Title

Uncovering the Role of Biophysical Cues in Decision Making in the Mammalian Spindle

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Uncovering the Role of Biophysical Cues in Decision Making in the Mammalian Spindle

by
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Dedicated to my family, the one I was born with and the one I've chosen,

who hold me up and keep me moving forward

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Statement Regarding Author Contributions

Chapter 2 of this dissertation is a reprint of work that was previously published: Chong MK, Rosas Salvans M, Tran V, Dumont S. Chromosome size-dependent polar ejection force impairs mammalian mitotic error correction. *Journal of Cell Biology* **223** (8): e202310010 (2024). I performed the experiments described in the study, analyzed the data, and wrote the manuscript under the guidance of Sophie Dumont, PhD. Vanna Tran performed some of the preliminary experiments and provided technical assistance and conceptual feedback and Miquel Rosas Salvans, PhD provided conceptual assistance.

Chapter 3 of this dissertation contains unpublished work that is a work in progress. I designed and performed the experiments and completed analysis described in this chapter with equal contribution from Miquel Rosas Salvans, PhD and under the guidance of Sophie Dumont, PhD.

Uncovering the Role of Biophysical Cues in Decision Making in the Mammalian Spindle

Megan Chong

Abstract

When a cell divides, it builds the mitotic spindle, a micron-scale bipolar structure made of dynamic microtubules that must attach to chromosomes and segregate them evenly into two daughter cells. Segregation relies on microtubule-generated force to capture and move chromosomes, yet how this force contributes to a cell's decision-making during division has been challenging to discern. In my thesis work, I have addressed how biophysical cues at the kinetochore contribute to two key decisions during mitosis: first, whether to hold on to chromosome attachments or let go and second, when to stop building the spindle and divide. The kinetochore is the macromolecular interface that connects chromosomes to spindle microtubules. For accurate chromosome segregation, sister kinetochores need to become bioriented or attach to opposite spindle poles. Biorientation occurs by a constant feedback loop, destabilizing incorrect attachments while reinforcing correct ones. How the kinetochore distinguishes between correct and incorrect attachments is not clear. In this work, I address the long-standing hypothesis that tension at the kinetochore dictates which attachments to maintain. Using live imaging to monitor outcomes, I directly perturb force on kinetochores globally by chromokinesin overexpression and locally by individual chromosome arm ablation. Together with experiments enriching for attachment errors, this work demonstrates that elevated force at the kinetochore promotes attachment stabilization, and this effect is not chromosome-agnostic but leads to impaired error correction on long chromosomes.

In parallel with local error correction decisions, the cell must globally prevent anaphase until all chromosomes are attached correctly. The spindle assembly checkpoint (SAC) generates a diffusible signal at unattached kinetochores to prevent premature segregation. The precise cues that trigger SAC satisfaction at an individual kinetochore and how the cell measures and integrates these signals is not well understood. Here, I use laser ablation to generate unpaired, sisterless kinetochores and monitor anaphase entry timing. I find a progressive, titratable delay in mitosis with increasing number of unpaired kinetochores, suggesting that without a sister kinetochore or a discrete opposing force, attachments may not be sufficiently stable to satisfy the SAC with normal dynamics. Still, these unpaired kinetochores are insufficient to prevent anaphase entirely, suggesting either that the SAC does satisfy eventually or that the cell cannot detect this low level of SAC signal. Altogether, I find that altering the physical landscape of the kinetochore by perturbing opposing force on it, either by manipulating chromokinesins or removing its sister, alters two critical pathways: error correction and the SAC. I find that force directly impacts the fidelity of cell division by signaling the error correction machinery and may alter dynamics of SAC satisfaction either directly or indirectly.

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Chapter 1: Introduction

Dynamic Assembly of the Mammalian Spindle

In studying biological order, from sub-cellular molecular machines to organismal body plans, it is tempting to search for a blueprint that shapes biological structures. Instead, closer investigation of mechanisms has revealed a common biological theme: order emerging from chaos. At all scales, rather than employ error-free processes, biology has optimized to harness stochasticity. Many robust and elegant patterns arise simply by layering regulatory elements on top of random events, amplifying desired outcomes at the expense of errors. Indeed, processes as diverse as enzyme allostery, bacterial chemotaxis, and body axis formation in embryogenesis employ rules to select for order within randomness (Kirschner et al., 2000). In mitosis, accurate segregation of the genome relies on such a process (Lampson and Grishchuk, 2017).

When a cell divides, it builds the mitotic spindle, a bipolar structure that must attach to all chromosome pairs and divide them evenly into two daughter cells. The fidelity of this process is critical to maintaining a healthy genome across the billions of cells that divide in human tissues every day and avoid disease (Santaguida and Amon, 2015). Spindle assembly is a dynamic mechanical process that relies on rules and checkpoints to ensure accuracy. The kinetochore is the macromolecular interface that connects chromosomes to spindle microtubules. It comprises ~100 protein species balanced in precise ratios, which bind to bundles of 15-25 microtubules that make up the mammalian kinetochore-fiber (k-fiber) (Johnston et al., 2010; McEwen et al., 1997). It must transmit and respond to force to move chromosomes and sense attachment status in order to signal to the cell when to enter anaphase. These mechanical and

biochemical roles are both critical for faithful chromosome segregation, but how these roles may synergize or feedback on one another is not well understood.

In this dissertation, I ask how and whether biophysical cues regulate decision making in mammalian cell division. First, in this introductory chapter, I review our current understanding of the kinetochore as a mechanical interface and decision-making hub. Then, in Chapter 2, I investigate the role of chromosome size and kinetochore tension on a cell's ability to correct errors that arise during mitosis to ensure accurate chromosome segregation. In Chapter 3, I probe the cellular conditions required for satisfaction of the spindle assembly checkpoint at individual kinetochores and globally across the cell. Finally, in chapter 4, I discuss open questions regarding how physical forces dictate key decisions cells make as they divide.

Principles of Kinetochore Decision-Making

The kinetochore is a highly multivalent protein interface with a precise stoichiometry. It is assembled hierarchically, with inner kinetochore proteins connected to centromeric histones and outer kinetochore proteins, which bind microtubules, built on top of this inner plate (Cheeseman and Desai, 2008). The composition of the kinetochore changes throughout the cell cycle. In early mitosis, before attaching to microtubules, the kinetochore assembles the fibrous corona (Jokelainen, 1967; Kops and Gassmann, 2020). This protein assembly at the outer edge of the kinetochore radiates outward, expanding the reach of the kinetochore's microtubule binding capacity. As attachments form, the corona disassembles, restricting microtubule binding from new directions (Sacristan et al., 2018). Simultaneously, the kinetochore recruits

new microtubule binding proteins, like Ska (Cheerambathur et al., 2017; Hanisch et al., 2006) and SKAP (Fang et al., 2009; Schmidt et al., 2010), to optimize kinetochore grip for smooth movement at late metaphase and anaphase. How this architecture gives rise to critical decision points during cell division is not well understood. In particular, we do not yet know how and whether physical cues, like tension at the kinetochore, can directly induce these changes in kinetochore composition that regulate error correction or anaphase entry.

The features that promote robust kinetochore-microtubule attachment may also contribute to accurate kinetochore decision-making during mitotic progression. For example, the molecular redundancy and tunability of the kinetochore-microtubule interface support its mechanical robustness. These same features may enhance the accuracy of mitotic decisions, including error correction and the spindle assembly checkpoint (SAC) (Long et al., 2019). Multivalency—the ability to modify kinetochore grip at many points in space and time—increases the input information at a single kinetochore (Volkov et al., 2018). This may reduce sensitivity to noise and improve overall mitotic outcomes. In comparison to yeast mitosis, which has fewer redundant features and binds to just 1-3 microtubules at metaphase, mammalian mitosis requires more time to assemble a spindle and so may require a higher threshold for determining if an attachment is correct (Peterson and Ris, 1976). The tunability of kinetochore grip, facilitated by the dynamic Ndc80 phosphorylation-dephosphorylation cycle (DeLuca et al., 2006, 2011) and recruitment of kinetochore maturation markers like Ska (Cheerambathur et al., 2017; Hanisch et al., 2006) and SKAP (Fang et al., 2009; Schmidt et al., 2010), may impact k-fiber lifetime directly and alter the ease with which

detachment can occur in the event of an incorrect attachment. Thus, mechanical features of the attachment are well-positioned to alter the decision-making landscape for kinetochores performing error correction or signaling the spindle assembly checkpoint.

Error correction

Mitotic error correction ensures all chromosomes are correctly attached to the spindle to avoid missegregation. As the spindle assembles, sister kinetochores need to become bioriented, attaching to opposite spindle poles, but correct bioriented attachments form alongside incorrect and incomplete attachments. How the kinetochore distinguishes between these correct and incorrect attachments remains unclear. The current model for error correction holds that it stabilizes correct attachments and destabilizes incorrect ones in a continual feedback loop (Sarangapani and Asbury, 2014). This stabilization occurs through dephosphorylation of Ndc80, the primary load-bearing complex in the mammalian kinetochore (Powers et al., 2009), and subsequent maturation of the attachment, which involves recruitment of late metaphase kinetochore proteins and eventual SAC satisfaction. Destabilization is thought to be mediated primarily by Aurora B kinase-regulated phosphorylation of Ndc80 (DeLuca et al., 2006, 2011), but the details of how this triggers detachment are still being elucidated. In early mitosis and in monopolar spindles, kinetochores with a high level of Ndc80 phosphorylation and a low k-fiber lifetime attach to the spindle and travel without detaching (Cameron et al., 2006). Kinetochore-microtubule attachment redundancy mediates this robust attachment state even at high phosphorylation states. Ndc80, is

present in excess, with just a small fraction (~30%) engaged with microtubules at metaphase (Yoo et al., 2018). This allows multiple points of contact with microtubule bundles, reinforcing the attachment interface and protecting it from spontaneous detachment. This redundancy may also reduce the sensitivity of kinetochore checkpoint signaling to detachment of individual microtubules, preventing unnecessary mitotic delays.

Full detachment from incorrect attachments, then, may require an additional cue. Recent work has shown high variability in inter-kinetochore tension may facilitate kinetochore detachments with increased frequency by providing the force to separate the kinetochore from the k-fiber (Parmar et al., 2023). Additionally, optogenetic activation of Aurora B kinase at kinetochores under high or low inter-kinetochore tension revealed phosphorylation triggered microtubule depolymerization under low force and detachment under high force (Ke et al., 2009). While detachment under force is an intuitive model, it is complicated by the long-standing tension hypothesis in error correction (Sarangapani and Asbury, 2014). Correct, bioriented attachments are under high tension from opposing force of the two halves of the spindle, while incorrect attachments are thought to experience lower force. Tension has long been thought to be the stabilizing cue signaling correct attachments through a catch-bond mechanism (Miller et al., 2019, 2016). However, direct perturbations of tension are difficult to perform in live cells, so we do not yet know the full effects of force at the kinetochore on overall attachment stability and error correction outcomes. Understanding how the physical features of this interface impact a kinetochore's propensity to hold onto k-fibers

will provide insight into how cells maintain genome integrity in different cell and tissue contexts with varying physical characteristics.

The spindle assembly checkpoint

The intact spindle assembly checkpoint (SAC) prevents anaphase until all chromosomes are properly bioriented, ensuring daughter cells receive exactly one copy of all chromosomes (Rieder et al., 1995). The SAC functions both locally and globally to prevent anaphase; it signals locally at unattached kinetochores, generating a diffusible WAIT anaphase signal, which propagates globally to inhibit the anaphase promoting complex (Musacchio, 2015). Similar to error correction, a long-held hypothesis posited that tension at the kinetochore served as a cue to distinguish unproductive attachments from bioriented ones and trigger SAC satisfaction. However, ample evidence now suggests that the SAC can, under certain circumstances, satisfy or stay silent in the absence of interkinetochore tension (Etemad et al., 2015; Long et al., 2019; O'Connell et al., 2008; Tauchman et al., 2015). Whether those circumstances arise in the course of normal spindle assembly is not known. Moreover, while tension may not be directly monitored or sensed by the SAC machinery at the kinetochore, what cues are monitored to trigger a SAC satisfaction decision and whether tension is required to generate those cues are not yet clear. Without directly monitoring kinetochore tension, discriminating between stable and premature kinetochore attachments may require monitoring either k-fiber lifetime or kinetochore maturation markers as a proxy for attachment stability or alternatively may rely on quick error correction outcomes.

In addition to regulating SAC satisfaction events at kinetochores, the cell must integrate SAC signals across all ninety-two kinetochores in the human cytoplasm. Ultimately, the anaphase entry decision relies not only on the ability to adequately detect stable attachments at a kinetochore but also the ability to detect cytoplasmic signal emanating from SAC-positive kinetochores and determine if it is above an unacceptable threshold. While SAC silencing at the kinetochore has been demonstrated to occur in a switch-like, all-or-none manner in mammalian cells (Kuhn and Dumont, 2017), the cytoplasmic SAC signal has been found to be graded based on the number of unattached kinetochores in the cell (Collin et al., 2013; Dick and Gerlich, 2013). Whether this graded response makes the cell permissive to anaphase entry with SAC-positive kinetochores and how this threshold for anaphase prevention may vary based on cell type or chromosome complement will be important for understanding how aneuploidies develop.

Objectives of the thesis

While recent work has begun to identify the molecular determinants of kinetochore attachment mechanics, conceptual and technical gaps still remain, particularly in our understanding of how these mechanics impact overall mitotic progression and chromosome segregation accuracy. In this work, I use quantitative live imaging and direct physical perturbations to ask how the mechanical features of the kinetochore-microtubule interface feed into its decision-making capacity and affect segregation outcomes.

In chapter 2, I find that chromosome size directly impacts error correction efficiency and provide direct evidence for the long-standing tension hypothesis. Our model for how differential tension on long chromosomes stabilizes both correct and incorrect attachments provides a framework for understanding how the mechanical landscape of the kinetochore can have wide-reaching impacts on cell division accuracy. In chapter 3, I find evidence of a clear but incomplete SAC defect in cells with 1-3 kinetochores that lack opposing force from sister k-fibers. This titratable delay in mitotic timing supports a model in which presence of a sister kinetochore supports SAC satisfaction with wildtype dynamics but is not required for anaphase entry. In Chapter 4, I discuss the intersection of these decision-making pathways in the literature and expand on possible future directions.

Together, the findings herein demonstrate that the physical and signaling roles of the kinetochore-microtubule interface are not merely coincident but act in synchrony to regulate mammalian spindle assembly and control segregation outcomes. Understanding that physical features play such a critical role in healthy mitosis may provide new insight on a whole host of new questions. Given the role of tension at the kinetochore in these key mitotic decisions, future work may explore how kinetochore material properties, chromatin compliance, tissue mechanics, and other physical features alter mitotic fidelity and shape karyotype evolution across species.

Chapter 2: Chromosome size-dependent polar ejection force impairs mammalian mitotic error correction

Abstract

Accurate chromosome segregation requires sister kinetochores to biorient, attaching to opposite spindle poles. To this end, the mammalian kinetochore destabilizes incorrect attachments and stabilizes correct ones, but how it discriminates between these is not yet clear. Here, we test the model that kinetochore tension is the stabilizing cue and ask how chromosome size impacts that model. We live image PtK2 cells, with just 14 chromosomes, widely ranging in size, and find that long chromosomes align at the metaphase plate later than short chromosomes. Enriching for errors and imaging error correction live, we show that long chromosomes exhibit a specific delay in correcting attachments. Using chromokinesin overexpression and laser ablation to perturb polar ejection forces, we find that chromosome size and force on arms determine alignment order. Thus, we propose a model where increased force on long chromosomes can falsely stabilize incorrect attachments, delaying their biorientation. As such, long chromosomes may require compensatory mechanisms for correcting errors to avoid chromosomal instability.

Introduction

The kinetochore is the multivalent interface that connects chromosomes to spindle microtubules at cell division. Accurate chromosome segregation requires biorientation, a state in which sister kinetochores attach to opposite spindle poles. To preserve genome integrity, each kinetochore must monitor chromosomes' attachment status and signal to the cell whether or not it is ready to enter anaphase (Musacchio and Desai, 2017). Correct, bioriented attachments form alongside incorrect attachments, which must be detected and corrected through a process called error correction. Both physical and biochemical features differ between correct and incorrect attachments and understanding how these cues govern error correction and how their detection varies across chromosomes is central to understanding mitotic fidelity (Lampson and Grishchuk, 2017).

