Interphase cohesin regulation ensures mitotic fidelity after genome reduplication

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ABSTRACT To ensure faithful genome propagation, mitotic cells alternate one round of chromosome duplication with one round of chromosome separation. Chromosome separation failure thus causes genome reduplication, which alters mitotic chromosome structure. Such structural alterations are well documented to impair mitotic fidelity following aberrant genome reduplication, including in diseased states. In contrast, we recently showed that naturally occurring genome reduplication does not alter mitotic chromosome structure in Drosophila papillar cells. Our discovery raised the question of how a cell undergoing genome reduplication might regulate chromosome structure to prevent mitotic errors. Here, we show that papillar cells ensure mitotic fidelity through interphase cohesin regulation. We demonstrate a requirement for cohesins during programmed rounds of papillary genome reduplication known as endocycles. This interphase cohesin regulation relies on cohesin release but not cohesin cleavage and depends on the conserved cohesin regulator Pds5. Our data suggest that a distinct form of interphase cohesin regulation ensures mitotic fidelity after genome reduplication.

INTRODUCTION When cycling cells skip chromosome separation and then reenter S-phase, the genome is reduplicated. Such cycles are referred to as endocycles. Endocycles generate polyploid cells, which are common throughout nature (Fox and Duronio, 2013; Orr-Weaver, 2015). Following developmental endocycles or in pathological conditions, some polyploid cells return to mitosis (Levan and Hauschka, 1953; Fox et al., 2010; Davoli and de Lange, 2012). Division of such genome-reduplicated cells can generate genome instability through a variety of mechanisms, such as multipolar division or the formation of diplochromosomes, a mitotic chromosome structure that is a form of polyteny in which all products of replication are held together in one chromosome. Such diplochromosomes lead to mitotic errors when cells divide (Vidwans et al., 2002; Hassel et al., 2014; Schoenfelder et al., 2014; Chen et al., 2016; Stormo and Fox, 2016, 2017). Diplochromosomes have also been observed in tumor models in mice (Davoli et al., 2010) and following chemotherapeutic drug treatments in human cell culture (Blakeslee and Avery, 1937; Sumner, 1998).

Previously, we developed parallel models of naturally occurring and experimentally induced endocycled Drosophila cell types (Stormo and Fox, 2016). One cell type, the rectal papillar precursors of the hindgut, undergo developmentally programmed endocycles before returning to mitosis (hereafter “papillar cells”). The second cell type, wing imaginal disc cells, can be induced to endocycle by transient heat-shock driven expression of the endocycle regulator Cdh1/fizzy-related (hereafter- “HS>fzr cells”). Both papillar and HS>fzr cells return to mitosis after endocycling, but chromosome configuration at anaphase onset is very different. In papillar cells, chromatids undergo preanaphase chromosome separation into Recent Sister pairs (SIRS) (Figure 1A) (Stormo and Fox, 2016). In contrast, chromatids in HS>fzr cells are arranged in diplochromosomes as anaphase begins (Figure 1A). Likely because of these structural differences, papillar cell mitosis is relatively error free, whereas HS>fzr cell mitosis is highly error prone. These results raised the question of what molecular mechanism accounts for the difference in chromosome structure between cells capable or incapable of SIRS.

One candidate regulator of reduplicated chromosome structure is the cohesin complex. Cohesins are responsible for holding sister
Cohesion regulation during mitotic cell cycles ensures that chromosomes are attached specifically to their sisters and not to other chromosomes. This interphase cohesin exit gate opening depends on the conserved cohesin regulator Pds5. These findings reveal new interphase cohesin regulation during endocycles and shed light on the structural regulation of chromosomes in genome reduplicated cells.

RESULTS
Cohesin cleavage is sufficient to separate reduplicated chromatids

Previous studies in genome-reduplicated cells have found cohesins to be dispensable for chromosome structure (Pauli et al., 2008). However, these studies focused on nonmitotic cells. Our previous work (Stormo and Fox, 2016) showed a major difference in mitotic fidelity between genome-reduplicated cells that are capable of SIRS and those that are not. We showed that tetraploid cells that are SIRS-deficient retain conjoined diplochromosomes at metaphase. However, we did not explore whether differential regulation of cohesins is responsible for the decreased mitotic fidelity in such cells with persistent diplochromosomes. We therefore examined the role of the cohesin complex in chromosome structure of mitotic polyploid cells, using our two previously established models.

