Review

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Catch and release: how do kinetochores hook the right microtubules during mitosis?

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Sport fishermen keep tension on their lines to prevent hooked fish from releasing. A molecular version of this angler's trick, operating at kinetochores, ensures accuracy during mitosis: the mitotic spindle attaches randomly to chromosomes and then correctly bioriented attachments are stabilized due to the tension exerted on them by opposing microtubules. Incorrect attachments, which lack tension, are unstable and release quickly, allowing another chance for biorientation. Stabilization of molecular interactions by tension also occurs in other physiological contexts, such as cell adhesion, motility, hemostasis, and tissue morphogenesis. Here, we review models for the stabilization of kinetochore attachments with an eye toward emerging models for other force-activated systems. Although attention in the mitosis field has focused mainly on one kinase-based mechanism, multiple mechanisms may act together to stabilize properly bioriented kinetochores and some principles governing other tension-sensitive systems may also apply to kinetochores.

"Esa! Esa! Shame upon on you!
You are but the pike, Kenozha,
You are not the fish I wanted
You are not the King of Fishes!"
–Henry Wadsworth Longfellow, *The Song of Hiawatha*

Tension-dependent stabilization of kinetochore–microtubule attachments

Mitosis research has been guided for over half a century by the idea that mechanical tension signals proper attachment of chromosomes to microtubules of the mitotic spindle and selectively stabilizes these attachments. Chromosomes are coupled to spindle microtubules via kinetochores, which are multiprotein complexes that form persistent attachments to growing and shortening microtubule tips, thereby harnessing the dynamics of the filaments to produce force and movement. Accurate mitosis requires all kinetochores to become properly 'bioriented', with replicated sister chromatids attached to opposite sides of the spindle (or with homologous chromosomes attached to opposite sides during the first meiotic division of gametogenesis). Dietz [1] was the first to recognize that chromosomes repeatedly reorient on the spindle, in a trial-and-error process that ceases only when proper biorientation is achieved because only this arrangement is stable [2,3]. Dietz also suggested a possible cause for this differential stability: mechanical tension. Bioriented chromosomes come under tension and their sister kinetochores are stretched apart by opposing spindle forces, whereas incorrectly attached chromosomes are relaxed (Figure 1A). Direct evidence that tension indeed confers stability to chromosome–spindle attachments came from classic micromanipulation experiments using grasshopper spermatocytes [3]. The idea has since become a central tenet of mitosis research.

An attractive molecular explanation for how tension may stabilize bioriented attachments began to emerge when genetic studies uncovered a kinase, Aurora B, whose activity prevents errors in chromosome segregation [4–7]. Aurora B phosphorylates key microtubule-binding elements within the kinetochore [5,8–11], reducing their biochemical affinity for [9,12], and promoting their detachment from, microtubules [12–16]. If the detachment-promoting activity of Aurora B is directed selectively toward kinetochores lacking tension and suppressed at kinetochores bearing tension, then it could explain why only relaxed attachments are unstable *in vivo*. Experiments in a variety of cell types are consistent with this idea (see especially [17–19]), but do not yet provide final proof (see below). Nevertheless, the hypothesis that tension suppresses Aurora B-triggered detachment has become so popular among mitosis researchers that it is difficult to find skepticism about it in current literature.

Meanwhile, numerous instances have been uncovered in other physiological contexts where mechanical tension stabilizes molecular interactions. In some cases, force acts via regulation of kinase enzymes [20,21], as proposed for kinetochores. Other cases involve specialized 'catch bonds' that are directly stabilized by force [22–28]. Force on a protein can also promote or inhibit its proteolytic

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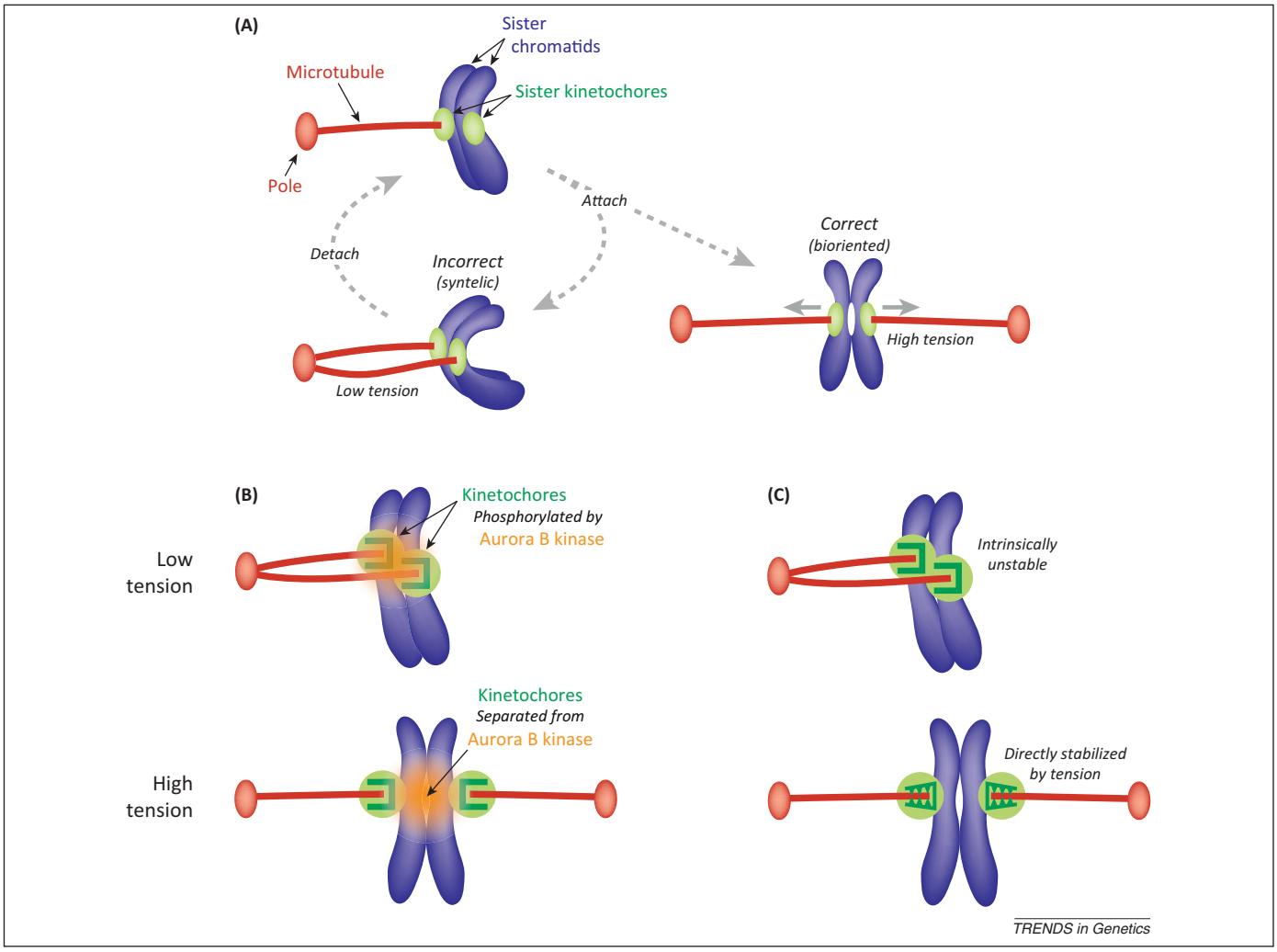

