Tissue Architecture Is Required for Chromosome Segregation Fidelity in Epithelia

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ABSTRACT

Chromosome segregation is classically viewed as a cell-intrinsic process. We tested this assumption by comparing chromosome segregation fidelity in various epithelial cells in their native tissue and as dissociated cells in culture and discovered that chromosome segregation fidelity is dependent on the tissue environment. Using organoid culture systems, we show that it is tissue architecture, the cell adhesion and cell polarity patterns that define a tissue, which is responsible for the heightened chromosome segregation fidelity in tissues and that disruption of tissue architecture leads to chromosome instability. Tissue architecture enhances the cell’s ability to correct erroneous microtubule-kinetochore attachments and this enhancement is especially important for maintaining chromosome stability in the liver, a polyploid tissue with high levels of erroneous attachments. Moreover, we find that neural progenitor cells, which proliferate in a region of the brain that lacks defined architecture, maintain their polarity and chromosome segregation fidelity even when removed from their tissue environment. We show that disruption of cell polarity in epithelia recapitulates the chromosome segregation defects observed in dissociated cells, arguing that cell polarity is the critical component of tissue architecture that facilitates chromosome segregation. Our data bring us to the surprising conclusion that the external environment influences chromosome segregation across many epithelial cell types. The function of epithelial tissues requires that cells maintain distinct adhesions and polarity in both interphase and mitosis. In relinquishing autonomy over their polarity, epithelial cells also rely on their native tissue for chromosome segregation fidelity. We propose that disruption of tissue architecture could explain the chromosome instability that characterizes and drives cancer. More broadly, our observations highlight the importance of context for even the most fundamental cellular processes and thus the need to use experimental systems that maximize physiologic relevance across all areas of cell biology.
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INTRODUCTION

The viability of any organism and a species as a whole is contingent upon the transmission of a complete copy of the genome from one cell to the next. This occurs through the sequential processes of DNA replication and chromosome segregation. Failure to execute these processes faithfully can alter anywhere from one to millions of base pairs of DNA sequence. Specifically, errors during chromosome segregation can lead to the gain or loss of entire chromosomes, a condition known as aneuploidy. Changes in chromosome copy number cause commensurate changes in gene expression, and given the hundreds of genes contained on any given chromosome, aneuploidy has profound effects on cellular physiology. Indeed, in most cases chromosome missegregation and the aneuploid state are associated with impaired cellular fitness. However, in rare cases aneuploidy appears to provide a fitness advantage, contributing to cellular transformation and cancer.

Given the importance of faithful chromosome segregation for cell and organism viability, this process is subject to meticulous execution and stringent regulation. Over the past century, investigations using model organisms and cell culture systems have revealed many of the players and mechanisms that are important for this process. While most of the discoveries made using these systems are presumably generalizable across contexts, there has yet to be a thorough investigation of chromosome segregation in mammalian tissues to confirm the universality of its mechanisms and regulation. In order to understand how these processes occur in tissues and how they might be deregulated in disease, it is critical to study chromosome segregation in the tissue context.

CHROMOSOME SEGREGATION

Mechanism

The partitioning of sister chromatids into separate daughter cells is mediated by interactions between the mitotic spindle and kinetochores. The mitotic spindle is a bipolar array of microtubules nucleated by centrosomes. The kinetochore is a protein
complex that assembles at the centromere of each sister chromatid (1). The inner kinetochore, compromised of multiple CENP proteins, associates with the centromeric histone H3 variant CENP-A throughout the cell cycle (Figure 1A, left panel). At the beginning of mitosis, additional proteins assemble on the inner kinetochore to form the outer kinetochore (Figure 1A, right panel). A key component of the outer kinetochore is the Ndc80 complex, a heterotetrameric complex comprised of Ndc80 (Hec1 in humans), Nuf2, Spc24, and Spc25. This complex forms a rod-like structure with a central $\alpha$-helical coiled coil tail and terminal globular head domains. One of the globular head domains, comprised of the N-termini of Ndc80 and Nuf2, enables the binding of microtubules to kinetochores (2,3). Here, the N-terminal calponin-homology domains of Ndc80 and Nuf2 and adjacent positively charged region of Ndc80 form electrostatic interactions with the negatively-charged C-terminal tail of tubulin. In some organisms, including mammals, multiple kinetochore complexes assemble in a tandem array along the centromere and thus any given chromosome can be bound by several (15-20) microtubules (4).
Figure 1. The kinetochore. A) Components of the inner kinetochore, which is present throughout the cell cycle (left panel), and the outer kinetochore, which assembles on the inner kinetochore at the beginning of mitosis (right panel). Adapted from Cheeseman 2014. B) The Ndc80 complex. Adapted from Tanaka 2010.

At the onset of mitosis, the two centrosomes begin nucleating microtubules to form the mitotic spindle. The microtubules then begin to “search and capture” kinetochores (5). Initially, the kinetochore makes lateral attachments with a microtubule (Figure 2A, top panel). Eventually, with the help of motor proteins and microtubule depolymerization, these lateral attachments are converted to end-on attachments, in
which the kinetochore binds to the plus-end of the microtubule (Figure 2A, bottom panel). As the two centrosomes move to opposite ends of the cell and the mitotic spindle becomes bipolar, the interactions between microtubules and kinetochores congress the chromosomes to the metaphase plate in preparation for the onset of anaphase and segregation of sister chromatids into separate daughter cells.

In order for sister chromatids to segregate into separate daughter cells, their kinetochores must attach to microtubules emanating from opposite spindle poles, a condition known as amphitelic attachment (Figure 2B, bottom left) (5). However, because the interaction between the kinetochores and microtubules is random, amphitelic attachment is not the only possible outcome of microtubule search and capture. Sister kinetochores can also become attached to microtubules originating from the same spindle pole, termed a syntelic attachment (Figure 2B, top right). Alternatively, only one sister kinetochore may attach to microtubules, forming a monotelic attachment (Figure 2B, top left). Finally, because a single sister chromatid can bind multiple microtubules, a merotelic attachment is possible, in which a sister chromatid is bound to microtubules emanating from opposite spindle poles (Figure 2B, bottom right). If sister chromatids are under syntelic, monotelic, or merotelic attachment, they will not segregate properly into daughter cells. In order to eliminate these aberrant attachments and form correct amphitelic attachments, the cell relies on the cooperative functions of error correction and the spindle assembly checkpoint.
Figure 2. **Microtubule-kinetochore attachment.** A) A lateral attachment of a kinetochore to a microtubule (top panel) and an end-on attachment of a kinetochore to a microtubule (bottom panel). B) Monotelic (top left), syntelic (top right), amphitelic (bottom left), and merotelic (bottom right) microtubule-kinetochore attachments. Adapted from Tanaka 2010.

**Regulation**

The error correction pathway eliminates aberrant attachments in favor of amphitelic attachments by destabilizing syntelic and monotelic attachments. The kinase Aurora B is central to this pathway. Aurora B is located in between sister kinetochores. Here, it can phosphorylate Ndc80 and other kinetochore proteins to disrupt their interaction with microtubules (6-8). Importantly, the extent to which aurora B can phosphorylate kinetochore substrates is inversely correlated with the distance of these substrates from the centromere (9). When kinetochore proteins are close to the inner centromere, Aurora B is able to phosphorylate these substrates and thereby destabilize microtubule-kinetochore attachments (Figure 3A, left panel). However, when
Kinetochore proteins are further from the inner centromere, Aurora B is less able to phosphorylate them and microtubule-kinetochore attachments are stabilized (Figure 3A, right panel). This becomes relevant for proper chromosome segregation because amphitelic attachment stretches the kinetochore sufficiently to reduce Aurora B phosphorylation and stabilize the attachment whereas monotelic and syntelic attachment do not (10).

Although error correction can enrich for amphitelic attachments, it alone cannot prevent anaphase onset in the presence of other aberrant attachments. The spindle assembly checkpoint accomplishes this function. Unattached kinetochores serve as substrates for spindle assembly checkpoint activation. When microtubules are not bound to Ndc80, the kinase Mps1 binds to Ndc80 to initiate the checkpoint (Figure 3B) (11). Mps1 phosphorylates kinetochore proteins, which serves to recruit other checkpoint components including Bub3, Bub1, BubR1, Mad1, and Mad2. Together, these components inhibit Cdc20, an essential activator of the anaphase-promoting complex, and thereby prevent anaphase onset. The error correction pathway and spindle assembly checkpoint therefore complement each other in order to ensure proper chromosome segregation. When a kinetochore is not under tension, as occurs with a monotelic or syntelic attachment, Aurora B phosphorylates the kinetochore to sever the attachment. This gives the kinetochore an opportunity to form an amphitelic attachment while simultaneously delaying mitotic progression.

