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REVIEW

Mechanisms driving acentric chromosome transmission

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Abstract The kinetochore-microtubule association is a core, conserved event that drives chromosome transmission during mitosis. Failure to establish this association on even a single chromosome results in aneuploidy leading to cell death or the development of cancer. However, although many chromosomes lacking centromeres, termed acentrics, fail to segregate, studies in a number of systems reveal robust alternative mechanisms that can drive segregation and successful poleward transport of acentrics. In contrast to the canonical mechanism that relies on end-on microtubule attachments to kinetochores, mechanisms of acentric transmission largely fall into three categories: direct attachments to other chromosomes, kinetochore-independent lateral attachments to microtubules, and long-range tether-based attachments. Here, we review these "non-canonical" methods of acentric chromosome transmission. Just as the discovery and exploration of cell cycle checkpoints provided insight into both the origins of cancer and new therapies, identifying mechanisms and structures specifically involved in acentric segregation may have a significant impact on basic and applied cancer research.

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Abbreviations

APC/C	Anaphase-promoting complex
PtK cells	*Potorous tridactylus* cells
UFBs	Ultrafine DNA bridges
CHMP4C	Charged multivesicular body protein 4C
ESCRT-III	Endosomal sorting complexes required for transport-III

Kinetochore-microtubule interactions drive poleward chromosome transmission

In order to produce genetically identical daughter cells following mitosis, a cell must first duplicate its genome and then equally partition genetic material to its daughter cells through chromatid segregation. To accomplish this, replicated chromosomes condense and align on the metaphase plate. Sister chromatids, held together by the cohesin ring complex, biorient through the formation of stable kinetochore-microtubule connections (Tanaka 2005). The spindle assembly checkpoint ensures that chromosomes establish microtubule-kinetochore associations prior to exiting metaphase. Anaphase entry is achieved by CDK1 activation of the anaphase-promoting complex (APC/C), resulting in cyclin degradation and proteolytic severing of cohesion, followed by

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separation and poleward segregation of sister chromatids (Rahal and Amon 2008).

The critical role of the kinetochore in driving poleward chromosome movement is highlighted by elegant experiments in which either the kinetochore is ablated or the chromosome arms are severed. Elimination of the kinetochore prevents poleward transport, while severing of the chromosome arms does not disrupt poleward transport of the kinetochore-bearing chromosome fragment (McNeill and Berns 1981; Uretz et al. 1954). In addition, mutations of the conserved kinetochore protein Ndc80 cease chromosome segregation in multiple organisms (for review, see Tooley and Stukenberg 2011). Chemical detachment of kinetochores from the chromosomes yields kinetochore fragments that still move poleward (Brinkley et al. 1988), demonstrating that kinetochores are sufficient for mediating the poleward movement of chromosomes. Kinetochore-mediated transport requires the formation of a mitotic spindle. The spindle is composed of three classes of microtubules: K-fibers, astral microtubules, and interpolar microtubules (Merdes and Cleveland 1997). K-fibers provide the major force driving chromosome segregation as they emanate from the spindle pole and connect directly to the kinetochore. During the initial stages of chromosome segregation, microtubule depolymerization at the kinetochore drives poleward motion (Inoué and Salmon 1995; Asbury 2017). Fluorescent labeling of microtubule segments reveals K-fibers also undergo poleward flux during late anaphase through enhanced depolymerization at the poles (Mitchison 1989). The minus end-directed motor protein dynein is associated with kinetochores (Bader and Vaughan 2010), although whether it provides a force driving chromosome segregation remains unclear.

The functional significance of kinetochore-microtubule attachments is underlined by the fact that chromosomes lacking centromeres, and therefore unable to build kinetochores, often fail to segregate in mitosis, resulting in the formation of harmful micronuclei (Fenech et al. 2011). DNA contained inside a micronucleus is lost during subsequent rounds of cell division or is reincorporated in the nucleus after having undergone a dramatic DNA damage event termed chromothripsis (Crasta et al. 2012; Zhang et al. 2015; Ly et al. 2017). Micronucleus loss leads to aneuploidy, often resulting in cell death. Chromothripsis can produce mutants in tumor suppressor genes and may be an initiating event in the development of cancer (Stephens et al. 2011).

The obvious conclusion from these observations is that kinetochores are essential for chromosome transmission. However, careful observations in multiple insect species, yeast, mammalian, and plant cells reveal examples of successful transmission of chromosomes lacking a centromere/kinetochore (see Table 1). The study of these chromosomes, known as acentrics, provides insights into kinetochore-independent mechanisms driving the behavior of intact chromosomes, including their congression and segregation (for review, see Fuge 1990; Maiato et al. 2017). Here, we will review past and recent studies of acentric transmission that provide insights into the underlying mechanisms.

Acentric chromosomes arise through a variety of mechanisms

Perhaps the most common means of acentric formation occurs when a cell enters and exits metaphase with an improperly or unrepaired double-stranded DNA break (Fenech et al. 2011). The unrepaired break generates two chromosome fragments: one containing a telomere, a centromere, and a broken end, and the other containing only a telomere and a broken end (Fig. 1a). Lacking its centromere, the acentric fragment cannot establish a kinetochore and attach to the mitotic spindle through canonical methods.

Acentrics are also generated through translocations, such as Robertsonian translocations. Robertsonian translocations result from translocations between acrocentric chromosomes (Fig. 1b). When both breakpoints are in the short arms, the products are a short acentric chromosome and a metacentric chromosome bearing two centromeres, one of which often becomes inactivated (Morin et al. 2017). Similarly, recombination between inverted repeats on two sister chromatids results in (1) a comparable acentric chromosome containing two telomeres but no centromere and (2) a dicentric chromosome (Titen and Golic 2008) (Fig. 1b). Lacking a kinetochore, these acentrics do not form canonical attachments with microtubules.

Finally, acentrics also arise through gene amplification. Commonly found in cancer cells, these small, circular acentric chromosomes bearing oncogenes are referred to as double minutes (Cox et al. 1965; Cowell 1982) (Fig. 1c). Displaced from their regulatory elements, the genes on these extrachromosomal elements undergo increased transcription and may provide a

fitness advantage for the cancer cell (Pauletti et al. 1990). As a consequence of being circular, double minutes lack telomeres and a centromere (Wahl 1989).

Acentrics resulting from double-stranded breaks contain an unrepaired broken end, while acentrics formed from translocation/recombination events and double minutes do not have broken ends. Similarly, acentrics from double-stranded breaks and from translocation/recombination events are linear and contain telomeres, while acentrics from double minutes are circular and lack telomeres. These differences influence the behavior and segregation mechanisms of acentrics during mitosis. Therefore, we will subsequently distinguish these types of acentrics as "acentric fragments," (Fig. 1a) "double-telomere acentrics," (Fig. 1b) or "double minutes" (Fig. 1c).

Acentric chromosome transmission occurs in a diversity of eukaryotes

Fixed studies of dividing grasshopper neuronal stem cells suggested early evidence of successful mitotic transmission of acentric chromosomes (Carlson 1938a). Cells were irradiated to generate acentric fragments and then fixed throughout mitosis. In metaphase cells, the acentrics were observed at the edge of the metaphase plate clearly separated from the main mass of centric chromosomes. In early anaphase cells, sister acentrics appeared to separate at the same time as undamaged chromosomes but had not moved poleward and instead remained at the edge of the metaphase plate. However, in late anaphase, acentrics appeared to have begun to move poleward. In telophase cells, some acentrics were interpreted as reincorporating into daughter nuclei while others had formed micronuclei (Carlson 1938a).

Since this initial observation, studies in a diversity of species including yeast, insects, mammals, and plants have documented acentric chromosome transmission (Table 1). For example, in *Saccharomyces pombe*, endonuclease-mediated excision of a centromere creates acentrics analogous to double-telomere acentrics. While most cells died after removal of the centromere, presumably due to failed transmission of the acentric, some colonies survived and grew, indicating the maintenance of these acentrics through division (Ishii et al. 2008). In human cancer cell lines under selective pressure, advantageous double minutes were maintained at a high copy number over an extended period of time (Pauletti et al. 1990). Furthermore, acentric fragments were observed to move off the metaphase plate and migrate poleward in *Scadoxus multiflorus* (previously known as *Haemanthus katherinae*) cells (Bajer 1958; for review, see Östergren et al. 1960). Collectively, these studies demonstrate the occurrence of successful transmission of acentrics to daughter cells through division.

Current evidence suggests that the efficiency of acentric chromosome transmission varies widely among species. For example, successful double-telomere acentric transmission in *Saccharomyces pombe* occurs in < 1% of divisions (Ishii et al. 2008) while successful acentric fragment transmission in *Drosophila melanogaster* occurs at a very high frequency of > 80% (Royou et al. 2010). The successful acentric transmission rates for other species appear to lie within this spectrum. Successful transmission of double minutes in human cells may occur at a more moderate frequency, as double minute transmission was estimated to occur in ~ 30% of anaphase cells studied (Kanda et al. 1998). Interestingly, analysis of fixed primary human lymphocytes revealed that even though ~ 12.5% of anaphase cells had divided with acentrics, only 5% of interphase cells had micronuclei (Falck et al. 2002), suggesting that successful acentric transmission might occur roughly 60% of the time. However, estimates of acentric transmission based on the comparisons between frequencies of fixed anaphase cells with acentrics and of fixed interphase cells with micronuclei are indirect, and could potentially exclude other possible outcomes for cells dividing with acentrics (Udroiu and Sgura 2020).

Nevertheless, the existence of systems with high rates of successful acentric transmission that have been directly observed through live imaging has enabled precise characterization of the mechanisms involved in poleward acentric movement. In general, these studies reveal three distinct strategies for successful acentric transmission: (1) direct association with centromere-containing chromosomes, (2) non-canonical association with microtubules, and (3) long-range tether-based interactions with centromere-containing chromosomes. Described in the next sections are our current understandings of the cellular mechanisms involved in each of these modes of acentric transmission.

