× for 5 min in 1× PBS + 0.1% Triton before mounting them in media containing 20 mM Tris (pH 8.0), 0.5% N-propyl gallate and 90% glycerol. The ratio of the total fluorescence intensities of BubR1-GFP to Ndc80 (**B**) or DAPI (**C**) were quantified as previously described.[20]

We next quantitatively examined the levels of a second checkpoint protein called BubR1 at stable syntelic attachments. In close agreement with previous findings,[8] unattached kinetochores in cells treated with colchicine to eliminate kinetochore-microtubule attachments exhibited a 3.2-fold increase in kinetochore-associated BubR1 relative to bioriented kinetochores in DMSO-treated control cells (**Fig. 2A and C**). In contrast, syntelically attached kinetochore pairs had 1.7-fold higher levels of BubR1 relative to their bioriented counterparts (**Fig. 2B**), which represents ~50% of the amount of BubR1 at unattached kinetochores. BubR1 levels at the bioriented and syntelic attachments (**Fig. 2B**) were measured after a two-hour MG132 treatment to arrest cells in metaphase—the same treatment that resulted in comparable depletion of kinetochore-associated Mad1 from both types of attachments.[20] Thus, a sub-population of BubR1 in S2 cells behaves differently than Mad1, which, given enough time, is lost from stable syntelic attachments. BubR1, like Mad1, may be stripped from kinetochores through dynein-mediated streaming along kinetochore fibers,[30,32] although this behavior has not been evident in Drosophila cells.[33] Our data suggest that only a subpopulation of BubR1 can be removed from stable syntelic attachments, possibly through dynein-mediated stripping, and that the remaining ~50% is regulated through different molecular mechanisms. In summary, kinetochore levels of BubR1 at stable syntelic attachments are lower than at unattached kinetochores but higher than at bioriented attachments, while the kinetics of Mad1 depletion from syntelic attachments are often slower than the kinetics at bioriented attachments (**Fig. 3**).

We next examined the behavior of ABK in NOD-expressing cells because the localization of BubR1 and the Mad proteins to kinetochores is regulated by ABK.[34,35] Active phosphorylated ABK localized properly to the inner centromere in both bioriented and stable syntelic attachments (**Fig. 2D**). Thus, neither the persistence of syntelic attachments nor the changes in kinetochore checkpoint protein levels at these attachments can be attributed to significant changes in ABK localization or activity following NOD overexpression.

Our NOD overexpression studies also had obvious implications for another important unresolved question in the field: How does tension stabilize kt-MT attachments? There are presently two major models for tension-dependent stabilization of kt-MT attachments. The models will be referred to here as (1) spatial positioning and (2) catch bond. Spatial positioning posits that kt-MT attachment affinity is increased by repositioning

attachment factors further away from ABK through a tension-dependent structural change within the kinetochore called intrakinetochore stretch.[21,36,37] For simplicity's sake, spatial positioning will be presented here although related models have been proposed that do not evoke spatial positioning of attachment factors relative to ABK.[38] Ultimately, these alternative models, like spatial positioning, involve tension-dependent silencing of ABK-mediated error correction. The catch bond model, on the other hand, postulates that tension increases the affinity of attachment factors for microtubules via purely mechanical means and independent of ABK.[15] The spatial positioning and catch bond models are not mutually exclusive, and it is believed that both contribute to tension-mediated stabilization of kt-MT attachments in cells, but it remains to be seen whether that is indeed the case and, if so, what the relative contribution of each mechanism is to kt-MT attachment stability.

In catch bonds, the interaction between two components becomes stronger when force is applied to them. Purified budding yeast kinetochore particles, lacking ABK, exhibit catch bond properties as the lifetime of their interactions with dynamic microtubules was found to increase with the application of increasing force using an optical trap.[15] Furthermore, increased tension caused the single microtubule to which a particle was attached to undergo fewer catastrophes and more frequent rescues. While the data strongly suggest that budding yeast kt-MT attachments act as catch-bonds and that tension regulates kt-MT dynamics, the molecular mechanisms underlying these observations are currently unknown. We now propose a mechano-molecular hypothesis to explain the kinetochore catch bond. We acknowledge that the model outlined here is speculative, and we do not consider it to be comprehensive or to preclude other potential catch bond mechanisms. Rather, we view the model as an important discussion point within the broader conversation surrounding the question of how tension stabilizes kt-MT attachments.