**Merotely**

Given the mechanism by which error correction detects aberrant attachments, merotelic attachments are problematic for the cell. Because a merotelically attached kinetochore is bound by microtubules emanating from opposite spindle poles, it is possible for the kinetochore to be stretched enough to avoid phosphorylation by Aurora B and thus not activate the spindle assembly checkpoint. This attachment can therefore persist into anaphase. Depending on the ratio of microtubules from each spindle pole bound to the kinetochore, the chromosome may lag behind the two main masses of segregating chromosomes and potentially segregate into the wrong daughter cell (12). If a lagging chromosome is separated sufficiently from the other segregating
chromosomes, it will not be incorporated into the main nucleus but instead form a separate nucleus when the chromosomes decondense and the nuclear envelope reforms in telophase.

Figure 3

A

B
Figure 3. Error correction and the spindle assembly checkpoint. A) Location of kinetochore proteins relative to the zone of Aurora B activity when the kinetochore is not (left panel) and is (right panel) under tension. B) Accumulation of spindle assembly checkpoint components at an unattached kinetochore to inhibit the anaphase-promoting complex (top panel). Entire figure adapted from Cheeseman 2014.

CHROMOSOME MISSEGREGATION
Causes

Given the challenges posed by merotelic attachments, it is perhaps not surprising that cell culture studies suggest that the most common naturally arising chromosome segregation defect is a merotelic attachment giving rise to a lagging chromosome and potentially a micronucleus (13-15). One study of a chromosomally stable human colorectal cancer cell line observed micronuclei forming after 0.13% of mitoses (16). In the majority (90%) of these cases, the micronucleus was in the correct daughter cell, indicating that although the chromosome was lagging, it did segregate into the proper daughter cell to produce euploid progeny. In the remaining 10% of cases, however, the micronucleus was in the incorrect daughter cell, indicating that the lagging chromosome had segregated into the same daughter cell as its sister chromatid to generate two aneuploid daughter cells. From this study, the basal rate at which chromosomes missegregate into incorrect daughter cells to generate aneuploid progeny was estimated to be once every $10^3$-$10^4$ cell divisions.

When the rate of chromosome missegregation is elevated above baseline, the condition is known as chromosome instability. This can arise by many mechanisms. Not surprisingly, disrupting components of the spindle assembly checkpoint leads to a dramatic increase in chromosome missegregation. For example, mutations in Bub1b, the gene encoding the checkpoint component BubR1, cause chromosome missegregation and aneuploidy in up to one-third of cells in mice and humans (17-19). Alternatively, there can be situations that create aberrant microtubule-kinetochore attachments that are not properly detected by error correction and the spindle assembly checkpoint. For example, DNA damage and chromosome rearrangements can produce chromosomes lacking centromeres (acentric chromosomes) or chromosomes with two centromeres (dicentric chromosomes), both of which can attach improperly to the
microtubules yet not activate the spindle assembly checkpoint (20,21). Additionally, defects in sister chromatid cohesion, which allow sister chromatids to be pulled more easily from one another, can effectively hide aberrant attachments from the error correction pathway (22). Finally, given the propensity for merotelic attachments to missegregate, any situation that increases the frequency of merotely will increase the rate of chromosome missegregation. Impaired formation of the mitotic spindle increases the frequency of merotelic attachments. This can happen if centrosome separation is delayed or if a cell enters mitosis with more than two centrosomes (23). The presence of supernumerary centrosomes is common in polyploid cells and cancer cells, and studies of such cells in culture have shown that these cells initiate mitosis with multipolar spindles and then cluster the centrosomes into two poles to divide in a bipolar fashion, a process that leads to a high frequency of merotelic attachments (Figure 4) (24,25).

**Figure 4**

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**Consequences**

*Figure 4. Mitosis in the presence of supernumerary centrosomes.* Mitotic progression in the presence of two (top panel) or more than two (bottom panel) centrosomes. In the presence of supernumerary centrosomes, cells begin mitosis with a multipolar spindle and eventually cluster the centrosomes into two poles, a process that increases the frequency of merotelic attachments.

**Chromosome Damage**
Errors in chromosome segregation can have significant consequences even if the chromosome does not ultimately missegregate into the incorrect daughter cell. If lagging chromosomes are near the spindle midzone at the onset of cytokinesis, they can become entrapped by the cleavage furrow and experience extensive DNA damage (26). Moreover, if the lagging chromosome becomes a micronucleus, it can experience even further DNA damage and possible chromosome rearrangement as micronuclei have impaired import of DNA replication and repair factors (27,28). Thus, regardless of where a lagging chromosome ultimately segregates, it can experience considerable DNA damage with significant consequences for cell fitness.

**Aneuploidy**

If a chromosome does segregate into the incorrect daughter cell, that daughter cell will now have an additional copy of the chromosome at the expense of the other daughter, and both of these cells will now be aneuploid. Changes in chromosome copy number lead to complimentary changes in the RNA and protein levels of genes encoded on that chromosome, and therefore aneuploidy can have dramatic impacts on cell physiology (29,30). Across model organisms, aneuploidy has been associated with numerous fitness defects. Aneuploid cells exhibit impaired growth, inefficient energy utilization, and proteotoxic stress (29,31-33). These adverse effects are believed to arise from the cumulative effect of hundreds of proteins being expressed outside of their physiologic range (34). Reflecting the adverse consequences of aneuploidy at the cellular level, aneuploidy present throughout an organism also has significant fitness consequences. In both mice and humans, organism-wide aneuploidy is almost always associated with embryonic lethality (35,36). In humans, only three single autosomal aneuploidies—trisomy 13, 18, and 21—can occasionally survive to term, but all of these cases are associated with severe developmental defects and reduced lifespan.

**Cancer**

In light of the many adverse consequences of aneuploidy in untransformed cells and organisms, it is perhaps surprising that aneuploidy is a widespread feature of cancer. Over 75% of solid tumors are aneuploid for at least one chromosome, and
many cancer cells exhibit high levels of chromosome instability (37). As with all other genomic alterations present in cancer, an outstanding question is whether aneuploidy is a driver or passenger in tumorigenesis. Mouse models of chromosome instability have produced conflicting results, with some models having increased rates of tumorigenesis while other models appear protected from cancer (18,38-40). It has been proposed that these differences might arise from differing degrees of chromosome missegregation, with high levels of chromosome missegregation being too disastrous for cells, but lower levels of chromosome missegregation allowing for adaptability and tumorigenesis (41). Indeed, analyses of the specific aneuploidies observed in tumors demonstrates a bias for the gain of chromosomes that have high numbers of oncogenes and loss of chromosomes that have many tumor suppressor genes (42,43). Thus, it is possible that low levels of chromosome instability, and thus aneuploidy, promote cancer by enabling cells to activate oncogenes, disrupt tumor suppressor genes, and adapt to other challenges of tumorigenesis. If chromosome instability and aneuploidy indeed drives cancer, it remains to be determined how cancer cells are able to tolerate the known adverse consequences of aneuploidy while they instead exploit it for proliferative advantage.

EXTERNAL REGULATION OF CHROMOSOME SEGREGATION

Much of our understanding of mammalian chromosome segregation—both its mechanism and fidelity—has been based on cell culture systems. These highly tractable systems have allowed for rigorous dissection of chromosome segregation and revealed key mediators and regulators of this process. These processes all occur within the cell, leading to the assumption that chromosome segregation fidelity is independent of the external environment. However, studies in cell culture systems have shown that extracellular forces can influence various aspects of chromosome segregation including its fidelity (44,45). Whether external influences are also physiologically relevant in the organism remains to be determined. Indeed, select observations argue that chromosome segregation is more complex and potentially context-dependent. Hepatocytes are polyploid and harbor supernumerary centrosomes. Studies of hepatocytes and other polyploid cells in culture have shown them to have high levels of
chromosome missegregation and aneuploidy (24,25,46,47). However, single cell sequencing of hepatocytes in the adult liver has failed to reveal appreciable levels of aneuploidy (19). In a similar vein, although chromosome instability is widespread in cancer cells, there is no universal explanation for its presence. Indeed, most chromosomally unstable cancer cells have fully functional spindle assembly checkpoints (48-50). Therefore, there is reason to suspect that chromosome segregation might harbor additional regulation or context dependence that cell culture systems have failed to reveal. In this thesis, I formally test whether chromosome segregation is cell autonomous.
METHODS

ANIMALS
C57BL/6J mice, Centrin 2-GFP mice, Cre-ER<sup>T2</sup> mice, and Itgb1<sup>flox</sup> mice were purchased from the Jackson Laboratory. Mx-Cre and Itgb1<sup>flox</sup> mice were obtained from Reinhard Fässler (Max Planck Institute of Biochemistry, Martinsried, Germany). H2B-mCherry mice were obtained from the RIKEN Center for Life Science Technologies (CLST) (Kobe, Japan). All animal procedures were approved by the local animal welfare committees (Massachusetts Institute of Technology Committee on Animal Care and Kantonales Veterinäramt Zürich).