Figure 1. Tension-dependent error avoidance during mitosis. **(A)** The accuracy of mitosis depends on trial-and-error and selective stabilization of correctly 'bioriented' attachments (i.e., those with sister kinetochores attached to microtubules emanating from opposite sides of the mitotic spindle). Bioriented kinetochores come under tension due to forces exerted on them by opposing microtubules, which somehow stabilizes the attachments. Conversely, a lack of tension on incorrectly attached kinetochores fails to stabilize them, so they release quickly, giving another chance for biorientation. For simplicity, we focus here on one type of incorrect attachment, called 'syntelic', with both sister kinetochores bound exclusively to microtubules from a single pole. Another incorrect attachment, 'merotelic', occurs when a single sister binds microtubules from both poles [83]. Although merotelics are geometrically distinct from syntelics, they may or may not be corrected by similar mechanisms [84]. **(B,C)** Two models for how tension may stabilize bioriented attachments. (B) Spatial separation model where a pool of Aurora B kinase located in-between the sister kinetochores (i.e., at the inner centromere) selectively phosphorylates the kinetochores of relaxed chromosomes, weakening their grip on the microtubules and promoting their release. Tension on correctly bioriented chromosomes causes them to stretch, spatially separating their kinetochores from Aurora B and preventing kinase-triggered detachment. Other mechanisms for tension-dependent suppression of Aurora B are also possible (Figure 2F,G). (C) Catch bond-like model where tension acts directly and independently of phosphoregulation on the kinetochore–microtubule interface, causing it to adopt a more stable configuration (Figure 2H).

cleavage [29,30], or enable its binding to another protein [32,33]. Although understanding of how tension-activated molecules contribute to cell morphogenesis is far from complete, it is clear that many different schemes have evolved for sensing and responding to mechanical force [34–36].

Here, we review current ideas about how tension stabilizes kinetochore–spindle attachments. Key experiments that form the basis of the popular kinase-based model are examined. We also consider alternative mechanisms suggested by work outside the mitosis field and by recent experiments where reconstituted kinetochore–microtubule attachments were directly manipulated *in vitro*. We do not discuss how the 'wait anaphase' (checkpoint) signals generated by kinetochores may be suppressed by tension, a topic already covered by several excellent reviews [37–39].

Evidence for tension-dependent suppression of Aurora B kinase

Aurora B is widely conserved, even across evolutionarily distant eukaryotes [40,41], and is clearly important for promoting proper attachments between chromosomes and spindle microtubules. Mutating it [4,5], depleting it from dividing cells [6], or inhibiting its activity [6,42] causes severe chromosome missegregation, although the spindle remains fully capable of attaching and pulling on kinetochores [5–7,17]. Many pairs of sister kinetochores in Aurora B-deficient cells fail to biorient and the cells accumulate erroneous attachments where both sisters are bound to microtubules emanating from the same spindle pole [5–7]. In normal cells, such aberrant configurations are short lived [1–3], but Aurora B deficiency makes them unusually stable [42]. Aurora B phosphorylates key microtubule-binding elements within

kinetochores *in vivo*, including the widely conserved Ndc80 and Knl1 subcomplexes [11,43,44] and the yeast Dam1 subcomplex [8]. Phosphorylation [9,12] and phosphomimetic mutations at Aurora B target sites [15,16,44] reduce the biochemical affinity of kinetochore subcomplexes for microtubules [9,12,44] and also accelerate detachment of subcomplexes [12,16] and larger kinetochore assemblies [15] from microtubules *in vitro*. Altogether, these data suggest that Aurora B promotes detachment of kinetochores from microtubules and that this activity is somehow restricted *in vivo* to erroneously attached kinetochores.

The idea that tension might regulate Aurora B arose from studies of yeast engineered to enter mitosis without prior replication of their DNA or without sister chromatid cohesion [7,45]. The resulting unpaired chromatids cannot biorient, so the spindle exerts little or no tension on them, and they associate with either of the two spindle poles randomly [7]. However, upon Aurora B inhibition, they associate almost exclusively with the 'old' pole, that is, the one leftover from the previous cell cycle (rather than the 'new' pole formed *de novo* during the most recent S phase). Aurora B activity is evidently needed to break their attachments to the old pole, which they inherit from G1. This Aurora B-dependent turnover is reminiscent of the classic observations in insect spermatocytes, where unpaired X chromosomes undergo rapid pole-to-pole movements [46] because they lack the tension normally required for stabilizing chromosome–spindle attachments in these cells [2,3]. The similarity suggests a unified explanation: Aurora B may promote kinetochore detachment universally (during insect, yeast, and perhaps all eukaryotic cell division), and its activity may be universally inhibited by tension.