We first tested whether cleavage of the Rad21 cohesin subunit is sufficient to dissociate the conjoined diplochromosome configuration found in HS>fzr wing cells. To do this, we took advantage of an established system that enables heat-shock-inducible Rad21 cleavage (Rad21<sup>TEV</sup>, Materials and Methods). We first confirmed that heat shock (Figure 2, A and G) and Rad21<sup>TEV</sup> alone has no effect on chromosome structure (Figure 2, B and G). TEV-protease expression also has no effect on diploid or polyploid mitotic chromosomes when Rad21 is wild type (Figure 2, C and G). We next combined induced endocycles and cohesin cleavage by driving expression of both HS>fzr and HS>TEV transgenes using a single heat shock in a rad21<sup>TEV</sup> animals. In these animals endocycling still occurs, resulting in tetraploid cells, but these chromosomes lack cohesion between sisters and instead unpaired chromatids are visible (Figure 2, D and G). These data strongly suggest that diplochromosomes are held together by cohesin in the same manner as wild-type mitotic chromosomes. Further, we find that cohesin cleavage is sufficient to dissociate the conjoined chromatids found in diplochromosomes.

We next performed a similar experiment in papillar cells, which lack conjoined metaphase chromosomes. It was possible that these cells are able to undergo SIRS because they lack standard cohesins. We tested whether papillar chromatid pairs are held together by cohesins. As in the wing disc, following Rad21<sup>TEV</sup> cleavage all chromosome cohesion is lost, and we observe individual chromatids (Figure 2, E vs. F and H). These data show that in both cells with induced endocycles, which result in diplochromosomes, and in papillar cell endocycles, which result in conjoined diplochromosomes at metaphase. However, we did not explore whether differential regulation of cohesins is responsible for the decreased mitotic fidelity in such cells with persistent diplochromosomes. We therefore examined the role of the cohesin complex in chromosome structure of mitotic polyploid cells, using our two previously established models.

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Here we investigate the role of cohesins on the structure of chromosomes in cells undergoing endocycles, using our two model cell types. Unlike other endocycled cells, such as the Drosophila salivary gland, these two cell types return to mitosis, which allows direct visualization of chromosome structure and the effects of cohesin regulation on mitosis. We find that in SIRS-capable papillar cells, cohesin exit gate opening during endocycles prevents formation of diplochromosomes. This interphase cohesin exit gate opening depends on the conserved cohesin regulator Pds5. These findings reveal new interphase cohesin regulation during endocycles and shed light on the structural regulation of chromosomes in genome reduplicated cells.
chromosomes, regardless of proximity within the nucleus or homology. Moreover, following SIRS, papillar chromosomes lose polytene structure and are attached to only a subset of their sister chromatids prior to anaphase (Figure 1A). We previously suggested that papillar chromatids were attached via cohesion with only the most recent sister chromatid (Stormo and Fox, 2016), which we had evidence for based on the symmetric appearance of random or radiation-induced chromosome breaks at the same location on adjacent chromatids (Bretscher and Fox, 2016). To further examine whether papillar chromatids were attached in recent rather than random sister pairs (Figure 3A, model 1 vs. model 2), we pulse-labeled chromatids with 5-bromo-2′-deoxyuridine (BrdU) (Figure 3A, Materials and Methods). Our data are consistent with papillar chromatids pairing with their recent sisters (Figure 3B).

We next sought to uncover the mechanism that enables papillar chromatids to establish cohesion with only their most recent sister. We hypothesized that, during papillar endocycles, cohesin is removed between each sister chromatid after each round of replication. Such interphase cohesin regulation could occur through one of two pathways. First, cohesins could be removed from papillar chromosomes during each endocycle by Rad21 destruction, similarly to its destruction at anaphase (Figure 3C, “Rad21 Cleavage”). Second, cohesins could be removed during each endocycle by exit gate...
opening (Figure 3C, “Exit gate opening”), similarly to how nonpericentric cohesin is removed in prophase.