The model focuses on the interface between kinetochore microtubules and a core attachment factor called Ndc80 (also known as highly expressed in cancer 1 [HEC1]) that localizes to the outer kinetochore. Microtubule structure and dynamics are key components of the model. Catastrophe, or the transition from assembly to disassembly, is accompanied by a dramatic change in the structure of protofilaments at the plus-end of the microtubule from straight to curved. Conversely, rescue events and subsequent polymerization most likely require a majority of protofilaments at the plus-end to be in a straight conformation. Thus, protofilament plus-ends adopt distinct structural states depending on the polymerization state of the microtubule: straight when the microtubule is polymerizing and curved when it is depolymerizing. Microtubule dynamics also produce force. Polymerization can generate a pushing force of ~3–4 piconewtons (pN) against a barrier[39] while measurements of the force produced by depolymerizing microtubules have suggested that each curving protofilament can generate up to 5 pN.[40]

Kinetochore microtubules in budding yeast must transition between polymerization and depolymerization since each kinetochore associates with a single microtubule,[41] assembly and disassembly only takes place at the plus-ends,[42] and pre-anaphase centromere movements occur at rates similar to those of growing and shrinking plus-ends of astral microtubules.[43] The fact that metaphase chromosomes oscillate in many vertebrate cell types indicates that kt-MTs also transition between polymerization and depolymerization at kinetochores bound to multiple microtubules. Interestingly, a majority of metazoan kt-MTs analyzed by electron tomography exist in a curved/depolymerizing configuration regardless of their oscillatory state.[44,45] Nonetheless, kt-MTs were also found to contain some straightened protofilaments with one study reporting that ~1/3 of kt-MT plus-ends were in a straightened/polymerizing state.[45] Our model presumes that, regardless of whether a kinetochore interacts with a single or multiple microtubules, (1) kt-MTs transition between assembly and disassembly and (2) the conformation of the kt-MT plus-ends and the extent of protofilament curvature changes accordingly, with depolymerizing ends being more curved and polymerizing ends being more straight.

The core kt-MT attachment factor Ndc80 interacts with microtubules through an N-terminal calponin homology domain via its so-called "toe."[46] Ndc80 can bind both α and β

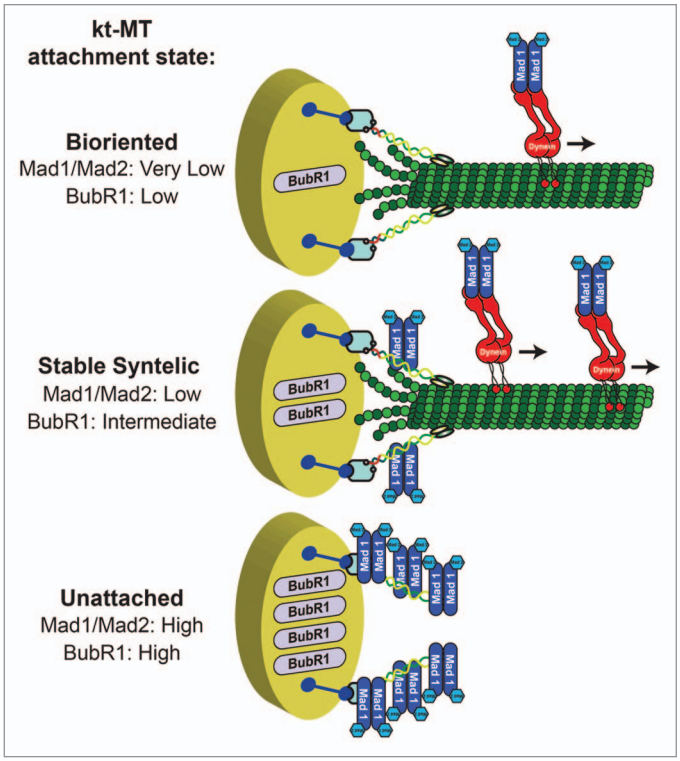

Figure 3. Graphical summary of Mad1 and BubR1 levels at bioriented, NOD-stabilized syntelic and unattached kinetochores. Unattached kinetochores have the highest levels of Mad1 and BubR1. Mad1 levels are very low at bioriented attachments while BubR1 is detectable but reduced ~3.2 fold relative to unattached kinetochores. Mad1 is stripped from syntelic attachments by dynein although it is depleted with slower kinetics than from bioriented attachments. Syntelic attachments have intermediate levels of BubR1, which are ~50% the amount seen at fully unattached kinetochores but ~1.7 fold higher than the levels at bioriented attachments.