Acentric transmission through direct association with normal centric chromosomes

In theory, the most straightforward way for an acentric to be transported to a daughter cell would be to connect with a centromere-containing chromosome. A connected acentric could then "ride" poleward as the centric chromosome is pulled by the mitotic spindle to the pole. No dedicated force would be required to act on the acentric, as acentric segregation would be dependent upon the kinetochore of the centric chromosome to which it was attached. In fact, acentrics forming functional connections with centric chromosomes during mitosis have been observed in multiple species. For example, studies in fission yeast have demonstrated that double-telomere acentrics form end-to-end fusions with centromere-containing chromosomes and are transmitted through multiple cell cycles (Ishii et al. 2008; Ohno et al. 2016). In human cells, double minutes also attach to segregating chromosomes (Kanda et al. 1998). Double minutes cluster together, and clusters stick to chromosome arms (Kanda et al. 1998). Thus, when centromere-containing chromosomes segregate poleward, double minutes are also transported to daughter cells (Kanda et al. 1998).

Acentric chromosome attachment to normal centric chromosomes can occur through homologous recombination, protein scaffolds, and possibly other mechanisms such as DNA catenation. For example, double-telomere acentrics attach to centric chromosomes in *Saccharomyces pombe* via non-canonical homologous recombination between sub-telomeric sequences on the acentric and centric chromosomes (Ohno et al. 2016). This recombination restores a centromere to the former acentric, allowing for the genetic material on the acentric to be transported poleward. This interchromosomal recombination occurs between the acentric and either of the other two centric chromosomes (Ishii et al. 2008). Fusion results in loss of telomeric sequences from both chromosomes involved (Ishii et al. 2008; Ohno et al.

Table 1 Summary of examples of acentric segregation poleward. Examples of proposed instances of acentric transmission. Examples are sorted based on the type of acentric they most resemble, the cell type the acentrics were observed in, and the potential mechanism of acentric segregation (segregation mode left blank if currently unknown)

Acentric category	Organism	Potential segregation mode	References
"Acentric fragment"	*Drosophila melanogaster*	Long-range tethers + microtubules	Royou et al. 2005; Royou et al. 2010; Kotadia et al. 2012; Derive et al. 2015; Karg et al. 2015; Bretscher and Fox 2016; Karg et al. 2017; Montembault et al. 2017; Warecki and Sullivan 2018; Warecki et al. 2020; Landmann et al. 2020
	Potorous tridactylus	Long-range tethers	Humphrey and Brinkley 1969; Liang et al. 1993; Ono et al. 2017
	Nephrotoma suturalis	Long-range tethers + microtubules	LaFountain Jr et al. 2001, 2002a, b
	Chortophaga viridifasciata		Carlson 1938a; Carlson 1938b
	Saccharomyces cerevisiae	Direct association with other chromosomes	Malkova et al. 1996; Galgoczy and Toczyski 2001; Melo et al. 2001; Kaye et al. 2004
	Scadoxus multiflorus	Microtubules	Bajer 1958; Bajer 1964; Bajer et al. 1987; Bajer and Vantard 1988; Khodjakov et al. 1996
	Cyclops strenuus		Stich 1953
"Double-telomere acentric"	*Saccharomyces pombe*	Direct association with other chromosomes	Ishii et al. 2008; Ohno et al. 2016
	Drosophila melanogaster		Titen and Golic 2008
	Pales ferruginea	Microtubules	Dietz 1972; Fuge 1975;
"Double minute acentric"	*Homo sapiens*	Direct association with other chromosomes	Kanda et al. 1998; Kanda et al. 2001a
	Amaranthus palmeri	Direct association with other chromosomes	Koo et al. 2018

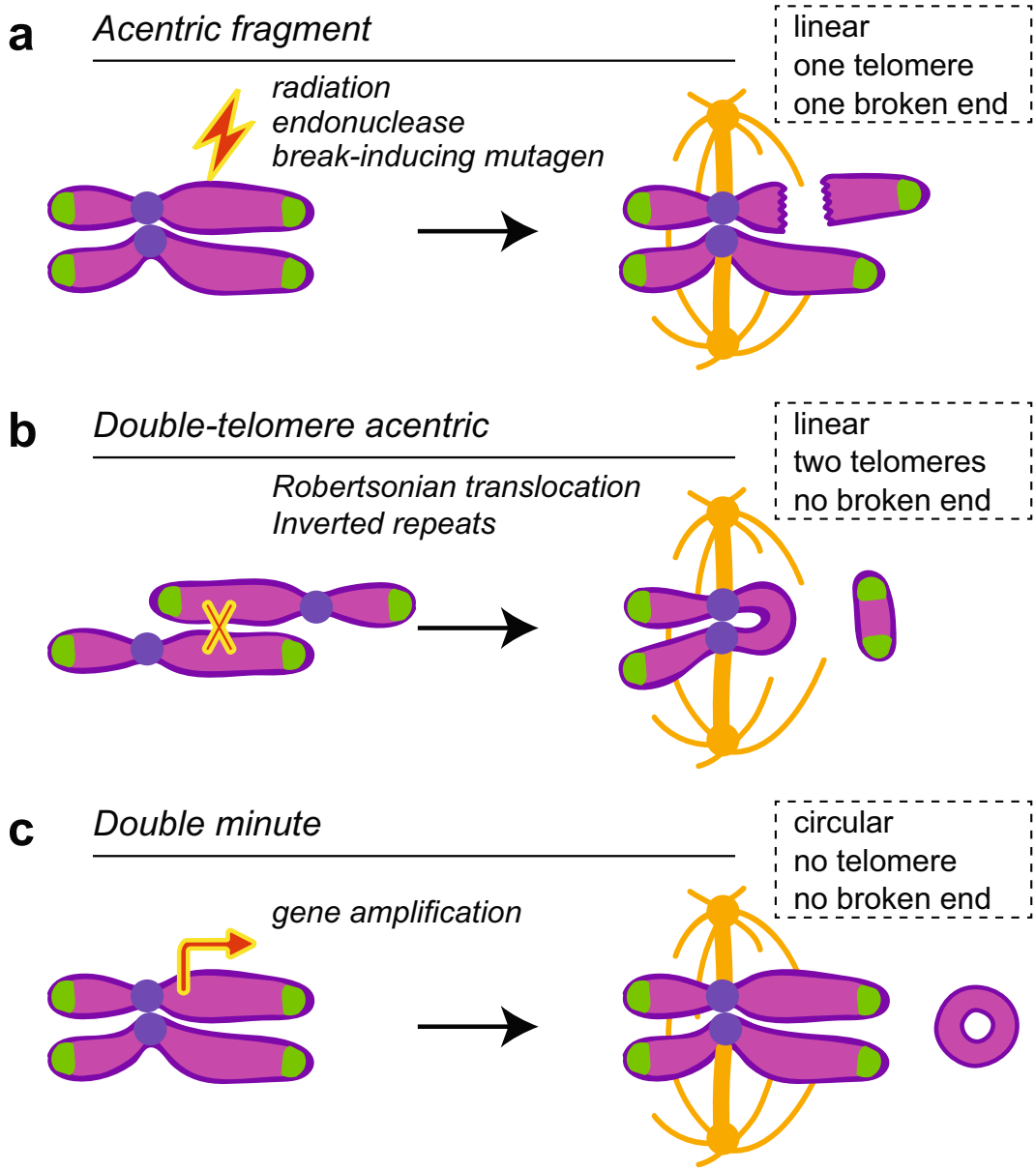

Fig. 1 Acentric chromosomes arise through distinct mechanisms. Because all of the classes described below lack a centromere, association with microtubules (orange) relies on kinetochore-independent mechanisms. **a** Acentric fragments arise from double-stranded DNA breaks that are unrepaired before entry into anaphase. These fragments are linear and contain one broken end, one telomere (green), and no centromere (blue). **b** Double-telomere acentrics arise from certain types of translocations, such as Robertsonian translocations and recombination between inverted repeats. This produces a dicentric chromosome and a linear acentric fragment possessing two telomeres (green) and no centromere (blue). **c** Double minutes arise from gene amplifications that create extrachromosomal DNA molecules. Double minutes are circular and lack both telomeres (green) and centromeres (blue)

2016). Because these fusion events are rare, occurring less than 1% of the time (Ishii et al. 2008; Ohno et al. 2016), the majority of cells in which the double-telomere acentric is induced cannot transmit the acentric and die. However, cells from the same surviving populations experience no subsequent growth defects (Ishii et al. 2008), suggesting that the required fusion event needs only to occur once for the acentric material to be competently transmitted through subsequent divisions. Consistent with this view, cells from surviving populations divided with elongated chromosome arms corresponding to the original acentric attached to a centric

chromosome (Ishii et al. 2008). Therefore, although homologous recombination between an acentric and centric chromosome might occur rarely, the result is a highly stable DNA molecule that can effectively be passed on through many divisions (Ohno et al. 2016) (Fig. 2a).

In contrast, connections between double minute acentrics and centric chromosomes may depend on protein scaffolds rather than homologous recombination. In mitotic cultured human cells, double minutes closely associate with each other and the arms of segregating centric chromosomes (Kanda et al. 1998; 2001a). The kinetochores of the centric chromosomes are then responsible for driving the poleward segregation of the double minutes. These associations persist through cytokinesis (Kanda et al. 1998), allowing double minutes to be successfully transmitted to daughters. The nature of the connection between double minutes and chromosome arms is unclear, although their behavior in mitosis suggests they possess an inherent "stickiness." It has been proposed that this stickiness is derived from scaffolding proteins associated with multiple copies of replication origins on double minutes (Kanda et al. 2001a). These scaffolding proteins might then mediate the connection between the double minutes and the arms of normal chromosomes. Interestingly, episomes of the Epstein-Barr virus, which are structurally similar to double minutes, likewise attach to mitotic chromosome arms, an association mediated by the viral EBNA-1 protein (Kanda et al. 2001b). In general, several other viral genomes utilize a similar strategy to segregate during mitosis (Feeney and Parish 2009). The distribution of double minutes to daughter cells is unequal (Kanda et al. 1998), which suggests the possibility that some divisions fail to segregate double minutes to both daughters. However, this strategy of acentric transmission is nevertheless successful, perhaps because unlike with other acentric types, double minutes are often present in high numbers within a cell (Kanda et al. 1998). In addition, double minute attachment to chromosomes is relatively common (Kanda et al. 1998). Thus, in contrast to connections formed through homologous recombination, acentric-centric connections based on protein scaffolding occur more frequently but are less permanent, requiring re-establishment every division cycle (Fig. 2b).