Current models for error correction propose that incorrect kinetochore-microtubule attachments are molecularly destabilized to promote detachment, while correct attachments are stabilized (Funabiki, 2019; Sarangapani and Asbury, 2014). A key regulator in this process is Aurora B kinase, which phosphorylates the kinetochore's primary loadbearing complex Ndc80C (Powers et al., 2009) to reduce its affinity for microtubules (Biggins and Murray, 2001; Cheeseman et al., 2006; DeLuca et al., 2011; Zaytsev et al., 2015). This phosphorylation decreases with kinetochore tension and is required for error correction to occur (DeLuca et al., 2006; Lampson et al., 2004). Moreover, applying ectopic tension to incorrect attachments is sufficient to delay their correction indefinitely in grasshopper spermatocytes (Nicklas and Koch, 1969), indicating a causative role for tension in distinguishing between correct and incorrect

attachments in some cell types. However, how the mammalian error correction machinery responds to acute changes in tension and what cellular sources of said tension are relevant remain poorly understood.

While tension at bioriented kinetochores is generated primarily by kinetochore-bound microtubules (k-fibers) exerting opposing poleward pulling force on sisters, non-kinetochore microtubules also exert force in the spindle that influences kinetochore behavior. Polar ejection force arises from non-kinetochore microtubules growing outward from spindle poles generating outward force on chromosome arms (Rieder et al., 1986). This polar ejection force, mediated both by microtubule polymerization and by two chromokinesins in mammalian cells (Kif22/Kid and Kif4a), contributes to chromosome congression and regulates the amplitude of kinetochore oscillations at metaphase (Barisic et al., 2014; Iemura and Tanaka, 2015; Ke et al., 2009; Levesque and Compton, 2001; Wandke et al., 2012). Overexpression of the Drosophila orthologue of Kif22/Kid induces attachment errors, which elude error correction (Cane et al., 2013b), suggesting that polar ejection forces can contribute to attachment stabilization, possibly by increasing kinetochore tension. In mammalian cells, tension perturbations are needed to understand how the error correction machinery responds to tension changes and how the magnitude of polar ejection force—which in principle can vary throughout mitosis (Ke et al., 2009; Thompson et al., 2022) and across different chromosomes—impacts kinetochore tension and attachment stability.

Segregation error rates vary not only across cell types but also across chromosomes within the same cell type. Indeed, long chromosomes have been shown to missegregate more frequently than short chromosomes in human cells (Klaasen et

al., 2022; Worrall et al., 2018). Many mechanisms could underlie this difference including differential nuclear positioning in interphase (Klaasen et al., 2022; Tovini and McClelland, 2019), distinct positions of long and short chromosomes along the metaphase plate (Rieder and Salmon, 1994; Wan et al., 2012), or variable biophysical features between long and short chromosomes. Indeed, given the biophysical nature of error detection models, error correction cues might be read differently based on the physical features of the chromosome or kinetochore in question, complicating our understanding of how tension impacts kinetochore-microtubule attachment stability. However, studying segregation outcomes is not sufficient to understand the dynamic formation of correct attachments. Instead, probing how efficiently chromosomes of different sizes become correctly bioriented sheds light not only on the cues driving error correction, but also on the mechanisms underlying chromosome-based differences in mitotic trajectories and segregation outcomes.

Here, we provide direct evidence for the tension model for error correction in mammalian cells and show that differential tension at chromosomes of different sizes impacts both their biorientation and error correction efficiency. In mammalian rat kangaroo (PtK2) cells, we show that long chromosomes become bioriented later and experience higher pushing force than short chromosomes in the same cell. By enriching for errors and letting them correct in live imaging of drug washouts, we show that long chromosomes are less efficient at correcting errors therefore delaying their biorientation. Increasing size-based force via chromokinesin Kif22/Kid overexpression increases the probability of chromosomes of any size becoming persistently stuck at spindle poles. Finally, with laser ablation, we cut chromosome arms and find that chromosome size,

rather than simply identity, sets biorientation efficiency or success rate. Here, biorientation efficiency does not set a strict order of events in chromosome alignment, but rather refers to the probability of successfully becoming bioriented. We propose a model in which elevated force on long chromosomes increases kinetochore tension, stabilizing kinetochore-microtubule attachments, thus prolonging the lifetime of erroneous attachments at long chromosomes. These findings provide a framework for understanding not just mechanisms of error detection, which may vary in response to chromosome-specific differences, but also aneuploidy and karyotype evolution.

Results and Discussion

Long chromosomes align less efficiently than short chromosomes and experience higher spindle pushing force.

To determine which physical features promote correct kinetochore-microtubule attachment in mitosis, and whether they vary across chromosomes, we assessed whether dividing rat kangaroo (PtK2) cells exhibit a bias in chromosome alignment efficiency. With just 14 chromosomes, widely ranging in size, PtK2 cells are particularly well suited to this question. We classified chromosomes by size into two, roughly even groups for easy identification: long chromosomes (≥7μm along the longest dimension by phase microscopy) and short ones (<7μm) (Fig. 2.1A & 2.1B). We imaged live spindle assembly in PtK2 cells expressing eYFP-Cdc20 to mark kinetochores and scored the frequency of early and late aligning chromosomes belonging to either the long or short chromosome group. Here, we define early aligning chromosomes as the first three chromosomes to begin oscillating at the metaphase plate, a marker of biorientation, and

late aligning chromosomes as the last three chromosomes to align (Fig. 2.1C). While long and short chromosomes were evenly represented in the early aligning group, long chromosomes were significantly overrepresented among late aligning chromosomes (Fig. 2.1D, 2.1E; Fisher's exact test p=0.0041). Thus, on average, long chromosomes biorient and form correct attachments later in spindle assembly than short chromosomes in the same cell, suggesting less efficient or delayed attachment formation. Such a delay could be due to chromosome position differences in the spindle, intrinsic differences in chromosome or kinetochore identity, and/or differences in the formation or correction of incorrect attachments that must be resolved prior to alignment.

A longstanding error correction model posits high tension across sister kinetochores as the primary cue that promotes formation of correct attachments while disfavoring incorrect ones (Funabiki, 2019; Sarangapani and Asbury, 2014). Thus, we sought to test whether different sized chromosomes are subject to differing forces. At correct attachments, tension is generated by the opposing force from k-fibers pulling sister kinetochores toward opposite spindle poles; in contrast, incorrect, syntelic attachments (with both sister kinetochores attached to the same spindle pole) may experience kinetochore tension when poleward pulling is counteracted by anti-poleward force along chromosome arms and this may, in turn, affect attachment stability (Fig. 2.1F). To test how polar ejection forces affect long and short chromosomes, we generated monopolar spindles by treating cells with Eg5 inhibitor STLC and tracked kinetochore movements with respect to the pole. Assuming roughly equal poleward pulling force at all chromosomes due to standard k-fiber size (McEwen et al., 1998,

1997), the distance from poles will be proportional to the strength of the outward polar ejection force. In live monopolar spindles, the kinetochores on long chromosomes were significantly farther from poles on average than short chromosomes (Fig 2.1G, 2.1H; unpaired t-test p=0.0005). Thus, long chromosomes biorient less efficiently but also experience higher pushing force than their shorter counterparts.

Long chromosomes correct errors less efficiently.

We hypothesized that increased pushing force at long chromosomes may generate enough tension to stabilize incorrect attachments, prolonging their lifetime and delaying congression. To test this, we enriched for errors in attachment using the Eg5 inhibitor STLC to generate monopolar spindles (Kapoor et al., 2000; Wu et al., 2018) and then washed out the drug, allowing spindle bipolarization and error correction to occur (Lampson et al., 2004). Following drug washout, as spindle poles separate, monotelic attachments (one attached sister kinetochore, one unattached), incorrect syntelic attachments (both sister kinetochores attached to the same spindle pole), and other complex attachment errors convert to bioriented attachments, which oscillate at the new metaphase plate (Fig. 2.2A). We measured the frequency of these attachment types after STLC washout by immunofluorescence and found that while ~80% of monopolar spindles exhibit 1-2 unambiguously monotelic kinetochores (Fig. 2.6), consistent with previous work (Kapoor et al., 2000), only ~20% of bipoles contain monotelic attachments (Fig. 2.6), suggesting most chromosome alignment events following drug washout represent error correction events, though we suspect these errors transit between attachment states from syntelic to monotelic and back. We

measured the time needed for chromosomes to start oscillating after drug washout and used it as a proxy for biorientation and error correction (Fig. 2.2B, 2.2C). Alignment times were normalized to measure rank order and account for high variance in time required to achieve bipolarity between different cells (Fig. 2.7). We find that long chromosomes become bioriented later than short chromosomes following drug washout (Fig. 2.2C & 2.2D; Mann-Whitney test p=0.0003). In principle, this could either reflect that long chromosomes have more trouble moving within the spindle than short ones or that long chromosomes correct errors less efficiently (i.e. have a lower success rate converting errors to bioriented attachments). To determine whether drag force limits chromosome movement within the spindle, we measured the velocity of kinetochore movement on long and short chromosomes in monopolar spindles (a prolonged prometaphase-like state) to avoid differences in kinetochore maturation that could affect kinetochore grip and speed of movement in bipoles. We did not see a significant difference in kinetochore speed between groups (Fig. 2.2E; p=0.318), consistent with previous findings in grasshopper cells (Nicklas, 1965). Thus, chromosome movement velocity does not impact biorientation. Instead, these data indicate that long chromosomes take longer to correct erroneous attachments. Indeed, we find clear instances of syntelic attachments in fixed, regularly cycling cells (Fig. 2.6), and together with our previous findings (Fig. 2.2D), this suggests that the observed alignment delay might be due to the higher forces long chromosomes experience.

High polar ejection force increases persistence of polar chromosomes and reduces size-effect.

To test if elevated force on long chromosomes is responsible for their delayed error correction, we globally increased polar ejection force and imaged spindle assembly. If elevated force on long chromosomes does not meaningfully contribute to kinetochore tension or attachment stability, increasing anti-poleward force should speed chromosome alignment and reduce dwell time of chromosomes near poles. Indeed, chromokinesins are known to promote chromosome alignment by pushing chromosomes towards the metaphase plate and their depletion slows the progression to metaphase in human cells (Wandke et al., 2012). Alternately, if polar ejection force on long chromosomes can stabilize incorrect attachments, increasing this force globally may result in more delayed chromosome alignment. To discriminate between these possibilities, we perturbed the force balance between poleward pulling at the kinetochore and anti-poleward pushing along chromosome arms by overexpressing the rat kangaroo chromokinesin Kif22/Kid tagged with a HaloTag (Fig. 2.3A). We confirmed chromokinesin overexpression (Kid OE) and proper localization by immunofluorescence, which showed significantly more total Kid on chromosomes in over expressing cells compared to control (Fig. 2.3B). Additionally, we measured the distance of kinetochores on long and short chromosomes from the spindle pole in monopoles and found short chromosomes are significantly further from the pole in Kid OE than in control (Fig. 2.8), indicating an increase in ejection force on these short chromosomes.

We imaged spindle assembly and quantified the number of polar chromosomes which persisted at poles for longer than 15 minutes (Fig. 2.3D). Here, we define polar chromosomes as those that did not move to the spindle center but rather oriented sister kinetochores toward the same spindle pole and took on a characteristic V shape of chromosomes under polar ejection force (Fig. 2.3D). Among control cells, only 53% of cells displayed persistently stuck polar chromosomes compared to 82% of Kid OE cells (Fig. 2.3E), and on average, Kid OE cells had significantly more chromosomes stuck at poles per cell (Fig. 2.3F; 0.9 ± 1.1 vs. 2.8 chromosomes ± 2.8, mean±SD; p=0.0202), consistent with observations in Drosophila S2 cells (Cane et al., 2013b). This effect was a specific result of increasing polar ejection force along chromosome arms as overexpressing Kid∆C, lacking 89 amino acids at the C terminus responsible for DNA binding, was indistinguishable from control cells (Fig. 2.3C, 2.3F, and Video 2.4). Moreover, when categorized by size, instance of short chromosomes stuck at poles increased significantly with Kid OE (Fig. 2.3F; 0.2 chromosomes vs. 1.2 chromosomes; p=0.0009), while long chromosomes in persistent polar attachments increased more modestly with no significant difference (0.7 chromosomes vs. 1.7 chromosomes; p=0.07). This distribution suggests a shift in the size-effect delaying chromosome alignment, where specifically increasing polar ejection force on short chromosomes (Fig. 2.8) pushes them over a tension threshold and leads to chromosomes of all sizes getting stuck in polar attachments.

Additionally, consistent with impaired error correction of chromosomes under elevated force, we observed polar chromosome attachments that do not resolve in the duration of imaging (Fig. 2.3G) and cells entering anaphase with chromosomes stuck at

poles as well as lagging chromosomes (Fig. 2.3H). This indicates the force balance between poleward pulling at the kinetochore and anti-poleward pushing by chromokinesins impacts the ability of cells to detect attachment errors. Despite this, errors that are detected seem to reach alignment on similar timescales to controls (Fig. 2.3G). Together, we find that globally increasing polar ejection force leads to an enrichment of chromosomes stuck near poles, consistent with a model in which polar ejection force contributes to kinetochore attachment stability even at polar, non-bioriented attachments.

Chromosome size determines chromosome biorientation efficiency.

To directly test the role of chromosome size and size-based force in chromosome alignment efficiency, we sought to acutely change chromosome size. To do so, we used laser ablation to cut chromosome arms and measured alignment efficiency. If chromosome size, either directly or indirectly, leads to differential alignment efficiency, shortening chromosome arms should promote earlier chromosome alignment with respect to other long chromosomes in the same cell (model 1; Fig. 2.4A). If, instead, intrinsic chromosome-specific differences, like kinetochore composition or size (Drpic et al., 2018), dictate chromosome alignment order, ablating chromosome arms should not affect alignment efficiency (model 2; Fig. 2.4A). We ablated long chromosomes in early prometaphase in cells with many unaligned long chromosomes (Fig. 2.4B, 2.4C). Ablated chromosomes congressed to the metaphase plate before other long, unablated chromosomes in the same cell did (Fig. 2.4D; Mann-Whitney test $p=0.002$), indicating that chromosome size, not simply identity, sets alignment efficiency

(model 1). This effect was specific to chromosome arm shortening and not due to damaging the arms as control ablations, which nick chromosomes at their ends or along their arms without shortening them (Fig. 2.4E & 2.4F), did not change alignment order (Fig. 2.4G). Given the indistinguishable speeds of kinetochores on long and short chromosomes that exclude any drag effect (Fig. 2.2E), we conclude that the expedited alignment of ablated chromosomes is the result of reducing polar ejection forces. We propose a model wherein increased polar ejection force on long chromosomes prematurely stabilizes incorrect or incomplete attachments, causing a delay in congression and biorientation (Fig. 2.5).

Elevated tension at long chromosomes stabilizes incorrect attachments and delays error correction.

Accurate chromosome segregation requires cells to be able to distinguish between correct and incorrect attachments, global features of the spindle, despite having only local cues. Here, we ask how the ability both to form bioriented attachments and to detect incorrect attachments varies across chromosomes in the same cell. We demonstrate that in regularly cycling PtK2 cells, with just 14 chromosomes widely ranging in size, the formation of correct, bioriented attachments is biased by chromosome size such that long chromosomes tend to be the last to become correctly attached (Fig. 2.1). Error correction models have long posited that the kinetochore relies on physical differences—specifically variable tension on kinetochores leading to differential kinetochore phosphorylation—to determine which attachments to reinforce (bioriented) and which to destabilize (syntelic) (Funabiki, 2019; Sarangapani and

Asbury, 2014). By enriching for attachment errors, we find that mammalian error correction efficiency varies based on the physical features of the chromosome in question (Fig. 2.2), and specifically perturbing chromosome size-based force reveals that is the result of the kinetochore sensing and responding to differences in tension (Figs. 2.3 & 2.4). Together, these findings suggest that while all chromosomes are competent to form both correct and incorrect attachments, long chromosomes more readily stabilize incorrect attachments, delaying their alignment and biorientation (Fig. 2.5). In addition to defining biophysical models for error correction, this work defines new questions about protective mechanisms for not just minimizing segregation error rates but also coordinating the threshold for error detection across different chromosomes in the cell.