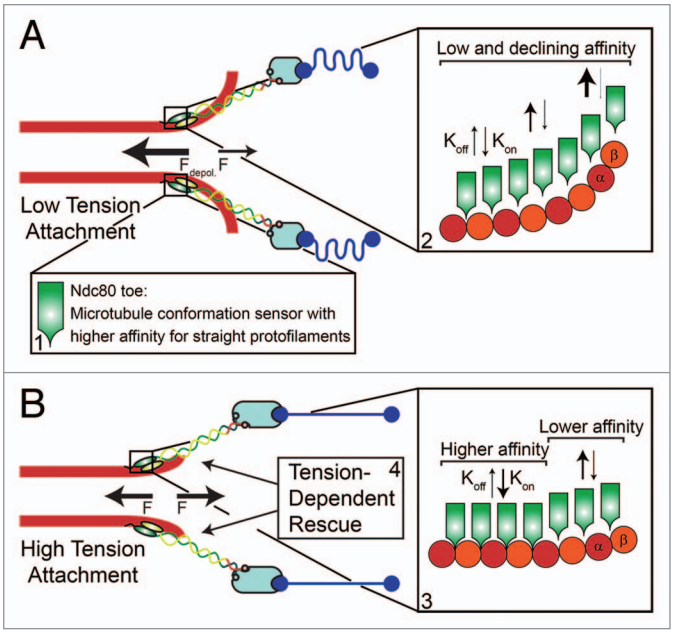

Figure 4. Mechano-molecular model of a kinetochore catch bond. (**A**) When Ndc80 makes initial end-on contact with a dynamic microtubule that has transitioned into a depolymerizing state, the kinetochore is pulled poleward by the force of microtubule depolymerization. Ndc80 maintains load-bearing interactions with the shrinking microtubule through biased diffusion that results from preferential binding of the Ndc80 toe (insert 1) to the straight regions of protofilaments at some distance from the curling plus-ends. While these initial interactions are load bearing we propose that the curved conformation of the depolymerizing plus-ends prevents high affinity Ndc80 toe interactions (insert 2). (**B**) When opposing forces produce kinetochore tension, internal kinetochore elements (light and dark blue) extend through stretching and/or reorientation and microtubule protofilaments straighten at the plus-end. The model posits that tension-dependent straightening of protofilaments increases the affinity of Ndc80 for the microtubule by presenting higher affinity binding sites for the Ndc80 toe (insert 3) and promotes rescue once protofilament straightening reaches a critical threshold (insert 4).

tubulin monomers[46,47]—a property that distinguishes it from most microtubule-associated proteins, which typically interact with α/β heterodimers. More specifically, the Ndc80 toe binds to a hinge point between each tubulin monomer that is proposed to pivot with protofilament curvature in a manner that reduces the affinity of Ndc80 for a curved lattice.[46] Accordingly, the Ndc80 complex exhibits greater affinity for straight microtubules than for curved microtubule substrates.[46,48] Thus, the Ndc80 toe is postulated to act as a microtubule conformation sensor that causes the Ndc80 complex to associate preferentially with straight protofilaments (**Fig. 4A**, inset 1).