The evidence that tension suppresses the detachment-promoting activity of Aurora B, although entirely circumstantial, is compelling. Formally, any property absent from correctly attached kinetochores but shared by unpaired and erroneously attached kinetochores could underlie their differential susceptibility to detachment by Aurora B. Whatever the key difference, it does not depend on precisely how kinetochore pairs are linked: bioriented kinetochore pairs are resistant to Aurora B-dependent turnover regardless of whether they are linked naturally, through replicated sisters bound by cohesion [7], or artificially, through a single 'dicentric' DNA molecule or through DNA entanglements (created by inhibiting topoisomerase) [17]. This adaptability again mirrors the situation in insect spermatocytes, where erroneous attachments can be artificially stabilized either by applying tension with a microneedle [3] or by arranging a pair of maloriented chromosomes such that they become mechanically interlocked [2]. Incorrect attachments can also be artificially stabilized in *Drosophila* S2 cells by overexpressing NOD [19], a kinesin-10 motor that localizes on chromosomes. Chromosome-anchored NOD is normally thought to help align chromosomes at the spindle equator by pushing them away from the poles. NOD overexpression may elevate this polar ejection force and significantly increase tension at kinetochores, thereby suppressing Aurora B and preventing erroneous attachments from releasing [19]. Given the similarity of these observations, it seems likely that tension somehow suppresses Aurora B. Notably, however, definitive evidence showing that direct application of mechanical tension is sufficient to suppress chromosome reorientation has only been obtained in meiotic grasshopper spermatocytes. Whether reorientation in these particular cells depends on Aurora B has not, to our knowledge, been proven. More generally, it remains unproven whether direct application of mechanical tension is sufficient to inhibit any Aurora B-dependent activity.

Is kinetochore phosphorylation sensitive to tension?

Immunostaining with the '3F3' antibody confirms that tension, either from a micromanipulation needle or from normal spindle forces, can inhibit kinetochore phosphorylation [47–49]. 3F3 antibody specifically detects phosphorylated kinetochore proteins [50], but probably not Aurora B substrates. (It recognizes spindle checkpoint proteins phosphorylated either by Plk1 or Mps1 [51–54].) Nevertheless, key ideas that could apply to Aurora B are illustrated by experiments where chromosomes from lysed cells are washed, directly manipulated, and then immunostained with 3F3. Relaxed kinetochores on the washed chromosomes are devoid of 3F3 phosphoepitope, but they can be rephosphorylated by incubation with ATP if a phosphatase inhibitor is also present [49]. Applying tension with a microneedle prevents rephosphorylation. The rephosphorylation of relaxed kinetochores by incubation with ATP shows that the chromosomes retain a complete phosphorylation system, including substrate and kinase. The requirement for a phosphatase inhibitor shows that a phosphatase is also retained and does not require tension for its activity. Rather, tension must prevent phosphorylation in this case by inhibiting the kinase, deforming the substrate, or repositioning the substrate relative to the kinase [49].

To our knowledge, such direct tests of tension sensitivity have not been performed using phosphospecific antibodies against *bona fide* Aurora B substrates. However, antibodies that recognize phosphorylation of several Aurora B substrates, including Ndc80 and Knl1, reveal correlations that are mostly consistent with tension-dependent suppression *in vivo* [11,44]. High levels of phosphorylation on Ndc80 and Knl1 correlate with unaligned, relaxed chromosomes. Low phosphorylation is seen at metaphase, when most kinetochore pairs are aligned, stretched apart, and probably bioriented. An exception is anaphase, when low tension correlates with low Ndc80 phosphorylation [11], presumably because Aurora B delocalizes from the chromosomes at this time (binding instead to microtubules in the spindle midzone, together with other members of the 'chromosomal passenger complex' [41]). More puzzlingly, in cells treated with nocodazole to depolymerize their microtubules, the level of phosphorylation on Ndc80 is low [11], whereas high levels are seen for Knl1 [44]. Tension should be absent after microtubule depolymerization, so the low phosphorylation of Ndc80 is incompatible with a strict model in which Aurora B always phosphorylates all its targets at relaxed kinetochores.

The spatial separation model for suppression of Aurora B activity

In mitotic animal cells, Aurora B localizes prominently on inner-centromeric chromatin, midway between sister

kinetochores. This pattern is the basis for an appealing 'spatial separation' model explaining how Aurora B may selectively phosphorylate the kinetochores of relaxed chromosomes: sister kinetochores on relaxed chromosomes are close enough to the inner centromere to be phosphorylated by Aurora B located there (Figure 1B). Tension stretches the chromosomes and this deformation spatially separates the kinetochores from centromeric Aurora B, thereby inhibiting their phosphorylation.

Aspects of this model have been tested using a Förster resonance energy transfer (FRET)-based biosensor for Aurora B [18,44]. When the sensor is targeted to kinetochores (by fusion with the kinetochore proteins, Mis12 or Ndc80), its behavior matches the predictions for native kinetochore substrates, reporting high phosphorylation on unaligned kinetochores or after drug treatments that relax the chromosomes (e.g., nocodazole or monastrol), and reporting low phosphorylation on kinetochores that are properly aligned and bioriented. If instead the sensor is targeted to inner centromeres (by fusion with the centromere-targeting domain of CENP-B) then its phosphorylation remains high, even on stretched chromosomes [18]. This observation indicates that the inner-centromeric Aurora B is constitutively active and that proximity of a substrate to this active pool is sufficient to cause its phosphorylation. The data are also nicely consistent with the spatial separation model, but a key question remains: in the normal physiological situation, is the Aurora B at the inner centromere directly responsible for phosphorylation of relaxed kinetochores?