Overall, this work provides insight into the biophysical mechanism of error correction and reveals that the dynamics of chromosome biorientation are not chromosome-agnostic. Indeed, long chromosomes exhibit distinct behaviors in spindle assembly, more frequently getting stuck at poles (Fig. 2.3), congressing later in mitosis (Fig. 2.1), and oscillating with lower amplitude than their short counterparts (Ke et al., 2009). Chromosome arm ablation speeds alignment (Fig. 2.4D) indicating these are effects of chromosome size and not simply identity. Still, long and short chromosomes move at similar speeds (Fig. 2.2E). While chromosome identity, kinetochore position, or early spindle position could affect the propensity to form errors and possibly contribute to the rate of their correction, our data show that the main factor regulating error correction efficiency or success rate is chromosome size. As such, we propose that differences in alignment order and tendency to dwell at spindle poles stem primarily

from chromosome size-based force specifically stabilizing attachments at long polar chromosomes rather than drag force or steric effects. Consistent with this, increasing polar ejection force increases the number of stuck polar chromosomes and reduces the effect of size on tendency to become persistently stuck at poles (Fig. 2.3E & 2.3F), indicating chromosome size and force influence the resolution of incorrect attachments but not necessarily the formation of correct ones. Regardless, stuck chromosomes that do align do so on a similar timescale to those in control cells (Fig. 2.3G), suggesting that beyond a certain tension threshold, additional tension will not slow alignment further but may impact the probability of error detection. This is consistent with work that shows that the tension state impacts kinetochore detachment probability (Chen et al., 2021; De Regt et al., 2022; Miller et al., 2019, 2016; Parmar et al., 2023; Sarangapani et al., 2013) and intra-kinetochore stretch, in particular, is the relevant feature for attachment stability (Drpic et al., 2015; Etemad et al., 2015; O'Connell et al., 2008; Tauchman et al., 2015). Additionally, attachment stability and error correction efficiency may also be linked to chromosome position in the spindle, leading to differential access to known regulators like Aurora A kinase (Eibes et al., 2023; Ye et al., 2015). Indeed, increasing dwell time of chromosomes near spindle poles may tip the balance to favor Aurora A-mediated error correction such that proximity to poles not only stabilizes errors on long chromosomes temporarily, but also activates the pathway required for their correction after some delay. Together, these findings support a model in which high force near poles increases the propensity to stabilize polar chromosome attachments (Fig. 2.5). In healthy cells, elevated polar ejection force at long chromosomes might come from more numerous chromokinesins, non-kinetochore microtubules polymerizing against a larger

chromatin surface and generating force, or both (Schneider et al., 2022). Regardless, this size dependent mechanism for error detection is especially interesting considering recent work showing higher rates of missegregation of large chromosomes in human cells (Klaasen et al., 2022; Worrall et al., 2018) and formation of complex attachment errors in polar chromosomes (Tovini and McClelland, 2019; Vukušić and Tolić, 2022).

Our model, that error correction dynamics depend on chromosome size and resultant kinetochore tension, may have wide ranging functional implications for segregation outcomes, and thus propensity toward aneuploidy. How this differential tension and attachment stability across chromosomes of different sizes alters molecular maturation of the kinetochore in Aurora B regulated processes such as Ndc80 phosphorylation (Sarangapani et al., 2021), SKA (Cheerambathur et al., 2017; Hanisch et al., 2006) and SKAP (Fang et al., 2009; Schmidt et al., 2010) recruitment, and checkpoint satisfaction (Etemad et al., 2015; Tauchman et al., 2015) remains an exciting open question. Relatedly, while we propose elevated polar ejection force opposes error correction at long chromosomes, it is also clear that polar ejection force is required for chromosome alignment (Iemura and Tanaka, 2015; Wandke et al., 2012). We see these two opposing roles as part of an important balancing act that drives evolution of kinetochore tension sensitivity. It is worth noting that missegregation rates were found to be similar across all chromosomes in PtK1 cells by fixed cell imaging (Torosantucci et al., 2009), yet these results are not inconsistent with a size-based model for error correction efficiency. Chromosome segregation errors rely on failure of both the error correction machinery and the spindle assembly checkpoint (SAC), but how tightly these pathways are coupled may vary across systems. Indeed, observations

of SAC satisfaction dynamics have been variable. While Drosophila S2 cells show partial SAC satisfaction at persistently incorrect attachments (Cane et al., 2013a), live monitoring of the SAC in PtK2 cells revealed switch-like loss of Mad1 from the kinetochore (Kuhn and Dumont, 2017). Thus, how and whether persistently stuck polar chromosomes signal the checkpoint in mammalian cells remains unclear. Our work demonstrates that however many errors do or do not slip through at anaphase, the dynamics of error correction do, indeed, vary in PtK2 cells with chromosome size (Fig. 2.2). Future work will be required to untangle the crosstalk between the SAC and error correction.

Characterizing not just the propensity of individual chromosomes for missegregation but also the features that tune that propensity up or down will inform our understanding of different organisms' or cell types' oncogenic capacity and tendency toward aneuploidy. Interesting evolutionary questions arise from non-random missegregation rates. Is there evolutionary pressure to maintain oncogenes on short chromosomes, which may be less likely to missegregate? Or instead, in organisms with long chromosomes carrying oncogenes, is there selective pressure to either homogenize chromosome size over evolutionary time or develop compensatory molecular mechanisms that will equalize missegregation rates? The size-dependence revealed here is likely just one of many features that impact error correction efficiency. Indeed, in different species, chromosome size (Klaasen et al., 2022; Tovini and McClelland, 2019; Worrall et al., 2018), kinetochore position (metacentric, telocentric, or holocentric) (Dumont et al., 2020), material properties (Cojoc et al., 2016), and size (Drpic et al., 2018), as well as chromatin material properties (Schneider et al., 2022) all

have the potential to impact segregation outcomes. Future work on these topics will shed light on how organisms employ either a low threshold for sensing incorrectness, compensatory mechanisms that tune error detection or correction capacity on a per chromosome basis, or both to avoid catastrophic chromosomal instability. Ultimately, the findings herein provide insight into the biophysical mechanism of error detection at the kinetochore and provide a framework through which to think more broadly about how biophysical processes must shape molecular mechanisms across evolution.

Figures and Figure Legends

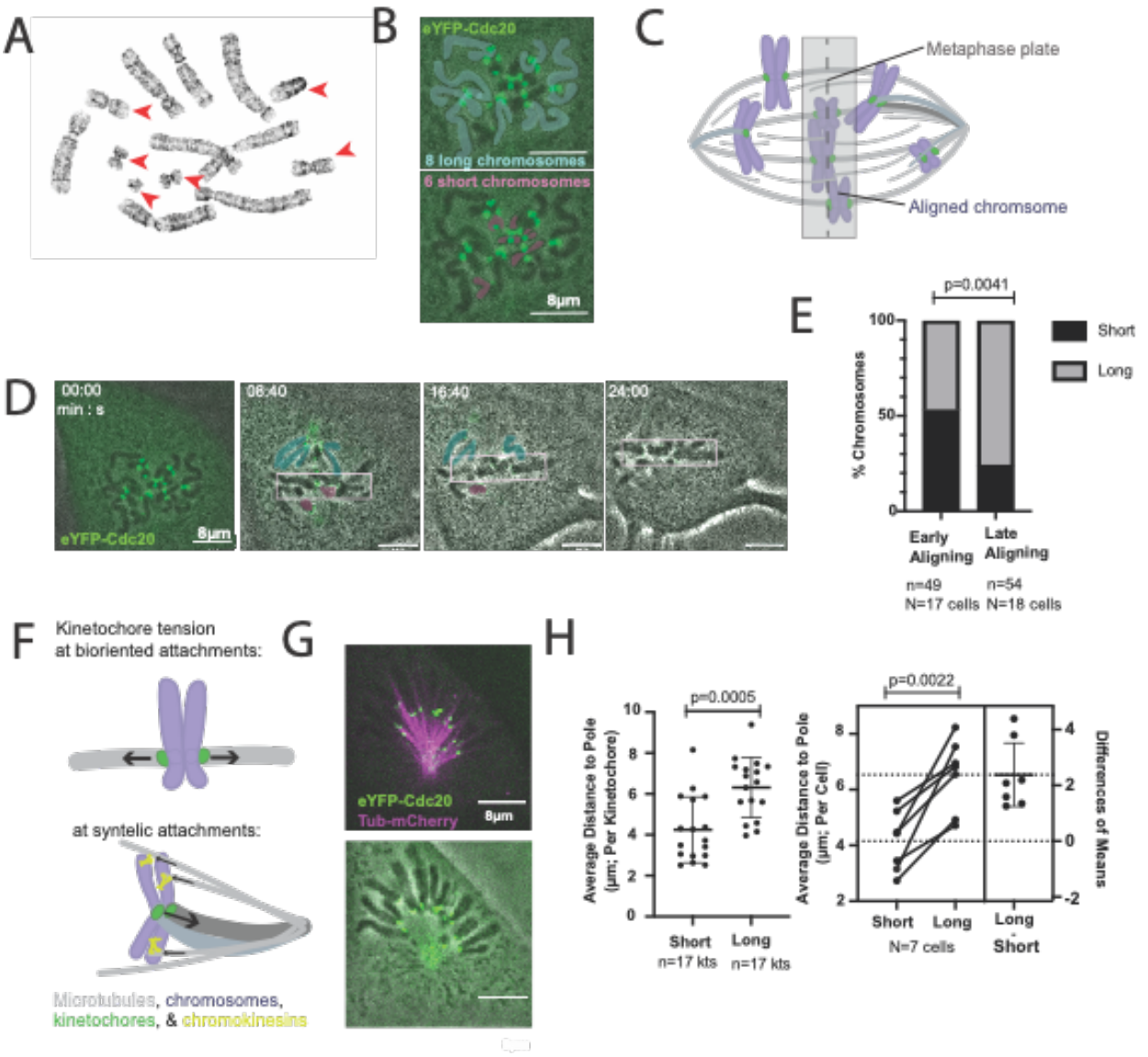

Figure 2.1. Long chromosomes align less efficiently than short chromosomes and experience higher spindle pushing force. (A) Chromosome spread of PtK2 cell line expressing eYFP-Cdc20. Red arrowheads indicate chromosomes classified as “short”. (B) Long (blue, top) and short (pink, bottom) chromosomes in a live mitotic PtK2 cell. Chromosomes were classified by phase contrast microscopy with the help of temporal tracking information. (C) Spindle assembly schematic depicting aligned, or congressed, chromosomes oscillating within the central gray box of the metaphase plate and unaligned chromosomes, in various attachment states. Alignment for (D)&(E) is defined by K-K stretch and oscillatory movement within the spindle center as indicated here. (D) Representative time-lapse imaging of spindle assembly of cell shown in (B) showing that some chromosomes align soon (Figure caption continued on the next page.)

(Figure caption continued from the previous page.) after onset of mitosis (pink box indicating oscillatory area of the metaphase plate) while others move to poles, leading to a delay in alignment. Two short chromosomes are highlighted in pink and three long chromosomes are highlighted in blue. See also Video 1. (E) Percent of long and short chromosomes, which are early aligning (the first three to begin oscillating in a given spindle) or late aligning (the last three). n denotes number of chromosomes counted while N denotes number of cells (Fisher's exact test). (F) For bioriented attachments, poleward pulling by sister k-fibers produces opposing force that generates kinetochore tension while for syntelic errors, poleward pulling is counteracted by polar ejection force along chromosome arms. To assess whether polar ejecton force scales with chromosome size, live imaging was performed on STLC-treated monopolar spindles in PtK2 cells expressing eYFP-Cdc20 and tubulin-mCherry (G) (see also Video 2) and the distance of kinetochores from the pole was used to evaluate the magnitude of pushing force (H) (Unpaired t test).

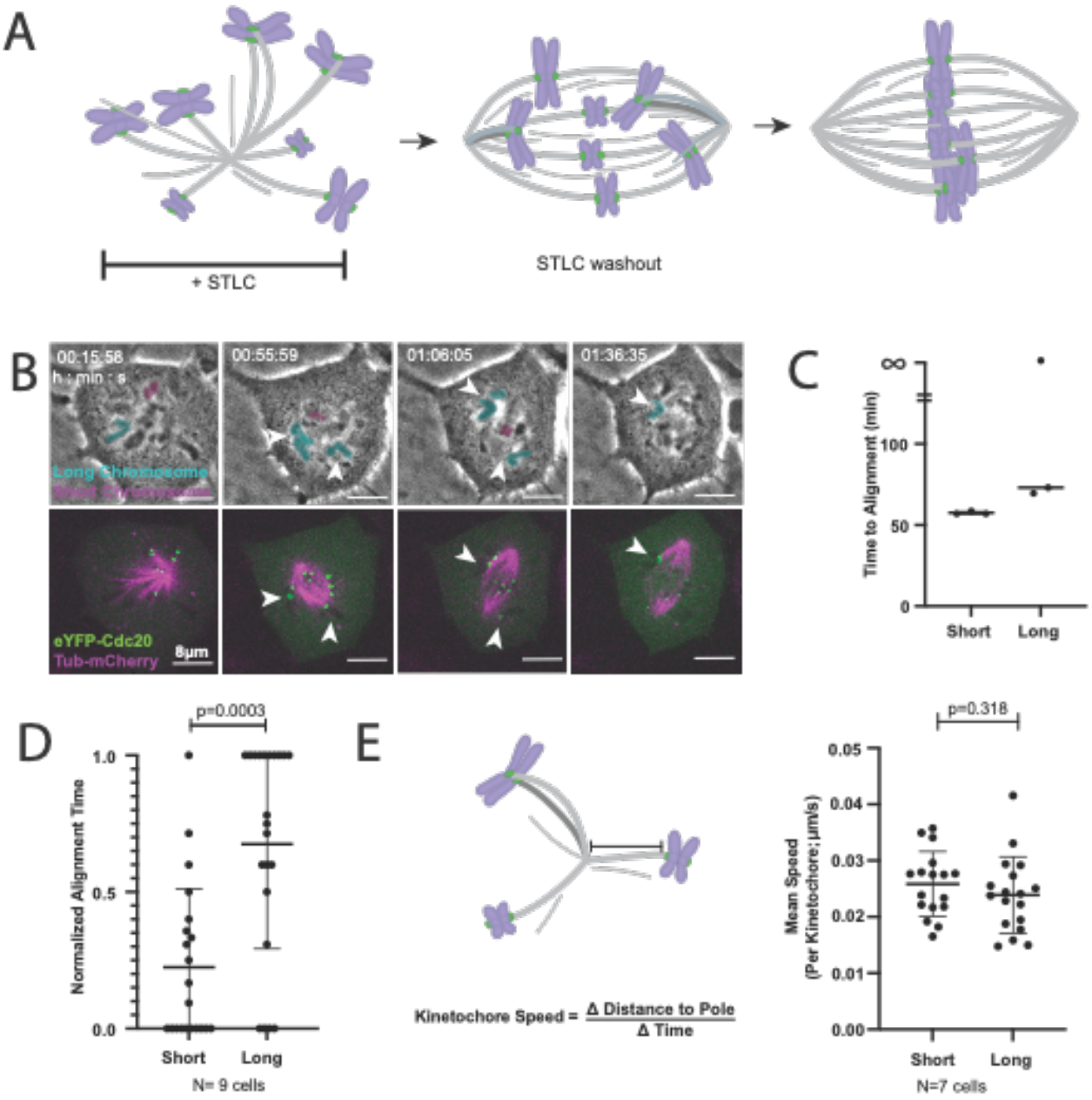

Figure 2.2. Long chromosomes correct errors less efficiently. (A) Real-time error correction scheme: STLC washout allows monopolar spindles, enriched in attachment errors, to recover bipolarity and perform error correction. (B) Representative time lapse of error correction assay in PtK2 cells expressing eYFP-Cdc20 and mCherry-α-tubulin. Arrowheads denote long chromosomes stuck in erroneous attachments. One short chromosome is highlighted in pink and three incorrectly attached long chromosomes are highlighted in blue. See also Video 3. (C) Raw alignment time of short chromosomes (<7µm) and long chromosomes (≥7µm) in cell shown in (B). (D) Normalized alignment time for population such that time t=0 is the (Figure caption continued on the next page.)