We propose that when the kinetochore makes initial end-on contact with a microtubule that has transitioned into a depolymerizing state, the Ndc80 complexes will bind weakly to the microtubule lattice with the highest affinity interactions being found along the straight portions of protofilaments at some distance from the highly curved plus-ends (**Fig. 4A**, inset 2). This early kt-MT interaction will result in the kinetochore moving poleward as the force generated by microtubule depolymerization dominates and Ndc80 complexes remain loosely associated with the shortening microtubule through biased diffusion.[46,49] The model next envisions that increasing levels of opposing force—for example, from chromosome biorientation—will reduce the curvature of kt-MT plus-ends (**Fig. 4B**). In this case, the interaction between Ndc80 and the microtubule would behave like a catch bond as tension-dependent straightening of protofilaments increases the affinity of Ndc80 for kt-MTs by creating more accessible binding sites for the Ndc80 toe (**Fig. 4B**, inset 3). We also propose that the microtubule would eventually transition into a polymerizing state once tension-mediated straightening of the protofilaments reaches a critical threshold (**Fig. 4B**, inset 4).

The full picture in dividing cells is certainly more complicated than our model. While the model focuses on Ndc80 to highlight a potential tension-dependent mechanism for increasing the kinetochore's affinity for microtubules, the catch bond mechanism almost certainly depends on other critical properties of the kinetochore that the model does not discuss. These include, but are not limited to: (1) the number of Ndc80 molecules, (2) the 3-dimensional arrangement of Ndc80 attachment sites on the lattice and (3) the number, arrangement and functions of other kinetochore associated proteins such as the Dam1 complex in budding yeast or the Ska complex in vertebrate cells. It should be noted that the model does not require that tension-dependent rescues be mediated by the Ndc80 complex; per se, but only that they depend on straightening of the protofilaments, which could be accomplished by other kinetochore components. Indeed, like the purified kinetochore particles, application of tension to purified Dam1 complex-microtubule attachments also increased the rescue rate and reduced the catastrophe frequency.[50] Regardless of what molecules impart tension-dependent straightening of the protofilament; our model proposes that the result will be the same—higher affinity interactions between the Ndc80 toe and the microtubule.

Kinetochore-microtubule attachment stability, especially during the initial establishment of end-on attachments, most likely depends on the combined inputs from phospho-regulation through spatial positioning and mechano-regulation via the catch bond pathway (**Fig. 5**). We propose that distinct contributions from the two pathways would yield a gradient of kt-MT attachment affinities—the highest affinity coming when Ndc80 is associated with straight protofilaments and dephosphorylated (**Fig. 5A**) and the lowest affinity interaction coming from phosphorylated Ndc80 with a prevalence of curved protofilaments in its vicinity (**Fig. 5D**). Since it is unclear whether one mechanism or the other dominates in modulating the affinity of Ndc80 for microtubules the exact order of affinities in our proposed attachment stability gradient is not clear; however, intermediate attachment affinities would be expected if Ndc80 was phosphorylated but protofilaments were straight (**Fig. 5B**) or if Ndc80 was dephosphorylated and protofilaments were curved (**Fig. 5C**). An additional layer of complexity exists for kinetochores that bind numerous microtubules as individual kt-MT interactions with varying affinities may exist within the same kinetochore.

Elevating PEFs via NOD overexpression has provided key insights into how tension produced at kinetochores upon