TISSUES
Proliferative mammary glands were harvested from pregnant females at 4.5 days gestation. Proliferative skin was harvested from embryos at 14.5 to 16.5 days gestation. Proliferative livers were harvested from pups at 8 to 10 days of age (development) and from adults 48 hours after partial hepatectomy (regeneration). Proliferative brains were harvested from embryos at 12.5 to 14.5 days gestation.

PARTIAL HEPATECTOMIES
Partial hepatectomies were performed on 8 week-old male mice as previously described with minor modifications (51). Instead of removing the entire median lobe and the gallbladder, only the right median lobe was removed while the left median lobe and gallbladder were spared. Mice were sacrificed 48 hours after surgery to process livers for immunofluorescence. For β1 integrin knockout experiments, Mx-Cre;Itgb1<sup>flox/flox</sup> or Itgb1<sup>flox/flox</sup> mice were injected intraperitoneally with poly-IC (250 mg, Amersham Biosciences) over three days and partial hepatectomies were performed 14 days after the final injection.

TISSUE DISSOCIATION
To isolate mammary epithelial cells, mammary glands from a single 6 to 8 week-old female mouse were harvested and digested in 10 mL DMEM-F12 with 2.5 mM L-
glutamine, 25 mM HEPES, 100 U/mL and 100 µg/mL penicillin-streptomycin, 20 µg/mL Liberase TM (Roche), and 150 U/mL collagenase III at 37 °C with 5% CO₂ for 16 hours. Digested tissue was resuspended with gentle trituration, diluted with 25 mL PBS, and pelleted at 1,000 RPM at room temperature for 5 minutes. The supernatant was aspirated to remove all but 5 mL of PBS and an equal volume of 0.25% trypsin-EDTA was added. Digested tissue was trypsinized at 37 °C with 5% CO₂ for 45 minutes with occasional agitation. Trypsin was inactivated by adding 25 mL of DMEM-F12 with 2.5 mM L-glutamine, 25 mM HEPES, 100 U/mL and 100 µg/mL penicillin-streptomycin, and 10% FBS. Cells were treated with 100 µL of 1 mg/mL DNase and pelleted at 1,000 RPM at room temperature for 5 minutes. Cells were resuspended in phenol red-free Mammary Epithelial Basal Medium (MEBM) with growth factors (Lonza). To isolate keratinocytes, neonatal (one day-old) mice were decapitated and cleaned with betadine followed by isopropyl alcohol. Skin was removed and floated on 0.25% trypsin in PBS at 4 °C for 16 hours. Epidermis was peeled from dermis, transferred to DMEM with 10% FBS, minced using a razor blade, and triturated using a serological pipette. Cells were centrifuged at 150 g at 4 °C for 5 minutes, resuspended in DMEM with 10% FBS, and passaged through at 70 µm mesh strainer. Cells were again pelleted, resuspended in Complete Defined Keratinocyte Serum-Free Medium (Thermo Fisher Scientific) and passaged through another 70 µm mesh strainer. To isolate neonatal hepatocytes, livers were isolated from neonatal (8-10 day-old) mice, cut into small pieces, and incubated in disruption solution (HBSS without calcium and magnesium, 25 mM HEPES, 0.5 mM EDTA, and 0.9 mM MgCl₂, pH 7.4) at 37 °C with shaking for 10 minutes. This step was repeated twice with fresh disruption solution. Liver pieces were then incubated in digestion solution (HBSS with calcium and magnesium, 25 mM HEPES, and 0.05% collagenase IV, pH 7.4) at 37 °C with shaking for 5 minutes. Remaining liver pieces were transferred to fresh digestion solution for another 5 minutes and the two digestion solutions were combined. The suspension was triturated with a serological pipette, passed through a 70 µm filter, and diluted in hepatocyte medium (DMEM with 4.5 g/L glucose, 10% FBS, 1X MEM Non-Essential Amino Acids (Thermo Fisher Scientific). Cells were pelleted at 30 g at 4 °C for 3 minutes, resuspended in 5 mL hepatocyte medium, and combined with 5 mL Percoll-HBSS (4.5 mL Percoll, 0.5 mL 10X HBSS).
Cells were pelleted at 150 g at 4 °C for 3 minutes, washed with hepatocyte media, and resuspended in SUM3 media (75% DMEM with 4.5 g/L glucose, 25% Waymouth’s MB 752/1, 0.5% FBS, 2 mM L-glutamine, 10 mM HEPES, 50 ng/mL epidermal growth factor, 1 µg/mL insulin, 30 nM sodium selenite, 10 µg/mL transferrin, 50 ng/mL somatotropin, 670 ng/mL triodo-L-thyronine). To isolate adult hepatocytes, mice were anesthetized with 2% isoflurane-oxygen delivered via nosecone. The liver was perfused with disruption solution for 10 minutes followed by digestion solution for 10 minutes by incising the portal vein with a 25G needle attached to a peristaltic pump. The inferior vena cava was incised immediately after perfusion began to allow fluid outflow. After perfusion was complete, the digested liver was transferred to 10 mL fresh disruption solution, shaken to liberate cells, triturated with a serological pipette, passaged through a 70 µm filter, and diluted with 10 mL hepatocyte medium. Cells were pelleted at 30 g at 4 °C for 3 minutes, resuspended in 12 mL hepatocyte medium, and combined with 12 mL Percoll-HBSS (10.8 mL Percoll, 0.5 mL 10X HBSS). Cells were pelleted at 150 g at 4 °C for 3 minutes, washed with hepatocyte medium, and resuspended in hepatocyte medium. To isolate neural progenitor cells, brains were isolated from embryos at 13.5 days gestation and minced into small pieces in PBS with 2% glucose. Brain pieces were transferred to 0.25% trypsin in EDTA and incubated at 37 °C for 15 minutes. An equal volume of NeuroCult proliferation medium with FBS (80% NeuroCult NSC Basal Medium, 10% NeuroCult NSC Proliferation Supplement, 10% FBS) (STEMCELL Technologies) was added to trypsin solution and tissue was triturated extremely gently using a P1000 pipette tip five times. Cells were centrifuged at 150 g at room temperature for 5 minutes. The pellet was triturated once in 1 mL NeuroCult proliferation medium with FBS, dissolved in an additional 2 mL complete embryonic NeuroCult proliferation medium with FBS, passaged through a 40 µm filter, and resuspended in NeuroCult proliferation medium with EGF (90% NeuroCult NSC Basal Medium, 10% NeuroCult NSC Proliferation Supplement, 20 ng/mL epidermal growth factor) (STEMCELL Technologies).

CELL CULTURE
To culture mammary epithelial cells in vitro, dissociated cells were cultured in MEBM with supplements on dishes coated with 30 μg/mL collagen I at 37 °C with 5% CO₂ for 24 hours. Cells were then washed with PBS three times and treated with 0.25% trypsin-EDTA at 37 °C for 45 seconds to remove contaminating fibroblasts. Remaining cells were then trypsinized with 0.25% trypsin-EDTA at 37 °C for 6 minutes and resuspended in DMEM-F12 with 2.5 mM L-glutamine, 25 mM HEPES, 100 U/mL and 100 μg/mL penicillin-streptomycin, and 10% FBS. Cells were pelleted at 1,000 RPM for 5 minutes, washed with PBS, resuspended in MEBM with supplements, 1 μM progesterone, 1 μg/mL prolactin, plated on coverslips coated with 30 μg/mL collagen I, and incubated at 37 °C with 5% CO₂ for 24 hours. To culture mammary epithelial cells as spheroids, cells were trypsinized and washed as above and resuspended in MEBM with supplements at 7 x 10⁵ cells per mL. Cells were then combined with four volumes of phenol red-free, growth factor-reduced Matrigel (Corning) and 100 μL gels were cast in individual wells of 24-well glass-bottom plates (Mat Tek). Gels were polymerized at 37 °C for 30 minutes before adding 500 μL of MEBM with supplements, 1 μM progesterone, 1 μg/mL prolactin. Gels were cultured at 37 °C with 5% CO₂ for 24 to 96 hours, with medium changed after 48 hours of culture. To deplete β1-integrin in spheroids, 100 nM 4-hydroxytamoxifen was added to the medium of Cre-ER<sup>T2;Itgb1<sup>±/±</sup>flox/flox</sup> or Cre-ER<sup>T2;Itgb1<sup>1/1</sup>flox/flox</sup> mammary epithelial cells for the 24 hours of culture in a monolayer and the first 48 hours of culture in Matrigel. To inhibit the spindle assembly checkpoint, 500 nM reversine (Cayman) was added to medium 2 hours prior to fixation or imaging. To eliminate F-actin, 400 nM latrunculin A (Sigma) was added to medium 2 hours prior to fixation. To culture keratinocytes, dissociated cells were cultured in Complete Defined Keratinocyte Serum-Free Medium (Thermo Fisher Scientific) on coverslips coated with 10 μg/mL fibronectin at 37 °C with 5% CO₂ for 24 hours. To culture neonatal hepatocytes, dissociated cells were cultured in SUM3 medium on coverslips coated with 30 μg/mL collagen I at 37 °C with 5% CO₂ for 48 hours. To culture adult hepatocytes, dissociate cells were cultured in SUM3 medium on coverslips coated with 30 μg/mL collagen I at 37 °C with 5% CO₂ for 48 hours. To culture neural progenitor cells, dissociated cells were cultured in NeuroCult proliferation medium with EGF on
coverslips coated with 5 µg/mL CellAdhere Laminin-521 (STEMCELL Technologies) at 37 °C with 5% CO₂ for 48 hours.