(Figure caption continued from the previous page.) time of the first chromosome oscillating at the metaphase plate following drug washout and t=1 is the time the last chromosome begins oscillating prior to anaphase (Mann-Whitney test). (E) Mean kinetochore speed of long and short chromosomes with respect to the pole in monopolar spindles. (Unpaired t test)

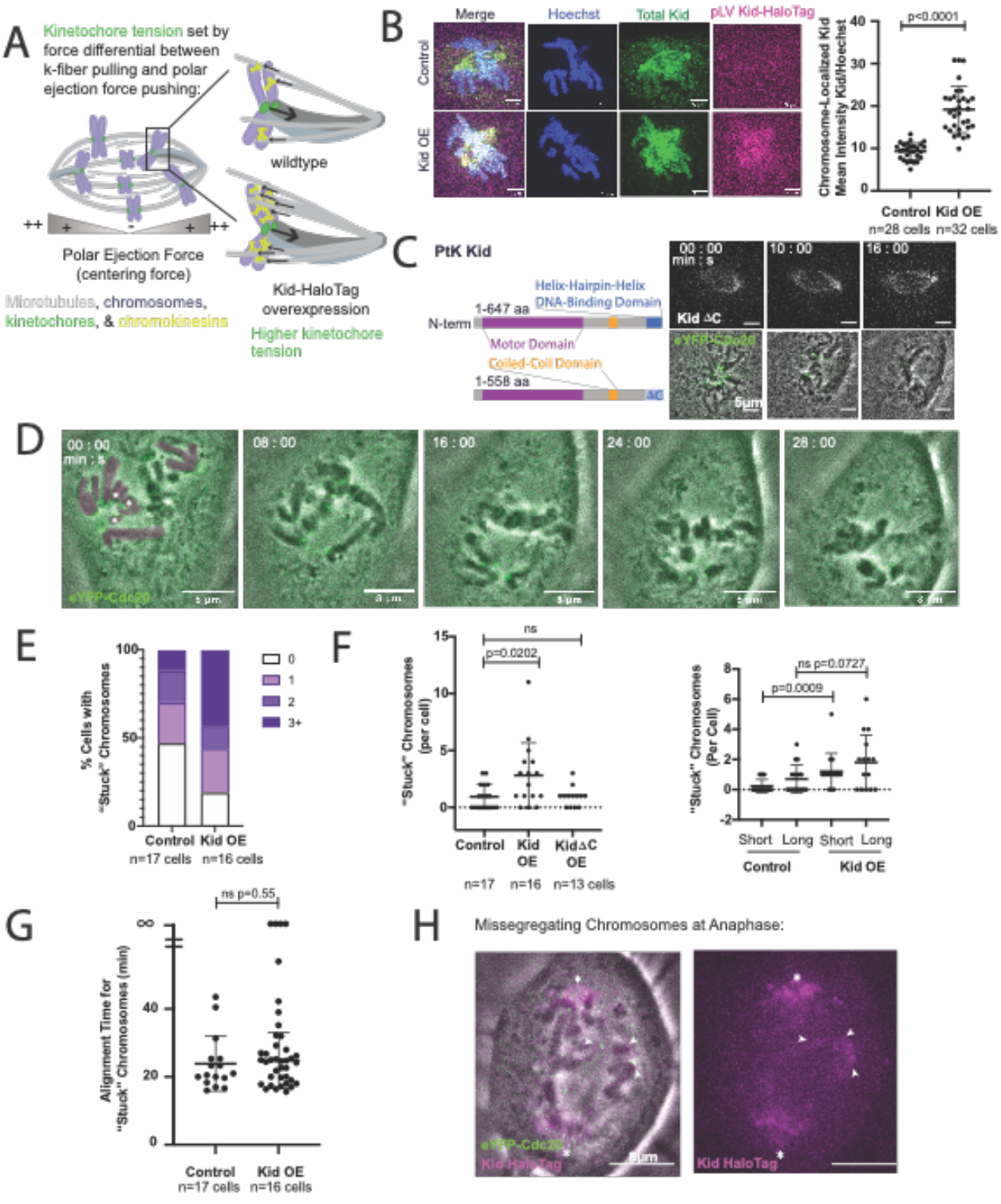

Figure 2.3. High polar ejection force increases persistence of polar chromosomes and reduces size effect. (A) Polar ejection forces are strongest near poles. Kid overexpression will increase both chromosome centering force exerted by the spindle and, for syntelic kinetochores, kinetochore tension. Tension is set by the force balance between poleward pulling at kinetochores (Figure caption continued on the next page.)

(Figure caption continued from the previous page.) and anti-poleward pushing along chromosome arms. (B) Immunofluorescence of fixed PtK2 cells transiently infected with Kid OE construct (pLV Kid-HaloTag) or not infected and intensity measurements for chromosome-localized Kid normalized to Hoechst signal for one experiment. Experiment was performed in triplicate obtaining similar results each time. (C) Schematic of the rat kangaroo chromokinesin Kid highlighting key domains, in particular the C terminal hairpin-helix-hairpin (HhH) domain responsible for non-specific DNA binding, which has been deleted in the Kid ΔC construct. Representative time lapse of Kid ΔC OE PtK2 cells expressing eYFP-Cdc20. See also Video 4. (D) Representative time lapse of Kid OE PtK2 cells expressing eYFP-Cdc20. Polar chromosomes are pseudo-colored in magenta for first frame (00:00) and short polar chromosomes are marked with an asterisk. See also Video 5. (E) Percent of cells with chromosomes stuck in polar attachments. Chromosomes were considered stuck if they had not reached alignment within 15 minutes of approaching spindle poles. (F) Number of chromosomes stuck near poles in control and Kid OE spindle assembly (Mann-Whitney test). (G) Time of alignment following pole approach for stuck polar chromosomes in control and Kid OE spindles. (H) Missegregating chromosomes at anaphase in Kid OE spindle from (C). Poles are marked with asterisks and lagging chromosomes denoted by arrowheads.

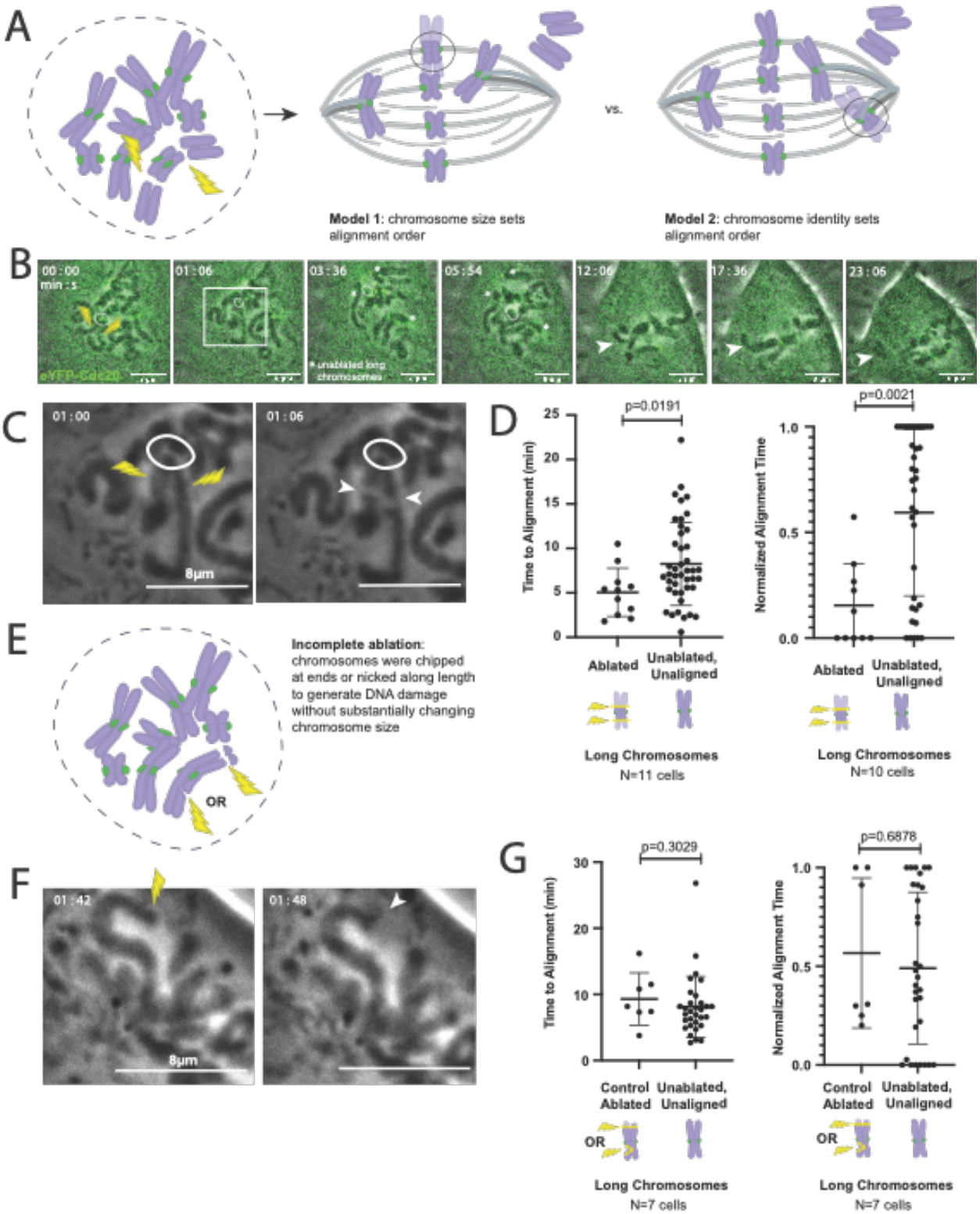

Figure 2.4. Chromosome size determines chromosome biorientation efficiency. (A) Chromosome arm ablation assay: chromosome arms of a long chromosome were cut shortly after nuclear envelope breakdown and kinetochores were followed throughout spindle assembly to determine (Figure caption continued on the next page.)

(Figure caption continued from the previous page.) the time of alignment with respect to other long, unaligned chromosomes in the same cell to test whether chromosome size (model 1; ablated chromosome aligns early) or chromosome identity (model 2; ablated chromosome still aligns late) determine alignment order. (B) Representative example of spindle assembly following chromosome arm ablation in PtK2 cells expressing eYFP-Cdc20. White circle indicates kinetochore on ablated chromosome, arrowheads mark ablated arms, asterisks denote unablated, unaligned long chromosomes. See also Video 6. (C) Zoom in phase contrast images of successful chromosome arm ablation in (B) indicated by white box. Ablation was considered complete if a clear space was observed between the kinetochore-containing fragment and the unattached arms (arrow heads) and the chromosome fragments were mechanically uncoupled in subsequent frames. (D) Alignment time of ablated and unablated, long chromosomes in raw time and normalized such that t=0 is the first chromosome to align following ablation and t=1 is the last chromosome to align prior to anaphase (Mann-Whitney test). (E) Control ablation scheme depicting how incomplete ablations were performed, either by chipping chromosome ends off or by nicking chromosome arms to damage DNA without changing chromosome size substantially. (F) Representative example of control ablation, chipping chromosome ends. White arrowhead marks chipped chromosome end. (G) Raw time to alignment for control ablations and other unablated long chromosomes in the same cell and normalized alignment time such that t=0 is the first chromosome to align following ablation and t=1 is the last chromosome to align prior to anaphase (Mann-Whitney test).

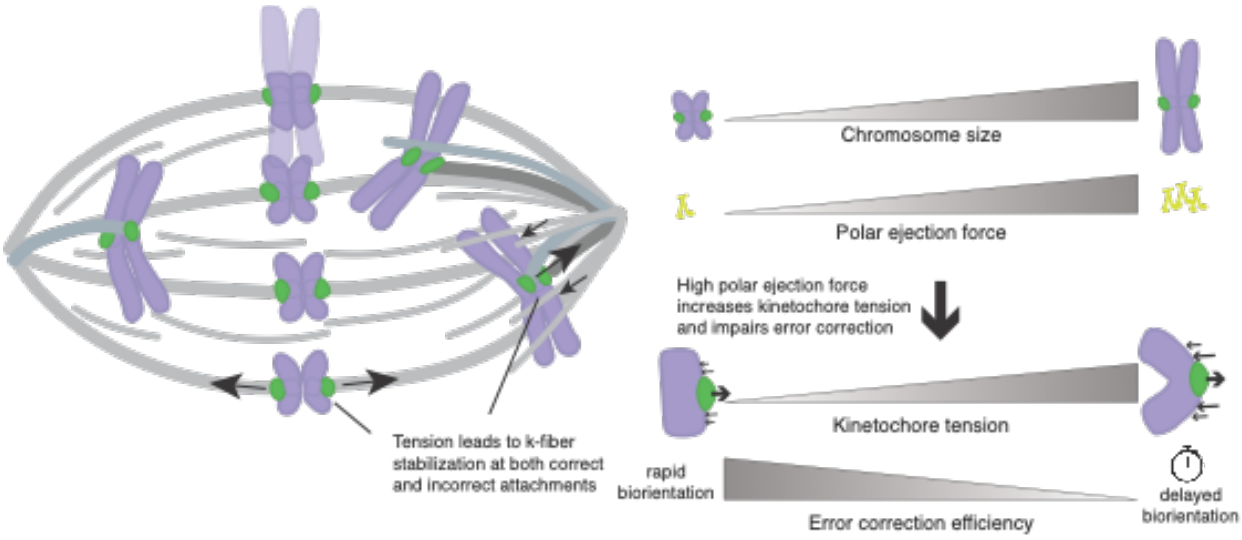

Figure 2.5. Model for differential tension and error correction efficiency at chromosomes of different sizes. Schematic of an assembling spindle depicting early alignment of short chromosomes and delayed congression of long chromosomes. Based on our findings, we propose that these delays are due to elevated polar ejection force and thus elevated kinetochore tension, which stabilizes both correct and incorrect attachments. Despite this stabilization, long polar chromosomes typically manage to biorient, suggesting an existing mechanism for detecting errors subject to modest kinetochore tension after some delay. Detection of these errors could occur via a graded tension threshold wherein low tension attachments are corrected immediately, moderate tension attachments after a delay, and high tension attachments after a longer delay. Alternatively, the error correction machinery could rely on a tension-independent mechanism for error detection in these cases.

Supplemental Figures and Figure Legends

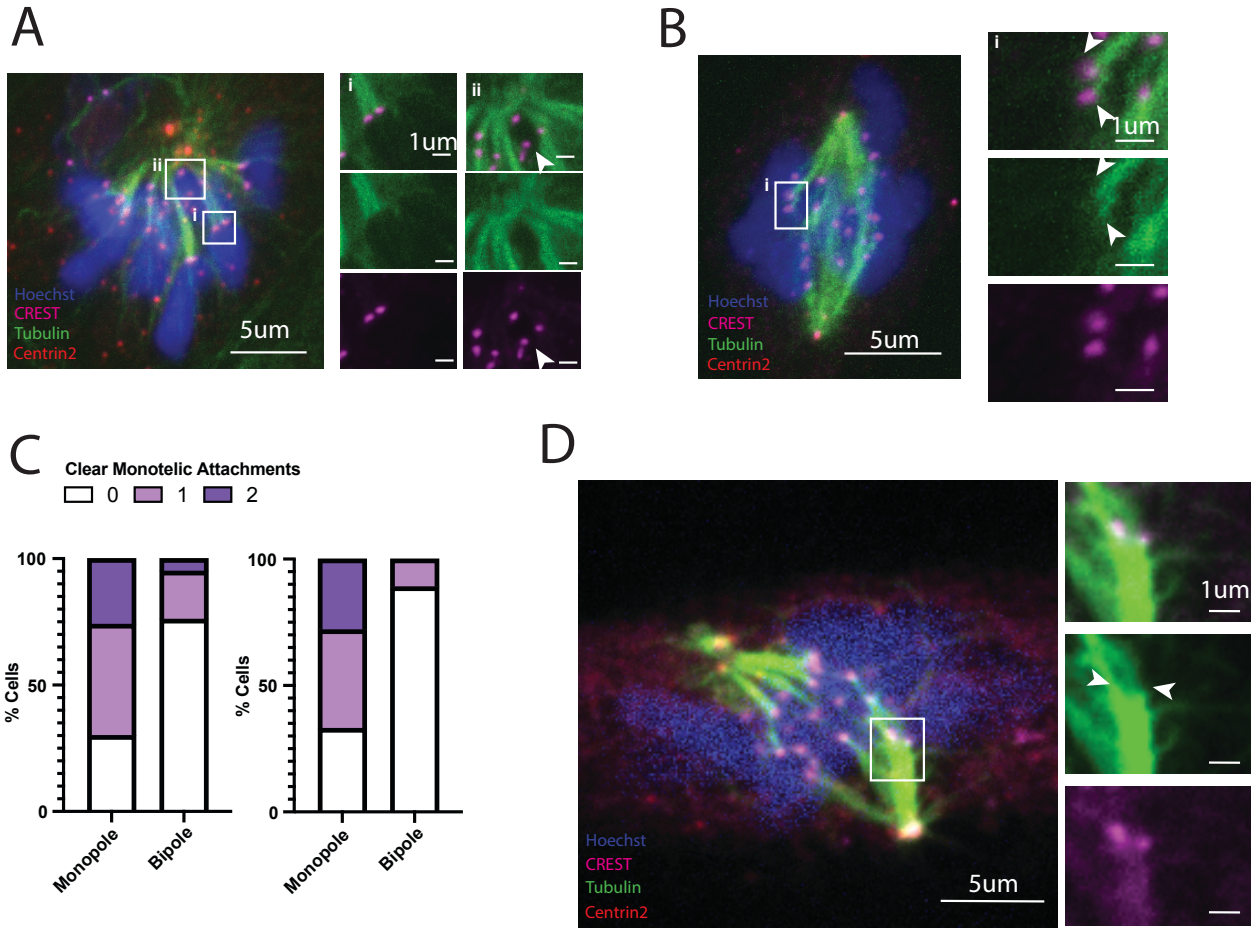

Figure 2.6. Chromosomes experiencing delayed biorientation may be delayed as a result of syntelic attachment in both STLC-washout and regularly cycling cells. (A) Immunofluorescence of a monopolar spindle in wildtype PtK2 cells depicting a clear syntelic attachment (i) and a clear monotelic attachment (ii) and (B) a bipolar spindle following STLC-washout again showing clear examples of syntelic (i) and monotelic (ii) attachments. (C) Proportion of cells displaying either clear monotelic attachments, in which 1-3 kinetochores were clearly unassociated with microtubules, or no monotelic attachments in which kinetochores were all associated with microtubules (in either end-on or lateral configuration) in two independent experiments (left, right). In all cases of cells with monotelic attachment, cells displayed 1-3 unattached kinetochores, but never more. (D) Immunofluorescence of a wildtype, regularly cycling PtK2 cell highlighting a naturally occurring syntelic attachment.