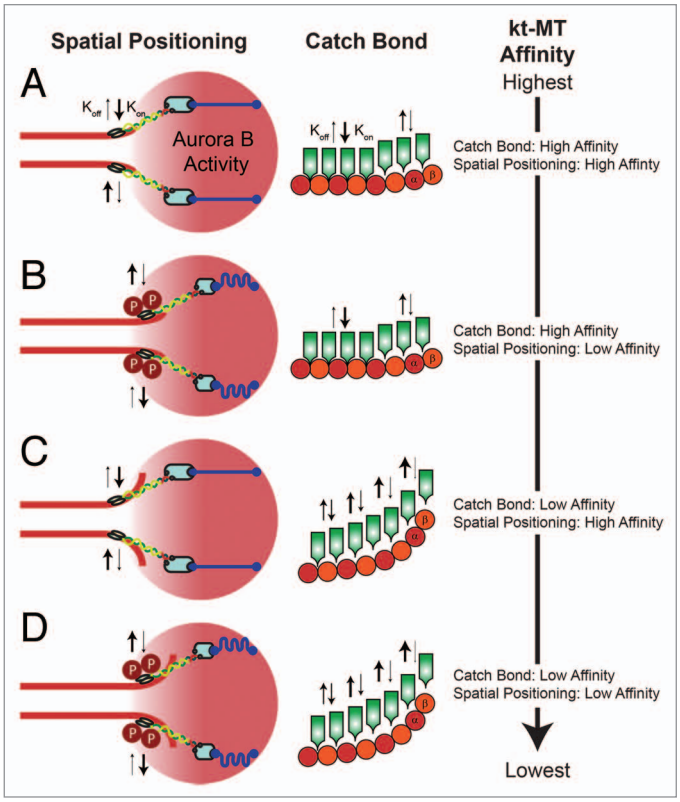

Figure 5. The combined contribution of spatial positioning and catch bond mechanisms could create a kt-MT attachment affinity gradient. (**A**) In spatial positioning, tension repositions the Ndc80 complex further away from ABK by stretching (or reorienting) internal kinetochore elements (light and dark blue) resulting in reduced phosphorylation and increasing the affinity of the complex for microtubules. In the proposed catch bond mechanism, tension straightens protofilaments thereby increasing the affinity of the Ndc80 toe for microtubules. Dephosphorylated Ndc80 associated with straight protofilaments produces the highest affinity kt-MT interaction. Lower affinity interactions would result if Ndc80 was phosphorylated but associated with straight protofilaments (**B**) or if Ndc80 was dephosphorylated and associated with curved protofilaments (**C**) although it is unclear which of these combinations would exhibit higher affinity. (**D**) The lowest affinity kt-MT interaction would result from phosphorylated Ndc80 associated with curved protofilaments.

chromosome biorientation regulates kt-MT attachment stability in unperturbed cells. Further experimentation employing the PEF assay[20] should promote a more refined understanding of the mechanisms involved in attachment stabilization and of the relative contributions of factors such as spatial positioning and catch bond interactions at the outer kinetochore.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interests were disclosed.

Acknowledgments

Live-cell spinning disk confocal imaging was done at the University of Massachusetts Amherst spinning disc confocal and super-resolution imaging user facility developed by co-PIs Jennifer L. Ross and Patricia Wadsworth with funding provided by an NSF Major Research Instrumentation (MRI) grant DBI #0923318. The work was funded by Honors Research Grants from the Commonwealth Honors College at UMASS Amherst to author PTM and support from the Charles H. Hood Foundation, Inc., Boston, MA to author TJM and Research Grant No.5-FY13–205 from the March of Dimes Foundation to TJM.

Supplemental Materials

Supplemental materials may be found here:
www.landesbioscience.com/journals/bioarchitecture/article/25734.