We first tested whether Rad21 destruction takes place during papillar endocycles. Normally, Rad21 is cleaved by Separase at the onset of anaphase. Our previous work found no evidence of mitosis during papillar endocycles (Fox et al., 2010), but we reasoned that it was possible for Separase to be regulated in a noncanonical manner in papillar cells, so that it was active during endocycles. To test this hypothesis, we first knocked down separase using two separate RNA interference (RNAi) lines (Materials and Methods). We then examined the structure of chromosomes in those cells during the first mitosis after papillar endocycles and SIRS. We find that in the absence of Separase, papillar chromosomes undergo SIRS normally, as chromosomes are arranged in pairs at metaphase that are indistinguishable from wild type (Figure 3, D vs. E and G). To ensure that knockdown of separase was successful, we performed live imaging on separase RNAi papillar chromosomes in metaphase with neat pairs of chromatids similarly to wild-type cells (Figure 4, A vs. B, 2:00, 8:00, and D). As an alternative approach, we expressed a previously established noncleavable Rad21 that lacks the Separase cleavage site (UAS>rad21NC; Materials...
FIGURE 4: Cohesin exit gate opening is required for mitotic fidelity and organ development in papillar cells. (A–C) Time lapse of papillar cell mitosis from the indicated genotypes. His-2av-GFP, magenta; CenpC-tomato, green; time, minutes from anaphase onset. Final panel depicts histone channel only; yellow arrows show spindle pole position. White double arrowhead indicates DNA bridge. Scale bar = 5 µm (main images), 1 µm (insets). (D) Quantification of the percentage of cells with aberrant metaphase and anaphases of the indicated genotypes (+SEM, from left to right, N = 12, 8, 27 metaphase and N = 12, 11, 39 cells per genotype). (E) Representative images of the adult hindgut of animals of the indicated genotypes (from N = 12 control and 8 SMC3-Rad21 animals). DAPI (DNA, white); papillae pseudocolored (green). Arrows indicate the location (or absence) of papillar structures. Yellow scale bar = 12.5 µm.

and Methods; Urban et al., 2014). Here, too, chromosome structure at the first mitosis is unaffected (Figure 3G). Together, these data strongly suggest that papillar cells do not require Rad21 cleavage to remove cohesion during endocycles.

As an alternative to cohesin cleavage, we reasoned that cohesin turnover via exit gate opening could account for the interphase cohesin regulation that we hypothesize is responsible for paired sister chromatids at papillary metaphase. To test this hypothesis, we used a previously established transgenic construct in which Rad21 and SMC3 are fused via a linker region (Eichinger et al., 2013) (Figure 3C). This fusion can load onto chromosomes normally but cannot be removed from chromatin because the fusion closes the exit gate. This construct is still cleaved normally at anaphase.

To test whether exit gate opening during endocycles was required for normal papillar chromosome structure, we expressed UAS-SMC3-Rad21 and looked at chromosomes during the first metaphase after SIRS. Chromosome structure is often substantially altered in these cells. Specifically, chromosomes persist as polytene chromosomes, suggesting SIRS does not take place (Figure 3, F, F’, and G). These chromosome phenotypes do not appear to disrupt papillar endocycles, as in metaphase spreads where the X centromeres are separate (as we described before for papillar cells; Stormo and Fox, 2016), we can count 8 X centromeres, indicating these cells are octoploid (Figure 3F’, asterisks in inset). The finding that cohesins can persist on papillar chromosomes without disrupting multiple endocycle S-phases is consistent with the observation that cohesin can remain associated with chromosomes during DNA replication (Rhodes et al., 2017). The chromosomal phenotype in papillar cells of SMC3-Rad21 animals suggests cohesin exit gate opening, most likely during endocycles, is important for SIRS.

We were surprised that, at the first metaphase post-SIRS, SMC3-Rad21 papillar cells displayed the haploid number of observed distinct chromosomes, as opposed to the diploid number. This implies that blocking cohesin exit gate opening can also promote ectopic homologue–homologue pairing. We had instead expected that homologues would remain separate at mitosis despite persistent sister-chromatid cohesion, because homologous chromosomes are not normally cohesed but are instead associated by somatic pairing mechanisms. In Drosophila, somatic homologue pairing is antagonized by condensins (Smith et al., 2013). At mitosis, condensins overcome the attractive forces of somatic pairing to drive homologues apart, but sisters remain attached by cohesins. We suspect SMC3-Rad21 expression antagonizes this condensin activity. Interestingly, cohesin and condensin II can antagonize each other’s functions in alignment of sister chromatids in cultured Drosophila cells (Senaratne et al., 2016).