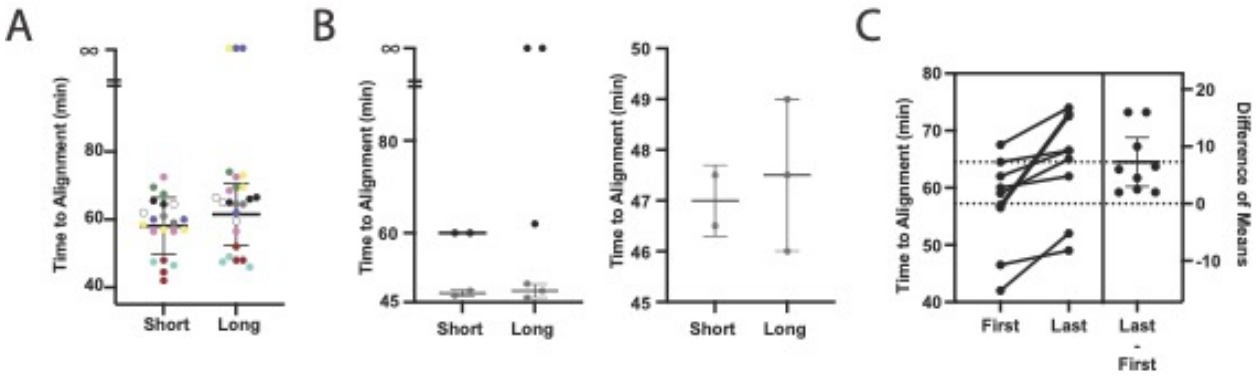

Figure 2.7. Time to biorientation after STLC washout varies widely between cells. (A) Raw time of chromosome alignment following STLC washout experiments for short and long chromosomes where colors correspond to chromosomes coming from the same cell. (B) Raw time of chromosome alignment in two independent cells from (A) (ex. #1 black and ex. #2 gray; left) and a zoomed in plot for ex. #2 (right) demonstrating that after drug washout, cells took variable amounts of time to achieve chromosome alignment initially. (C) Time of first detectable oscillating chromosome following STLC washout and last to begin oscillating prior to anaphase within the same cell, showing that while there is variability in the duration of the error correction process (~15 minute spread the time between the first and last chromosome alignment), most variability arises in the time between drug washout and the time of first chromosome alignment (~30 minute spread). This variable time before beginning correction of attachment errors following STLC washout indicates a need for time normalization in order to compare correction efficiency across cells.

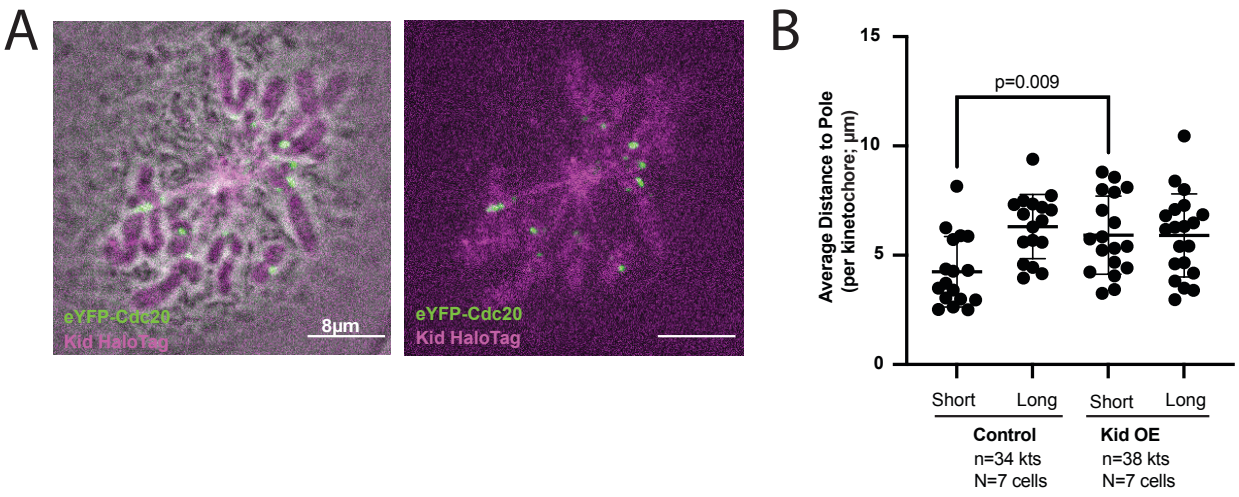

Figure 2.8. Chromokinesin overexpression increases polar ejection force experienced by short chromosomes. (A) eYFP-Cdc20 PtK2 cells overexpressing Kid (Kid OE) were treated with STLC and monopolar spindles were followed and analyzed. Kid-HaloTag was visualized using JF549 dye. See also Video S1. (B) The average distance of kinetochores from poles was used to assess the magnitude of pushing force on long and short chromosomes in Kid OE cells compared to control cells. (Unpaired t test).

Materials and Methods

Cell culture

PtK2 cells expressing human eYFP-Cdc20 (gift from Jagesh Shah, Harvard Medical School, Boston, MA) and wildtype PtK2 cells (ATCC) were cultured at 37˚ C and 5% CO_2 in MEM (Invitrogen) supplemented with sodium pyruvate (Invitrogen), non-essential amino acids (Invitrogen), penicillin/streptomycin, and 10% qualified and heat-inactivated fetal bovine serum. Karyotyping and cytogenic analysis were performed on G-banded metaphase cells from the eYFP-Cdc20 PtK2 line by Cell Line Genetics. Cells were transfected using Viafect (Promega) and imaged 72 hours after transfection with mCherry-α-tubulin (gift from M. Davidson, Florida State University, Tallahassee, FL). Transfection reactions were prepared in a 100μl reactions using a 1:6 ratio of DNA to Viafect in OptiMEM media. Overexpression experiments were done with transient lentiviral infection. The coding sequence for the rat kangaroo chromokinesin Kif22/Kid was obtained from the PtK transcriptome (Udy et al., 2015) and cloned into a puromycin resistant lentiviral backbone. Rat kangaroo KidΔC (89 amino acids deleted at the C terminus) was designed based on predicted secondary structure of Kid (Paysan-Lafosse et al., 2023) and homology of rat kangaroo Kid to human Kid at a helix-hairpin-helix (HhH) domain known to be involved in non-specific DNA binding (Tokai et al., 1996). Lentivirus was generated in Hek293T cells, and PtK2 cells were infected 48-72 hours prior to imaging spindle assembly.

Microscopy

Live imaging was done using an inverted (Eclipse Ti-E; Nikon) spinning-disk confocal microscope (CSU-X1; Yokogawa Electric Corporation) with Di01-T405/488/568/647 dichroic head (Semrock), 100x 1.45 Ph3 oil objective, 405 nm (100 mW), 488 nm (120 mW), 561 nm (150 mW), 642 nm (100 mW) diode lasers, emission filters (ET455/50M, ET525/50M, ET630/75M, and ET690/50M; Chroma Technology Corp.), and either an iXon3 camera (Andor Technology) (Fig. 2.1G, 2.2B, 2.3B, 2.3C, 2.3D, 2.3H, 2.4B, 2.4C, 2.4E, 2.6A, 2.6B, 2.6D, 2.8A) or a Zyla 4.2 sCMOS camera (Andor Technology) (Fig. 2.1B, 2.1D). Cells were plated for imaging on 35-mm dishes with #1.5 poly-D-lysine coated coverslips (MatTek Corporation). For all live experiments except drug washouts, images were collected at bin=1, while drug washout and monopole movies were collected at bin=2 on MetaMorph (7.8, MDS Analytical Technologies) or Micro-Manager (2.0.0). Cells were imaged in phase contrast and fluorescence in a single z-plane (Fig. 2.3, 2.8, 2.4), six z-planes spaced 0.35μm apart (Fig. 2.1G, 2.2), or three z-planes spaced 0.35μm apart (Fig. 2.1B, 2.1D). Cells were imaged in a humidified stage-top incubation chamber (Tokai Hit) at 37°C (or 30°C for monopole and STLC washout experiments (Fig. 2.1G and Fig. 2.2) with 5% CO_2. Cells expressing HaloTag constructs were labelled with 200nM JF549 or JF646 (Promega) for 30 minutes prior to imaging.

Immunofluorescence

For all immunofluorescence experiments described below, cells were seeded on poly-L-lysine coated coverslips. For immunofluorescence to validate chromokinesin

overexpression, cells were transiently infected with lentivirus driving expression of PtK Kid-HaloTag for 48 hours. Cells were then labeled with HaloTag dye JF549 and fixed in 99.8% methanol for 3 minutes at -20°C and permeabilized in TBS1x (Tris buffered saline) + 2% BSA + 0.1% Triton for 30 minutes (IF Buffer hereafter). For immunofluorescence after STLC-washout experiments, cells were treated with 7.5μM STLC for 1 hour, then washed out of solution (5x media exchanges) and incubated at 37°C for 55 – 85 minutes prior to fixation in 99.8% methanol for 3 minutes at -20°C and permeabilization in IF Buffer. For immunofluorescence of regularly cycling cells, these were cold-treated by incubating in 4°C media for 5 minutes prior to the same fixation and permeabilization conditions. For all above experiments, primary antibodies were incubated overnight at 4°C (chromokinesin overexpression) or for one hour at room temperature (STLC-washout and regularly cycling cells) at the following concentrations in IF Buffer: mouse anti-α-tubulin (DM1α, 1:1000, Sigma T6199), rabbit anti-rat kangaroo-Kid (3μg/ml, Genscript), human anti-CREST (1:25, Antibodies Incorporated 15-234-0001), rat anti-tubulin (1:2000, MCA77G, Bio-Rad; RRID: AB325003), rabbit anti-centrin2 (1:1000, Millipore, ABE480). The anti-rat kangaroo Kid antibody was raised in rabbits against the full Kid protein translated from the coding sequence obtained from the rat kangaroo transcriptome (Udy et al., 2015). Coverslips were washed three times in IF buffer (5 minutes each) before incubating with secondary antibodies (1:500 in IF buffer, 30 minutes at room temperature): goat anti-rabbit IgG Alexa Fluor 488 (A11008, Invitrogen) for Kid OE, and goat anti-rabbit IgG Alexa Fluor 647 (A21245, Invitrogen), goat anti-human IgG Alexa Fluor 561 (A21090, Invitrogen), goat anti-mouse 488 (A11001, Invitrogen), and goat anti-rat 488 (A11006, Invitrogen) for STLC-washout and

regularly cycling cells. Samples were washed three times in IF buffer, incubated with Hoechst 33342 (1μg/ml, H3570, Invitrogen) for one minute, and washed in IF buffer once more prior to mounting on slides with ProLong Gold Antifade Mountant (p36934, Thermo Fisher).

Laser ablation

Laser ablation experiments were done using 514 nm ns-pulsed laser light and a galvo-controlled MicroPoint Laser System (Andor, Oxford Instruments) operated through Micro-Manager. Spindle assembly was imaged with single z-planes in both phase contrast and 488 nm (to visualize kinetochores expressing eYFP-Cdc20) to identify unaligned long chromosomes (by phase contrast). Chromosomes were selected to ablate if they were relatively isolated (a stretch of chromosome not overlapping with others), not at the spindle center, and if most of the chromosome arms were in focus at a single z-plane (little to no tilt). Ablations were performed by firing the laser at 4-8 discrete points across the width of chromosome arms (40-80 pulses of 3ns at 20 Hz) and successful chromosome arm ablations were verified by a visible continuous gap between chromosome segments and mechanical uncoupling of ablated arms and the kinetochore-containing fragment (i.e., moving in different directions). Similarly, control ablations were considered successful if a noticeable change could be seen at the site of laser targeting (a loss of roundness at chromosome ends or visible damage along chromosome arms).

Drug treatments and washouts

To generate monopolar spindles, Eg5 motor activity was inhibited by addition of S-trityl-L-cysteine (STLC; 7.5μM). Monopoles were imaged and kinetochores tracked for no more than 45 minutes. Error correction was assessed by STLC washout, imaging every four minutes for the first 40-60 minutes until spindles appeared roughly bipolar but had not reached metaphase, then every 20 seconds thereafter.

Image analysis

Kinetochores were manually tracked using the MTrackJ plugin on FIJI (Schindelin et al., 2012) with a combination of eYFP-Cdc20 and phase contrast to follow individual kinetochores and measure the size of chromosomes. For kinetochores that left the imaging plane for a short period during a movie, rough position (polar or at the metaphase plate) was recorded using the phase contrast channel. Chromosome size was measured in 2-dimensions using the segmented line tool on FIJI on single z plane phase contrast images to measure the longest dimension of a chromosome. In a crowded spindle, kinetochore tracks were used to find the clearest frame for size measurement. Long chromosomes were considered those ≥7μm and short chromosomes were those <7μm in length.

For alignment order (Fig. 2.1D) and alignment time (Fig. 2.2C, 2.3D, 2.3E, 2.4D), the metaphase plate was defined as the region between poles in which oscillating chromosomes resided. Oscillating chromosomes were defined as chromosomes with K-K distance of ≥1.8μm moving periodically toward one spindle pole, then the other. Distance to pole (Fig. 2.1G) was calculated in monopolar spindles by finding the

shortest distance between kinetochore position and pole position (found by MTrackJ using mCherry-α-tubulin fluorescence) and speed of kinetochore movement (Fig. 2.4E) was determined by measuring the change in kinetochore-to-pole distance over the change in time.

For immunofluorescence, brightness and contrast for each channel were scaled identically within each experiment. A threshold mask (Yen method) was applied using the Hoechst signal (for chromosome selection) to determine the area in which we would measure fluorescence intensity of our protein of interest. Fluorescence intensity was measured of both the chromokinesin Kid and the reference (Hoechst) and normalized by the intensity of the reference (Fig. 2.3B). Three independent experiments were performed and quantified separately, yielding comparable results.

Statistical analyses were performed in Graphpad Prism 9. For parametric datasets (Fig. 2.1G), the student's t test was used. For non-parametric datasets (Fig. 2.2C, 2.3B, 2.3E, 2.3F, 2.4D), the Mann-Whitney was used. Fisher's exact test was used in Fig. 2.1D.

Chapter 3: Defining the spindle assembly checkpoint's input cues at individual kinetochores and how its output signals are integrated across many kinetochores

Abstract

The spindle assembly checkpoint (SAC) ensures accurate chromosome segregation at cell division, generating a diffusible signal at unattached kinetochores to inhibit the anaphase promoting complex. Tension at kinetochores has been implicated not as a direct SAC input cue, but a requirement to stabilize kinetochore-microtubule attachments. The precise input cues the SAC monitors and how the cell integrates SAC output signals across its many kinetochores to regulate anaphase entry are not yet clear. Here, we address these questions by laser ablating kinetochores to generate unpaired sisters in human RPE1 cells and monitoring the cell's ability to enter anaphase. In nocodazole-treated mitotic cells (without microtubules), we ablate 0, 1, or 2 kinetochores, wash out nocodazole to allow spindle formation and measure progress to anaphase. We find that neither 1, 2, nor 3 sister-less kinetochores is sufficient to prevent anaphase. However, we see a progressive delay to anaphase entry with increasing number of unpaired kinetochores, suggesting that the strength of the SAC scales with the number of kinetochores lacking a sister. We are now asking to what extent and with what dynamics these unpaired kinetochores silence the SAC, which will shed light on the cellular sources of input SAC satisfaction cues. In parallel, we are assessing the tolerance of anaphase entry to individual signaling kinetochores and questioning how this tolerance scales with chromosome complement. This work may have important implications for understanding how specific aneuploidies form and propagate in disease.