**LIVE IMAGING**

Mammary epithelial cells from Centrin 2-GFP;H2B-mCherry mice were combined with Matrigel as described above and gels were cast in 35 mm glass-bottom dishes (MatTek). Dishes were imaged under an incubated stage set to 37 °C with 5% CO₂ (Pathology Devices). Images were acquired using a CSU-22 spinning disc confocal head (Yokogawa) with Borealis modification (Andor) mounted on an Axiovert 200M microscope (Zeiss) with a 40X water immersion objective, an Orca-ER CCD camera (Hamamatsu), and MetaMorph acquisition software (Molecular Devices). Only cells that entered mitosis after imaging began were considered for analysis. If the cell did not enter anaphase before imaging ceased, it was analyzed only if it had been in mitosis for at least 60 minutes. In these cases, the duration of prometaphase to anaphase was the time interval from the start of mitosis to the end of imaging.

**IMMUNOSTAINING**

Tissues were fixed in 4% paraformaldehyde in PBS for 16-24 hours. Fixed tissues were washed with PBS, cryoprotected with 30% sucrose, and frozen in O. C. T. Compound (Tissue-Tek). Slides with 30 µm thick sections were prepared using a cryostat and stored at -80°C until use. Slides were dried at room temperature for 4-8 hours, rehydrated in PBS for 15 minutes and boiled in sodium citrate buffer (10 mM tri-sodium citrate dihydrate, 0.05% Tween 20, pH 6.0) for 20 minutes. Slides were washed in PBS, dried briefly, and sections outlined with a hydrophobic pen. Sections were incubated with extraction buffer (1% Triton X-100 in PBS) for 15 minutes followed by incubation in blocking solution (3% BSA, 0.3% Triton X-100 in PBS) for 1 hour at room temperature. Sections were incubated with primary antibodies diluted in blocking solution at room temperature for 16-24 hours. Sections were washed three times with blocking solution for 10 minutes each and then incubated with secondary antibodies diluted in blocking solution with 5 µg/mL Hoechst 33342 (Thermo Fisher Scientific) at room temperature for 1-2 hours. Sections were washed with blocking solution for 5 minutes twice and once
with PBS for 5 minutes. Sections were mounted with ProLong Gold Antifade Reagent (Life Technologies). To immunostain cultured cells, cells were washed with PBS and fixed with 4% PFA in PBS at room temperature for 5 minutes followed by ice-cold methanol at −20°C for 5 minutes. Fixed cells were washed with PBS for 5 minutes, permeabilized with 0.1% Triton X-100 in PBS (0.1% PBST) for 10 minutes, and blocked with 4% BSA in 0.1% PBST for 20 minutes. Primary antibodies were diluted in blocking solution and applied for 30 minutes. Cells were washed with 0.1% PBST for 5 minutes thrice. Secondary antibodies were diluted in blocking solution with 5 µg/mL Hoechst 33342 dye (Thermo Fisher Scientific) and applied for 30 minutes. Cells were washed with 0.1% PBST for 5 minutes twice followed by PBS for 5 minutes. All incubations were performed at room temperature. Cells were mounted with ProLong Gold Antifade Mountant (Thermo Fisher Scientific). To immunostain spheroids, gels were washed with PBS and fixed with 4% PFA in PBS at room temperature for 10 minutes followed by ice-cold methanol (or 1:1 methanol:acetone for p150) at −20 °C for 10 minutes. Fixed gels were washed with PBS for 5 minutes, permeabilized with 0.5% Triton X-100 in PBS (0.5% PBST) for 20 minutes, and blocked with 10% goat serum, 0.2% BSA, 0.2% Triton X-100, 0.1% Tween 20 in PBS for 1 hour with rocking. Primary antibodies were diluted in blocking solution and applied for 16-24 hours with rocking. Gels were washed with 0.2% BSA, 0.2% Triton X-100, 0.1% Tween-20 for 10 minutes thrice. Secondary antibodies were diluted in blocking solution with 5 µg/mL Hoechst 33342 dye (Thermo Fisher Scientific) and applied for 1-2 hours. Gels were washed for 10 minutes twice followed by PBS for 10 minutes. All incubations were performed at room temperature in the original glass-bottom wells. Gels were coated with ProLong Gold Antifade Mountant (Thermo Fisher Scientific). The following primary antibodies were used: α-Tubulin-FITC (clone DM1A, 1:1,000, Sigma), Ytubulin (clone GTU88, 1:500, Sigma), keratin 14 (clone Poly19053, 1:1,000, BioLegend), cytokeratin 8 (clone EP1628Y,1:1,000, Abcam), pan-cadherin (ab6529, 1:500, Abcam), CENP-C (gift from Iain Cheeseman,1:10,000), ZO-1 (61-7300, 1:100, Thermo Fisher Scientific), α6 integrin (clone MAB1378, 1:250, Millipore), and p150 (1:100, BD Biosciences). All secondary antibodies were various AlexaFluor conjugates (1:1,000, Thermo Fisher Scientific). The Ytubulin and p150 antibodies were directly conjugated using the AlexaFluor 568 Antibody Labeling Kit.
AlexaFluor 568 Phalloidin (1:500, Thermo Fisher Scientific) was added to secondary antibody solution when desired to visualize F-actin. Images were acquired using a CSU-22 spinning disc confocal head (Yokogawa) with Borealis modification (Andor) mounted on an Axiovert 200M microscope (Zeiss) with a 63X oil immersion objective (Zeiss), an Orca-ER CCD camera (Hamamatsu), and MetaMorph acquisition software (Molecular Devices). To quantify F-actin, four equally spaced lines were drawn through each cell and the phalloidin intensity was measured at each point on the cortex and at two equidistant points in the cytoplasm (ImageJ).

**FLUORESCENCE IN SITU HYBRIDIZATION (FISH)**

Liver sections were immunostained for pan-cadherin as described above. After final washes, sections were fixed with 2% (wt/vol) paraformaldehyde in PBS for 10 minutes and washed three times with PBS for 5 minutes each. Hydrophobic ink was removed and slides were incubated in 2× SSC for 5 minutes, followed by incubation in 50% (vol/vol) formamide, 2× SSC for 2 hours. Fluorescently labeled probes targeting different regions of mouse chromosome 16 (RP23-354F11 and RP23-18M23) and human chromosome 7 (RP11-243E12 and RP11-377B19) (Empire Genomics) were diluted in hybridization buffer as per the manufacturer’s instructions and applied to sections. Sections were sealed with a coverslip and rubber cement and incubated in the dark at 45 °C for 2 hours to allow probes to infiltrate section. This was followed by incubation at 85 °C for 5 minutes to denature the DNA. Hybridization was performed in the dark at 37 °C for 48 hours. Slides were then washed with 0.4× SSC containing 0.3% Nonidet P-40 for 2 minutes at 73 °C, followed by 2× SSC containing 0.1% Nonidet P-40 for 1 minutes at room temperature. Slides were incubated in 0.05 µg/mL DAPI in 2× SSC for 30 minutes and mounted with ProLong Gold Antifade Reagent (Life Technologies). Images were acquired on a spinning disk confocal microscope (PerkinElmer) and analyzed using the Volocity software package (PerkinElmer).