Another possibility is that acentrics could potentially remain connected to chromosomes through DNA catenation. Interestingly, catenation tightly links sister acentric fragments to one another in metaphase *Allium cepa* cells (Giménez-Abián et al. 2002). Topoisomerase II activity is required to resolve these catenations and allow sister acentric fragments to separate from one another (Giménez-Abián et al. 2002). After separation, acentric fragments do not appear to segregate and instead form micronuclei (Giménez-Abián et al. 2002). Nevertheless, it is possible to imagine a situation in which a double-stranded break occurring in G2 could result in one intact sister chromatid and one sister chromatid broken into a centric and an acentric fragment. Unresolved catenation could then hold the acentric fragment close to the intact sister chromatid's arm. During anaphase, the acentric would be transported poleward with the intact sister chromatid, driven by the intact sister chromatid's kinetochore.

Acentric transmission through direct association with microtubules

Early in mitosis, acentrics often move outward from the poles either towards the metaphase plate or the cell periphery. Studies indicate this movement is primarily driven by microtubules. Localization of acentric fragments to the edge of the metaphase plate was first observed in grasshopper neuroblasts (Carlson 1938a). In these cells, acentric fragments were indistinguishable from centric chromosomes until metaphase when they became positioned on the periphery of the metaphase plate (Carlson 1938a). Subsequent observations in diverse cell types revealed similar patterns of antipolar acentric fragment movement. These include prometaphase II of crane fly spermatocytes (LaFountain Jr et al. 2002a), prometaphase *Potorous tridactylus* (PtK) cells (Humphrey and Brinkley 1969; Liang et al. 1993; Khodjakov and Rieder 1996), human cells (Barisic et al. 2014), *Scadoxus multiflorus* cells (Bajer 1958; Bajer and Östergren 1963), *Taricha granulosa* cells (Rieder et al. 1986), and *Drosophila* neuroblasts (Royou et al. 2010; Karg et al. 2017). Antipolar localization to either the periphery or the metaphase plate is also observed for double minutes (Kanda et al. 2001a) and double-telomere acentrics (Fuge 1975). It is worth noting that in the early stages of division, in some systems, acentrics also move poleward before moving back towards the metaphase plate (Stich 1953; Bajer 1958; Khodjakov et al. 1996; LaFountain Jr et al. 2001, 2002a). Experiments in

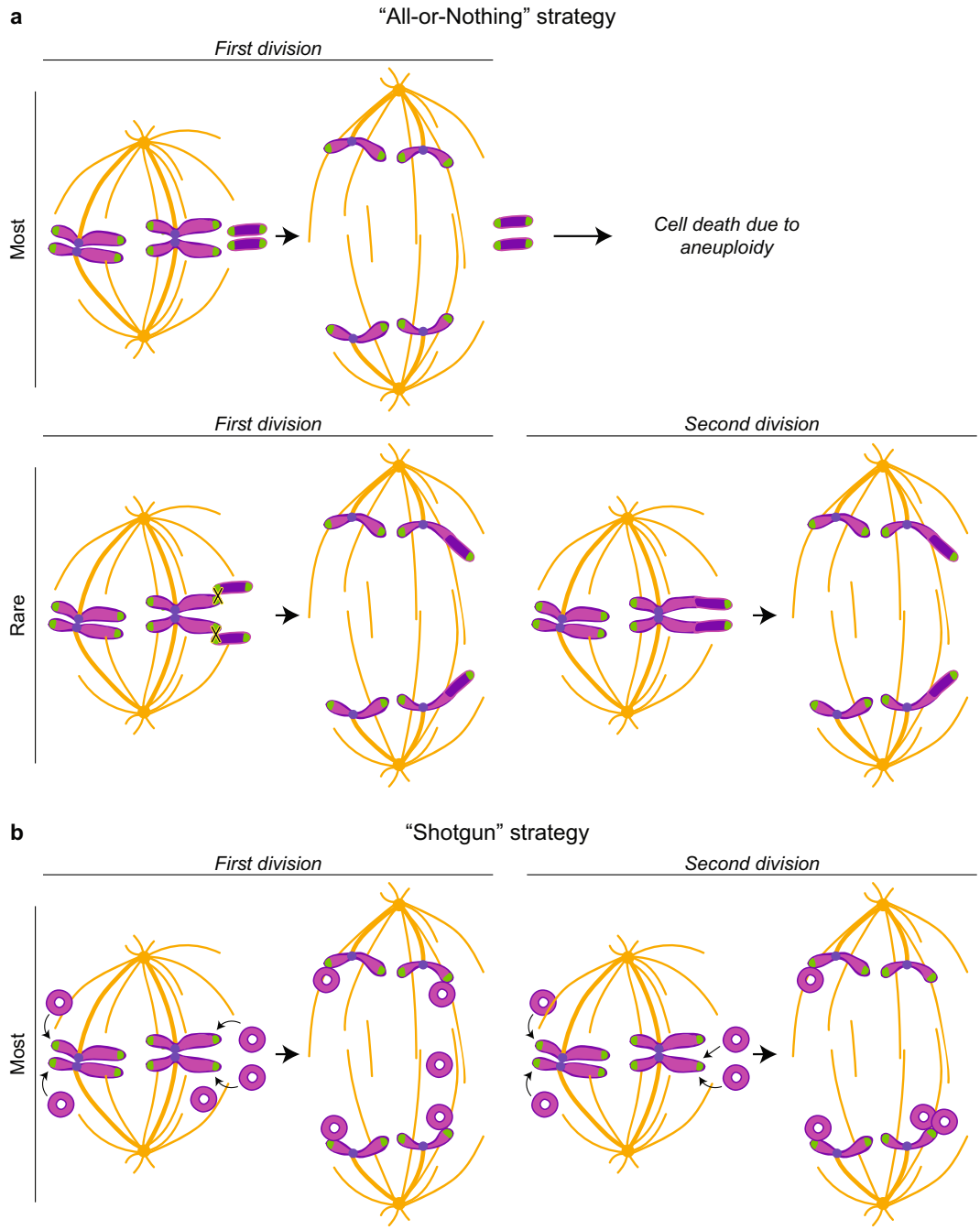

Fig. 2 Acentric transmission through association with normal chromosomes. **a** The "all-or-nothing" strategy: this is exemplified in instances in which rare recombination events occur between subtelomeric sequences on both the double-telomere acentric and centromere-containing chromosome. Although a rare event, this recombinant chromosome results in stable incorporation of the acentric fragment into an intact centromere-containing chromosome and stable mitotic transmission through multiple generations (Ishii et al. 2008; Ohno et al. 2016). **b** The "shotgun" strategy: this is exemplified by the segregation behavior of double minute chromosomes. Double minutes readily, but randomly, associate with the arms of normal chromosomes during mitosis. However, the association is temporary and must be re-established each time the cell divides (Kanda et al. 1998). This can result in unequal segregation or loss of some double minutes

Nephrotoma suturalis spermatocytes indicate that in some systems this initial acentric fragment movement poleward is mediated by microtubule flux (LaFountain Jr et al. 2001).

The simplest explanation for early, antipolar movement is through the plus-ends of growing microtubules contacting and exerting a force on the acentric. Known as polar ejection forces, they arise from the combined action of polymerizing microtubules and chromokinesins and push chromosome arms towards the metaphase plate in normal mitosis (Brouhard and Hunt 2005). Acentric chromosome fragments usually align at the outer edge of the metaphase plate encompassed by microtubules (Karg et al. 2017). Drug inhibition of microtubule flux or depletion of key chromokinesins result in failure of acentrics to properly localize to the periphery (Ault et al. 1991; Kanda et al. 2001a; Barisic et al. 2014; Karg et al. 2017). These data suggest that polar ejection forces are the key determinant for localizing acentrics to the periphery of the metaphase plate prior to anaphase.

After anaphase onset, in some systems, acentrics generally move poleward. Acentric segregation is often delayed relative to segregation of the normal centric chromosomes (Carlson 1938a; Fuge 1975; Khodjakov et al. 1996; Kanda et al. 2001a; LaFountain Jr et al. 2001, 2002a; Royou et al. 2010; Bretscher and Fox 2016). Studies in a variety of cell types demonstrate that poleward transport of acentrics relies on microtubules. Drug inhibition of microtubule flux in *Nephrotoma suturalis* spermatocytes slows poleward acentric fragment movement (LaFountain Jr et al. 2001). In late anaphase of *Drosophila* neuroblasts, microtubule bundles encompass poleward-moving acentric fragments (Karg et al. 2017). Laser ablation of these microtubules results in a cessation of acentric poleward movement (Karg et al. 2017). As acentrics are initially positioned at the outer edge of the metaphase plate, it is likely that the microtubules responsible for mediating acentric transport are primarily interpolar microtubules (Karg et al. 2017). This conclusion is supported by the fact that mutants specifically disrupting interpolar microtubules disrupt poleward migration of acentrics (Karg et al. 2017).

Acentrics exhibit extensive lateral associations with microtubules, as opposed to the canonical end-on kinetochore attachments observed in normal chromosomes. In *Drosophila* neuroblasts, robust microtubule bundles laterally encompass acentric fragments (Karg et al. 2017). Similar lateral microtubule bundles are also associated with double-telomere acentrics in *Pales ferruginea* (Fuge 1975) and acentric fragments in *Scadoxus multiflorus* (Bajer and Vantard 1988). These associations are reminiscent of the normal meiotic poleward chromosome segregation in *Caenorhabditis elegans* oocytes (Dumont et al. 2010). As with acentrics, in *C. elegans* oocytes, kinetochores are not required for chromosome segregation (Dumont et al. 2010; Muscat et al. 2015). In these meiotic divisions, lateral associations with microtubule bundles instead drive chromosome orientation and transport poleward (Wignall and Villeneuve 2009; Dumont et al. 2010; Muscat et al. 2015).

While lateral microtubule-acentric associations have been observed numerous times, the mechanisms by which these associations direct poleward force on acentrics remain unclear, although motor proteins are likely involved. In *C elegans* female meiosis, lateral microtubule-associated chromosomes rely on the minus end-directed motor protein dynein to propel their poleward segregation (Muscat et al. 2015). However, how dynein would localize to an acentric chromosome remains unclear. Studies in *Drosophila* neuroblasts reveal accurate acentric fragment segregation requires the chromokinesin KIF4A/Klp3A (Karg et al. 2017). However, how a plus end-directed motor contributes to acentric movement poleward towards the minus ends of microtubules remains to be determined. Because Klp3A depletion in these cells results in diminished arrays of interpolar microtubules (Karg et al. 2017), as described above, it is likely that Klp3A's effect on acentric segregation is indirect. Since KIF4A/Klp3A is needed for proper microtubule flux (Wandke et al. 2012), one possibility is that poleward acentric fragment transport requires microtubule flux. It is also possible that microtubule sliding during anaphase B transmits force from the elongating spindle to the acentrics via the interpolar microtubules. Additionally, an unknown chromokinesin could associate with the acentric and drive it poleward.