Introduction

When a cell divides, chromosomes must accurately attach to the spindle, sense their attachment status, and signal to the cell whether they are ready to enter anaphase (Musacchio and Desai, 2017). The kinetochore serves as both the mechanical interface mediating attachment and the signaling platform regulating anaphase entry. The spindle assembly checkpoint (SAC) prevents anaphase entry until all kinetochores are stably attached to the spindle in order to maintain genome integrity (Musacchio, 2015). Prior to microtubule attachment, kinetochores recruit Mad1 and Mad2, which assemble a signaling platform that generates a diffusible anaphase-prevention signal (Chen et al., 1998; De Antoni et al., 2005; Maldonado and Kapoor, 2011). The formation of stable end-on microtubule attachments, but not lateral ones, induces SAC satisfaction or silencing through dynein-mediated SAC stripping (Howell et al., 2001; Kuhn and Dumont, 2017; Wojcik et al., 2001). Determining which cellular conditions are required to generate and detect stable attachments capable of satisfying the SAC will expand our understanding of how aneuploidy is avoided in different cell and tissue contexts.

As kinetochore-microtubule attachments mature, the molecular composition of the kinetochore changes, lengthening k-fiber lifetime and stabilizing the attachment. Microtubule-binding proteins array in a sheet across the kinetochore and bind bundles of 15-25 microtubules that constitute a mammalian kinetochore-fiber (k-fiber) (McEwen et al., 1997; Zaytsev et al., 2015, 2014). Kinetochores binding microtubules at their plus-ends trigger dynein stripping of Mad1/2 from kinetochores to the spindle poles. How these layered signaling platforms determine which microtubule attachments warrant dynein-mediated stripping and which do not has not been well characterized. While it is

an attractive model, inter-kinetochore tension is not thought to be required for SAC satisfaction in the case of hyper-stable kinetochore-microtubule attachments (Etemad et al., 2015; Kuhn and Dumont, 2019; Tauchman et al., 2015). However, low-tension attachments such as those formed in prometaphase and present in monopolar spindles do not reliably satisfy the SAC, so the questions of how tension impacts formation of stable attachments and what cellular conditions generate stable attachments remain open. It may be that kinetochore-microtubule attachment maturation triggers SAC satisfaction through recruitment of molecular cues or lengthening of microtubule association time. Further, the dynamics of SAC satisfaction have been shown to be switch-like rather than graded at individual kinetochores in mammalian rat kangaroo (PtK2) cells (Kuhn and Dumont, 2017), but how and whether these dynamics may vary across cell type and species is unclear.

The SAC operates both locally at individual kinetochores and globally in the cytoplasm to trigger an anaphase entry decision. How permissive the cytoplasmic anaphase entry decision is may vary based on species, cell type, and chromosome complement (Collin et al., 2013; Dick and Gerlich, 2013; Rieder et al., 1995). Understanding how the cell integrates local SAC signals to generate a global anaphase entry decision will provide a framework for dissecting how individual chromosome and kinetochore features can impact overall mitotic accuracy and, ultimately, tissue health. This will provide a lens through which to understand evolutionary pressures shaping mitotic regulatory mechanisms and genome health.

Here, I show that the spindle assembly checkpoint can detect defects in kinetochore attachments when kinetochores lack the opposing force from their sisters,

but these defects are sufficient only to stall anaphase, not to prevent it. Cells with 1 – 3 sisterless kinetochores experience a titratable mitotic delay of 20 min maximum, negligible compared to mitotic delays observed in cells with active SAC signal at the kinetochore. Additionally, cells do not exhibit lagging chromosomes at anaphase, suggesting unpaired, sisterless kinetochores are unlikely to satisfy the SAC as a result of merotelic attachment formation. Instead, we propose a model wherein sisterless kinetochores form attachments with lower stability than control bioriented kinetochores, but which are sufficiently stable to satisfy the SAC with altered dynamics. These findings provide an important starting point for understanding what cues are required to generate stable attachments during spindle assembly and how those cues contribute to inducing a robust anaphase entry decision.

Results

Unpaired sisterless kinetochores induce a titratable metaphase delay but do not prevent anaphase.

To determine what cues are required at the kinetochore to form stable attachments and enter anaphase, we assessed whether kinetochores were capable of adequately preventing anaphase in the absence of their sister. We generated mitotic cells without a spindle by treating RPE1 cells with nocodazole to depolymerize microtubules, ensuring all kinetochores had an active SAC signal, and used laser ablation to produce unpaired, sisterless kinetochores (Fig. 3.1A). Then, we performed drug washouts and live imaging and measured mitotic progression. To control for any effects of photodamage or incomplete nocodazole washout, all experiments were

performed in parallel with control ablations, which were subject to equivalent ablations in a region of the cytoplasm containing no kinetochores (Fig. 3.1B). We found that cells with 0-3 individual kinetochore ablations entered anaphase with increasing time spent in metaphase that scaled with the number of unpaired sisterless kinetochores. In controls (0 unpaired kinetochores), 61.7% of all cells had entered anaphase after 6 - 14 min. or less spent in metaphase, while at the same point, just 32.5% of single ablated cells (1 unpaired kinetochore), 41.7% of double ablated cells (2 unpaired kinetochores), and 33% for triple ablated cells (3 unpaired kinetochores).

Eventual anaphase entry in cells with ablated kinetochores could reflect a defect in SAC signaling, either at the kinetochore or across the cell, or a defect in attachment of unpaired, sisterless kinetochores that triggered SAC satisfaction. To confirm unpaired, sisterless kinetochores were not forming merotelic attachments in the majority of cells, we assessed the instance of lagging chromosomes at anaphase following nocodazole washout. We saw 10% of cells with lagging chromosomes at anaphase in control cells compared to 12% in single ablated, 22% in double ablated and 0% in triple ablated cells (Fig. 3.1F). These populations are not statistically different ($p=0.46$), indicating that anaphase entry was unlikely to be the result of forming new merotelic attachments at sisterless kinetochores. Additionally, in ablated cells, the count of lagging kinetochores in some cases outnumbered that of sisterless kinetochores, suggesting these laggards may represent natural formation in merotelic attachments as a result of nocodazole washout. While extended metaphase duration in cells with kinetochore ablations suggests a SAC signaling defect, eventual anaphase entry suggests that defect is not complete. Thus, we conclude either cells can enter

anaphase with as many as three kinetochores actively signaling the SAC or that SAC satisfaction dynamics at these unpaired sisterless kinetochores is altered (Fig. 3.2).

Discussion

Faithful chromosome segregation requires tight regulation of anaphase entry timing, avoiding segregation until all chromosomes are properly bioriented. Here, we asked how this process is regulated at individual kinetochores and globally across the cell by eliminating opposing force from sister kinetochores. We found that cells with unpaired, sisterless kinetochores entered anaphase but experienced a progressive delay in the metaphase to anaphase transition that scaled with the number of unpaired kinetochores. This work suggests that opposing force from sister kinetochores contributes to the formation of stable attachments capable of silencing the SAC but are not required for eventual anaphase entry. Altogether, this leads to a model wherein unpaired kinetochores can form sufficiently stable attachments to satisfy the SAC, either locally at the kinetochore or globally across the cytoplasm, albeit with altered dynamics.

Overall, this work provides insight into the physical conditions required to satisfy the SAC individually at kinetochores and across the cell in a global anaphase entry decision. While generating unpaired sisterless kinetochores delays anaphase and extends the duration of metaphase, it does not prevent segregation altogether (Fig. 3.1C & 3.1D). In the absence of opposing force from a sister kinetochore, then, SAC signaling is defective. Unlike studies of mitosis in unreplicated genomes (O'Connell et al., 2008), we do not find that acutely generated unpaired, sisterless kinetochores form merotelic attachments often (Fig. 3.1F). While it is clear that ablation causes this

extension in metaphase duration, we have not yet identified the source of this delay. It may be that the global anaphase entry decision has a loose detection threshold such that a single kinetochore signaling the SAC can prevent anaphase for only a brief time and that buffer period extends with more signaling kinetochores. This is partially consistent with existing data that report SAC signal strength is graded across the cell (Collin et al., 2013; Dick and Gerlich, 2013). However, anaphase entry in the presence of even few SAC-positive kinetochores takes several hours in these instances while we see only minutes-long delays.

Instead, we propose the SAC may be satisfied with altered dynamics at unpaired kinetochores. This altered satisfaction could involve partial satisfaction of unpaired kinetochores (Fig. 3.2), though the remaining signal at a kinetochore would need to be less than one third of a fully active kinetochore to account for anaphase in the triple ablation condition. While non-human mammalian cells silence the SAC in an all-or-none fashion (Kuhn and Dumont, 2017), there is precedent for partial SAC satisfaction in Drosophila (Cane et al., 2013a). Alternatively, SAC satisfaction could be transient, stochastically turning on and off at an individual kinetochore over time (Fig. 3.2). This transient satisfaction could create progressive delays with increasing numbers of sisterless kinetochores by preventing anaphase until transient satisfaction overlaps across all sisterless kinetochores. Finally, SAC satisfaction at these sisterless kinetochores may simply occur at a lower rate, such that these lower tension attachments lose SAC signal steadily over many minutes (Fig. 3.2), delaying anaphase onset until satisfaction is complete.

Future work should aim to understand how the SAC signals at individual sisterless kinetochores and establish integration mechanisms of kinetochore signals across the cell. Opposing force has been shown to promote kinetochore-microtubule attachment stability even in the absence of biorientation (Chong et al., 2024). Probing the role of polar ejection forces in stabilizing sisterless kinetochore attachments and facilitating metaphase delay will help elucidate the cues the mammalian SAC machinery monitors and whether dynamics or efficiency varies across different chromosomes based on size or kinetochore position. Additionally, how kinetochore maturation markers, especially Ska and SKAP, which are recruited to kinetochores at metaphase (Cheerambathur et al., 2017; Fang et al., 2009; Hanisch et al., 2006; Schmidt et al., 2010), influence SAC satisfaction dynamics remains an exciting open question. These molecular indicators of mature, stable attachments may serve as cues to trigger SAC satisfaction in the absence of directly sensing tension. Alternately, the kinetochore may monitor k-fiber lifetime—or duration of association with Ndc80 and any given microtubule—to measure the stability of an attachment. Finally, it remains possible that SAC satisfaction occurs at most long-lived kinetochore-microtubule attachments and relies on error correction to facilitate detachment from unproductive kinetochores and trigger re-activation of the SAC. This model would represent a surprising paradigm shift in our understanding of the SAC, but careful characterization of SAC satisfaction dynamics has not been done in monopolar spindles to test it.

Characterizing the role of biophysical cues in SAC satisfaction provides insight into the mechanisms safeguarding the genome from aneuploidy. This work reveals new design principles underlying the molecular regulation of SAC satisfaction at the

kinetochore and the evolutionary forces shaping chromosome segregation outcomes and aneuploidy.

Figures and Figure Legends

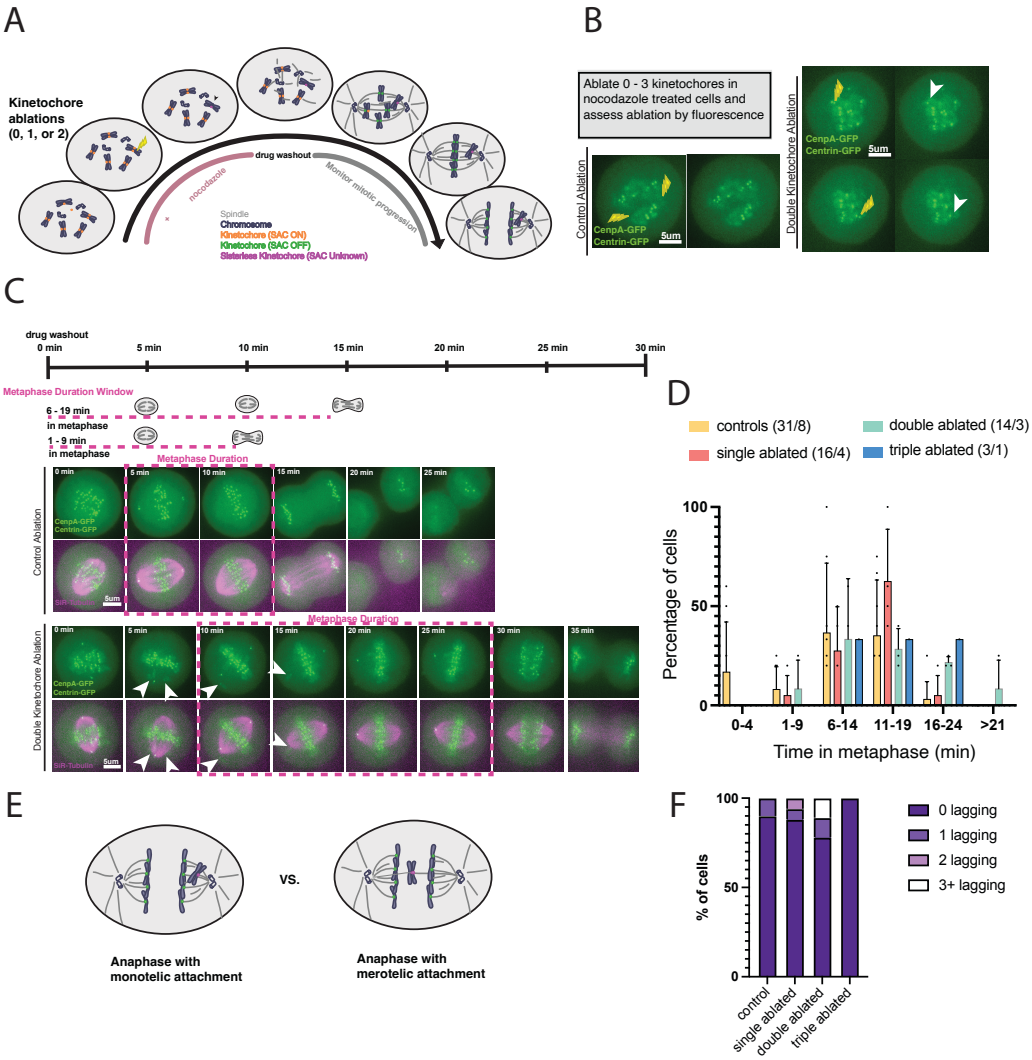

Figure 3.1. Unpaired sisterless kinetochores induce a titratable metaphase delay but do not prevent anaphase. (A) Laser ablation and drug washout schematic: nocodazole treatment ensures the SAC is active at all kinetochores when ablations occur and drug washout allows spindle recovery following kinetochore ablation to measure mitotic progression. (B) Representative examples of control and double kinetochore ablations. Lightning bolts denote location of ablation target, white arrowheads denote ablated kinetochore. (C) Representative time-lapse of RPE1 cells with control and double kinetochore ablations imaged every 5 min after nocodazole washout. Metaphase duration window is calculated based on number of frames at metaphase prior to anaphase onset and denoted with magenta dotted lines. White arrowheads denote unpaired sisterless kinetochores. (D) Metaphase duration for cells shown in (C) for control, single, double, and triple ablations. Numbers in parentheses are denoted (n/N) where n=number of cells (Figure caption continued on the next page.)

(Figure caption continued from the previous page.) and N=number of independent experiments. (E) Model for anaphase with unpaired, sisterless kinetochore in either monotelic (left) or merotelic (right) attachment depicting how merotely would induce lagging chromosomes at anaphase. (F) Percent of cells with lagging chromosomes following ablations.