We next analyzed the consequence of the disrupted mitotic chromosome structure phenotype of SMC3-Rad21 on papillar cell mitosis by performing live imaging. SMC3-Rad21 papillar cells fail to form a proper metaphase plate with pairs of sister chromatids bioriented. Instead, polytene chromosomes are still evident until anaphase (Figure 4C, –2:00). Subsequently, anaphase of SMC3-Rad21 papillar cells is highly error prone (Figure 4C, 6:00, D). Given
these mitotic defects, we examined the consequence of such error-prone divisions on tissue development by examining adult hindgut structure. Unlike in control animals, which display four rectal papillar structures in adults, SMC3-Rad21 animals completely lack obvious rectal papillar structures (Figure 4E). This suggests that SMC3-Rad21 cells do not survive the extremely aberrant mitotic divisions and thus fail to produce cells which are normally required for their construction (Fox et al., 2010; Schoenfelder et al., 2014). In contrast, the rest of the hindgut, which is formed by multiple rounds of diploid mitoses (Fox and Spradling, 2009; Sawyer et al., 2017) and also expresses SMC3-Rad21 under the samebyn-Gal4 driver, appears unaffected. We hypothesize that the lack of tissue-level phenotype unaffected by pds5 RNAi, as evidenced by our ability to count ~8 separate fourth chromosomes in otherwise polyten e pds5 RNAi cells (Figure 5B, arrow). These results strongly suggest that pds5-mediated cohesin exit gate opening at centromeres is key in interphase cohesin regulation during the premitotic endocycles of papillar cells. Deficiencies in this mechanism contribute to mitotic errors in cells with genome reduplication.

**DISCUSSION**

Cohesins typically hold together all products of S-phase through interphase until cleavage of the complex at anaphase. Here our results imply that cohesin removal mechanisms can be repurposed in
cells undergoing endocycles to prevent subsequent mitotic infidelity. In Drosophila papillar cells, our results suggest that chromosomes lose cohesion and then reestablish it with the most recent sister chromatid during endocycles (Figure 5F). We thus propose that this cohesin regulation occurs during interphase but involves a repurposing of the cohesin exit gate opening mechanism normally used during prophase. Our work also reveals a role for cohesin exit gate opening in antagonizing pairing between homologous chromosomes in cells that endocycle (Figure 5F).

Does such interphase cohesin regulation that we propose to occur in papillary cells also occur in other polyploid cell types? Drosophila ovarian nurse cells partially separate chromatids but do not proceed to a full mitosis (Hammond and Laird, 1985; Dej and Spradling, 1999) and thus may also undergo some degree of interphase cohesin regulation. In nonmitotic polyploid cells such as salivary gland cells, the cohesin complex is present and dynamic (Gause et al., 2010; Cunningham et al., 2012). However, because these cells are nonmitotic, chromatids are never separated enough to observe constraint by cohesins. Our work here suggests that interphase cohesin regulation is definitely not a property of all polyploid cells, as cells with induced endocycles contain diplochromosomes, where four chromatids remain cohesed (Figure 2). The difference between naturally occurring and ectopically induced endocycles may account for mitotic defects associated with the latter. Additionally, we also uncover a surprising role for cohesin exit gate opening in antagonizing homologue pairing. While lack of this anti-pairing mechanism may contribute to the phenotypes we see in papillary cells with compromised exit gate opening, we note that cells with ectopic diplochromosomes do not pair (Figure 2) and exhibit similar mitotic defects to papillary cells with defective exit gate opening (Stormo and Fox, 2016). Future work can examine the connection between cohesin exit gate opening and known homologue pairing regulators such as condensins. Further, we note that while we heavily favor an interphase model of cohesin regulation, it is possible that only the cohesins between recent sister chromatids, and not any other cohesins, somehow resist the prophase pathway specifically during SIRS. Such a mechanism would still likely involve some differential marking (such as acetylation) of the cohesins between recent sisters, which would likely have to occur in the last endocycle (i.e., would still be an interphase mechanism).