**RNA SEQUENCING**

To isolate RNA from spheroids, RNA STAT-60 (Amsbio) was added to monolayers and spheroids, homogenized with a rubber policeman or 1 mL syringe plunger, and
triturated with a P1000 pipette tip. RNA was then purified by chloroform extraction according to RNA STAT-60 instructions. RNA libraries were prepared using TruSeq (Illumina) and sequenced on a HiSeq 2000 (Illumina). For quality control purposes, BEDTools (version 2.25.0) was used to count the reads falling into genes, coding regions, intronic regions, 5' or 3' UTRs, flanking 3 kb genic regions, and intergenic regions. Other basic statistics including mapping rates, ratio of sense versus anti-sense reads, and rRNA percentages were also collected for each sample. To quantify gene expression, RSEM (version 1.2.30) was used to estimate gene expression levels based on mm9 UCSC known gene annotations. The count table was imported into DESeq2 (version 1.10.1) for differential gene expression test. The gene expression (Log2FPKM) table was first filtered to remove lowly expressed and low variance genes (average <1 and variance <0.2), and the rest were used for clustering analysis using Ward's method in SpotFire and GSEA (2.2.2) analysis against MSigDB hallmark gene sets.

**SINGLE CELL SEQUENCING**

To isolate nuclei from regenerated livers, livers were harvested from mice ten days after partial hepatectomy, pressed through a 40 µM filter into sucrose buffer (250 mM sucrose, 5 mM MgCl₂, 10 mM Tris-Cl pH 7.4), centrifuged at 600 g at 4°C for 10 minutes, resuspended in sucrose buffer, and centrifuged and resuspended again. To isolate nuclei from hepatocytes expanded in culture, hepatocytes were cultured in SUM3 medium for four days, trypsinized, washed with PBS, incubated in 0.2X PBS on ice for 10 minutes, lysed with a dounce homogenizer for 8 strokes, centrifuged at 1,000 g at 4 °C for 10 minutes, resuspended in sucrose buffer, and centrifuged and resuspended again. To isolate neural progenitor cells, neural progenitor cells were cultured in NeuroCult proliferation medium with EGF for four days, washed with PBS, dissociated with StemPro Accutase (Thermo Fisher Scientific), resuspended in NeuroCult proliferation medium, and gently triturated. To isolate single nuclei or neural progenitor cells, 0.25 x 10⁴ nuclei or cells were added to 20 mL of sucrose buffer or media in a 15 cm plate. Single cells and nuclei were isolated using a homemade microaspirator and transferred to 8 µL water in a 96-well plate. The microaspirator
needle was cleaned with 10% (vol/vol) bleach followed by water after transferring each cell or nucleus. Cells and nuclei were lysed and genomic DNA amplified using the GenomePlex Single Cell Whole Genome Amplification Kit (Sigma). All reagents were added using aerosol-resistant pipette tips in a laminar flow hood. After amplification, 4 µL of each sample were analyzed by agarose gel electrophoresis. Samples producing a smear ranging from 100 to 1,000 bp were sequenced. Samples were cleaned with paramagnetic beads (Beckman Coulter), normalized to 0.2 ng/µL, and libraries prepared using NexteraXT (Illumina) preformed at 1/12 reaction volume on a Mosquito HV (TTP Labtech). Samples were sequenced using a HiSeq2000 (Illumina) using 40 nucleotide single-end reads. Sequence reads were trimmed to 40 nucleotides and aligned to the mouse genome (mm9) using BWA (0.6.1) with default options. HMMcopy (0.1.1) was used to detect copy number alterations by estimating copy number in 500-kb bins controlling for mappability [downloaded from UCSC Genome Bioinformatics (http://hgdownload.cse.ucsc.edu/goldenPath/hg19/encodeDCC/wgEncodeMapability/ or http://hgdownload.cse.ucsc.edu/goldenPath/mm9/encodeDCC/wgEncodeMapability/)] and GC content (calculated by HMMcopy gcCounter). Standard deviations (SDs) of the corrected read copies (log₂ based) from HMMcopy were computed within sliding windows (30 adjacent 500-kb bins) for all chromosomes, and the average was calculated for each chromosome. The average SDs of the three autosomes with highest variability were averaged to generate a variability score (VS). Cells with a VS exceeding 0.34 were excluded from analysis. In order to detect aneuploidy in the context of a diploid or tetraploid cell or nucleus, a log₂ ratio exceeding 0.25 was considered a chromosome gain and a log₂ ratio below -0.3 was considered a chromosome loss.
RESULTS

TISSUE ARCHITECTURE IS REQUIRED FOR CHROMOSOME SEGREGATION FIDEILTY IN EPITHELIA

To explore the autonomy of chromosome segregation, we selected three epithelial cell types: mammary epithelial cells, keratinocytes, and neonatal hepatocytes, and examined their mitoses in their native tissue and after the cells had been dissociated and expanded in culture (Figure 1A). We quantified lagging chromosomes in anaphase, defined as whole chromosomes with an intact centromere in between the main masses of segregating chromosomes, as they are alleged to be the most common chromosome segregation defect in mammalian cells (15). We never observed lagging chromosomes in tissues (Figure 1B). Surprisingly, we observed a significant increase in lagging chromosomes after cells had been dissociated from their resident tissue and expanded in culture for less than 48 hours (Figure 1B,C). Importantly, this increase in chromosome missegregation was not secondary to polyploidization or the presence of supernumerary centrosomes, as there was little to no increase in the number of cells harboring supernumerary centrosomes within the first 48 hours of culture (Figure S1A). We conclude that chromosome segregation fidelity in epithelia is dependent on the tissue environment.

The cell culture environment differs from the tissue environment in several ways. When cells are dissociated from their tissue and expanded in culture they lose their canonical adhesions to other cells and the extracellular matrix leading to a loss of their intrinsic polarity and they are exposed to a drastically different atmosphere and concentration of growth factors compared to the tissue. Any of these alterations could presumably influence chromosome segregation. To test whether loss of tissue architecture caused chromosome segregation defects, we cultured mammary epithelial cells in Matrigel as this leads to the formation of spheroids that resemble the acini of the mammary gland (52). After 48 hours in Matrigel, cells had formed small clusters that lacked pronounced apicobasal polarity (henceforth immature spheroids, Figure 1D). However, by 96 hours in Matrigel the majority of cells had formed spheroids with a single cell layer surrounding a central lumen (henceforth mature spheroids). The cells in mature spheroids harbored apicobasal polarity as evidenced by the basal localization of
the cell-matrix adhesion protein α6 integrin and the apical ribbon of the tight junction protein ZO-1 (Figure 1D). Notably, while mitotic cells in immature spheroids had lagging chromosomes at a frequency similar to dissociated mammary epithelial cells, chromosome segregation fidelity was almost completely rescued in mature spheroids (Figure 1E). As was true for dissociated cells, the increase in chromosome segregation defects in immature spheroid was not secondary to polyploidization or supernumerary centrosomes (Figure S1B). We conclude that tissue architecture—the patterns of cell-cell adhesion, cell-matrix adhesion, and cell polarity that define the organization of epithelial cells in a tissue—is required for accurate chromosome segregation and that disruption of this architecture can lead to chromosome instability.

**TISSUE ARCHITECTURE ENHANCES THE CORRECTION OF ERRONEOUS MICROTUBULE-KINETOCHORE ATTACHMENTS**

Lagging chromosomes are the product of merotelic microtubule-kinetochore attachments (13,14). These erroneous attachments are prevalent during early stages of mitosis but are typically corrected by anaphase onset through the cooperative activities of error correction and the spindle assembly checkpoint (53,54). Thus, the increased frequency of lagging chromosomes that we observe upon disruption of tissue architecture could be caused by increases in the frequency of merotely in early mitosis, impaired correction of merotely as mitosis progresses, impaired function of the spindle assembly checkpoint, or a combination thereof. The immature and mature spheroids provide an ideal system for investigating these putative mechanisms as the spheroids differ only in their duration of culture in Matrigel and the presence of architecture.

We first asked whether the effect of tissue architecture on chromosome segregation was via changes in gene expression or post-transcriptional changes. To this end, we performed RNA sequencing on spheroids after 48 and 96 hours of culture in Matrigel, when the majority of spheroids are immature and mature, respectively. Gene expression analysis revealed that spheroids after 48 and 96 hours in Matrigel were transcriptionally more similar to each other than to dissociated mammary epithelial cells cultured for the same duration (Figure S2A). Hallmark gene sets enrichment analysis revealed that genes involved in epithelial-mesenchymal transition were up-regulated in spheroids cultured for 48 hours compared to spheroids cultured for 96
hours, perhaps reflecting the absence of epithelial architecture (Figure S2B). Hallmark gene sets enriched in spheroids cultured for 96 hours included E2F targets, consistent with the observed increase in mitotic index in spheroids at 96 hours compared to 48 hours (Figure S2B). Indeed, the expression of genes promoting proliferation, such as Ccnb1, was 1.2-fold higher in spheroids after 96 hours compared to 48 hours (Figure 2A). Expression of genes involved in kinetochore-microtubule attachment, error correction, and the spindle assembly checkpoint was also elevated in spheroids grown for 96 hours compared to 48 hours, but the fold increase in these genes was similar to that of proliferative genes indicating that the expression of these genes per mitotic cell is equivalent in the two types of spheroids (Figure 2A). We conclude that tissue architecture influences chromosome segregation in a post-transcriptional manner.