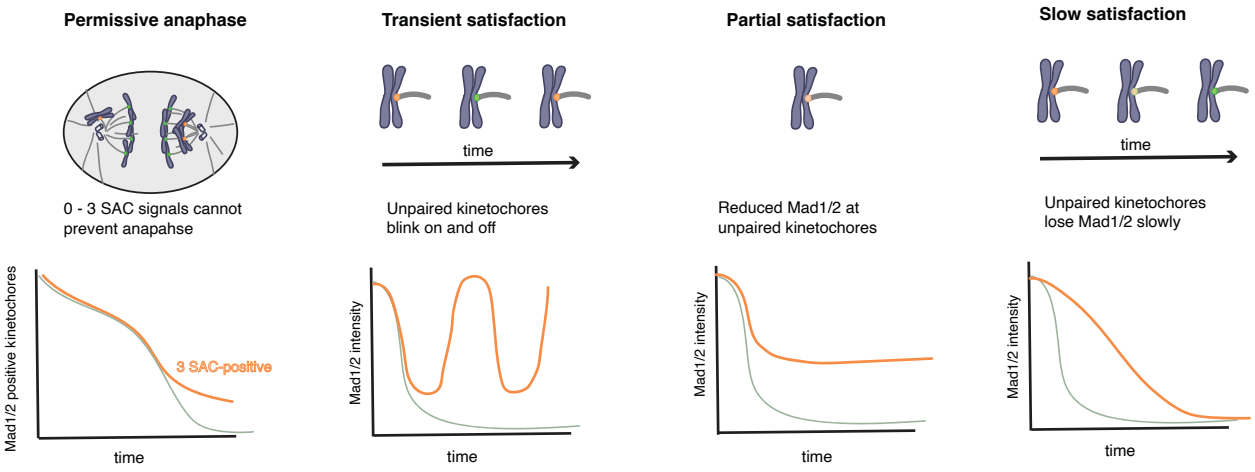

Figure 3.2. Model for SAC satisfaction at unpaired sisterless kinetochores. Extended metaphase duration may reflect different defects of the spindle assembly checkpoint. First, permissive anaphase in which the global checkpoint cannot prevent anaphase in the presence of 0 – 3 kinetochores signaling the SAC. Second, transient satisfaction in which individual sisterless kinetochores satisfy and reactivate over time, delaying anaphase onset. This delay would increase with number of sisterless kinetochores as they asynchronously oscillate between active and satisfied. Third, it could reflect partial but incomplete removal of Mad1 and Mad2 at individual kinetochores. Or fourth, stripping of SAC components may occur at a slower rate than in unperturbed control cells. All of these defects could produce a similar titratable delay in anaphase onset with increasing number of unpaired kinetochores.

Materials and Methods

Cell culture

RPE1 cells expressing human CenpA-GFP and human Centrin-GFP (gift from Alexey Khodjakov, Wadsworth Center) were cultured at 37˚ C and 5% CO_2 in DMEM/F12 (11320, Thermo Fisher) supplemented with penicillin/streptomycin, and 10% qualified and heat-inactivated fetal bovine serum.

Microscopy

Live imaging was done using an inverted (Eclipse Ti-E; Nikon) spinning-disk confocal microscope (CSU-X1; Yokogawa Electric Corporation) with Di01-T405/488/568/647 dichroic head (Semrock), 100x 1.45 Ph3 oil objective, 405 nm (100 mW), 488 nm (120 mW), 561 nm (150 mW), 642 nm (100 mW) diode lasers, emission filters (ET455/50M, ET525/50M, ET630/75M, and ET690/50M; Chroma Technology Corp.), and an iXon3 camera (Andor Technology). Cells were plated for imaging on 35-mm dishes with #1.5 poly-D-lysine coated coverslips (MatTek Corporation). Images were collected every 10 s during ablations and every 5 min after drug washout at bin=1.

Laser ablations and drug washouts

To synchronize cells and ensure the SAC was active at all kinetochores, microtubule polymerization was inhibited by addition of nocodazole (2μM) and cells were labeled with SiR-Tubulin (Cytoskeleton Inc., 1:10,000) and were incubated for no more than 60 minutes prior to imaging. Laser ablation experiments were done using 514 nm ns-pulsed laser light and a galvo-controlled MicroPoint Laser System (Andor, Oxford

Instruments) operated through Micro-Manager. Control and kinetochore ablations were performed in parallel while cells were in nocodazole by targeting the laser to fire 20-40 pulses of 3ns at 20 Hz per kinetochore. Control ablations targeted an area of the cytoplasm near chromosomes without destroying any kinetochores. Nocodazole was washed out of the imaging plate using 10x2mL media exchanges, and cells were imaged every five minutes until anaphase.

Image analysis

Mitotic duration was determined by identifying metaphase spindles manually, where metaphase was defined as a state in which all chromosomes were attached to the spindle and aligned tightly at the midpoint between two spindle poles. Anaphase was identified by sister kinetochore separation. Metaphase duration was calculated based on a 5 min imaging interval such that cells found to be at metaphase for 0 frames prior to anaphase were determined to have a metaphase duration of 0-4 min, 1 frame at metaphase prior to anaphase had a metaphase duration of 1-9 min, and so on. Lagging chromosomes were assessed in cells with a clear view of anaphase and kinetochores were considered lagging if they were noticeably set away from the main chromosome mass in the direction of the spindle midzone.

Chapter 4: Conclusions and Future Directions

Physical Features Affecting Kinetochore Decision-Making

In this dissertation, I have asked how biophysical cues contribute to key decisions cells make during division: whether to hold on (error correction) and when to divide (the spindle assembly checkpoint). Because it is so hard to separate force at the kinetochore from microtubule binding, this has been historically challenging to address but understanding the role of physical cues in regulating mitosis provides a frame of reference for understanding cell division in context. Indeed, cell division is a distinctly mechanical process so the physical landscape in which it occurs may be critical for understanding what maintains chromosome segregation fidelity.

In Chapter 2, I asked how the capacity to form correct attachments and resolve incorrect ones varied across chromosomes of different sizes and what role tension played in promoting correct biorientation. I investigated this question in mammalian rat kangaroo (PtK2) cells and used laser ablation and chromokinesin overexpression to perturb tension at kinetochores. I found that long chromosomes experience higher polar ejection force than short ones, which promotes attachment stabilization and delays error correction when incorrect attachments do form. This provided direct evidence that force at the kinetochore leads to attachment stabilization and improves accuracy of chromosome segregation. Thus, the kinetochore-microtubule interface relies on its mechanics to perform its regulatory function.

Many open questions remain about the physical features affecting error correction outcomes in mammalian mitosis. How chromatin compliance (Schneider et al., 2022), kinetochore material properties (Cojoc et al., 2016), and kinetochore position (Dumont et al., 2020) affect error correction efficiency will be important for

contextualizing the evolution of karyotypes across species. Additionally, the role of tissue and extracellular matrix in providing a strong foundation for the spindle to transmit force most effectively is only just beginning to be understood (Knouse et al., 2018). Understanding these extracellular effects may reveal cell type and tissue-specific differences in aneuploidy propensity. In addition to the physical signals regulating error correction, how error correction impacts other processes, including kinetochore maturation and spindle assembly checkpoint satisfaction remain open questions.

In Chapter 3, I addressed the roles of signal detection and signal integration in the anaphase entry decision. I used human RPE1 cells to ask whether kinetochores required opposing force from a sister kinetochore in order to satisfy the spindle assembly checkpoint (SAC) and enter anaphase. I found that 1 – 3 unpaired, sisterless kinetochores delayed anaphase onset in a graded manner but did not prevent it, reflective of a SAC defect at the kinetochore in the absence of a sister. This defect likely stemmed from altered satisfaction dynamics, either at the kinetochore or across the cytoplasm. Immediate future directions will focus on measuring SAC satisfaction at high time resolution in these unpaired, sisterless kinetochores and assessing integration mechanisms for incorporating signals from multiple kinetochores across the cell.

This work has also opened the door for questions of how SAC satisfaction dynamics vary across different chromosomes. By pairing kinetochore ablations with chromosome arm ablations and chromokinesin overexpression (Chong et al., 2024), future experiments could identify direct effects of force on detection of stable attachments. Additionally, while SAC satisfaction has been demonstrated to occur in a stepwise manner in mammalian PtK2 cells (Kuhn and Dumont, 2017), how this may

differ across systems is unknown (Cane et al., 2013a). Careful characterization of SAC satisfaction dynamics in human cells in both bipolar and monopolar spindles will provide insight into two questions: first, whether partial removal of Mad 1 and Mad2 is biologically relevant in human cells and second, whether SAC satisfaction always occurs stably or can transiently oscillate between active and silent. Such transient oscillation may be contingent on the specific mechanism of SAC satisfaction, which can occur in both dynein-dependent and -independent ways. This work may also provide insight into how long-lived k-fibers must be in order to induce stable SAC satisfaction by measuring k-fiber lifetime in monopoles and bipoles. These details of SAC satisfaction mechanism determine the susceptibility of the system to noise. Additionally, this thesis sets the groundwork for characterizing the cytoplasmic detection threshold for the SAC (i.e. the number of SAC-signaling kinetochores required to prevent anaphase onset altogether), which may vary across species and with chromosome complement.

Crosstalk in kinetochore signaling pathways safeguards the genome

How these two critical signaling roles at the kinetochore, error correction and the spindle assembly checkpoint, interface with one another remains a pressing open question. While both are required to maintain accuracy of chromosome segregation, whether their precision and efficiency align under all circumstances is not clear. Because SAC satisfaction occurs independently in sister kinetochores (Kuhn and Dumont, 2017) but biorientation, the desired outcome of error correction, reflects the attachment status of both sisters simultaneously, it stands to reason that the SAC may occasionally satisfy at incorrect attachments only to reactivate following error correction-

induced detachment. By characterizing the specific cues each pathway senses, we will better be able to predict when these two pathways may diverge, leading to missegregation. Ultimately, quantitative analysis paired with creative mechanical perturbations will be critical for this, allowing a clearer picture of the design principles that dictate cellular decision-making in the context of mitosis and across cell biology.

References

Barisic, M., Aguiar, P., Geley, S., Maiato, H., 2014. Kinetochore motors drive congression of peripheral polar chromosomes by overcoming random arm-ejection forces. Nat. Cell Biol. 16, 1249–1256. https://doi.org/10.1038/ncb3060

Biggins, S., Murray, A.W., 2001. The budding yeast protein kinase Ipl1/Aurora allows the absence of tension to activate the spindle checkpoint. Genes Dev. 15, 3118–3129. https://doi.org/10.1101/gad.934801

Cameron, L.A., Yang, G., Cimini, D., Canman, J.C., Kisurina-Evgenieva, O., Khodjakov, A., Danuser, G., Salmon, E.D., 2006. Kinesin 5–independent poleward flux of kinetochore microtubules in PtK1 cells. J. Cell Biol. 173, 173–179. https://doi.org/10.1083/jcb.200601075

Cane, S., McGilvray, P.T., Maresca, T.J., 2013a. Insights from an erroneous kinetochore-microtubule attachment state. BioArchitecture 3, 69–76. https://doi.org/10.4161/bioa.25734

Cane, S., Ye, A.A., Luks-Morgan, S.J., Maresca, T.J., 2013b. Elevated polar ejection forces stabilize kinetochore–microtubule attachments. J. Cell Biol. 200, 203–218. https://doi.org/10.1083/jcb.201211119

Cheerambathur, D.K., Prevo, B., Hattersley, N., Lewellyn, L., Corbett, K.D., Oegema, K., Desai, A., 2017. Dephosphorylation of the Ndc80 Tail Stabilizes Kinetochore-Microtubule Attachments via the Ska Complex. Dev. Cell 41, 424-437.e4. https://doi.org/10.1016/j.devcel.2017.04.013

Cheeseman, I.M., Chappie, J.S., Wilson-Kubalek, E.M., Desai, A., 2006. The Conserved KMN Network Constitutes the Core Microtubule-Binding Site of the Kinetochore. Cell 127, 983–997. https://doi.org/10.1016/j.cell.2006.09.039

Cheeseman, I.M., Desai, A., 2008. Molecular architecture of the kinetochore–microtubule interface. Nat. Rev. Mol. Cell Biol. 9, 33–46. https://doi.org/10.1038/nrm2310

Chen, G.-Y., Renda, F., Zhang, H., Gokden, A., Wu, D.Z., Chenoweth, D.M., Khodjakov, A., Lampson, M.A., 2021. Tension promotes kinetochore–microtubule release by Aurora B kinase. J. Cell Biol. 220, e202007030. https://doi.org/10.1083/jcb.202007030

Chen, R.-H., Shevchenko, A., Mann, M., Murray, A.W., 1998. Spindle Checkpoint Protein Xmad1 Recruits Xmad2 to Unattached Kinetochores. J. Cell Biol. 143, 283–295. https://doi.org/10.1083/jcb.143.2.283

Chong, M.K., Rosas-Salvans, M., Tran, V., Dumont, S., 2024. Chromosome size-dependent polar ejection force impairs mammalian mitotic error correction. J. Cell Biol. 223, e202310010. https://doi.org/10.1083/jcb.202310010

Cojoc, G., Roscioli, E., Zhang, L., García-Ulloa, A., Shah, J.V., Berns, M.W., Pavin, N., Cimini, D., Tolić, I.M., Gregan, J., 2016. Laser microsurgery reveals conserved viscoelastic behavior of the kinetochore. J. Cell Biol. 212, 767–776. https://doi.org/10.1083/jcb.201506011

Collin, P., Nashchekina, O., Walker, R., Pines, J., 2013. The spindle assembly checkpoint works like a rheostat rather than a toggle switch. Nat. Cell Biol. 15, 1378–1385. https://doi.org/10.1038/ncb2855

De Antoni, A., Pearson, C.G., Cimini, D., Canman, J.C., Sala, V., Nezi, L., Mapelli, M., Sironi, L., Faretta, M., Salmon, E.D., Musacchio, A., 2005. The Mad1/Mad2 Complex as a Template for Mad2 Activation in the Spindle Assembly Checkpoint. Curr. Biol. 15, 214–225. https://doi.org/10.1016/j.cub.2005.01.038

De Regt, A.K., Clark, C.J., Asbury, C.L., Biggins, S., 2022. Tension can directly suppress Aurora B kinase-triggered release of kinetochore-microtubule attachments. Nat. Commun. 13, 2152. https://doi.org/10.1038/s41467-022-29542-8

DeLuca, J.G., Gall, W.E., Ciferri, C., Cimini, D., Musacchio, A., Salmon, E.D., 2006. Kinetochore Microtubule Dynamics and Attachment Stability Are Regulated by Hec1. Cell 127, 969–982. https://doi.org/10.1016/j.cell.2006.09.047

DeLuca, K.F., Lens, S.M.A., DeLuca, J.G., 2011. Temporal changes in Hec1 phosphorylation control kinetochore–microtubule attachment stability during mitosis. J. Cell Sci. 124, 622–634. https://doi.org/10.1242/jcs.072629

Dick, A.E., Gerlich, D.W., 2013. Kinetic framework of spindle assembly checkpoint signalling. Nat. Cell Biol. 15, 1370–1377. https://doi.org/10.1038/ncb2842

Drpic, D., Almeida, A.C., Aguiar, P., Renda, F., Damas, J., Lewin, H.A., Larkin, D.M., Khodjakov, A., Maiato, H., 2018. Chromosome Segregation Is Biased by Kinetochore Size. Curr. Biol. 28, 1344-1356.e5. https://doi.org/10.1016/j.cub.2018.03.023

Drpic, D., Pereira, A.J., Barisic, M., Maresca, T.J., Maiato, H., 2015. Polar Ejection Forces Promote the Conversion from Lateral to End-on Kinetochore-Microtubule

Attachments on Mono-oriented Chromosomes. Cell Rep. 13, 460–468. https://doi.org/10.1016/j.celrep.2015.08.008

Dumont, M., Gamba, R., Gestraud, P., Klaasen, S., Worrall, J.T., De Vries, S.G., Boudreau, V., Salinas-Luypaert, C., Maddox, P.S., Lens, S.M., Kops, G.J., McClelland, S.E., Miga, K.H., Fachinetti, D., 2020. Human chromosome-specific aneuploidy is influenced by DNA -dependent centromeric features. EMBO J. 39, e102924. https://doi.org/10.15252/embj.2019102924

Eibes, S., Rajendraprasad, G., Guasch-Boldu, C., Kubat, M., Steblyanko, Y., Barisic, M., 2023. CENP-E activation by Aurora A and B controls kinetochore fibrous corona disassembly. Nat. Commun. 14, 5317. https://doi.org/10.1038/s41467-023-41091-2

Etemad, B., Kuijt, T.E.F., Kops, G.J.P.L., 2015. Kinetochore–microtubule attachment is sufficient to satisfy the human spindle assembly checkpoint. Nat. Commun. 6, 8987. https://doi.org/10.1038/ncomms9987

Fang, L., Seki, A., Fang, G., 2009. SKAP associates with kinetochores and promotes the metaphase-to-anaphase transition. Cell Cycle 8, 2819–2827. https://doi.org/10.4161/cc.8.17.9514

Funabiki, H., 2019. Correcting aberrant kinetochore microtubule attachments: a hidden regulation of Aurora B on microtubules. Curr. Opin. Cell Biol. 58, 34–41. https://doi.org/10.1016/j.ceb.2018.12.007

Hanisch, A., Silljé, H.H.W., Nigg, E.A., 2006. Timely anaphase onset requires a novel spindle and kinetochore complex comprising Ska1 and Ska2. EMBO J. 25, 5504–5515. https://doi.org/10.1038/sj.emboj.7601426

Howell, B.J., McEwen, B.F., Canman, J.C., Hoffman, D.B., Farrar, E.M., Rieder, C.L., Salmon, E.D., 2001. Cytoplasmic dynein/dynactin drives kinetochore protein transport to the spindle poles and has a role in mitotic spindle checkpoint inactivation. J. Cell Biol. 155, 1159–1172. https://doi.org/10.1083/jcb.200105093