With respect to molecular mechanisms of cohesin regulation, our results also revealed differences between pds5 knockdown and SMC3-Rad21 expression in papillary cells. We propose that SMC3-Rad21 represents a situation where cohesin complexes cannot be removed by the prophase pathway at onset of mitosis, and therefore chromosome arms remain attached. In contrast, pds5 knockdown blocks cohesin release during endocycles but not during prophase. Our results reveal a differential sensitivity for cohesin regulation at chromosome arms and centromeres in this RNAi condition. This could reflect that Pds5 in papillary cells is more essential for centromeric cohesion than for arm cohesion. Along these lines, kinase activity of polo kinase as well as Aurora B phosphorylation of SA1 and 2 participate with Pds5 and Wapl in the prophase pathway to remove arm cohesion (Sumara et al., 2002; Gimenez-Abian et al., 2004; Hauf et al., 2005; Kueng et al., 2006; Shintomi and Hirano, 2009), and it is possible that in pds5 RNAi animals these other arm cohesin regulators are still able to separate papillary polytene chromosome arms.

Future work can address regulation of pericentric cohesins during papillary cell endocycles. In mitotic cells, pericentromeric cohesin is not removed during the prophase pathway, because that region is protected by Shugoshin (Moore et al., 1998; Lee et al., 2004; Watanabe, 2005). Shugoshin directly antagonizes Wapl, a partner of Pds5 (Hara et al., 2014). If Pds5 is required to remove cohesins during endocycles, then how does it bypass Shugoshin? One possibility is that Shugoshin is not present in these cells during endocycles. If so, then this would allow the prophase pathway to clear cohesins from the entire chromosome, including centromeres, during each endocycle.

In disease, continued study of chromosome structure after genome reduplication is important because diplochromosomes are induced by common cancer therapeutics such as topoisomerase inhibitors (Hande, 1998; Sumner, 1998). Our data suggest that if cells prone to diplochromosomes regulated cohesins differently, so that only paired recent sisters were present at metaphase, then the rate of mitotic errors and aneuploidy in these cells would dramatically drop. Given our identification here of interphase cohesin regulation during papillary endocycles, papillary cells represent a valuable system for further study of cohesin regulation. Additionally, the importance of mitotic genome reduplicated cells in disease suggests that understanding chromosome structure in these cells may give insight into new therapies.

MATERIALS AND METHODS

Drosophila stocks

Stocks were obtained from the Bloomington Drosophila Stock Center (stock number in parentheses): w118 (3605); His-2av-GFP (24163); vtdex814 (26165); vtdex58; rad21.271TEV-myc (27613); UAS-NLS-V5-TEV-NLS (27605); HS-NLS-V5-TEV-NLS (27612); pds5-RNAi (35632, previously validated by Kusch (2015) to cause meiotic recombination phenotypes); Gal80T5 (7018); the Vienna Drosophila Stock Center (Dietzl et al., 2007): see-RNAi (v45091), previously validated to phenocopy see mutants in larval neuroblasts; Cipressa et al., 2016; see-RNAi (v106237); or were kind gifts: tomato-Cenp-C, HS-fzr (Sigrist and Lehner, 1997); byro-gal4 (Singer et al., 1996); UAS-SMC3-vtd-GFP (Eichinger et al., 2013); UAS-rad2113C (Urban et al., 2014).

Drosophila culture and genetics

All flies were raised on standard media (Archen Scientific, Durham, NC). All experiments involving a UAS transgene (including RNAi) were performed at 29°C to maximize Gal4-mediated transgene expression. Heat shocks to induce fzr or TEV expression were performed on third instar larvae. Animals for these experiments were heat shocked in a vial at 37°C (water bath) for 20 min. In experiments involving inducible transgenic Rad21 cleavage, endogenous Rad21 was removed using two null rad21 mutant alleles in trans (vtdex414 and vtdex58). These mutant alleles were rescued by a ubiquitously expressed rad21 transgene containing a tobacco etch virus (TEV) cleavage site (rad21113E, Pauli et al., 2008). This construct was then cleaved either in all cells (using a heat shock promoter, HS-TEV) or specifically in our cell type of choice using a UAS promoter (UAS-TEV). Animals were examined 10 h after HS-fzr expression, as this is the time where we previously established that mitosis resumes after heat shock in these animals.