To identify which aspect(s) of chromosome segregation is influenced by tissue architecture, we first performed live imaging of spheroids prepared from Centrin 2-GFP;H2B-mCherry mice to simultaneously monitor centrosome and chromosome movements in mitosis. Notably, the time from prometaphase onset to anaphase onset was significantly higher in the immature spheroids compared to the mature spheroids (Figure 2B, first and third columns). This difference was largely due to an increased duration of metaphase, the time from bipolar centrosomes to anaphase onset, suggesting that cells were arresting prior to anaphase as a consequence of spindle assembly checkpoint activation (Figure 2B, ninth and eleventh columns). Indeed, this difference between immature and mature spheroids was eliminated by treatment with the spindle assembly checkpoint inhibitor reversine (Figure 2B). These results indicate that the increase in lagging chromosomes in the absence of tissue architecture is not a consequence of defects in spindle assembly checkpoint function but rather due to an increased prevalence of merotelic attachments that prolongs checkpoint activation and, in spite of this, increases the frequency of lagging chromosomes in anaphase.

Increased merotely could result from generating more merotelic attachments in early mitosis or reduced correction of these attachments as mitosis progresses. Impaired spindle formation, either from delayed centrosome separation or the presence of supernumerary centrosomes, can increase the frequency of merotely in early mitosis (23-25). Disruption of tissue architecture did not increase the frequency of
supernumerary centrosomes (Figure S1A, B) nor did it increase the time from prometaphase onset to the formation of a bipolar spindle (Figure 2B, fifth and seventh columns). Moreover, there was no difference in the time required for chromosomes to congress to a metaphase plate after the onset of mitosis (Figure 2C). There was therefore no reason to suspect that disruption of tissue architecture increases the formation of merotelic attachments in early mitosis. To directly assess the prevalence of merotelic attachments in early mitosis, we induced precocious anaphase using reversine and quantified lagging chromosomes in mature and immature spheroids. The frequency of cells with lagging chromosomes was only mildly increased in immature spheroids compared to mature spheroids in the presence of reversine (Figure 2C) and the fold increase was far less than that observed in untreated spheroids (Figure 1E). Together these results argue that there is no dramatic increase in the frequency of merotely in early mitosis in the absence of tissue architecture, leaving the final possibility that loss of tissue architecture impairs the correction of merotelic attachments.

Error correction involves Aurora B detecting and dissociating erroneous microtubule-kinetochore attachments to produce an unbound kinetochore that activates the spindle assembly checkpoint and serves as a substrate for future microtubule binding. We noted that in 12% (3 of 26) of immature spheroid mitoses, we observed individual chromosomes being expelled from the metaphase plate and moving towards the spindle pole (Figure 2E, top panel, arrowheads). We never observed such events in mature spheroids (0 of 30, Figure 2E, bottom panel). This increase frequency of chromosome expulsion, coupled to the known increase in spindle assembly checkpoint activation, indicates that Aurora B is actively severing erroneous attachments but that proper attachments are not readily established in the immature spheroids. We speculate that tissue architecture alters the cytoskeleton in a post-transcriptional manner to facilitate proper microtubule-kinetochore as mitosis progresses.

**TISSUE ARCHITECTURE IS ESPECIALLY IMPORTANT FOR CHROMOSOME SEGREGATION FIDELITY IN POLYPLOID EPITHELIA**

If tissue architecture indeed facilitates the correction of merotelic attachments, it should be especially important in situations where merotely is increased. The adult liver
provides an ideal system for testing this prediction as adult hepatocytes are polyploid and harbor supernumerary centrosomes. Although adult hepatocytes are normally quiescent, they have tremendous regenerative capacity and proliferate extensively after a partial hepatectomy. We performed partial hepatectomies on adult mice and compared the mitoses of adult hepatocytes during regeneration to those of diploid neonatal hepatocytes during liver development. We confirmed that polyploid hepatocytes harboring supernumerary centrosomes indeed proliferate during liver regeneration (Figure S3A). Consistent with reports of hepatocytes and other cell types harboring supernumerary centrosomes in culture, these hepatocytes entered mitosis with multipolar spindles but then clustered centrosomes to enter anaphase with a bipolar spindle (24,25,46,47) (Figure 3A, S3B). However, in contrast to the cell culture studies, we observed very few lagging chromosomes during anaphase in regenerating adult livers (Figure 3B). Importantly, in our hands the surprisingly high accuracy of chromosome segregation in polyploid hepatocytes was unique to the tissue environment. We observed a dramatic increase in the frequency of lagging chromosomes when we dissociated adult hepatocytes from liver and expanded them in culture for 48 hours (Figure 3B-D). This increase in lagging chromosomes was not due to defects in centrosome clustering as we observed only a 2% increase in the frequency of multipolar spindles in anaphase in dissociated adult hepatocytes (Figure S3B). In summary, we find that polyploid hepatocytes harboring supernumerary centrosomes have high chromosome segregation fidelity in their native liver but this is completely lost upon disruption of tissue architecture. This observation supports our conclusion that tissue architecture facilitates the correction of merotelic attachments, as epithelial cells harboring supernumerary centrosomes are especially dependent on the tissue environment for chromosome segregation fidelity. This observation also explains the conflicting reports of high chromosome missegregation and aneuploidy among hepatocytes in culture but low levels of aneuploidy in the liver (19,46,47).

**CELL POLARITY IS CRITICAL FOR CHROMOSOME SEGREGATION FIDELITY**

All of the epithelial cells we analyzed thus far divide in the context of an epithelium, maintaining their native adhesion, polarity, and shape as they do so.
However, not all cell types divide in the context of an epithelium. For example, embryonic neural progenitor cells migrate into the ventricular zone to undergo division (55). Given the importance of tissue architecture for chromosome segregation, one would expect neural progenitor cells to have increased chromosome segregation defects or to have adopted autonomous mechanisms to ensure chromosome segregation fidelity. We analyzed the mitoses of neural progenitor cells both in the embryonic brain and after they had been dissociated and expanded in culture (Figure 4A). Neural progenitor cells in the embryonic brain had only a slight, insignificant increase in lagging chromosomes compared to the other tissues we studied (Figure 4B). Most importantly, chromosome segregation defects did not increase when the cells were dissociated and expanded in culture for 48 hours (Figure 4B). This prompted us to determine how neural progenitor cells retain chromosome segregation fidelity independent of the tissue environment as this could lend further insight into the specific contributions provided by tissue architecture. Like the other dissociated cell types, dissociated neural progenitors lost adhesion with neighboring cells (Figure 1A, 4A). However, unlike the other dissociated cell types, dissociated neural progenitor cells retained a shape that was reminiscent of the cells in the tissue. This shape was defined by two features. First, the neural progenitor cells in culture remained rounded, in contrast to the other epithelial cell types that became flat in culture. Second, the neural progenitor cells in culture remained bipolar in shape, in contrast to the other epithelial cell types that lost polarity and became radially symmetric (Figure 1A, 4A). We explored whether either cell rounding or polarity were important for chromosome segregation.

Cell rounding is driven by actomyosin contractility at the cell cortex (56,57). In tissues, all cell types had an enrichment of cortical actin. In culture, only neural progenitor cells retained this enrichment (Figure 4C, S4A). To test the importance of cortical actin, we treated dissociated neural progenitor cells and adult hepatocytes with latrunculin A to depolymerize actin (Figure S4B). Loss of cortical actin did not lead to chromosome segregation defects in neural progenitor cells nor did it exacerbate the chromosome segregation defects in adult hepatocytes, indicating that cortical actin is not required for chromosome segregation fidelity in these cells (Figure 4D). Moreover, cortical actin enrichment was not sufficient for chromosome segregation fidelity, as immature
spheroids had the highest enrichment of cortical actin (Figure S4A). Epithelial cell polarity is driven by extracellular matrix cues transduced intracellularly by integrins (58). To test the importance of polarity, we depleted β1 integrin in both spheroids and liver (59,60). Depleting β1 integrin in spheroids caused cells to lose polarity and occlude the lumen (Figure 4E). Loss of β1 integrin in liver did not result in a striking change in morphology but did disrupt hepatocyte polarity (Figure 4E). Notably, in both contexts, loss of β1 integrin increased the frequency of lagging chromosomes to levels reminiscent of dissociated epithelial cells. Based on these results, it appears that the cell polarity imparted by tissue architecture is the critical component facilitating chromosome segregation fidelity.