While both acentric fragments and double-telomere acentrics rely to some degree on attachments to microtubules for their poleward movement (Fuge 1975; Bajer and Vantard 1988; LaFountain Jr et al. 2001; Karg et al. 2017), double minutes seemingly do not. This conclusion is based on the fact that drug inhibition of microtubule flux disrupts the initial peripheral localization of double minutes but not their subsequent poleward movement (Kanda et al. 2001a).

Acentric transmission through long-range DNA tether/thread-based associations

Acentric fragments are also capable of connecting to centric chromosomes through long-range connections (LaFountain Jr et al. 2002b; Royou et al. 2010; Bretscher and Fox 2016; Ono et al. 2017). Known as DNA tethers, they are thought to connect the broken ends of the centric and acentric fragments (Royou et al. 2010). For example, in *Drosophila* larval neuroblasts, DNA tethers contain histones and a number of associated proteins, including the chromosome passenger complex components Aurora B kinase and INCENP; the cell cycle kinases BubR1, Bub3, and Polo; and the APC/C cofactor Cdc20 (Royou et al. 2010; Derive et al. 2015). The tethers are extremely efficient at promoting mitotic acentric fragment transmission. Despite induction of large acentric fragments in ~ 80% of larval cells, the larvae develop to adults, exhibiting no reduction in viability (Royou et al. 2010). Live analysis reveals that, although delayed, sister acentrics separate, move poleward, and are incorporated into daughter telophase nuclei (Royou et al. 2010). Functional studies reveal that the DNA tether and its associated proteins are essential for the proper mitotic segregation of the acentrics. Reductions in BubR1 or Polo activity result in abnormal positioning of the acentric on the metaphase plate and ultimately failed acentric segregation (Royou et al. 2010). Although the mechanism for how these proteins contribute to acentric transport remains unclear, interactions between BubR1 kinase and Bub3 lead to localized inhibition of APC/C near the tether and the acentric (Derive et al. 2015), suggesting that delayed acentric segregation may be functionally important for poleward movement.

As the tether links the acentric fragment to its centric partner, the tether may provide the force driving acentric poleward transport. However, studies revealing acentric fragments often move poleward with their telomeres leading would argue against a connection to the broken end of the acentric fragment providing this poleward pulling force (Karg et al. 2017). Additionally, it is possible that acentric fragment segregation is dependent upon the kinetochore of the centric chromosome to which the tether connects. As discussed above, microtubules provide a major transporting force for these acentric fragments (Karg et al. 2017). While the role of the tether in generating/transmitting force on/to the acentric fragment is unclear, it may be that the tether is required to maintain the acentric in the vicinity of the metaphase spindle, enabling the acentric to associate with interpolar microtubules during anaphase. In addition, as described below, the tether provides a distinct, essential role facilitating incorporation of the late-segregating acentric into the telophase nucleus (Karg et al. 2015; Warecki and Sullivan 2018).

Efficient transmission of acentrics has also been observed in the polyploid *Drosophila* papillary cells (Bretscher and Fox 2016). Acentric fragments, either generated naturally or through X-irradiation, exhibit a delayed but successful poleward migration and incorporation into daughter nuclei (Bretscher and Fox 2016). In contrast to neuroblasts, in papillary cells, DNA tethers are not observed and proper segregation does not rely on BubR1 (Bretscher and Fox 2016). Instead, acentric transmission relies on FANC2D, FANC1, and Bloom helicase activity (Bretscher and Fox 2016). Previous studies demonstrated that these proteins are associated with ultrafine DNA bridges (UFBs) (Liu et al. 2014). UFBs contain DNA, but in contrast to DNA tethers, do not contain histones or stain with DAPI (Liu et al. 2014). UFBs connect centromeres, telomeres, or fragile sites of separating sister chromatids (Liu et al. 2014). UFBs may arise due to unresolved catenations or due to incomplete replication (Liu et al. 2014) or from entanglements caused by homologous recombination (Chan and West 2018). It is thought that FANC2D, FANC1, and Bloom helicase resolve UFBs as sister chromatids separate (Liu et al. 2014). Therefore, it is possible that similar connections provide a link between acentric fragments and centric chromosomes in *Drosophila* papillary cells. Whether this UFB-like link drives acentric poleward transport or, like the DNA tethers, provides an alternative role remains to be determined.

Long-range tethers and threads are present in diverse cell types. For example, in *Nephrotoma suturalis* spermatocytes, acentric fragments are connected to centric chromosomes through telomere-telomere tethers (LaFountain Jr et al. 2002b). Similar connections are proposed to occur between acentric fragments and centric chromosomes in PtK_2 cells (Ono et al. 2017). The makeup of these tethers is unknown. In addition, fragile sites that result in lagging chromosome sections connected to main nuclei bear remarkable resemblance to acentric fragments and tethers. For example, in several genera of bluegrass, fragile sites result in lagging chromosome sections in anaphase that lack centromeres but remain connected to centric chromosomes by a thin

DNA thread (Rocha et al. 2017a; Rocha et al. 2017b). Aurora B and INCENP-coated DNA threads are also observed connecting spatially distant chromosomes during early meiosis in *Drosophila* oocytes (Hughes et al. 2009; Hughes and Hawley 2014). These threads are composed of heterochromatin and must be resolved by Topoisomerase II activity for proper division, suggesting they result from catenation (Hughes et al. 2009; Hughes and Hawley 2014).

It is tempting to speculate that the tethers connecting acentric fragments and centric chromatin must originate from the broken ends of the acentric and centric fragments. However a number of recent observations suggest tethers/threads form a variety of connections. For example, in *Drosophila* neuroblasts, the DNA binding protein barrier-to-autointegration factor, BAF, which localizes to acentric-centric tethers, is also observed localizing to tethers between sister acentrics segregating to opposing daughters (Warecki et al. 2020). Furthermore, in crane fly spermatocytes undergoing meiosis, acentric fragments generated by cutting a trailing chromosome arm travel across the equator of the cell to the opposing daughter cell (LaFountain Jr et al. 2002b). Laser ablation studies indicate this movement is mediated by a cytologically undetectable tether between the telomeres of segregating sister chromatids (LaFountain Jr et al. 2002b). Analogous experiments have shown a similar telomere-telomere tether exists in PtK_2 cells as well (Ono et al. 2017). Laser severing of chromosome arms in anaphase resulted in some acentric fragments that regularly moved to the opposing pole. A second laser cut between the telomeres of the acentric fragment and its sister chromatid halted the acentric movement (Ono et al. 2017). Taken together, these examples indicate that tethers may not only originate from the broken ends of acentric fragments but also from their telomeres.

Cellular adaptations facilitating acentric transmission

Acentric incorporation into telophase nuclei has been observed in many cell types (Carlson 1938a; Liang et al. 1993; Kanda et al. 2001a; LaFountain Jr et al. 2002a; Royou et al. 2010). Acentric segregation is often associated with striking temporal and spatial modifications during anaphase, telophase, and cytokinesis. These include delays in and/or modification of cytokinesis and nuclear envelope reassembly (Kotadia et al. 2012; Karg et al. 2015; Montembault et al. 2017). Acentrics are also associated with spindle elongation, cell elongation, and expansion of the myosin-based contractile ring (Kotadia et al. 2012; Montembault et al. 2017). These modifications during the final stage of the cell cycle likely serve to promote proper segregation and incorporation of acentrics into daughter nuclei, preserving genome integrity (Fig. 3).

Late-segregating acentrics risk blocking the ingressing cleavage furrow, resulting in furrow regression and aneuploidy. Consequently, it has been proposed that mammalian and yeast cells have evolved a mechanism known as the abscission checkpoint that delays cytokinesis until lagging chromatin has cleared the midzone (for review, see Petsalaki and Zachos 2019). According to this model, in the abscission checkpoint, Aurora B kinase phosphorylates charged multivesicular body protein 4C (CHMP4C), a key component of the endosomal sorting complexes required for transport (ESCRT)-III complex (Carlton et al. 2012). CHMP4C phosphorylation results in its sequestration in the center of the midbody in a complex with ANCHR and Vps4, another ESCRT-III component (Petsalaki and Zachos 2019). Although still unclear, it is possible that the formation of this complex at the midbody prevents Vps4 from performing membrane remodeling events at the ingression sites that are required for cytokinesis completion (Petsalaki and Zachos 2019). Further research on the proposed checkpoint is required to define its mechanism and the nature of the cytokinesis delay.