References

1. Thompson SL, Compton DA. Examining the link between chromosomal instability and aneuploidy in human cells. J Cell Biol 2008; 180:665-72; PMID:18283116; http://dx.doi.org/10.1083/jcb.200712029
2. Nezi L, Musacchio A. Sister chromatid tension and the spindle assembly checkpoint. Curr Opin Cell Biol 2009; 21:785-95; PMID:19846287; http://dx.doi.org/10.1016/j.ceb.2009.09.007
3. Santaguida S, Musacchio A. The life and miracles of kinetochores. EMBO J 2009; 28:2511-31; PMID:19629042; http://dx.doi.org/10.1038/emboj.2009.173
4. Cheeseman IM, Desai A. Molecular architecture of the kinetochore-microtubule interface. Nat Rev Mol Cell Biol 2008; 9:33-46; PMID:18097444; http://dx.doi.org/10.1038/nrm2310
5. Cheeseman IM, Chappie JS, Wilson-Kubalek EM, Desai A. The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. Cell 2006; 127:983-97; PMID:17129783; http://dx.doi.org/10.1016/j.cell.2006.09.039
6. DeLuca JG, Gall WE, Ciferri C, Cimini D, Musacchio A, Salmon ED. Kinetochore microtubule dynamics and attachment stability are regulated by Hec1. Cell 2006; 127:969-82; PMID:17129782; http://dx.doi.org/10.1016/j.cell.2006.09.047
7. Kiyomitsu T, Obuse C, Yanagida M. Human Blinkin/AF15q14 is required for chromosome alignment and the mitotic checkpoint through direct interaction with Bub1 and BubR1. Dev Cell 2007; 13:663-76; PMID:17981135; http://dx.doi.org/10.1016/j.devcel.2007.09.005
8. Hoffman DB, Pearson CG, Yen TJ, Howell BJ, Salmon ED. Microtubule-dependent changes in assembly of microtubule motor proteins and mitotic spindle checkpoint proteins at PtK1 kinetochores. Mol Biol Cell 2001; 12:1995-2009; PMID:11451998; http://dx.doi.org/10.1091/mbc.12.7.1995
9. Chao WC, Kulkarni K, Zhang Z, Kong EH, Barford D. Structure of the mitotic checkpoint complex. Nature 2012; 484:208-13; PMID:22437499; http://dx.doi.org/10.1038/nature10896
10. Hardwick KG, Johnston RC, Smith DL, Murray AW. MAD3 encodes a novel component of the spindle checkpoint which interacts with Bub3p, Cdc20p, and Mad2p. J Cell Biol 2000; 148:871-82; PMID:10704439; http://dx.doi.org/10.1083/jcb.148.5.871
11. Sudakin V, Chan GK, Yen TJ. Checkpoint inhibition of the APC/C in HeLa cells is mediated by a complex of BUBR1, BUB3, CDC20, and MAD2. J Cell Biol 2001; 154:925-36; PMID:11535616; http://dx.doi.org/10.1083/jcb.200102093
12. King JM, Nicklas RB. Tension on chromosomes increases the number of kinetochore microtubules but only within limits. J Cell Sci 2000; 113:3815-23; PMID:11034909
13. Li X, Nicklas RB. Mitotic forces control a cell-cycle checkpoint. Nature 1995; 373:630-2; PMID:7854422; http://dx.doi.org/10.1038/373630a0
14. Nicklas RB, Koch CA. Chromosome micromanipulation. 3. Spindle fiber tension and the reorientation of mal-oriented chromosomes. J Cell Biol 1969; 43:40-50; PMID:5824068; http://dx.doi.org/10.1083/jcb.43.1.40
15. Akiyoshi B, Sarangapani KK, Powers AF, Nelson CR, Reichow SL, Arellano-Santoyo H, et al. Tension directly stabilizes reconstituted kinetochore-microtubule attachments. Nature 2010; 468:576-9; PMID:21107429; http://dx.doi.org/10.1038/nature09594
16. Kitajima TS, Ohsugi M, Ellenberg J. Complete kinetochore tracking reveals error-prone homologous chromosome biorientation in mammalian oocytes. Cell 2011; 146:568-81; PMID:21854982; http://dx.doi.org/10.1016/j.cell.2011.07.031
17. Lampson MA, Renduchitala K, Khodjakov A, Kapoor TM. Correcting improper chromosome-spindle attachments during cell division. Nat Cell Biol 2004; 6:232-7; PMID:14767480; http://dx.doi.org/10.1038/ncb1102