Two recent observations suggest that the Aurora B directly involved in error correction is distinct from the prominent pool at the inner centromere. First, an antibody that specifically recognizes the phosphorylated active form of Aurora B labels not only the inner-centromeric pool between sister kinetochores, but also the outer kinetochore [11]. The outer-kinetochore population of Aurora B diminishes as kinetochores become properly aligned and less phosphorylated on Ndc80, whereas the inner-centromeric pool remains prominent. The correlation between Ndc80 phosphorylation levels and enrichment of outer-kinetochore Aurora B suggests that this population, rather than the inner-centromeric pool, is responsible for phosphorylating Ndc80. A second recent observation is that Aurora B supports normal cell growth [55] and accurate chromosome segregation [56] even when its targeting to inner centromeres is disrupted (by mutations that interfere with the binding of INCENP to the centromere-targeting factor, survivin). Whether inner-centromere localization of Aurora B is completely abolished in this case remains uncertain. However, if localization at the inner centromere is truly dispensable, then tension-dependent stretching of inner-centromeric chromatin cannot be the basis for suppression of Aurora B, and a key assumption of the spatial separation model would be wrong. Clearly, even with impressive advancements in understanding of Aurora B, we lack a complete picture of how tension confers stability to kinetochore–microtubule attachments.

Mechanically regulated molecular systems outside of mitosis

The notion that mechanical force can regulate molecular interactions and cellular activities is pervasive in many fields besides mitosis. In a growing number of cases, tension-dependent molecular behaviors have been demonstrated by the application of force directly to purified proteins. What can those of us interested in chromosome segregation learn from studies of other micromechanical systems (and vice versa)?

One lesson is that nature has produced a variety of molecular mechanisms for sensing mechanical force (Figure 2A–E). There are examples where phosphorylation is regulated by force, similar to the proposal for kinetochores, but not necessarily via spatial separation of the kinase from its substrates. Adherent cells, for example, sense their mechanical environment through a series of biochemical events that includes tension-dependent phosphorylation of the focal adhesion protein p130Cas [57]. Tension acts in this case by 'substrate priming': the relaxed p130Cas substrate domain is normally resistant to phosphorylation, but becomes susceptible under load (Figure 2E) [20]. Another phosphoregulatory mechanism occurs in muscle cells, where a kinase domain within the giant elastic protein titin senses mechanical strain and initiates a cascade of downstream biochemical events to control transcription of adaptive genes [58]. Force is transmitted directly through titin kinase itself, causing its activation by pulling an autoinhibitory domain away from its active (ATP-binding) site (Figure 2D) [21]. More generally, force on a molecule can expose a previously buried 'cryptic' binding site for another molecule. Thus, stretching of the focal adhesion protein talin activates its binding to vinculin (Figure 2A) [32], and stretching an F-actin network activates the binding of β-integrin to the actin cross-linking protein, filamin [33]. Of course, force can also disrupt binding sites. The stretching that activates binding of β-integrin to filamin also causes the simultaneous unbinding of another filamin-binding protein, FliGAP [33].

Tension-dependent stabilization outside of mitosis

As in mitosis, the concept that force can stabilize load-bearing molecular structures is central to many other areas of biology and the effect has been demonstrated in various molecular systems. Tension-dependent stabilization probably explains how fibrils of collagen, the most abundant structural protein in vertebrates, are preferentially oriented along directions of load transmission [59]. Tension stabilizes collagen fibrils indirectly, by rendering them resistant to cleavage by collagenolytic enzymes [30], possibly because it inhibits the partial unfolding of the triple-helical structure of collagen (Figure 2C) [60]. Tension can also cause stabilization more directly, via specialized molecular interactions called 'catch bonds' (Box 1) [34,35]. Catch bonds were first demonstrated in single molecule experiments involving selectins [22], adhesion molecules that support tethering and rolling of leukocytes on vascular endothelium during inflammation. Lifetimes of individual selectin–ligand bonds initially increase and then decrease with tension, giving rise to a biphasic lifetime versus force curve [22,24]. Around the same time, it was also discovered that adhesion of fimbriated bacteria to host cells is enhanced by hydrodynamic force [23], in part because a protein at the tip of the fimbria, fimH, forms catch bonds with mannosylated glycoproteins on the host cell surface.