For papillary cell experiments, we used the hindgut specific byro-gal4 driver to express transgenes. For all transgenes except for UAS TEV, we expressed these transgenes throughout development. To avoid prolonged UAS-TEV expression, we used previously established methods that rely on Gal80ts to repress Gal4 expression (Fox and Spradling, 2009; Fox et al., 2010) to confine expression of UAS-TEV to the period of endocycles (second larval instar) and not mitosis (which occurs much later: hours 24–48 post-puparium formation at 22°C).
Chromosome cytology

Chromosome preparations were performed as previously described (Gatti et al., 1994; Fox et al., 2010). We used enriched for meta-phase cells by first incubating tissue in colcemid (Sigma, St. Louis, MO) at 50 μg/ml for 20 min in phosphate-buffered saline (PBS). Imaging was performed on a Zeiss Axio Imager 2 with a 63× oil immersion lens.

Live imaging

Live-imaging preparations were prepared as previously described (Prasad et al., 2007; Fox et al., 2010). Imaging was performed on a spinning disk confocal (Yokogawa CSU10 scanhead) on an Olympus IX-70 inverted microscope using a 60×/1.3 NA PlanSapo Silicon oil, 488 and 568 nm Krypton laser lines for excitation, and an Andor Ixon3 897 512 electron-multiplying charge-coupled device camera. The system was controlled by MetaMorph 7.7. Images were analyzed in ImageJ (Schneider et al., 2012).

BrdU feeding and staining

To determine whether papillar chromatids are associated with most recent sisters, we fed 1 mg/ml BrdU dissolved in PBS + food coloring for 1 h during the second instar stage, when papillar cells endocycle twice to reach 8C ploidy. The goal of this experiment was to occasionally label only the second-to-last S-phase in these cells so that one-fourth of all DNA strands at mitosis were BrdU labeled at mitosis. If one-fourth of DNA strands contain BrdU, we would expect one half of chromatids to be labeled and each chromosome to contain one labeled chromatid if recent sisters are paired (Figure 3A, Recent Sisters). In contrast, if sister chromatids are randomly paired, then we would see chromatids in which neither chromatid was labeled, as well as chromatids in which both chromatids were labeled, in the same cell (Figure 3A, Random Sisters). To ensure pulse labeling of chromosomes, larvae were washed in PBS after feeding, and animals with no food coloring in their gut were discarded.

To image BrdU in metaphase spreads, chromosome cytology and BrdU antibody staining were performed as described previously (Rat anti-BrdU, Serotec 1:100, clone 3J9) was performed based on Sullivan and Karpen (2001) with slight modifications. In brief, hour 24–48 post-puparium formation (at 22°C) animals were dissected. Dissected tissue was incubated in 0.5% sodium citrate for 15 min then fixed on a coverslip in 11:1:2 methanol:acetic acid:H2O. Fix was removed and replaced with 10 μl of 45% acetic acid. The coverslip was then squashed on a positively charged slide (VWR, Radnor, PA) and then frozen in liquid nitrogen until the coverslip could be removed using a razor blade. Slides were then transferred to 95% ethanol at −20°C. All subsequent steps were performed directly on the slide, and tissue denaturation and BrdU antibody staining were performed as described previously (Fox and Spradling, 2009).

Statistics

All statistics were computed in Prism 7 (GraphPad, La Jolla, CA). Metaphase spreads were blinded and then scored. Metaphase spreads were compared with wild type using a chi-squared test on total counts. For live imaging, cells were averaged within animals, and then mean and standard error were calculated by averaging between animals. Means were compared using one-way analysis of variance (ANOVA). NS, not significant for p > 0.05, *p < 0.05, **p < 0.01, ***p < 0.001.

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REFERENCES

Blakeslee AF, Avery AG (1937). Methods of inducing doubling of chromosomes in plants by treatment with colchicine*. J Hered 28, 393–411.

Bretschger HS, Fox DT (2016). Proliferation of double-strand break-resistant polyploid cells requires Drosophila FANCDD2. Dev Cell 37, 444–457.