Iemura, K., Tanaka, K., 2015. Chromokinesin Kid and kinetochore kinesin CENP-E differentially support chromosome congression without end-on attachment to microtubules. Nat. Commun. 6, 6447. https://doi.org/10.1038/ncomms7447

Johnston, K., Joglekar, A., Hori, T., Suzuki, A., Fukagawa, T., Salmon, E.D., 2010. Vertebrate kinetochore protein architecture: protein copy number. J. Cell Biol. 189, 937–943. https://doi.org/10.1083/jcb.200912022

Jokelainen, P.T., 1967. The ultrastructure and spatial organization of the metaphase kinetochore in mitotic rat cells. J. Ultrastruct. Res. 19, 19–44. https://doi.org/10.1016/S0022-5320(67)80058-3

Kapoor, T.M., Mayer, T.U., Coughlin, M.L., Mitchison, T.J., 2000. Probing Spindle Assembly Mechanisms with Monastrol, a Small Molecule Inhibitor of the Mitotic Kinesin, Eg5. J. Cell Biol. 150, 975–988. https://doi.org/10.1083/jcb.150.5.975

Ke, K., Cheng, J., Hunt, A.J., 2009. The Distribution of Polar Ejection Forces Determines the Amplitude of Chromosome Directional Instability. Curr. Biol. 19, 807–815. https://doi.org/10.1016/j.cub.2009.04.036

Kirschner, M., Gerhart, J., Mitchison, T., 2000. Molecular “Vitalism.” Cell 100, 79–88. https://doi.org/10.1016/S0092-8674(00)81685-2

Klaasen, S.J., Truong, M.A., van Jaarsveld, R.H., Koprivec, I., Štimac, V., de Vries, S.G., Risteski, P., Kodba, S., Vukušić, K., de Luca, K.L., Marques, J.F., Gerrits,

E.M., Bakker, B., Foijer, F., Kind, J., Tolić, I.M., Lens, S.M.A., Kops, G.J.P.L., 2022. Nuclear chromosome locations dictate segregation error frequencies. Nature 607, 604–609. https://doi.org/10.1038/s41586-022-04938-0

Knouse, K.A., Lopez, K.E., Bachofner, M., Amon, A., 2018. Chromosome Segregation Fidelity in Epithelia Requires Tissue Architecture. Cell 175, 200-211.e13. https://doi.org/10.1016/j.cell.2018.07.042

Kops, G.J.P.L., Gassmann, R., 2020. Crowning the Kinetochore: The Fibrous Corona in Chromosome Segregation. Trends Cell Biol. 30, 653–667. https://doi.org/10.1016/j.tcb.2020.04.006

Kuhn, J., Dumont, S., 2019. Mammalian kinetochores count attached microtubules in a sensitive and switch-like manner. J. Cell Biol. 218, 3583–3596. https://doi.org/10.1083/jcb.201902105

Kuhn, J., Dumont, S., 2017. Spindle assembly checkpoint satisfaction occurs via end-on but not lateral attachments under tension. J. Cell Biol. 216, 1533–1542. https://doi.org/10.1083/jcb.201611104

Lampson, M., Grishchuk, E., 2017. Mechanisms to Avoid and Correct Erroneous Kinetochore-Microtubule Attachments. Biology 6, 1. https://doi.org/10.3390/biology6010001

Lampson, M.A., Renduchitala, K., Khodjakov, A., Kapoor, T.M., 2004. Correcting improper chromosome–spindle attachments during cell division. Nat. Cell Biol. 6, 232–237. https://doi.org/10.1038/ncb1102

Levesque, A.A., Compton, D.A., 2001. The chromokinesin Kid is necessary for chromosome arm orientation and oscillation, but not congression, on mitotic spindles. J. Cell Biol. 154, 1135–1146. https://doi.org/10.1083/jcb.200106093

Long, A.F., Kuhn, J., Dumont, S., 2019. The mammalian kinetochore–microtubule interface: robust mechanics and computation with many microtubules. Curr. Opin. Cell Biol. 60, 60–67. https://doi.org/10.1016/j.ceb.2019.04.004

Maldonado, M., Kapoor, T.M., 2011. Constitutive Mad1 targeting to kinetochores uncouples checkpoint signalling from chromosome biorientation. Nat. Cell Biol. 13, 475–482. https://doi.org/10.1038/ncb2223

McEwen, B.F., Ding, Y., Heagle, A.B., 1998. Relevance of kinetochore size and microtubule-binding capacity for stable chromosome attachment during mitosis in PtK1 cells. Chromosome Res. 6, 123–132. https://doi-org.ucsf.idm.oclc.org/10.1023/A:1009239013215

McEwen, B.F., Heagle, A.B., Cassels, G.O., Buttle, K.F., Rieder, C.L., 1997. Kinetochore Fiber Maturation in PtK1 Cells and Its Implications for the Mechanisms of Chromosome Congression and Anaphase Onset. J. Cell Biol. 137, 1567–1580. https://doi.org/10.1083/jcb.137.7.1567

Miller, M.P., Asbury, C.L., Biggins, S., 2016. A TOG Protein Confers Tension Sensitivity to Kinetochore-Microtubule Attachments. Cell 165, 1428–1439. https://doi.org/10.1016/j.cell.2016.04.030

Miller, M.P., Evans, R.K., Zelter, A., Geyer, E.A., MacCoss, M.J., Rice, L.M., Davis, T.N., Asbury, C.L., Biggins, S., 2019. Kinetochore-associated Stu2 promotes

chromosome biorientation in vivo. PLOS Genet. 15, e1008423. https://doi.org/10.1371/journal.pgen.1008423

Musacchio, A., 2015. The Molecular Biology of Spindle Assembly Checkpoint Signaling Dynamics. Curr. Biol. 25, R1002–R1018. https://doi.org/10.1016/j.cub.2015.08.051

Musacchio, A., Desai, A., 2017. A Molecular View of Kinetochore Assembly and Function. Biology 6, 5. https://doi.org/10.3390/biology6010005

Nicklas, R.B., 1965. CHROMOSOME VELOCITY DURING MITOSIS AS A FUNCTION OF CHROMOSOME SIZE AND POSITION. J. Cell Biol. 25, 119–135. https://doi.org/10.1083/jcb.25.1.119

Nicklas, R.B., Koch, C.A., 1969. CHROMOSOME MICROMANIPULATION. J. Cell Biol. 43, 40–50. https://doi.org/10.1083/jcb.43.1.40

O'Connell, C.B., Lončarek, J., Hergert, P., Kourtidis, A., Conklin, D.S., Khodjakov, A., 2008. The spindle assembly checkpoint is satisfied in the absence of interkinetochore tension during mitosis with unreplicated genomes. J. Cell Biol. 183, 29–36. https://doi.org/10.1083/jcb.200801038

Parmar, S., Gonzalez, S.J., Heckel, J.M., Mukherjee, S., McClellan, M., Clarke, D.J., Johansson, M., Tank, D., Geisness, A., Wood, D.K., Gardner, M.K., 2023. Robust microtubule dynamics facilitate low-tension kinetochore detachment in metaphase. J. Cell Biol. 222, e202202085. https://doi.org/10.1083/jcb.202202085

Paysan-Lafosse, T., Blum, M., Chuguransky, S., Grego, T., Pinto, B.L., Salazar, G.A., Bileschi, M.L., Bork, P., Bridge, A., Colwell, L., Gough, J., Haft, D.H., Letunić, I., Marchler-Bauer, A., Mi, H., Natale, D.A., Orengo, C.A., Pandurangan, A.P.,

Rivoire, C., Sigrist, C.J.A., Sillitoe, I., Thanki, N., Thomas, P.D., Tosatto, S.C.E., Wu, C.H., Bateman, A., 2023. InterPro in 2022. Nucleic Acids Res. 51, D418–D427. https://doi.org/10.1093/nar/gkac993

Peterson, J.B., Ris, H., 1976. Electron-microscopic study of the spindle and chromosome movement in the yeast *Saccharomyces Cerevisiae*. J. Cell Sci. 22, 219–242. https://doi.org/10.1242/jcs.22.2.219

Powers, A.F., Franck, A.D., Gestaut, D.R., Cooper, J., Gracyzk, B., Wei, R.R., Wordeman, L., Davis, T.N., Asbury, C.L., 2009. The Ndc80 Kinetochore Complex Forms Load-Bearing Attachments to Dynamic Microtubule Tips via Biased Diffusion. Cell 136, 865–875. https://doi.org/10.1016/j.cell.2008.12.045

Rieder, C., Salmon, E., 1994. Motile kinetochores and polar ejection forces dictate chromosome position on the vertebrate mitotic spindle. J. Cell Biol. 124, 223–233. https://doi.org/10.1083/jcb.124.3.223

Rieder, C.L., Cole, R.W., Khodjakov, A., Sluder, G., 1995. The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitory signal produced by unattached kinetochores. J. Cell Biol. 130, 941–948. https://doi.org/10.1083/jcb.130.4.941

Rieder, C.L., Davison, E.A., Jensen, L.C., Cassimeris, L., Salmon, E.D., 1986. 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. 103, 581–591. https://doi.org/10.1083/jcb.103.2.581

Sacristan, C., Ahmad, M.U.D., Keller, J., Fermie, J., Groenewold, V., Tromer, E., Fish, A., Melero, R., Carazo, J.M., Klumperman, J., Musacchio, A., Perrakis, A., Kops,

G.J., 2018. Dynamic kinetochore size regulation promotes microtubule capture and chromosome biorientation in mitosis. Nat. Cell Biol. 20, 800–810. https://doi.org/10.1038/s41556-018-0130-3

Santaguida, S., Amon, A., 2015. Short- and long-term effects of chromosome mis-segregation and aneuploidy. Nat. Rev. Mol. Cell Biol. 16, 473–485. https://doi.org/10.1038/nrm4025

Sarangapani, K.K., Akiyoshi, B., Duggan, N.M., Biggins, S., Asbury, C.L., 2013. Phosphoregulation promotes release of kinetochores from dynamic microtubules via multiple mechanisms. Proc. Natl. Acad. Sci. 110, 7282–7287. https://doi.org/10.1073/pnas.1220700110

Sarangapani, K.K., Asbury, C.L., 2014. Catch and release: how do kinetochores hook the right microtubules during mitosis? Trends Genet. 30, 150–159. https://doi.org/10.1016/j.tig.2014.02.004

Sarangapani, K.K., Koch, L.B., Nelson, C.R., Asbury, C.L., Biggins, S., 2021. Kinetochore-bound Mps1 regulates kinetochore–microtubule attachments via Ndc80 phosphorylation. J. Cell Biol. 220, e202106130. https://doi.org/10.1083/jcb.202106130

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019

Schmidt, J.C., Kiyomitsu, T., Hori, T., Backer, C.B., Fukagawa, T., Cheeseman, I.M., 2010. Aurora B kinase controls the targeting of the Astrin–SKAP complex to bioriented kinetochores. J. Cell Biol. 191, 269–280. https://doi.org/10.1083/jcb.201006129

Schneider, M.W.G., Gibson, B.A., Otsuka, S., Spicer, M.F.D., Petrovic, M., Blaukopf, C., Langer, C.C.H., Batty, P., Nagaraju, T., Doolittle, L.K., Rosen, M.K., Gerlich, D.W., 2022. A mitotic chromatin phase transition prevents perforation by microtubules. Nature 609, 183–190. https://doi.org/10.1038/s41586-022-05027-y

Tauchman, E.C., Boehm, F.J., DeLuca, J.G., 2015. Stable kinetochore–microtubule attachment is sufficient to silence the spindle assembly checkpoint in human cells. Nat. Commun. 6, 10036. https://doi.org/10.1038/ncomms10036

Thompson, A.F., Blackburn, P.R., Arons, N.S., Stevens, S.N., Babovic-Vuksanovic, D., Lian, J.B., Klee, E.W., Stumpff, J., 2022. Pathogenic mutations in the chromokinesin KIF22 disrupt anaphase chromosome segregation. eLife 11, e78653. https://doi.org/10.7554/eLife.78653

Tokai, N., Fujimoto-Nishiyama, A., Toyoshima, Y., Yonemura, S., Tsukita, S., Inoue, J., Yamamota, T., 1996. Kid, a novel kinesin-like DNA binding protein, is localized to chromosomes and the mitotic spindle. EMBO J. 15, 457–467. https://doi.org/10.1002/j.1460-2075.1996.tb00378.x

Torosantucci, L., De Santis Puzzonia, M., Cenciarelli, C., Rens, W., Degrassi, F., 2009. Aneuploidy in mitosis of PtK1 cells is generated by random loss and nondisjunction of individual chromosomes. J. Cell Sci. 122, 3455–3461. https://doi.org/10.1242/jcs.047944

Tovini, L., McClelland, S., 2019. Impaired CENP-E Function Renders Large Chromosomes More Vulnerable to Congression Failure. Biomolecules 9, 44. https://doi.org/10.3390/biom9020044

Udy, D.B., Voorhies, M., Chan, P.P., Lowe, T.M., Dumont, S., 2015. Draft De Novo Transcriptome of the Rat Kangaroo Potorous tridactylus as a Tool for Cell Biology. PLOS ONE 10, e0134738. https://doi.org/10.1371/journal.pone.0134738

Volkov, V.A., Huis In 'T Veld, P.J., Dogterom, M., Musacchio, A., 2018. Multivalency of NDC80 in the outer kinetochore is essential to track shortening microtubules and generate forces. eLife 7, e36764. https://doi.org/10.7554/eLife.36764

Vukušić, K., Tolić, I.M., 2022. Polar Chromosomes—Challenges of a Risky Path. Cells 11, 1531. https://doi.org/10.3390/cells11091531

Wan, X., Cimini, D., Cameron, L.A., Salmon, E.D., 2012. The coupling between sister kinetochore directional instability and oscillations in centromere stretch in metaphase PtK1 cells. Mol. Biol. Cell 23, 1035–1046. https://doi.org/10.1091/mbc.e11-09-0767

Wandke, C., Barisic, M., Sigl, R., Rauch, V., Wolf, F., Amaro, A.C., Tan, C.H., Pereira, A.J., Kutay, U., Maiato, H., Meraldi, P., Geley, S., 2012. Human chromokinesins promote chromosome congression and spindle microtubule dynamics during mitosis. J. Cell Biol. 198, 847–863. https://doi.org/10.1083/jcb.201110060

Wojcik, E., Basto, R., Serr, M., Scaërou, F., Karess, R., Hays, T., 2001. Kinetochore dynein: its dynamics and role in the transport of the Rough deal checkpoint protein. Nat. Cell Biol. 3, 1001–1007. https://doi.org/10.1038/ncb1101-1001

Worrall, J.T., Tamura, N., Mazzagatti, A., Shaikh, N., van Lingen, T., Bakker, B., Spierings, D.C.J., Vladimirou, E., Foijer, F., McClelland, S.E., 2018. Non-random Mis-segregation of Human Chromosomes. Cell Rep. 23, 3366–3380. https://doi.org/10.1016/j.celrep.2018.05.047

Wu, W., Jingbo, S., Xu, W., Liu, J., Huang, Y., Sheng, Q., Lv, Z., 2018. S-trityl-L-cysteine, a novel Eg5 inhibitor, is a potent chemotherapeutic strategy in neuroblastoma. Oncol. Lett. https://doi.org/10.3892/ol.2018.8755

Ye, A.A., Deretic, J., Hoel, C.M., Hinman, A.W., Cimini, D., Welburn, J.P., Maresca, T.J., 2015. Aurora A Kinase Contributes to a Pole-Based Error Correction Pathway. Curr. Biol. 25, 1842–1851. https://doi.org/10.1016/j.cub.2015.06.021

Yoo, T.Y., Choi, J.-M., Conway, W., Yu, C.-H., Pappu, R.V., Needleman, D.J., 2018. Measuring NDC80 binding reveals the molecular basis of tension-dependent kinetochore-microtubule attachments. eLife 7, e36392. https://doi.org/10.7554/eLife.36392

Zaytsev, A.V., Mick, J.E., Maslennikov, E., Nikashin, B., DeLuca, J.G., Grishchuk, E.L., 2015. Multisite phosphorylation of the NDC80 complex gradually tunes its microtubule-binding affinity. Mol. Biol. Cell 26, 1829–1844. https://doi.org/10.1091/mbc.E14-11-1539

Zaytsev, A.V., Sundin, L.J.R., DeLuca, K.F., Grishchuk, E.L., DeLuca, J.G., 2014. Accurate phosphoregulation of kinetochore–microtubule affinity requires unconstrained molecular interactions. J. Cell Biol. 206, 45–59. https://doi.org/10.1083/jcb.201312107

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