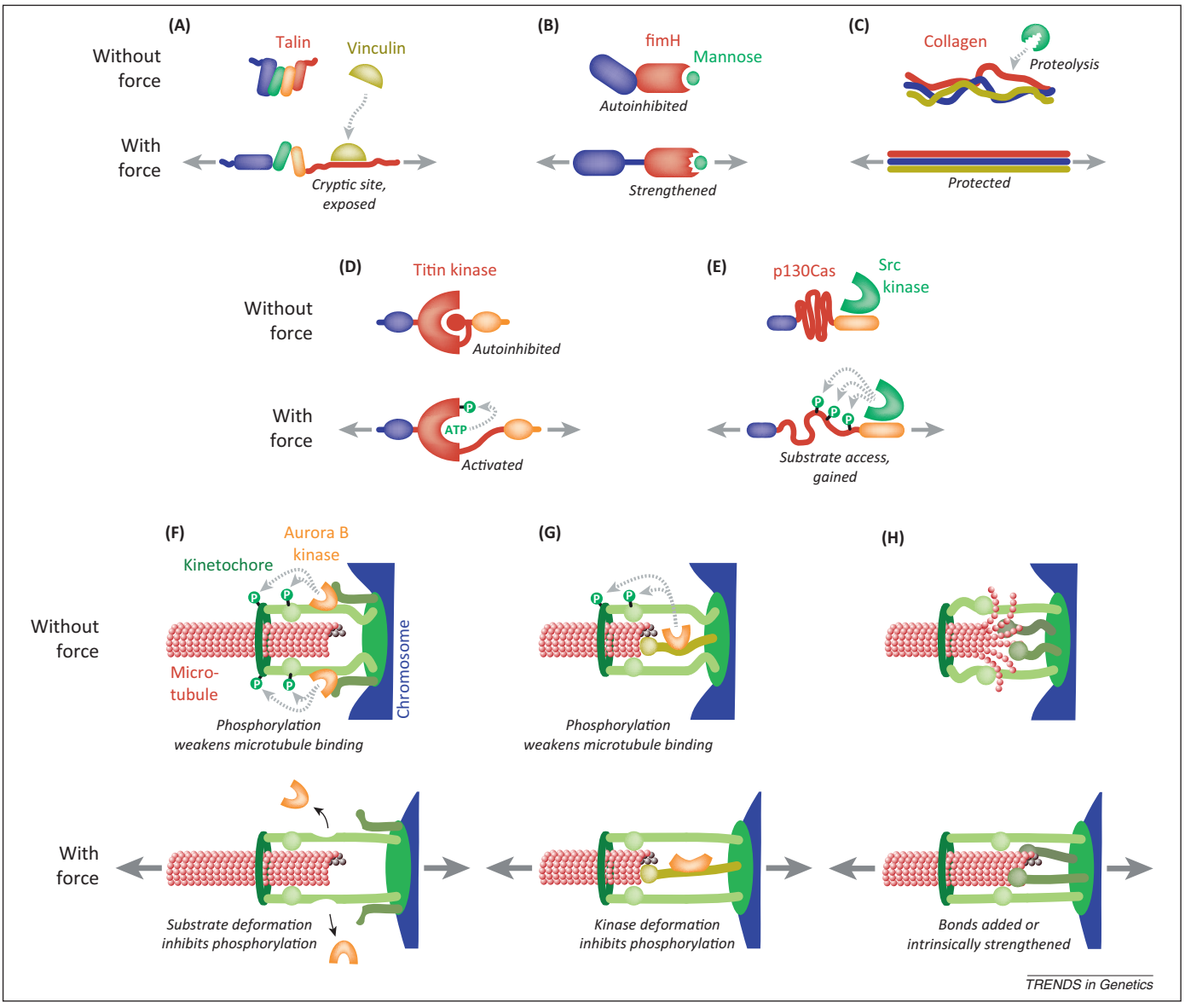

Figure 2. Gallery of mechanosensitive molecules and models for mechanosensation at kinetochores. **(A)** Force exerted on talin exposes a cryptic binding site for vinculin [32]. **(B)** Force strengthens the fimH–mannose bond by pulling away an autoinhibitory domain [61]. **(C)** Force on collagen protects it from proteolytic cleavage [30]. **(D)** Force activates titin kinase by pulling away an autoinhibitory domain [21]. **(E)** Force on p130Cas promotes its phosphorylation by Src kinase [20]. **(F–H)** Speculative models for how force-dependent deformations could strengthen a kinetochore–microtubule attachment. (F) Kinetochore tension could deform Aurora B substrates, preventing the kinase from weakening their grip on the microtubule. (G) If Aurora B at the kinetochore–microtubule interface bears mechanical load, then the kinase itself could be inhibited by load. (H) Tension can also stabilize the kinetochore–microtubule interface directly [66], perhaps by altering the conformation of the microtubule tip or the kinetochore microtubule-binding elements in a way that strengthens existing bonds or promotes formation of additional bonds.

Multiple force-sensitive molecules are often combined

A second lesson that mitosis aficionados can learn from studies of other mechanically regulated systems is that multiple tension-controlled molecules often participate together in the same cellular process. Thus, bacterial adhesion is enhanced not only by fimH catch bonds at fimbrial tips [61], but also by mechanically responsive fimA molecules, which form a helical polymer comprising most of the length of the fimbriae. The fimA polymer acts as a near-perfect shock absorber, uncoiling and recoiling dynamically to maintain the optimal force at the tip (i.e., the force where fimH–mannose bonds are longest lived) [62]. Likewise, the blood clotting potential of von Willebrand Factor is regulated not only by the catch/flex bonds it forms with platelet glycoproteins [31,63,94], but also by a proteolytic cleavage process that is enhanced by shear forces [29,95]. Mechanosensation by adherent cells via integrins is perhaps the most multifaceted example currently known. A dizzying number of force-sensitive molecules participate, including (i) force-activated binding of integrin to actin via filamin [33]; (ii) force-stabilized binding of integrin to fibronectin [64]; (iii) force-activated self-assembly of fibronectin [65]; (iv) force-activated binding of vinculin to talin [32]; and, possibly, (v) force-dependent regulation of focal adhesion kinase [36]. The cooperation of so many mechanically regulated proteins in one pathway may seem surprising. However, the experimental tools for studying force-sensitive molecules are relatively new. As they become more widespread, coincidence of multiple force sensors in a single pathway may turn out to be the norm rather than an exception.

Box 1. A conflagration of catch bonds

Bell [85] first theorized that force would accelerate the dissociation of receptor–ligand bonds by tilting their energy landscape and lowering the energetic barrier for dissociation. Such interactions are known as 'slip bonds', and their lifetime typically decreases exponentially with tension. Later, it was proposed that tension could also do the opposite (i.e., prolong bond lifetime) by triggering a conformational change that tightens the ligand-binding pocket [86]. This counter-intuitive behavior can be likened to a finger trap gag toy: the harder one pulls, the more stable the interaction becomes. Since the initial discovery that force stabilizes selectin–ligand and fimH–mannose bonds (Figure 2B and main text), catch bonds have been found in many other biological contexts. Two prominent examples are the binding of integrin to fibronectin [64], which supports cell adhesion to the extracellular matrix, and the binding of von Willebrand factor via its A1 domain to platelet glycoprotein Ib [63,94], which initiates blood clotting preferentially in areas of high flow.