Drosophila neuroblasts rely on the alternative strategy of spindle and cell elongation for clearing late-segregating acentric fragments from the cleavage plane (Kotadia et al. 2012). It is likely that chromatin remaining on the metaphase plate during late anaphase provides signals that drive these adaptations. Support for this idea comes from the observation that in the presence of lagging acentrics, myosin and likely the cleavage furrow are both broadened, and there is an increased flow of myosin from the cleavage furrow to the cortices of daughter cells (Montembault et al. 2017). Spindle elongation, broadening of the cleavage furrow, and enriched myosin at cell cortices require RhoGEF activity (Kotadia et al. 2012; Montembault et al. 2017), which localizes to the overlapping midzone microtubules via the centralspindlin motor complex (Somers and Saint 2003). Delayed nuclear sequestration of RhoGEF in divisions with acentrics is important for

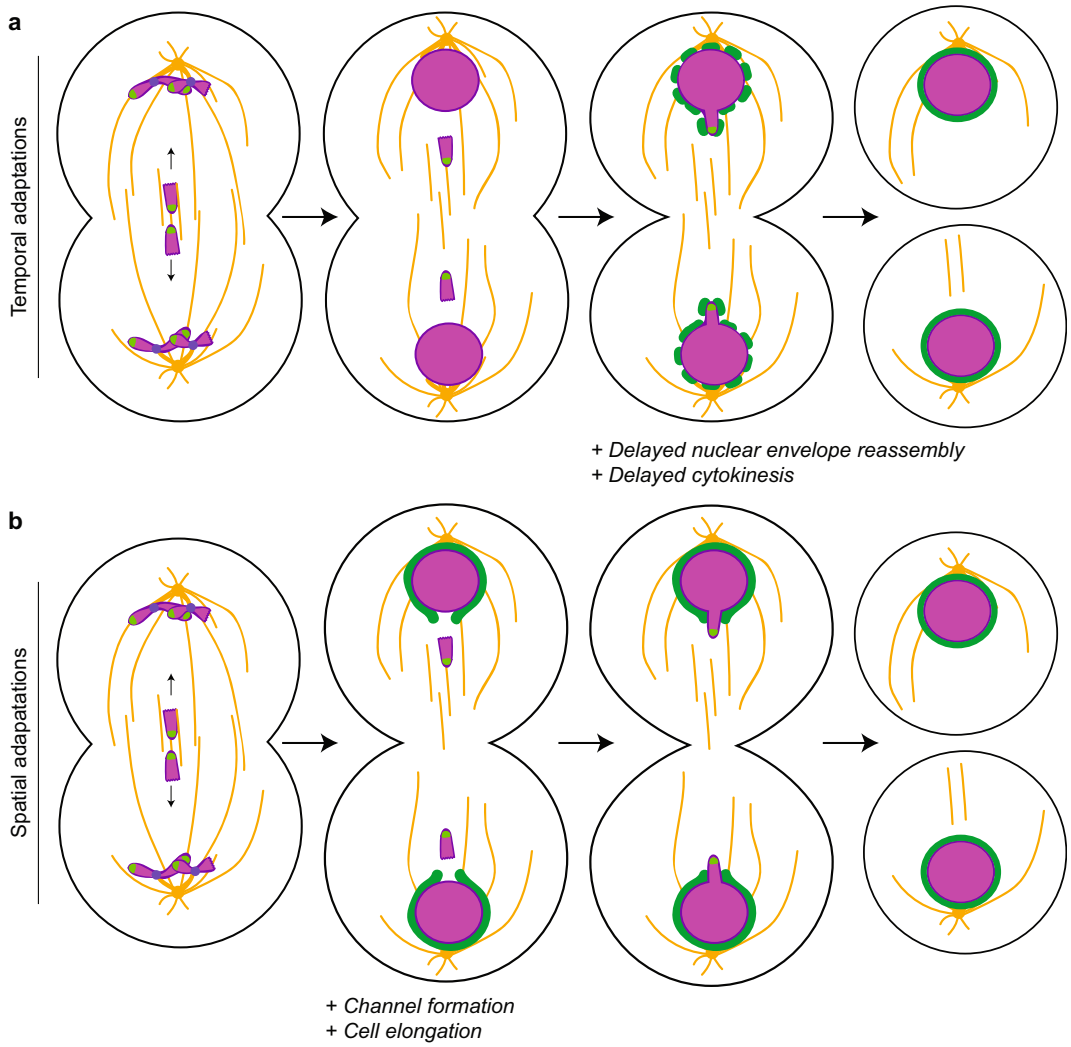

Fig. 3 Cellular adaptations that facilitate successful mitotic transmission of acentrics. **a** Temporal adaptations: global delays in nuclear envelope reassembly (dark green) provide more time for acentrics to rejoin daughter nuclei at the poles (Montembault et al. 2017). In addition, delayed cytokinesis provides enough time for lagging chromatin, including late-segregating acentric fragments, to clear the metaphase plate before cleavage furrow ingression (Petsalaki and Zachos 2019). **b** Spatial adaptations: highly specific channels in the nascent nuclear envelope (dark green) form to provide a passageway for acentrics to enter daughter nuclei (Karg et al. 2015). In addition, cell elongation provides enough space for late-segregating acentrics to clear the site of cleavage furrow ingression (Kotadia et al. 2012; Montembault et al. 2017)

driving these adaptations (Montembault et al. 2017). In addition, because the lagging acentric fragments in *Drosophila* neuroblasts are encompassed by microtubules, it is possible that these overlapping microtubules result in an increased sequestering of RhoGEF at the midzone during late anaphase, although this remains to be demonstrated.

While spindle and cell elongation facilitate clearing of late-segregating acentrics from the cleavage plane, additional adaptations are required to ensure they arrive at the poles prior to the completion of nuclear envelope assembly. Otherwise, acentrics would be "locked out" and form highly mutagenic micronuclei (Fenech et al. 2011). In general, nuclear envelope assembly begins first on the poleward-facing sides of daughter nuclei before completion on the midzone-facing sides of the nuclei (Gerlich et al. 2001). This would provide a small amount of extra time for acentrics to enter daughter nuclei. In addition, midzone-localized Aurora B kinase inhibits nuclear envelope reassembly on late-segregating acentrics until they move away from the midzone (Afonso et al. 2014) (Fig. 4a). In *Drosophila*

neuroblasts, incorporation into daughter nuclei of the late-segregating acentric fragments is also accomplished by both global and highly localized delays in nuclear envelope reassembly (Karg et al. 2015; Montembault et al. 2017). For example, in response to lagging chromatin, nuclear envelope reassembly is globally delayed on daughter nuclei (Montembault et al. 2017).

However, a global delay is not always sufficient to provide time for late-segregating acentrics to reach daughter nuclei before completion of nuclear envelope assembly (Warecki et al. 2020). In these cases, the acentric and its associated DNA tether induce formation of localized channels in the envelopes of the newly formed daughter nuclei that permit acentric passage and incorporation into daughter nuclei (Karg et al. 2015) (Fig. 4b). These channels form through all layers of the nuclear envelope (Warecki et al. 2020) and are dependent upon the activity of a pool of Aurora B kinase localized to the tether connecting acentrics to daughter nuclei (Karg et al. 2015; Warecki and Sullivan 2018). Channel formation is not due to a physical blockage of nuclear envelope reassembly by the tether, as inhibition of Aurora B activity disrupts channel formation without affecting the tether (Karg et al. 2015). The final step of acentric fragment incorporation requires fusion of the nuclear membrane on acentrics to the nuclear membrane on daughter nuclei and is thought to provide the final driving force to allow acentrics to enter daughter nuclei (Warecki et al. 2020).

The acentric fragment is associated with nuclear membrane but absent of nuclear lamin and nuclear pore complexes (Afonso et al. 2014; Karg et al. 2015; Warecki et al. 2020). This inhibition is likely due to the pools of Aurora B localizing at the midzone and to the acentric. The spatial inhibition of nuclear envelope assembly has led to the proposal of an Aurora B–mediated checkpoint surveilling the anaphase-to-telophase transition (Afonso et al. 2014; Afonso et al. 2019). It has also been argued that the spindle mediates this inhibition as opposed to Aurora B (Liu et al. 2018) (Fig. 4a). In human cancer cell lines, lagging whole chromosomes associate with nuclear membrane but not with nuclear lamin or nuclear pore complexes (Liu et al. 2018). In this case, the spindle is proposed to mediate this inhibition (Liu et al. 2018). More research is required to differentiate between these two models. As segregating acentric fragments are encompassed by microtubules (Karg et al. 2017), it would be worthwhile to determine what role these microtubules play in the exclusion of certain nuclear envelope components from the late-segregating acentric (Fig. 4).

It is interesting to note that these cellular adaptations may produce synergistic interactions. For example, the delays in nuclear envelope reassembly that allow acentric entry into nuclei are also believed to be responsible for the delayed nuclear sequestration of RhoGEF that leads to a broadening of the cleavage furrow (Montembault et al. 2017). In addition, the abscission checkpoint is also activated when nuclear pore complex assembly is defective (Mackay et al. 2010), as it is on lagging chromatin (Afonso et al. 2014; Karg et al. 2015; Liu et al. 2018) and locally on the main nuclei at the site of channels (Karg et al. 2015; Warecki et al. 2020).

Unexplored issues in acentric transmission

Acentric transmission studies have primarily focused on congression and poleward transport. As described below, much remains unknown concerning the mechanisms driving these events. In addition, there are a number of other unique aspects of the mitotic behavior of acentrics that remain unexplored and are likely to provide insight into core mechanisms of chromosome transmission.

Spatial and temporal separation of sister acentrics

Acentrics are often physically separated from centromere-containing chromosomes on the metaphase plate. In addition, acentric segregation is often considerably delayed relative to their normal centric chromosomes. This delay may be a direct consequence of the physical separation. For example, the signals that release cohesin may be much weaker at the edge of the metaphase plate. Once cohesin is removed, catenated DNA remains binding sister chromosomes. Kinetochore microtubules may provide force that efficiently resolves these catenations in conjunction with Topoisomerase II activity (Holm et al. 1985; Baxter et al. 2011). Could the severely delayed separation of sister acentrics observed in some systems result from delays in resolving catenated sister DNA? In some instances, it appears that kinetochore-mediated forces are not required to resolve catenations between sister acentrics in a timely manner

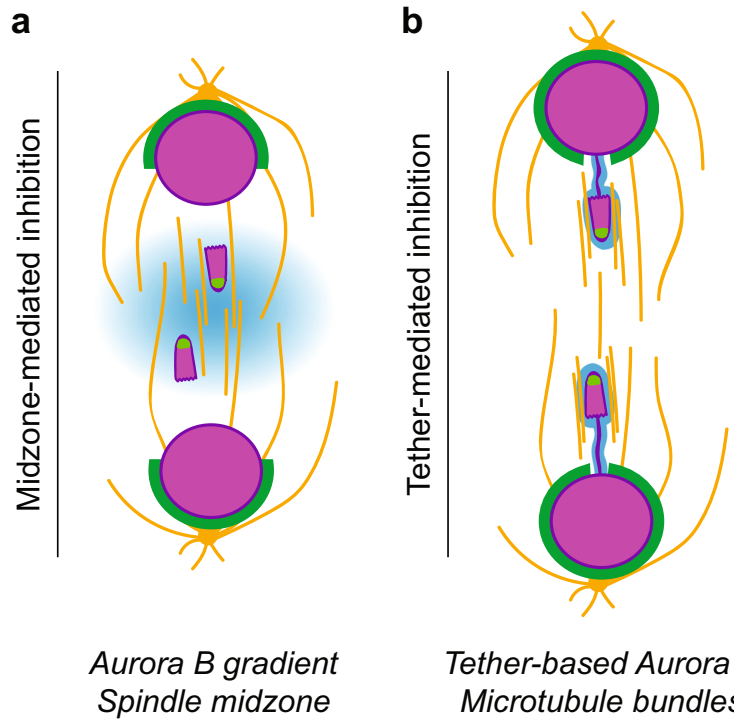

Fig. 4 Local midzone and tether-localized Aurora B signaling delays nuclear envelope reassembly in the presence of an acentric. **a** Midzone-mediated inhibition: recruitment of lamin and nuclear pore complexes is inhibited by an Aurora B gradient (blue) emanating from the midzone (Afonso et al. 2014). Inhibition may also be mediated by the high concentrations of spindle microtubules (orange) at the midzone (Liu et al. 2018). Low inhibitory Aurora B and microtubule concentrations at the poles facilitate recruitment of lamin and nuclear pore complexes and completion of nuclear envelope reassembly. **b** Tether-mediated inhibition: In *Drosophila* neuroblasts, a pool of Aurora B (blue) highly localized to the severely-delayed acentric fragment and its associated DNA tether (Royou et al. 2010) is responsible for localized inhibition of nuclear envelope assembly (Karg et al. 2015; Warecki and Sullivan 2018). The daughter nuclei, which are free of Aurora B, can recruit lamin and nuclear pore complexes. Acentrics and tethers, which are coated with Aurora B, cannot. Importantly, tether-based Aurora B activity explains the formation of channels in the nuclear envelope of daughter nuclei, which are far from the midzone. Late-segregating acentrics are surrounded by microtubule arrays (Karg et al. 2017). The contribution of these arrays to inhibition is currently unknown