18. Cimini D, Wan X, Hirel CB, Salmon ED. Aurora kinase promotes turnover of kinetochore microtubules to reduce chromosome segregation errors. Curr Biol 2006; 16:1711-8; PMID:16950108; http://dx.doi.org/10.1016/j.cub.2006.07.022
19. Nicklas RB, Ward SC. Elements of error correction in mitosis: microtubule capture, release, and tension. J Cell Biol 1994; 126:1241-53; PMID:8063861; http://dx.doi.org/10.1083/jcb.126.5.1241
20. Cane S, Ye AA, Luks-Morgan SJ, Maresca TJ. Elevated polar ejection forces stabilize kinetochore-microtubule attachments. J Cell Biol 2013; 200:203-18; PMID:23337118; http://dx.doi.org/10.1083/jcb.201211119
21. Maresca TJ, Salmon ED. Welcome to a new kind of tension: translating kinetochore mechanics into a wait-anaphase signal. J Cell Sci 2010; 123:825-35; PMID:20200228; http://dx.doi.org/10.1242/jcs.064790
22. Afshar K, Barton NR, Hawley RS, Goldstein LS. DNA binding and meiotic chromosomal localization of the Drosophila nod kinesin-like protein. Cell 1995; 81:129-38; PMID:7720068; http://dx.doi.org/10.1016/0092-8674(95)90377-1
23. Theurkauf WE, Hawley RS. Meiotic spindle assembly in Drosophila females: behavior of nonexchange chromosomes and the effects of mutations in the nod kinesin-like protein. J Cell Biol 1992; 116:1167-80; PMID:1740471; http://dx.doi.org/10.1083/jcb.116.5.1167
24. Zhang P, Knowles BA, Goldstein LS, Hawley RS. A kinesin-like protein required for distributive chromosome segregation in Drosophila. Cell 1990; 62:1053-62; PMID:2144792; http://dx.doi.org/10.1016/0092-8674(90)90383-P
25. Rieder CL, Davison EA, Jensen LC, Cassimeris L, Salmon ED. Oscillatory movements of monooriented chromosomes and their position relative to the spindle pole result from the ejection properties of the aster and half-spindle. J Cell Biol 1986; 103:581-91; PMID:3733881; http://dx.doi.org/10.1083/jcb.103.2.581
26. Rieder CL, Salmon ED. Motile kinetochores and polar ejection forces dictate chromosome position on the vertebrate mitotic spindle. J Cell Biol 1994; 124:223-33; PMID:8294508; http://dx.doi.org/10.1083/jcb.124.3.223
27. Khodjakov A, Rieder CL. Kinetochores moving away from their associated pole do not exert a significant pushing force on the chromosome. J Cell Biol 1996; 135:315-27; PMID:8896591; http://dx.doi.org/10.1083/jcb.135.2.315
28. Skibbens RV, Skeen VP, Salmon ED. Directional instability of kinetochore motility during chromosome congression and segregation in mitotic newt lung cells: a push-pull mechanism. J Cell Biol 1993; 122:859-75; PMID:8349735; http://dx.doi.org/10.1083/jcb.122.4.859
29. Howell BJ, Hoffman DB, Fang G, Murray AW, Salmon ED. Visualization of Mad2 dynamics at kinetochores, along spindle fibers, and at spindle poles in living cells. J Cell Biol 2000; 150:1233-50; PMID:10995431; http://dx.doi.org/10.1083/jcb.150.6.1233
30. Howell BJ, McEwen BF, Canman JC, Hoffman DB, Farrar EM, Rieder CL, et al. Cytoplasmic dynein/dynactin drives kinetochore protein transport to the spindle poles and has a role in mitotic spindle checkpoint inactivation. J Cell Biol 2001; 155:1159-72; PMID:11756470; http://dx.doi.org/10.1083/jcb.200105093
31. Howell BJ, Moree B, Farrar EM, Stewart S, Fang G, Salmon ED. Spindle checkpoint protein dynamics at kinetochores in living cells. Curr Biol 2004; 14:953-64; PMID:15182668; http://dx.doi.org/10.1016/j.cub.2004.05.053
32. Whyte J, Bader JR, Tauhata SB, Raycroft M, Hornick J, Pfister KK, et al. Phosphorylation regulates targeting of cytoplasmic dynein to kinetochores during mitosis. J Cell Biol 2008; 183:819-34; PMID:19029334; http://dx.doi.org/10.1083/jcb.200804114
33. Buffin E, Lefebvre C, Huang J, Gagou ME, Karess RE. Recruitment of Mad2 to the kinetochore requires the Rod/Zw10 complex. Curr Biol 2005; 15:856-61; PMID:15886105; http://dx.doi.org/10.1016/j.cub.2005.03.052