Catch bond-like behavior is also common in ATP-powered motor proteins. Many muscle and nonmuscle myosins attach to actin filaments more stably when force opposing their motion is applied [25–28]. Opposing force generally prolongs the attachment lifetime of myosin by slowing its release of ADP and thereby preventing it from binding ATP, which is normally required for detachment of myosin from actin. The result is a 'latch' effect that allows these myosins to consume less ATP while sustaining loads for long durations (e.g., while maintaining vascular tone). A particularly dramatic example is myosin1b, whose actin-attachment lifetime increases >75-fold in response to small opposing loads (<2pN) [28]. Similarly, opposing force applied to the kinesin motor domain favors its tight binding to microtubules [87], in this case by accelerating its release of ADP (which has the opposite effect on kinesin as on myosin). This tension-dependent stabilization probably helps to coordinate the hand-over-hand stepping of the twin motor domains of kinesin over the microtubule lattice [88]. Some of the earliest demonstrations of force-stabilized motor-filament attachments [25] pre-date the initial discovery of catch bonds. Most have not been described using the term 'catch bond', but their similarity is obvious.

Kinetochore-microtubule attachments display catch bond-like behavior

Given the diversity of force-sensing mechanisms across biology, it is natural to ask whether selective stabilization of proper kinetochore–microtubule attachments during mitosis relies solely on tension-dependent regulation of Aurora B, as is generally assumed, or whether other tension-dependent effects are also involved. Until recently, addressing this question would have been prohibitively difficult due to the lack of suitable *in vitro* assays for applying precisely controlled forces to kinetochore–microtubule interactions. Reconstitution of kinetochore–microtubule coupling using recombinant subcomplexes [13,16] and native kinetochore particles isolated from budding yeast [15,66] has made direct tests possible for the first time.

One way to study purified kinetochore particles *in vitro* is to link them to polystyrene beads, which serve as artificial cargoes (mimicking the chromosomes) and as handles to apply force (Figure 3A). Using a servo-controlled laser trap, bead-linked kinetochore particles can be attached to the tips of individual microtubules grown from coverslip-anchored seeds. The particles track with growing and shortening filament tips even when tension is applied continuously with the trap, to mimic the physiological situation. In addition, similar to kinetochores *in vivo*, the purified particles can maintain persistent, load-bearing tip attachments through periods of microtubule growth and shortening [15,66]. To measure the effect of tension on attachment lifetime, the laser trap can be programed to operate as a force 'clamp', applying a chosen level of force for the duration of each event. A large number of events is recorded, and mean attachment lifetimes are calculated at each force by dividing the total observation time by the number of detachments. Considering that the purified kinetochore particles lack detectable Aurora B kinase [66], one might expect the lifetime to decrease monotonically with force (similar to a typical 'slip bond'; Box 1). However, attachment lifetimes initially increase and then decrease with tension, lending a biphasic shape to the lifetime versus force curve (Figure 3C) [66]. These results indicate the existence of a direct, catch bond-like stabilization mechanism that may act in parallel with Aurora-based phosphoregulation to help ensure mitotic accuracy.

Catch bonds are often described by a two-state kinetic scheme where the bond switches between a weak and a strong state and force favors adoption of the strong state [24,67,68]. As force increases from zero, the average bond lifetime initially grows because the bond spends an increasing fraction of time in the strong state. Eventually, a critical force is reached, above which the lifetime decreases because the strong state is overpowered. This same model can describe the behavior of kinetochore–microtubule attachments (Figure 3B). Microtubule tips switch between two states, growth and shortening. Kinetochore particles detach from growing tips more slowly than from shortening tips (Figure 3E) [15,66], so tip growth corresponds to a strongly attached state. Moreover, tension decreases the likelihood that a growing tip will begin shortening (an event called a 'catastrophe') and increases the likelihood that a shortening tip will resume growth (a 'rescue'), thereby causing the kinetochore-tip attachments to spend more time in the strongly attached state [66]. The resulting lifetime versus force relations for both kinetochore-microtubule attachments and canonical catch bond systems are well described by mathematically equivalent functions. However, the two systems differ in at least one way: canonical catch bond systems typically switch rapidly between their weakly and strongly bound states, and because the associated conformational changes are subtle, it is not usually possible to directly measure kinetic rates for switching or detachment specifically from the strongly or weakly bound states. Given that microtubule tips *in vitro* switch relatively infrequently between growth and shortening, distinguishing between the two states is straightforward, and the specific kinetic rates can be directly measured [66].

Alternative mechanisms for regulation of kinetochore–spindle attachment stability

The catch bond-like behavior observed using reconstituted kinetochore–microtubule attachments arises because their attachment stability depends on microtubule tip dynamics. This dependence implies more generally that altering microtubule tip dynamics by any means could affect kinetochore attachment stability. The core microtubule-binding kinetochore elements, Ndc80 and Dam1, for example,