Chen S, Stout JR, Dharmia S, Yde S, Calvi BR, Walczak CE (2016). Transient endoreplication down-regulates the kinesin-14 HSET and contributes to genomic instability. Mol Biol Cell 27, 2911–2923.

Cipressa F, Moriciani P, Bosso G, Mannini L, Galati A, Raffa GD, Cacchione S, Musio A, Cenci G (2016). A role for Separase in telomere protection. Nat Commun 7, 10405.

Cunningham MD, Gause M, Cheng Y, Noyes A, Dorsett D, Kenison JA, Kassis JA (2012). Wapl antagonizes cohesion binding and promotes Polycimb-group silencing in Drosophila. Development 139, 4172–4179.

Davoli T, de Lange T (2012). Telomere-driven tetraploidization occurs in human cells undergoing crisis and promotes transformation of mouse cells. Cancer Cell 21, 765–776.

Davoli T, Denchi EL, de Lange T (2010). Persistent telomere damage induces bypass of mitosis and tetraploidy. Cell 141, 81–93.

Dej KJ, Spradling AC (1999). The endocycle controls nurse cell polypolytene chromosome structure during Drosophila oogenesis. Development 126, 293–303.

Dietl G, Chen D, Schnorrer F, Su KC, Barinova Y, Fellner M, Gasser B, Kinsey K, Oppel S, Scheiblauer S, et al. (2007). A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature 448, 151–156.

Dorsett D, Eisenberg JC, Misulovin Z, Martens A, Redding B, McKim K (2005). Effects of sister chromatid cohesion proteins on cut gene expression during wing development in Drosophila. Development 132, 4743–4753.

Eichert CS, Kurze A, Oliveira RA, Nasmyth K (2013). Disengaging the Smc3/kleisin interface releases cohesin from Drosophila chromosomes during interphase and mitosis. EMBO J 32, 656–665.

Eng T, Guacci V, Koshland D (2015). Interallelic complementation provides functional evidence for cohesion–cohesion interactions on DNA. Mol Biol Cell 26, 4224–4235.

Fox DT, Duronio RJ (2013). Endoreplication and polyploidy: insights into development and disease. Development 140, 3–12.

Fox DT, Gall JG, Spradling AC (2010). Error-prone polyploid mitosis during normal Drosophila development. Genes Dev 24, 2294–2302.

Fox DT, Spradling AC (2009). The Drosophila hindgut lacks constitutively active adult stem cells but proliferates in response to tissue damage. Cell Stem Cell 5, 290–297.

Gatti M, Bonacorsi S, Pimpinelli S (1994). Looking at Drosophila mitotic chromosomes. In: Methods in Cell Biology, ed. SBG Lawrence and AF Eric, Amsterdam: Elsevier/Academic Press, 371–391.

Gause M, Misulovin Z, Bilyeu A, Dorsett D (2010). Dosage-sensitive regulation of cohesin chromosome binding and dynamics by Nipped-B, Pds5, and Wapl. Mol Cell Biol 30, 4940–4951.

Giménez-Ablan JF, Sumara I, Hrotta T, Hauf S, Gerlich D, la Torre de C, Ellenberg J, Peters JM (2004). Regulation of sister chromatid cohesion between chromosome arms. Curr Biol 14, 1187–1192.

Haering CH, Faras A-M, Arumugam P, Metson J, Nasmyth K (2008). The cohesin ring concatenates sister DNA molecules. Nature 454, 297–301.

Hammond MR, Laird CD (1985). Chromosome structure and DNA replication in nurse and follicle cells of Drosophila melanogaster. Chromosoma 91, 267–278.

Hande KR (1998). Etoposide: four decades of development of a topoisomerase II inhibitor. Eur J Cancer 34, 1514–1521.

Hara K, Zheng G, Qu Q, Liu H, Ouyang Z, Chen Z, Tomchick DR, Yu H (2014). Structure of cohesin subcomplex pinpoints direct shugoshin–Wapl antagonism in centromeric cohesion. Nat Struct Mol Biol 21, S84–S870.