(Giménez-Abián et al. 2002; Paliulis and Nicklas 2004). Nevertheless, it will be of great interest to determine both cohesin and DNA decatenation dynamics on severely delayed-separating acentrics relative to neighboring centric chromosomes. For acentric fragments, which result from damaged chromosomes, their physical and temporal separation from the normal chromosome complement may be adaptive, preventing inappropriate interactions between the two and preserving genomic stability. Might acentric fragments, which possess a broken end, be treated differently than other acentric types that have no breaks? Double minutes are also pushed to the periphery of metaphase cells (Kanda et al. 2001a). This suggests that physical separation of acentrics from centric chromosomes during metaphase may be a more general phenomenon, though more research is needed.

Accuracy of sister acentric segregation

Although acentric segregation is often delayed, acentric separation and partitioning to daughter cells can be surprisingly accurate in some systems. For example, *Drosophila* neuroblasts and papillary cells that divide with acentric fragments often produce euploid daughters (Royou et al. 2010; Bretscher and Fox 2016). Without kinetochores, it is unclear how sister acentric fragments are guided correctly to opposing poles. In *Drosophila* neuroblasts, each acentric is connected to its centric partner via a DNA tether (Royou et al. 2010). While lateral microtubule interactions are required for poleward transport of acentric fragments (Karg et al. 2017), perhaps the tether is important for connecting sister acentrics with their proper nuclei to ensure an equal partitioning of acentrics to each daughter cell.

Additionally, as the tether provides a connection between the acentric fragment and a centric chromosome, perhaps acentric transmission in *Drosophila* neuroblasts is so successful partly due to canonical kinetochore-based mechanisms acting on the connected centric chromosome. Acentric segregation does not always occur so accurately though. For example, partitioning of double minutes in human cells and acentric fragments in *Chortophaga viridifasciata*, *Scadoxus multiflorus*, and *Saccharomyces cerevisiae* appears to be more random (Kanda et al. 1998; Carlson1938a; Khodjakov et al. 1996; Kaye et al. 2004). The mechanisms that allow for accurate acentric partitioning in some cases but not others are yet to be identified.

Kinetochore-independent poleward transport forces

Much remains unknown about the forces driving acentrics poleward in most systems. For acentric fragments connected to centric chromosomes through long-range DNA tethers, it is possible that the tether provides a pulling force, although this seems unlikely. Analysis of acentric-microtubule interactions seems to suggest that interpolar microtubules are the ones important for acentric poleward transport (Fuge 1975; Karg et al. 2017). Given the close lateral association between acentrics and microtubules (Fuge 1975; Karg et al. 2017), it is likely that microtubule motor proteins drive the poleward transport of acentrics, although which ones remain unidentified. Although the chromokinesin Klp3a is required for proper acentric segregation in *Drosophila* neuroblasts, its action appears to be indirect, instead being required to form the overlap interpolar microtubules on which the acentric travels (Karg et al. 2017). Minus end-directed dynein plays a role in the poleward segregation of holocentric but kinetochore-less chromosomes in *C. elegans* oocytes (Muscat et al. 2015). Given this, might dynein provide the poleward force driving some acentric transport? If so, it is unclear how dynein would localize to an acentric chromosome.

Origin, composition, and function of the DNA tether and its relationship to UFBs

The origin, composition, and function of the DNA tether connecting acentric fragments to centric chromosomes and its relationship to UFBs are still unknown. When in the cell cycle the tethers form, if they form after S-phase, or whether they are composed of existing DNA or result from unscheduled DNA replication remains to be determined. Potential insight into this comes from studies in *Lolium* species, where fragile sites result in lagging acentric chromosome sections that are connected to centric chromosomes through DNA threads (Rocha et al. 2017a; Rocha et al. 2017b). This tether is composed of the genetic material of the fragile site (Rocha et al. 2017a). Unlike UFBs (Chan et al. 2007; Ke et al. 2011), tethers in *Drosophila* neuroblasts contain histones (Royou et al. 2010), suggesting a fundamental difference between the two structures. Cell cycle kinases and chromosome passenger proteins associate with the tether (Royou et al. 2010; Derive et al. 2015), and it is likely that additional proteins remain to be identified. As described above, insight has been gained on the function of some of these proteins, but much remains unknown. In addition, it would be interesting if poleward-moving acentrics with seemingly no connection to centric chromosomes were in fact connected to centric chromosomes by difficult-to-observe UFBs. If so, the presence of connections to centric chromosomes may be the defining feature that differentiates the small class of acentrics capable of segregation from the majority of acentrics that do not move poleward.

Fate of acentric fragments successfully incorporated into daughter nuclei

The fate of acentric fragments once successfully incorporated into telophase nuclei has not been directly investigated. The best outcome is that they re-associate with their centric counterpart and are subsequently repaired. Alternatively, continued failure to repair the double-stranded break that generated the acentric fragment may result in apoptosis (Brodsky et al. 2004; Titen and Golic 2008; Nowsheen and Yang 2012). The worst-case scenario would be for the acentric fragment to be unrepaired through the next cell cycle, fail to enter into the daughter nucleus, form a micronucleus, and undergo chromothripsis (Zhang et al. 2015).

Studying acentric segregation to identify forces acting on centric chromosomes

Studying acentric segregation reveals forces acting on centric chromosomes that might otherwise be difficult to detect due to the presence of kinetochore-mediated forces. For example, the telomere-telomere tethering observed between acentric fragments and chromosomes

segregating to the opposite pole (LaFountain Jr et al. 2002b; Ono et al. 2017) has been suggested to occur between intact centric chromosomes as well (for review, see Paliulis and Forer 2018). In these cases, the tethers are proposed to provide resistance to the poleward motion of the segregating chromosomes. Additionally, the lateral connections observed between acentrics and interpolar microtubules (Fuge 1975; Karg et al. 2017) may also occur between microtubules and the arms of centric chromosomes (for review see Fuge 1990). These connections likely provide a kinetochore-independent poleward force on centric chromosomes. With this in mind, as acentric transmission is further explored, the mechanisms and forces revealed likely influence the dynamics of centric chromosome arms as well.

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References

Afonso O, Matos I, Pereira AJ, Aguiar P, Lampson MA, Maiato H (2014) Feedback control of chromosome separation by a midzone Aurora B gradient. Science 345:332–336. https://doi.org/10.1126/science.1251121

Afonso O, Castellani CM, Cheeseman LP, Ferreira JG, Orr B, Ferreira LT, Chambers JJ, Morais-de-Sá E, Maresca TJ, Maiato H (2019) Spatiotemporal control of mitotic exit during anaphase by an aurora B-Cdk1 crosstalk. eLife 8:e47646. https://doi.org/10.7554/eLife.47646

Asbury CL (2017) Anaphase a: disassembling microtubules move chromosomes toward spindle poles. Biology (Basel) 6:E15. https://doi.org/10.3390/biology6010015

Ault JG, DeMarco AJ, Salmon ED, Rieder CL (1991) Studies on the ejection properties of asters: astral microtubule turnover influences the oscillatory behavior and positioning of mono-oriented chromosomes. J Cell Sci 99:701–710

Bader JR, Vaughan KT (2010) Dynein at the kinetochore: timing, interactions and functions. Semin Cell Dev Biol 21:269–275. https://doi.org/10.1016/j.semcdb.2009.12.015

Bajer A (1958) Cine-micrographic studies on chromosome movements in beta-irradiate cells. Chromosoma 9:319–331

Bajer A (1964) Cine-micrographic studies on dicentric chromosomes. Chromosoma 15:630–651. https://doi.org/10.1007/bf00319996

Bajer A, Östergren G (1963) Observation on transverse movements within the phragmoplast. Hereditas (Lund) 50:179–195

Bajer A, Vantard M (1988) Microtubule dynamics determine chromosome lagging and transport of acentric fragments. Mutat Res 201:271–281. https://doi.org/10.1016/0027-5107(88)90016-4

Bajer A, Vantard M, Molè-Bajer J (1987) Multiple mitotic transports expressed by chromosome and particle movement. Fortschr Zool 34:171–186

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

Baxter J, Sen N, López Martínez V, Monturus de Carandini ME, Schvartzman JB, Diffley JFX, Aragón L (2011) Positive supercoiling of mitotic DNA drives decatenation by topoisomerase II in eukaryotes. Science 331:1328–1332. https://doi.org/10.1126/science/1201538

Bretscher HS, Fox DT (2016) Proliferation of double-strand break-resistant polyploid cells requires Drosophila FANCD2. Dev Cell 37:444–457. https://doi.org/10.1016/j.devcel.2016.05.004

Brinkley BR, Zinkowski RP, Mollon WL, Davis FM, Pisegna MA, Perhouse M, Rao PN (1988) Movement and segregation of kinetochores experimentally detached from mammalian chromosomes. Nature 336:251–254. https://doi.org/10.1038/336251a0