To test whether the chromosome segregation defects that we observed led to genomic alterations, we sequenced single hepatocyte nuclei from hepatocytes after proliferating in the liver during regeneration and from hepatocytes that had been expanded in culture for an equivalent number of population doublings. Compared to liver before regeneration, there was a slight and insignificant increase in the frequency of aneuploid chromosomes after regeneration (19)(Figure 4G). However, expanding hepatocytes as dissociated cells in culture led to a five-fold increase in the frequency of aneuploidy, nearly consistent with the six-fold increase in the frequency of lagging chromosomes (Figure 4G, 3B). Neural progenitor cells expanded in culture for an equivalent number of population doublings did not accumulate any aneuploidy, consistent with them not manifesting chromosome segregation defects as dissociated cells (Figure 4G). We conclude that tissue architecture, through its enhanced correction of merotelic attachments, can endow genomic stability even to polyploid epithelia. This resolves the conflicting reports of high levels of chromosome missegregation and aneuploidy in hepatocytes in culture but low levels of aneuploidy in the liver (19,46,47).
DISCUSSION

REQUIREMENT OF TISSUE ARCHITECTURE FOR CHROMOSOME SEGREGATION FIDELITY

We formally tested whether the external environment influences chromosome segregation fidelity in tissues. We found this to be true for epithelial cells. By analyzing epithelial cell mitosis in tissues and in culture, we found that epithelial cells cannot segregate chromosomes faithfully outside of their canonical tissue architecture. The cells rely on the polarity imparted by the native tissue to correct erroneous microtubule-kinetochore attachments. It therefore appears that epithelial cells are in a tradeoff with their native tissue. In order for a tissue to function properly, epithelial cells must adopt distinct adhesion and polarity and these features must be maintained through mitosis. Epithelial cells relinquish autonomy with respect to adhesion and polarity, and these features influence intracellular processes such as chromosome segregation. When tissue architecture is disrupted, these cells are unable to adapt, and chromosome missegregation ensues. This intimate relationship between epithelial architecture and chromosome stability is perhaps why biology has favored separating cell division and tissue morphogenesis, for example by inhibiting cell division during gastrulation (61,62). In this vein, it is interesting to note that appreciable levels of chromosome missegregation have been observed in cleavage-stage embryos, where cells proliferate rapidly in the absence of tissue structure (63-65).

Although we find tissue architecture to be important for chromosome segregation in all of the epithelial cell types we studied, dividing in the context of a tissue is not the only path toward chromosome segregation fidelity. Indeed, we find that embryonic neural progenitor cells, which naturally relocate to the ventricular zone to divide, not only have high chromosome segregation fidelity in the brain but also retain their polarity and chromosome segregation fidelity outside of the tissue environment. It remains to be determined what allows neural progenitor cells to be autonomous in their cellular polarity and chromosome segregation fidelity when other epithelial cell types are not. More importantly, it will be interesting to determine how cell polarity specifically affects error correction to increase chromosome segregation fidelity.
DISRUPTION OF TISSUE ARCHITECTURE AS A SOURCE OF CHROMOSOME INSTABILITY IN CANCER

Our findings have significant relevance to chromosome missegregation in cancer. Elevated chromosome missegregation, a state known as chromosome instability, is a defining feature of epithelial tumors and believed to drive both tumor development and metastasis (37,66). In spite of its ubiquity, a universal explanation for chromosome instability in cancer is lacking. Disruption of the chromosome segregation machinery does not appear to play a significant role, as chromosomally unstable cancer cell lines often have fully functional spindle assembly checkpoints (48-50). Certainly, disruption of tissue architecture occurs at all stages of carcinogenesis. Epithelial cells lose polarity during initial transformation in a primary tumor and epithelial architecture is further disrupted when cells undergo epithelial-mesenchymal transition and metastasize (67,68). Here, we show that disruption of epithelial architecture, whether by mechanical or genetic means, leads to chromosome instability. Therefore, the disruption of epithelial architecture that occurs during carcinogenesis could provide a universal path to chromosome instability regardless of the mutational background and in the presence of otherwise functional mitotic machinery. We highlight that the degree of chromosome instability caused by disruption of tissue architecture would be especially conducive to tumorigenesis. The frequencies of lagging chromosomes resulting from disruption of tissue architecture are considerably less than those resulting from disruption of the mitotic machinery. Indeed, studies have shown that moderate levels of chromosome missegregation are conducive to tumorigenesis, whereas more significant chromosome instability is incompatible with cell viability (41).

We attempted to investigate a relationship between EMT and chromosome instability in our system however this was complicated by the fact that inducing EMT also inhibits proliferation in untransformed cells. However, we note that the immature spheroids, which were chromosomally unstable, had an EMT gene expression signature. Moreover, we note that many studies have correlated chromosome instability with EMT, arguing that chromosome instability leads to EMT (69,70). However, our observations lead us to propose that the correlation reflects causation in the opposite direction, which is that loss of the epithelial phenotype leads to chromosome instability.
IMPORTANCE OF CONTEXT FOR FUNDAMENTAL CELLULAR PROCESSES

Our findings have important implications for all aspects of cell biology. We find that chromosome segregation is not faithfully recapitulated by dissociated cells in culture. Chromosome stability is critical for cell viability. This stresses the importance of culture systems that recapitulate tissue architecture for any pursuits involving long-term or many-fold expansion of epithelial cells outside of their native tissue. More broadly, our findings reveal high context dependence for even the most fundamental cellular processes. Cell culture systems have become a standard method for investigating mammalian biology due to the ease with which they can be experimentally manipulated and observed. However, these systems must be approached with caution. We expect that chromosome segregation is not the only fundamental cellular process that is not faithfully recapitulated in the cell culture environment.
SUMMARY

In this thesis, I formally tested whether chromosome segregation in tissues is influenced by external factors. By analyzing the mitoses of epithelial cells in their native tissue and as dissociated cells, I discovered that their chromosome segregation fidelity is dependent on the tissue environment. Through experiments in organoid culture systems, I showed that cell polarity is the critical component of tissue architecture facilitating chromosome segregation, and that tissue architecture ensures chromosome segregation fidelity by enhancing the correction of merotelic microtubule-kinetochore attachments as mitosis progresses. I showed that tissue architecture is especially important for chromosome segregation fidelity in polyploid hepatocytes, as these cells can segregate chromosomes faithfully in the liver but have dramatic chromosome segregation defects as dissociated cells. Together, these findings lead to the surprising conclusion that chromosome segregation in epithelial cells is a cell non-autonomous process. This has important implications for disease, as disruption of tissue architecture could explain the chromosome instability that defines and drives cancer. Moreover, this conclusion highlights the context-dependence of fundamental cellular processes and cautions against the exclusive use of cell culture systems for deciphering mammalian biology.
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**FIGURES**

**FIGURE 1**

**A.** Images of proliferative epithelial cells in tissue and dissociated cells from mammary gland, skin, and neonatal liver immunostained for epithelial markers (keratin 8 for mammary epithelial cells, keratin 14 for keratinocytes, and pan-cadherin for hepatocytes), αtubulin, and γtubulin. Scale bars, 10 µm.

**B.** Prevalence of lagging chromosomes in anaphase of epithelial cells from tissue and dissociated cells from mammary gland, skin, and neonatal liver. Error bars indicate SD.

**C.** ZO-1 α6 integrin Hoechst

**D.** Immature Mature

**E.** Percent of Anaphases with Lagging Chromosomes

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**Figure 1.** Tissue architecture is required for chromosome segregation fidelity in epithelia.

A. Images of proliferative epithelial cells in tissue and dissociated cells from mammary gland, skin, and neonatal liver immunostained for epithelial markers (keratin 8 for mammary epithelial cells, keratin 14 for keratinocytes, and pan-cadherin for hepatocytes), αtubulin, and γtubulin. Scale bars, 10 µm.

B. Prevalence of lagging chromosomes in anaphase of epithelial cells from tissue and dissociated cells from mammary gland, skin, and neonatal liver. Error bars indicate SD.
p = 0.007 (mammary gland tissue versus dissociated cells), 0.01 (skin tissue versus dissociated cells), and 0.03 (neonatal liver tissue versus dissociated cells) by one-tailed Fisher's exact test. n > 75 anaphases per condition.

C. Images of lagging chromosomes (arrowheads) in anaphase of dissociated keratinocyte (left) and neonatal hepatocyte (right) immunostained for CENP-C. Scale bars, 5 µm.

D. Images of immature and mature spheroids after 48 and 96 hours of culture in Matrigel, respectively, immunostained for ZO-1 and α6 integrin. Scale bars, 10 µm.

E. Prevalence of lagging chromosomes in anaphases of immature and mature spheroids after 48 (immature spheroids) or 96 (mature spheroids) hours of culture in Matrigel. Error bars indicate SD. p = 0.04 by one-tailed Fisher's exact test. n > 100 anaphases per condition.
Supplemental Figure 1. Chromosome segregation defects in the absence of tissue architecture are not secondary to supernumerary centrosomes.