34. Ditchfield C, Johnson VL, Tighe A, Ellston R, Haworth C, Johnson T, et al. Aurora B couples chromosome alignment with anaphase by targeting BubR1, Mad2, and Cenp-E to kinetochores. J Cell Biol 2003; 161:267-80; PMID:12719470; http://dx.doi.org/10.1083/jcb.200208091
35. Hauf S, Cole RW, LaTerra S, Zimmer C, Schnapp G, Walter R, et al. The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint. J Cell Biol 2003; 161:281-94; PMID:12707311; http://dx.doi.org/10.1083/jcb.200208092
36. Maresca TJ, Salmon ED. Intrakinetochore stretch is associated with changes in kinetochore phosphorylation and spindle assembly checkpoint activity. J Cell Biol 2009; 184:373-81; PMID:19193623; http://dx.doi.org/10.1083/jcb.200808130
37. Uchida KS, Takagaki K, Kumada K, Hirayama Y, Noda T, Hirota T. Kinetochore stretching inactivates the spindle assembly checkpoint. J Cell Biol 2009; 184:383-90; PMID:19188492; http://dx.doi.org/10.1083/jcb.200811028
38. Campbell CS, Desai A. Tension sensing by Aurora B kinase is independent of survivin-based centromere localization. Nature 2013; 497:118-21; PMID:23604256; http://dx.doi.org/10.1038/nature12057
39. Dogterom M, Yurke B. Measurement of the force-velocity relation for growing microtubules. Science 1997; 278:856-60; PMID:9346483; http://dx.doi.org/10.1126/science.278.5339.856
40. Grishchuk EL, Molodtsov MI, Ataullakhanov FI, McIntosh JR. Force production by disassembling microtubules. Nature 2005; 438:384-8; PMID:16292315; http://dx.doi.org/10.1038/nature04132
41. Winey M, Mamay CL, O'Toole ET, Mastronarde DN, Giddings TH Jr., McDonald KL, et al. Three-dimensional ultrastructural analysis of the Saccharomyces cerevisiae mitotic spindle. J Cell Biol 1995; 129:1601-15; PMID:7790357; http://dx.doi.org/10.1083/jcb.129.6.1601
42. Maddox PS, Bloom KS, Salmon ED. The polarity and dynamics of microtubule assembly in the budding yeast Saccharomyces cerevisiae. Nat Cell Biol 2000; 2:36-41; PMID:10620805; http://dx.doi.org/10.1038/71357
43. Pearson CG, Maddox PS, Salmon ED, Bloom K. Budding yeast chromosome structure and dynamics during mitosis. J Cell Biol 2001; 152:1255-66; PMID:11257125; http://dx.doi.org/10.1083/jcb.152.6.1255
44. McIntosh JR, Grishchuk EL, Morphew MK, Efremov AK, Zhudenkov K, Volkov VA, et al. Fibrils connect microtubule tips with kinetochores: a mechanism to couple tubulin dynamics to chromosome motion. Cell 2008; 135:322-33; PMID:18957206; http://dx.doi.org/10.1016/j.cell.2008.08.038
45. VandenBeldt KJ, Barnard RM, Hergert PJ, Meng X, Maiato H, McEwen BF. Kinetochores use a novel mechanism for coordinating the dynamics of individual microtubules. Curr Biol 2006; 16:1217-23; PMID:16782013; http://dx.doi.org/10.1016/j.cub.2006.04.046
46. Alushin GM, Ramey VH, Pasqualato S, Ball DA, Grigorieff N, Musacchio A, et al. The Ndc80 kinetochore complex forms oligomeric arrays along microtubules. Nature 2010; 467:805-10; PMID:20944740; http://dx.doi.org/10.1038/nature09423
47. Wilson-Kubalek EM, Cheeseman IM, Yoshioka C, Desai A, Milligan RA. Orientation and structure of the Ndc80 complex on the microtubule lattice. J Cell Biol 2008; 182:1055-61; PMID:18794333; http://dx.doi.org/10.1083/jcb.200804170
48. Schmidt JC, Arthanari H, Boeszoermenyi A, Dashkevich NM, Wilson-Kubalek EM, Monnier N, et al. The kinetochore-bound Ska1 complex tracks depolymerizing microtubules and binds to curved protofilaments. Dev Cell 2012; 23:968-80; PMID:23085020; http://dx.doi.org/10.1016/j.devcel.2012.09.012
49. Powers AF, Franck AD, Gestaut DR, Cooper J, Gracyzk B, Wei RR, et al. The Ndc80 kinetochore complex forms load-bearing attachments to dynamic microtubule tips via biased diffusion. Cell 2009; 136:865-75; PMID:19269365; http://dx.doi.org/10.1016/j.cell.2008.12.045
50. Franck AD, Powers AF, Gestaut DR, Gonen T, Davis TN, Asbury CL. Tension applied through the Dam1 complex promotes microtubule elongation providing a direct mechanism for length control in mitosis. Nat Cell Biol 2007; 9:832-7; PMID:17572669; http://dx.doi.org/10.1038/ncb1609