Brodsky MH, Weinert BT, Tsang G, Rong YS, McGinnis NM, Golic KG, Rio DC, Rubin GM (2004) Drosophila melongaster MNK/Chk2 and p53 regulate multiple DNA repair and apoptotic pathways following DNA damage. https://doi.org/10.1128/mcb.24.3.1219-1231.2004

Brouhard GJ, Hunt AJ (2005) Microtubule movements on the arms of mitotic chromosomes: polar ejection forces quantified in vitro. Proc Natl Acad Sci U S A 102:13903–13908. https://doi.org/10.1073/pnas.0506017102

Carlson JG (1938a) Mitotic behavior of induced chromosomal fragments lacking spindle attachments in the neuroblasts of the grasshopper. Proc Natl Acad Sci U S A 24:500–507. https://doi.org/10.1073/pnas.24.11.500

Carlson JG (1938b) Some effects of X-radiation on the neuroblast chromosomes of the grasshopper, Chortophaga viridifasciata. Genetics 23:596–609

Carlton JG, Caballe A, Agromayor M, Kloc M, Martin-Serrano J (2012) ESCRT-III governs the Aurora B-mediated abscission checkpoint through CHMP4C. Science 336:220–225. https://doi.org/10.1126/science.1217180

Chan YW, West SC (2018) A new class of ultrafine anaphase bridges generated by homologous recombination. Cell Cycle 17:2101–2109. https://doi.org/10.1080/15384101.2018.1515555

Chan KL, North PS, Hickson ID (2007) BLM is required for faithful chromosome segregation and its localization defines a class of ultrafine anaphase bridges. EMBO J 26:3397–3409. https://doi.org/10.1038/sj.emboj.7601777

Cowell JK (1982) Double minutes and homogeneously staining regions: gene amplification in mammalian cells. Annu Rev Genet 16:21–59. https://doi.org/10.1146/annurev.ge.16.120182.000321

Cox D, Yuncken C, Spriggs AI (1965) Minute chromatin bodies in malignant tumours of childhood. Lancet 286:55–58. https://doi.org/10.1016/s0140-6736(65)0-131-5

Crasta K, Ganem NJ, Dagher R, Lantermann AB, Ivanova EV, Pan Y, Nezi L, Protopopov A, Chowdhury D, Pellman D (2012) DNA breaks and chromosome pulverization from errors in mitosis. Nature 482:53–58. https://doi.org/10.1038/nature10802

Derive N, Landmann C, Montembault E, Claverie MC, Pierre-Elies P, Goutte-Gattat D, Founounou N, McCusker D, Royou A (2015) Bub3-BubR1-dependent sequestration of Cdc20Fizzy at DNA breaks facilitates the correct segregation of broken chromosomes. J Cell Biol 211:517–532. https://doi.org/10.1083/jcb.201504059

Dietz R (1972) Anaphase behavior of inversions in living cranefly spermatocytes. Chromosom Today 3:70–85

Dumont J, Oegema K, Desai A (2010) A kinetochore-independent mechanism drives anaphase chromosome separation during acentrosomal meiosis. Nat Cell Biol 12:894–901. https://doi.org/10.1038/ncb2093

Falck GC, Catalán J, Norppa H (2002) Nature of anaphase laggards and micronuclei in female cytokinesis-blocked lymphocytes. Mutagenesis 17:111–117. https://doi.org/10.1093/mutage/17.2.111

Feeney KM, Parish JL (2009) Targeting mitotic chromosomes: a conserved mechanism to ensure viral genome persistence. Proc Biol Sci 276:1535–1544. https://doi.org/10.1098/rspb.2008.1642

Fenech M, Kirsch-Volders M, Natarajan AT, Surralles J, Crott JW, Parry J, Norppa H, Eastmond DA, Tucker JD, Thomas P (2011) Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis 26:125–132. https://doi.org/10.1093/mutage/geq052

Fuge H (1975) Anaphase transport of akinetochoric fragments in tipulid spermatocytes. Electron microscopic observations on fragment-spindle interactions. Chromosoma 52:149–158. https://doi.org/10.1007/bf00326264

Fuge H (1990) Non-kinetochore transport phenomena, microtubule-chromosome associations, and force transmission in nuclear division. Protoplasma 158:1–9. https://doi.org/10.1007/BF01323267

Galgoczy DJ, Toczyski DP (2001) Checkpoint adaptation precedes spontaneous and damage-induced genomic instability in yeast. Mol Cell Biol 21:1710–1718. https://doi.org/10.1128/MCB.21.5.1710-1718.2001

Gerlich D, Beaudouin J, Gebhard M, Ellenberg J, Eils R (2001) Four-dimensional imaging and quantitative reconstruction to analyse complex spatiotemporal processes in live cells. Nat Cell Biol 3:852–855. https://doi.org/10.1038/ncb0901-852

Giménez-Abián JF, Clark DJ, Giménez-Martín G, Weingartner M, Giménez-Abián MI, Carballo JA, Díaz de la Espina SM, Bögre L, De la Torre C (2002) DNA catenations that link sister chromatids until the onset of anaphase are maintained by a checkpoint mechanism. Eur J Cell Biol 81:9–16. https://doi.org/10.1078/0171-9335-00226

Holm C, Goto T, Wang JC, Botstein D (1985) DNA topoisomerase II is required at the time of mitosis in yeast. Cell 41:553–563. https://doi.org/10.1016/s0092-8674(85)80028-3

Hughes SE, Hawley RS (2014) Topoisomerase II is required for the proper separation of heterochromatic regions during Drosophila melanogaster female meiosis. PLoS Genet 23: e1004650. https://doi.org/10.1371/journal.pgen.1004650

Hughes SE, Gilliland WD, Cotitta JL, Takeo S, Collins KA, Hawley RS (2009) Heterochromatic threads connect oscillating chromosomes during prometaphase I in Drosophila oocytes. PLoS Genet 5:e1000348. https://doi.org/10.1371/journal.pgen.1000348

Humphrey RM, Brinkley BR (1969) Ultrastructural studies of radiation-induced chromosome damage. J Cell Biol 42: 745–753. https://doi.org/10.1083/jcb.42.3.745

Inoué S, Salmon ED (1995) Force generation by microtubule assembly/disassembly in mitosis and related movements. Mol Biol Cell 6:1619–1640. https://doi.org/10.1091/mbc.6.12.1619

Ishii K, Ogiyama Y, Chikashige Y, Soejima S, Masuda F, Kakuma T, Hiraoka Y, Takahashi K (2008) Heterochromatin integrity affects chromosome reorganization after centromere dysfunction. Science 321:1088–1091. https://doi.org/10.1126/science.1158699

Kanda T, Sullivan KF, Wahl GM (1998) Histone-GFP fusion protein enables sensitive analysis of chromosome dynamics in living mammalian cells. Curr Biol 8:377–385. https://doi.org/10.1016/s0960-9882(98)70156-3

Kanda T, Otter M, Wahl GM (2001a) Mitotic segregation of viral and cellular acentric extrachromosomal molecules by chromosome tethering. J Cell Sci 114:49–58

Kanda T, Otter M, Wahl GM (2001b) Coupling of mitotic chromosome tethering and replication competence in epstein-barr virus-based plasmids. Mol Cell Biol 21:3576–3588. https://doi.org/10.1128/MCB.21.10.3576-3588.2001

Karg T, Warecki B, Sullivan W (2015) Aurora B-mediated localized delays in nuclear envelope formation facilitate inclusion of late-segregating chromosome fragments. Mol Biol Cell 26:2227–2241. https://doi.org/10.1091/mbc.E15-01-0026

Karg T, Elting MW, Vicars H, Dumont S, Sullivan W (2017) The chromokinesin Klp3a and microtubules facilitate acentric chromosome segregation. J Cell Biol 216:1597–1608. https://doi.org/10.1083/jcb.201604079

Kaye JA, Melo JA, Cheung SK, Vaze MB, Haber JE, Toczyski DP (2004) DNA breaks promote genomic instability by impeding proper chromosome segregation. Curr Biol 14: 2096–2106. https://doi.org/10.1016/j.cub.2004.10.051

Ke Y, Huh JW, Warrington R, Li B, Wu N, Leng M, Zhang J, Ball HL, Li B, Yu H (2011) PICH and BLM limit histone association with anaphase centromeric DNA threads and promote their resolution. EMBO J 30:3309–3321. https://doi.org/10.1038/emboj.2011.226

Khodjakov A, Rieder CL (1996) Kinetochores moving away from their associated pole do not exert a significant pushing force on the chromosome. J Cell Biol 135:315–327. https://doi.org/10.1083/jcb.135.2.315

Khodjakov A, Cole RW, Bajer AS, Rieder CL (1996) The force for poleward chromosome motion in Haemanthus cells acts along the length of the chromosome during metaphase but only at the kinetochore during anaphase. J Cell Biol 132:1093–1104. https://doi.org/10.1083/jcb.132.6.1093

Koo DH, Molin WT, Saski CA, Jiang J, Putta K, Jugulam M, Friebe B, Gill BS (2018) Extrachromosomal circular DNA-based amplification and transmission of herbicide resistance in crop weed *Amaranthus palmeri*. Proc Natl Acad Sci U S A 115:3332–3337. https://doi.org/10.1073/pnas.1719354115

Kotadia S, Montembault E, Sullivan W, Royou A (2012) Cell elongation is an adaptive response for clearing long chromatid arms from the cleavage plane. J Cell Biol 199:745–753. https://doi.org/10.1083/jcb.201208041

LaFountain JR Jr, Oldenbourg R, Cole RW, Rieder CL (2001) Microtubule flux mediates poleward motion of acentric chromosome fragments during meiosis in insect spermatocytes. Mol Biol Cell 12:4054–4065. https://doi.org/10.1091/mbc.12.12.4054

LaFountain JR Jr, Cole RW, Rieder CL (2002a) Polar ejection forces are operative in crane-fly spermatocytes, but their action is limited to the spindle periphery. Cell Motil Cytoskeleton 5:16–26. https://doi.org/10.1002/cm.10011

LaFountain JR Jr, Cole RW, Rieder CL (2002b) Partner telomeres during anaphase in crane-fly spermatocytes are connected by an elastic tether that exerts a backward force and resists poleward motion. J Cell Sci 115:1541–1549