A. Quantification of centrosome number (γtubulin foci) in mitotic epithelial cells from mammary gland, skin, and neonatal liver in tissue and dissociated cells. n = 50 mitoses per condition.

B. Quantification of centrosome number (γtubulin foci) in mitoses of immature and mature spheroids after 48 (immature spheroids) or 96 (mature spheroids) hours of culture in Matrigel. n = 50 mitoses per condition.
FIGURE 2

**A.** Average expression (log$_2$ FPKM) of genes involved in proliferation and chromosome segregation in spheroids cultured for 48 or 96 hours in Matrigel. Error bars indicate SD. 

**B.** Duration of different aspects of mitosis in Centrin 2-GFP;H2B-mCherry immature and mature spheroids in the absence (-) and presence (+) of 500 nm reversine.

**C.** Time of chromosome congression, defined as the time spanning the appearance of individual chromosomes to their congression to a metaphase plate, in Centrin 2-GFP;H2B-mCherry immature and mature spheroids. n > 25 mitoses per condition.

**Figure 2. Tissue architecture enhances the correction of erroneous microtubule-kinetochore attachments.**

**A.** Average expression (log$_2$ FPKM) of genes involved in proliferation and chromosome segregation in spheroids cultured for 48 or 96 hours in Matrigel. Error bars indicate SD. 

**B.** Duration of different aspects of mitosis in Centrin 2-GFP;H2B-mCherry immature and mature spheroids in the absence (-) and presence (+) of 500 nm reversine. Prometaphase was defined as the first frame when individual chromosomes could be observed. Bipolar was defined as the first frame when centrin foci were bipolar. Anaphase was defined as the first frame that chromosomes were pulled toward opposite spindle poles. $p = 0.04$ (prometaphase to anaphase in immature versus mature spheroids) by two-tailed unpaired t-test. n > 16 mitoses per condition.

**C.** Time of chromosome congression, defined as the time spanning the appearance of individual chromosomes to their congression to a metaphase plate, in Centrin 2-GFP;H2B-mCherry immature and mature spheroids. n > 25 mitoses per condition.
D. Quantification of lagging chromosomes in anaphases of immature and mature spheroids in the presence of 500 nm reversine. n > 35 anaphases per condition.
E. Time lapse images of mitotic cells in Centrin 2-GFP;H2B-mCherry immature (top panel) and mature (bottom panel) spheroids. Dots represent positions of Centrin 2-GFP foci when they could be visualized. Arrowheads mark chromosomes expelled from metaphase plate in immature spheroids. Number indicates minutes since prometaphase onset. Only H2B-mCherry channel is shown.
Supplemental Figure 2. Immature and mature spheroids are transcriptionally more similar than dissociated cells of equivalent duration in culture.

A. Ward’s clustering of dissociated mammary epithelial cells grown in culture and as spheroids for 48 and 96 hours based on expression (log₂ FPKM) of protein-coding genes by RNAseq.

B. Hallmark gene sets enriched in spheroids after 48 and 96 hours of culture in Matrigel using a false discovery rate (FDR) cutoff of 5%. The FDR for each gene set is indicated in parentheses.
Figure 3. Tissue architecture is especially important for chromosome segregation fidelity in polyploid epithelia.

A. Images of hepatocytes in prometaphase, metaphase, and anaphase during neonatal development and adult liver regeneration following partial hepatectomy immunostained for pan-cadherin, α-tubulin, and γ-tubulin. Scale bars, 5 μm.

B. Prevalence of lagging chromosomes in anaphases of hepatocytes during neonatal development and adult regeneration in the liver and as dissociated cells. Error bars indicate SD. p = 0.0004 (adult regeneration in liver versus as dissociated cells) by two-tailed Fisher’s exact test. n > 50 anaphases per condition.

C. Images of proliferative hepatocytes following partial hepatectomy in liver and adult hepatocytes expanded as dissociated cells immunostained for pan-cadherin, α-tubulin, and γ-tubulin. Scale bars, 10 μm.

D. Images of lagging chromosomes (arrowheads) in anaphase of dissociated adult hepatocytes immunostained for CENP-C. Scale bars, 5 μm.
Supplemental Figure 3. Polyploid hepatocytes proliferate during liver regeneration and divide in a bipolar fashion.

A. Ploidy of hepatocytes in interphase, prometaphase, and anaphase during neonatal development and adult regeneration in the liver as inferred by chromosome 16 FISH (interphase) and centrosome number (γ-tubulin foci, prometaphase and anaphase). n = 100 for interphase FISH, n > 50 for γ-tubulin foci in prometaphase, n > 25 for γ-tubulin foci in anaphase.

B. Quantification of bipolar and multipolar anaphases in hepatocytes during neonatal development, adult liver regeneration, and as dissociated cells. n > 50 anaphases per condition.
FIGURE 4

A. Tissue vs Dissociated cells

αTubulin, βTubulin, Hoechst

B. Percent of Anaphases with Lagging Chromosomes

C. Tissue vs Dissociated cells

Embryonic brain

D. Percent of Anaphases with Lagging Chromosomes

DMSO, Latrunculin A (400 nM)

E. Cre-ER²; Itgb1⁺/⁺ vs Cre-ER²; Itgb1⁺/+poly-IC

ZO-1, α6 Integrin, Hoechst

F. Percent of Anaphases with Lagging Chromosomes

Cre-ER²; Iggb1⁺⁺, Cre-ER²; Iggb1⁺/+poly-IC

G. Percent of Anaphases with Lagging Chromosomes

Before regeneration, After regeneration, Dissociated cells, Tissue, Dissociated cells

Adult hepatocyte nuclei, Neural progenitor cells
Figure 4. Cell polarity is critical for chromosome segregation fidelity
A. Images of proliferative cells in tissue and dissociated cells from embryonic brain immunostained for α-tubulin and γ-tubulin. Scale bars, 10 µm.
B. Prevalence of lagging chromosomes in anaphase of embryonic brain cells in tissue and as dissociated cells. Error bars indicate SD. n > 100 anaphases per condition.
C. Images of proliferative cells from adult liver and embryonic brain in tissue and as dissociated cells stained for phalloidin to label F-actin and Hoechst. Scale bars, 10 µm.
D. Prevalence of lagging chromosomes in dissociated cells from embryonic brain and adult liver in presence of DMSO or 400 nM latrunculin A. Error bars indicate SD. n > 100 anaphases per condition.
E. Images of control and β1 integrin knockout spheroids after 96 hours of culture in Matrigel immunostained for ZO-1 and α6 integrin and images of adult control and β1 integrin knockout livers stained for hematoxylin and eosin. Scale bars, 10 µm (top panel) and 200 µM (bottom panel).
F. Prevalence of lagging chromosomes in anaphase of control (Cre-ER\textsuperscript{T2}; Itgb1\textsuperscript{+/F}) and β1 integrin knockout (Cre-ER\textsuperscript{T2}; Itgb1\textsuperscript{F/F}) spheroids after 96 hours of culture in Matrigel and control (Igtb1\textsuperscript{F/F} + poly-IC) and β1 integrin knockout (Mx-Cre; Igtb1\textsuperscript{F/F} + poly-IC) livers 48 hours after partial hepatectomy. +4-OHT = 100 nM 4-hydroxytamoxifen added to culture media to activate Cre-ER\textsuperscript{T2}. +poly-IC = 250 mg poly-IC injected into mice twice over three days two weeks prior to partial hepatectomy to activate Mx-Cre. Error bars indicate SD. p = 0.05 (control versus β1 integrin knockout spheroids) and 0.02 (control versus β1 integrin knockout regeneration) by one-tailed Fisher’s exact test. n > 100 anaphases per condition.
G. Percent of aneuploid chromosomes in adult hepatocyte nuclei from liver before regeneration, after regeneration, and after expansion as dissociated cells, and neural progenitor cells from embryonic brain and after expansion as dissociated cells determined by single nucleus or cell sequencing. Data for liver before regeneration and embryonic brain are from Knouse et al. 2014. p = 0.025 (adult hepatocyte nuclei after regeneration versus after expansion as dissociated cells) by two-tailed Fisher’s exact test. n > 25 nuclei or cells per condition.
Supplemental Figure 4. Epithelial cells lose cortical actin enrichment when dissociated from their native tissue.

A. Ratio of cortical to cytoplasmic actin inferred by phalloidin staining in tissue and dissociated cells from mammary gland, neonatal liver, adult liver, and embryonic brain as well as in immature and mature mammary spheroids. p = 0.0029 (mammary gland), <0.0001 (mammary spheroids, neonatal liver, and adult liver), and 0.0017 (embryonic brain) by two-tailed unpaired t-test. n > 10 cells per sample.

B. Images of proliferating cells dissociated from adult liver and embryonic brain after treatment with 400 nM latrunculin A stained for phalloidin and Hoechst.