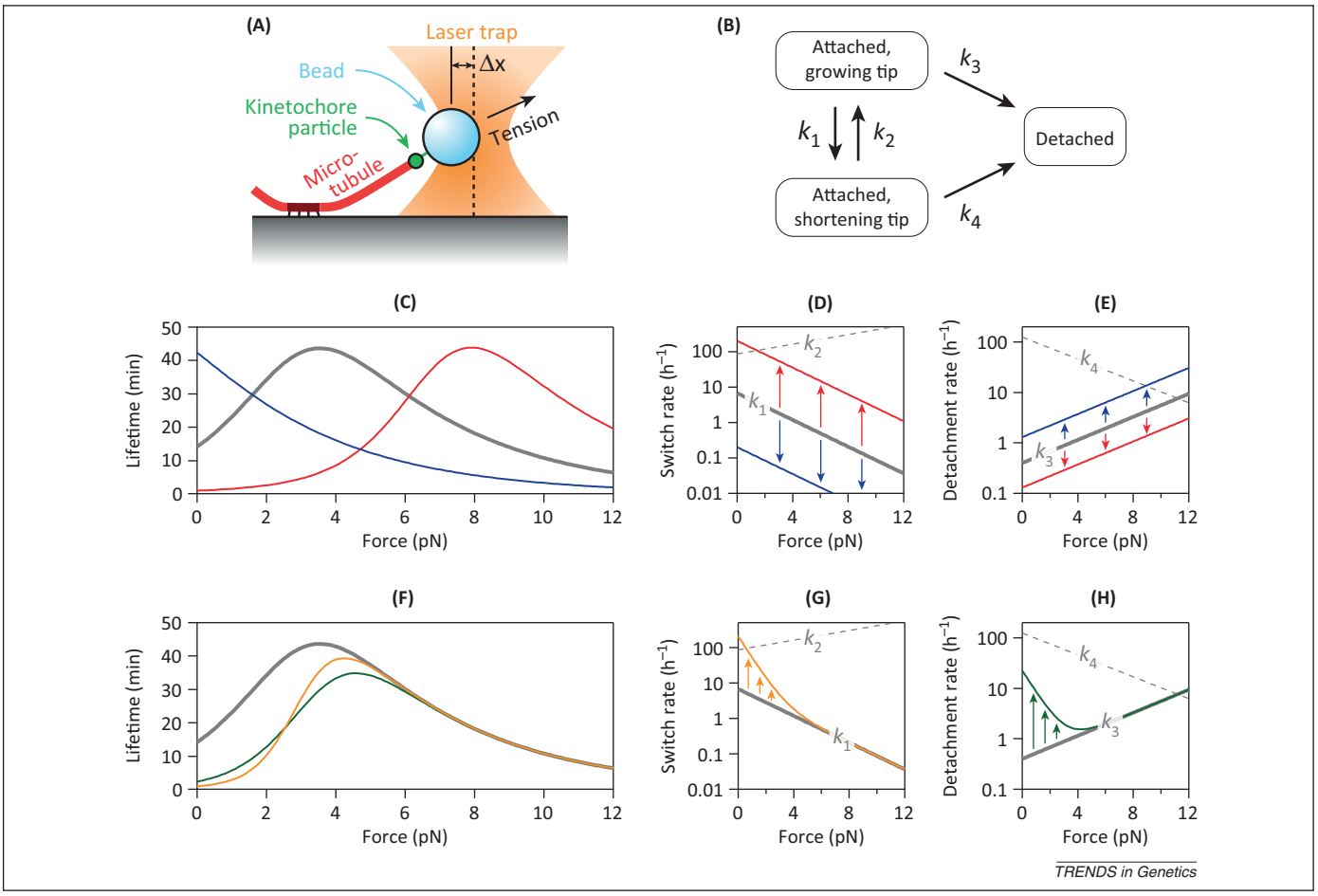

Figure 3. Catch bond-like behavior of kinetochore–microtubule attachments, and how it may be regulated. **(A)** Schematic of laser trap assay. A bead decorated with native kinetochore particles or recombinant kinetochore subcomplexes is attached to the dynamic tip of a coverslip-anchored microtubule. As the microtubule tip grows and shortens, the kinetochore-bead moves with it. During bead movement, the laser trap can be automatically steered to keep a fixed bead-trap distance (Δx), thereby maintaining a constant tensile force on the kinetochore–microtubule interface. **(B)** A kinetochore-attached microtubule tip can grow (assemble) or shorten (disassemble), with transitions between these states governed by the rates of catastrophe, k_1, and rescue, k_2. The kinetochore can detach from either state, with rates k_3 and k_4. Given that k_4 is generally much faster than k_3, the overall rate of detachment can be reduced by inhibiting catastrophe (k_1) or promoting rescue (k_2). **(C)** The mean lifetime of reconstituted kinetochore–microtubule attachments initially increases and then decreases with force in a catch bond-like manner (gray curve, adapted from [66]). In principle, the lifetime versus force relation could be tuned to selectively stabilize relaxed attachments (blue) or those bearing higher loads (red). **(D,E)** Force inhibits catastrophe (k_1, solid gray line), promotes rescue (k_2, dashed gray), accelerates detachment during growth (k_3, solid gray), and slows detachment during shortening (k_4, dashed gray). Simultaneously adjusting the rates of catastrophe and detachment during growth across all forces as shown (blue and red lines in D and E) would shift the lifetime versus force relation leftward and rightward (blue and red curves in C). **(F–H)** Selective phosphorylation of relaxed kinetochores by Aurora B could accelerate detachment [green curve in (H)] or induce catastrophe [orange curve in (G)] at kinetochores that bear low forces (e.g., <4pN). In either case, the net effect would be to sharpen the lifetime versus force curve, increasing its sensitivity to force in the low-force regime where tension prolongs attachment [green and orange curves in (F)].

affect tip dynamics in ways that promote microtubule growth [16], which in turn promotes attachment stability. Tip stabilization by Ndc80 and Dam1 is partially reversed by phosphomimetic mutations at Aurora B target sites on these subcomplexes, suggesting that Aurora B promotes kinetochore release not only directly, by accelerating detachment, but also indirectly, by destabilizing kinetochore-attached microtubule tips [15,16]. Other candidates for affecting attachment stability via this mechanism are microtubule regulators of the Kinesin-13 [69] and Kinesin-8 families [70], and plus end-binding proteins, such as XMAP215 and EB1 [71].