Landmann C, Pierre-Elies P, Goutte-Gattat D, Montembault E, Claverie M-C, Royou A (2020) The Mre11-Rad50-Nbs1 complex mediates the robust recruitment of Polo to DNA lesions during mitosis in. Journal of Cell Science 133 (13): jcs244442

Leimbacher P-A, Jones SE, A-MK. Shorrocks, de Marco Zompit M, Day M, Blaauwendraad J, Bundschuh D, Bonham S, Fischer R, Fink D, Kessler BM, Oliver AW, Pearl LH, Blackford AN, Stucki M (2019) MDC1 Interacts with TOPBP1 to Maintain Chromosomal Stability during Mitosis. Molecular Cell 74 (3):571–583.e8

Liang H, Wright WH, Cheng S, He W, Berns MW (1993) Micromanipulation of chromosomes in PTK2 cells using laser microsurgery (optical scalpel) in combination with laser-induced optical force (optical tweezers). Exp Cell Res 204:110–120. https://doi.org/10.1006/excr.1993.1015

Liu Y, Nielsen CF, Yao Q, Hickson ID (2014) The origins and processing of ultrafine anaphase DNA bridges. Curr Opin Genet Dev 26:1–5. https://doi.org/10.1016/j.ge.2014.03.003

Liu S, Kwon M, Mannino M, Yang N, Renda F, Khodjakov A, Pellman D (2018) Nuclear envelope assembly defects link mitotic errors to chromothripsis. Nature 561:551–555. https://doi.org/10.1038/s41586-018-0534-z

Ly P, Teitz LS, Kim DH, Shoshani O, Skaletsky H, Fachinetti D, Page DC, Cleveland DW (2017) Selective Y centromere inactivation triggers chromosome shattering in micronuclei and repair by non-homologous end joining. Nat Cell Biol 19:68–75. https://doi.org/10.1038/ncb3450

Mackay DR, Makise M, Ullman KS (2010) Defects in nuclear pore assembly Lead to activation of an Aurora B-mediated abscission checkpoint. J Cell Biol 191:923–931. https://doi.org/10.1083/jcb.201007124

Maiato H, Gomes AM, Sousa F, Barisic M (2017) Mechanisms of chromosome congression during mitosis. Biology (Basel) 6:E13. https://doi.org/10.3390/biology6010013

Malkova A, Ivanov EL, Haber JE (1996) Double-strand break repair in the absence of RAD51 in yeast: a possible role for break-induced DNA replication. Proc Natl Acad Sci U S A 93:7131–7136. https://doi.org/10.1073/pnas.93.14.7131

McNeill PA, Berns MW (1981) Chromosome behavior after laser microirradiation of a single kinetochore in mitotic PtK2 cells. J Cell Biol 88:543–553. https://doi.org/10.1083/jcb.88.3.543

Melo JA, Cohen J, Toczyski DP (2001) Two checkpoint complexes are independently recruited to sites of DNA damage in vivo. Genes Dev 15:2809–2821. https://doi.org/10.1101/gad.903501

Merdes A, Cleveland DW (1997) Pathways of spindle pole formation: different mechanisms; conserved components. J Cell Biol 138:953–956. https://doi.org/10.1083/jcb.138.5.953

Mitchison TJ (1989) Polewards microtubule flux in the mitotic spindle: evidence from photoactivation of fluorescence. J Cell Biol 109:637–652. https://doi.org/10.1083/jcb.109.2.637

Montembault E, Claverie MC, Bouit L, Landmann C, Jenkins J, Tsankova A, Cabernard C, Royou A (2017) Myosin efflux promotes cell elongation to coordinate chromosome segregation with cell cleavage. Nat Commun 8:326. https://doi.org/10.1038/s41467-017-00337-6

Morin SJ, Eccles J, Iturriaga A, Zimmerman RS (2017) Translocations, inversions and other chromosome rearrangements. Fertil Steril 107:19–26. https://doi.org/10.1016/j.fertnstert.2016.10.013

Muscat CC, Torre-Santiago KM, Tran MV, Powers JA, Wignall SM (2015) Kinetochore-independent chromosome segregation driven by lateral microtubule bundles. eLife 4:e06462. https://doi.org/10.7554/eLife.06462

Nowsheen S, Yang ES (2012) The intersection between DNA damage response and cell death pathways. Exp Oncol 34:243–254

Ohno Y, Ogiyama Y, Kubota Y, Kubo T, Ishii K (2016) Acentric chromosome ends are prone to fusion with functional chromosome ends through a homology-directed rearrangement. Nucleic Acids Res 44:232–244. https://doi.org/10.1093/nar/gkv997

Ono M, Preece D, Duquette ML, Forer A, Berns MW (2017) Mitotic tethers connect sister chromosomes and transmit "cross-polar" force during anaphase A of mitosis in PtK2 cells. Biomed Opt Express 8:4310–4315. https://doi.org/10.1364/BOE.8.004310

Östergren G, Molè-Bajer J, Bajer A (1960) An interpretation of transport phenomena at mitosis. Ann N Y Acad Sci 90:381–408. https://doi.org/10.1111/j.1749-6632.1960.tb23258.x

Paliulis LV, Forer A (2018) A review of "tethers": elastic connections between separating partner chromosomes in anaphase. Protoplasma 255:733–740. https://doi.org/10.1007/s00709-017-1201-1

Paliulis LV, Nicklas RB (2004) Micromanipulation of chromosomes reveals that cohesion release during cell division is gradual and does not require tension. Curr Biol 14:2124–2129. https://doi.org/10.1016/j.cub.2004.11.052

Pauletti G, Lai E, Attardi G (1990) Early appearance and long-term-persistence of the submicroscopic extrachromosomal elements (amplisomes) containing the amplified DHFR genes in human cell lines. Proc Natl Acad Sci U S A 87: 2955–2959. https://doi.org/10.1073/pnas.87.8.2955

Petsalaki E, Zachos G (2019) Building bridges between chromosomes: novel insights into the abscission checkpoint. Cell Mol Life Sci 76:4291–4307. https://doi.org/10.1007/s00018-019-03224-z

Rahal R, Amon A (2008) Mitotic CDKs control the metaphase-anaphase transition and trigger spindle elongation. Genes Dev 22:1534–1548. https://doi.org/10.1101/gad.1638308

Rieder CL, Davison EA, Jensen LC, Cassimeris L, Salmon ED (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

Rocha LC, Jankowska M, Fuchs J, Mittelmann A, Techio VH, Houben A (2017a) Decondensation of chromosomal 45S rDNA sites in *Lolium* and *Festuca* genotypes does not result in karyotype instability. Protoplasma 254:285–292. https://doi.org/10.1007/s00709-016-0942-6

Rocha LC, Silva GA, Bustamante FO, Silveira RA, Mittlemann A, Techi VH (2017b) Dynamics of 45S rDNA sites in the cell cycle: fragile sites and chromosomal stability in *Lolium* and *Festuca*. Genet Mol Res 16. https://doi.org/10.4328/gmr16019156

Royou A, Macias H, Sullivan W (2005) The Drosophila Grp/Chk1 DNA damage checkpoint controls entry into anaphase. Curr Biol 15:334–339. https://doi.org/10.1016/j.cub.2005.02.026

Royou A, Gagou ME, Karess R, Sullivan W (2010) BubR1- and Polo-coated DNA tethers facilitate poleward segregation of acentric chromatids. Cell 140:235–245. https://doi.org/10.1016/j.cell.2009.12.043

Somers WG, Saint R (2003) A RhoGEF and Rho family GTPase-activating protein complex link the contractile ring to cortical microtubules at the onset of cytokinesis. Dev Cell 4:29–39. https://doi.org/10.1016/s1534-5807(02)00402-1

Stephens PJ, Greenman CD, Fu B, Yan G, Bignell GR, Mudie LJ, Pleasance ED, Lau KW, Beare D, Stebbings LA et al (2011) Massive genomic rearrangement acquired in a single catastrophic event during cancer development. Cell 144:27–40. https://doi.org/10.1016/j.cell.2010.11.055

Stich H (1953) Stoffe und Ströungen in der Spindel von Cyclops strenuous. Chromosoma 6:199–236. https://doi.org/10.1007/BF01259940

Tanaka TU (2005) Chromosome bi-orientation on the mitotic spindle. Philos Trans R Soc Lond Ser B Biol Sci 360:581–589. https://doi.org/10.1098/rstb.2004.1612

Titen SW, Golic KG (2008) Telomere loss provokes multiple pathways to apoptosis and produces genomic instability in Drosophila melanogaster. Genetics 180:1821–1831. https://doi.org/10.1534/genetics.108.093625

Tooley J, Stukenberg PT (2011) The Ndc80 complex: integrating the kinetochore's many movements. Chromosom Res 19: 377–391. https://doi.org/10.1007/s10577-010-9180-5

Udroiu I, Sgura A (2020) Quantitative relationships between acentric fragments and micronuclei: new models and implications for curve fitting. Int J Radiat Biol 96:197–205. https://doi.org/10.1080/09553002.2020.1683638

Uretz RB, Bloom W, Zirkle RE (1954) Irradiation of parts of individual cells. II Effects of an ultraviolet microbeam focused on parts of chromosomes. Science 120:197–199. https://doi.org/10.1126/science.120.3110.197

Wahl GM (1989) The importance of circular DNA in mammalian gene amplification. Cancer Res 49:1333–1340

Wandke C, Barisic M, Sigl R, Rauch V, Wolf F, Amaro AC, Tan CH, Pereira AJ, 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

Warecki B, Sullivan W (2018) Micronuclei formation is prevented by Aurora B-mediated exclusion of HP1a from late-segregating chromatin in *Drosophila*. Genetics 210:171–187. https://doi.org/10.1534/genetics.118.301031

Warecki B, Ling X, Bast I, Sullivan W (2020) ESCRT-III-mediated membrane fusion drives chromosome fragments through nuclear envelope channels. J Cell Biol 219: e201905091. https://doi.org/10.1083/jcb.201905091

Wignall SM, Villeneuve AM (2009) Lateral microtubule bundles promote chromosome alignment during acentrosomal oocyte meiosis. Nat Cell Biol 11:839–844. https://doi.org/10.1038/ncb1891

Zhang CZ, Spektor A, Cornils H, Francis JM, Jackson EK, Liu S, Meyerson M, Pellman D (2015) Chromothripsis from DNA damage in micronuclei. Nature. 522:179–184. https://doi.org/10.1038/nature14493