In principle, dividing cells might exploit the interplay between tension, microtubule tip dynamics, and kinetochore attachment stability in interesting ways. Simultaneously promoting catastrophes while inhibiting detachment during assembly, for example, is predicted to shift the lifetime versus force curve rightward (Figure 3C, red curve), so that the optimum force (where attachment lifetime is longest) occurs at higher tension. Conversely, inhibiting catastrophes while promoting detachment during assembly would shift the curve leftward. If the rate changes were large enough, then the catch-bond effect would be abolished, and lifetime would decrease monotonically with force (Figure 3C, blue curve). We speculate that such shifts in the lifetime versus force relation may be biologically important. A leftward shift could allow stable attachment of relaxed kinetochores, perhaps promoting the formation of initial attachments during spindle assembly. A subsequent rightward shift could selectively destabilize kinetochores that have failed to biorient. A cell could tune the lifetime versus force relation for all kinetochores simultaneously, by globally modulating the dynamics of all kinetochore-attached microtubules [72]. The relation for a particular kinetochore could be tuned by post-translational modifications or by local binding of cofactors that alter detachment rates or the dynamics of attached microtubules.

Aurora B on the horizon: new concepts in the light of mechanobiology

As discussed above, current models for how Aurora B promotes accurate chromosome segregation are dominated by two concepts: that Aurora B phosphorylation causes kinetochore detachment from the spindle, and that tension suppresses this activity, possibly by spatially separating centromere-bound kinase from its substrates in the kinetochore. These relatively simple ideas emerged before a complete picture of the kinetochore was available and are probably inadequate to explain fully the dynamics of kinetochore phosphorylation and error correction *in vivo* [11,56]. We now know that the core kinetochore comprises >80 proteins, arranged in at least eight subcomplexes that assemble hierarchically into a large structure [73,74]. Why all this molecular complexity? One possibility is that it enables kinetochores to sense and respond in a sophisticated manner to a variety of mechanical and biochemical cues.

We imagine two alternative mechanisms, besides spatial separation, by which mechanical tension could suppress Aurora B phosphorylation of kinetochores (Figure 2F,G). First, tension could deform the substrates of Aurora B within the kinetochore to render them inaccessible to phosphorylation (or to render them more susceptible to dephosphorylation by phosphatases). Consistent with this view, kinetochores exhibit deformations that correlate with their attachment state [75–78] and could affect Aurora B kinase substrate access [39]. Tension-dependent control of substrate access at kinetochores would be similar to the protection of tension-bearing collagen molecules against proteolytic cleavage [30,59] and to the priming of tension-bearing p130Cas for phosphorylation by Src kinase [20]. A second possibility is that tension on Aurora B itself, or on its activator INCENP, could directly inhibit its kinase activity (Figure 2G). Aurora B and INCENP bind not only to chromatin and kinetochores (as discussed above), but also to microtubules [79,80], so they could bear some mechanical load at kinetochore–microtubule attachment sites. Direct mechanical control of Aurora B kinase activity would be similar to the load-dependent activation of titin kinase [21].

Assuming that Aurora B is indeed regulated by tension, how might it work together with the intrinsic catch bond-like behavior of kinetochores? The intrinsic catch bond-like behavior by itself produces only a modest stabilization *in vitro* (at the optimum force the mean attachment lifetime is increased approximately threefold relative to zero force) and the degree to which it will help stabilize biorientation *in vivo* is uncertain (Box 2). However, if Aurora B phosphorylation has a strong enough effect on the rates of kinetochore detachment and microtubule switching, and if its effects are sufficiently inhibited by tension, then the lifetime versus force relation could be substantially sharpened. (Two examples of such sharpening are depicted in Figure 3F–H.) Another intriguing possibility is that kinetochores may include canonical catch bonds that are stabilized by force even without changes in microtubule switching. If canonical catch bonds exist between kinetochores and microtubules, they could have a profound influence on attachment stability (Figure 2H) [81] and also chromosome movement [82].

Box 2. How tension-dependent modulation of microtubule tip dynamics could help stabilize biorientation

If both sister kinetochores attach to the tips of microtubules emanating from the same spindle pole (syntelic, Figure 1A, main text), then opposing spindle forces will not develop and tension on both tip attachments will be low. Both tip attachments will tend to remain in the disassembling state and, consequently, both will be weak (Figure 3E, main text, $k_4 >> k_3$). Correctly bioriented sister kinetochores will come under tension. In cells such as yeast, where microtubule minus ends are anchored statically at the spindle poles (i.e., in cells without poleward flux), the growth of tips attached to one bioriented sister kinetochore must be balanced by shortening of those attached to the other sister. This balance is probably achieved through tension-dependent modulation of tip dynamics [89–91]. The attachment on the growing side will be dramatically stabilized (Figure 3E, main text, $k_3 << k_4$), and this effect alone will reduce the likelihood relative to the syntelic case that the pair will revert to a singly attached ('monotelic') state. Although the attachment on the shortening side will be weaker than its sister, it may still be moderately stabilized relative to a syntelic attachment, because tension moderately inhibits detachment during shortening (Figure 3E, main text, k_4 decreases with force). In many cell types, a continuous poleward flux of microtubules occurs, driven by traction forces and balanced by disassembly at the poles [92,93]. In such cells, the growth of tips attached to bioriented kinetochores is not a zero-sum game: poleward flux may allow the simultaneous assembly of tips attached to both sisters. In this case, attachments on both sides would be dramatically stabilized and the pair would be far less likely to revert to a singly attached state.

Concluding remarks

In many ways, our understanding of how kinetochores 'catch and hold' the correct microtubules but release erroneous attachments remains in its infancy. Various lines of evidence suggest that erroneous attachments are selectively eliminated through tension-dependent control of the spatial separation between Aurora B kinase and its kinetochore substrates. Considering the molecular complexity of kinetochores, the importance of mechanical cues during mitosis, and the diversity of force sensors in other areas of biology, it would not be surprising to find numerous other tension-sensing mechanisms operating at kinetochores as well. Precise mechanical manipulation of reconstituted kinetochore–microtubule attachments should enable direct tests of the popular kinase-based model and facilitate the search for additional tension-sensing mechanisms.

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