Hassel C, Zhang B, Dixon M, Calvi BR (2014). Induction of endocycles represses apoptosis independently of differentiation and predisposes cells to genome instability. Development 141, 112–123.
Hauf S, Rostinger C, Koch B, Dittrich CM, Mechtler K, Peters, J-M (2005). Dissociation of cohesin from chromosome arms and loss of arm cohesin during early mitosis depends on phosphorylation of SA2. PLoS Biol 3, e69.

Ivanov D, Nasmyth K (2005). A topological interaction between cohesin rings and a circular minichromosome. Cell 122, 849–860.

Kueng S, Hegemann B, Peters BH, Lipp JU, Schleiffer A, Mechtler K, Peters, J-M. (2006). Wapl controls the dynamic association of cohesin with chromatin. Cell 127, 955–967.

Kusch T (2015). Brca2–Pds5 complexes mobilize persistent meiotic recombination sites to the nuclear envelope. J Cell Sci 128, 717–727.

Lee JY, Dej KJ, Lopez JM, Orr-Weaver TL (2004). Control of centromere localization of the MEI-S332 cohesion protection protein. Curr Biol 14, 1277–1283.

Lengronne A, McIntyre J, Katou Y, Kanoh Y, Hopfner KP, Shirahige K, Uhlmann F (2006). Establishment of sister chromatic cohesion at the S. cerevisiae replication fork. Mol Cell 23, 787–799.

Levan A, Hauschka TS (1953). Endomitotic reduplication mechanisms in asciates tumors of the mouse. J Nat Cancer Inst 14, 1–43.

Moore DP, Page AW, Tang TT, Kerrebrock AW, Orr-Weaver TL (1998). The cohesion protein MEI-S332 localizes to condensed meiotic and mitotic centromeres until sister chromatids separate. J Cell Biol 140, 1003–1012.

Murayama Y, Uhlmann F (2015). DNA entry into and exit out of the cohesin ring by an interlocking gate mechanism. Cell 163, 1628–1640.

Naarlyth K, Haering CH (2009). Cohesin: its roles and mechanisms. Annu Rev Genet 43, 525–558.

Orr-Weaver TL (2015). When bigger is better: the role of polyploidy in aneuploidy in the Drosophila rectum. Development 141, 3551–3560.

Senarathne TN, Joyce EF, Nguyen SC, Wu CT (2016). Investigating the interplay between sister chromatic cohesion and homolog pairing in Drosophila nuclei. PLoS Genet 12, e1006169.

Shintomi K, Hirano T (2009). Releasing cohesin from chromosome arms in early mitosis: opposing actions of Wapl–Pds5 and Sgo1. Genes Dev 23, 2224–2236.

Sigrist SJ, Lehner CF (1997). Drosophila fizzy-related down-regulates mitotic cyclins and is required for cell proliferation arrest and entry into endocycles. Cell 90, 671–681.

Singer JB, Harbecke R, Kusch T, Reuter L, Lengyel JA (1996). Drosophila brachymertron regulates gene activity and morphogenesis in the gut. Development 122, 3707–3718.

Skibbers RV (2016). Of rings and rods: regulating cohesin entrapment of DNA to generate intra- and intermolecular tethers. PLoS Genet 12, e1006337.

Smith HF, Roberts MA, Nguyen HQ, Peterson M, Hartl TA, Wang X-J, Klebba JE, Rogers GC, Bosco G (2013). Maintenance of interphase chromosome compaction and homolog pairing in Drosophila is regulated by the condensin Cap-H2 and its partner Mrg15. Genetics 195, 127–146.

Stigler J, Çamdere GO, Koshland DE, Greene EC (2016). Single-molecule imaging reveals a collapsed conformational state for DNA-bound cohesin. Cell Rep 15, 988–998.

Stormo BM, Fox DT (2016). Distinct responses to reduplicated chromosomes require distinct Mad2 responses. Elife 5, e15204.

Stormo BM, Fox DT (2017). Polytene: still a giant player in chromosome research. Chromosome Res 25, 201–214.

Sullivan B, Karpen G (2001). Centromere identity in Drosophila is not determined in vivo by replication timing. J Cell Biol 154, 683–690.

Sumara I, Vorlaufer E, Stukenberg PT, Kelm O, Redemann N, Nigg EA, Peters J-M (2002). The dissociation of cohesin from chromosomes in prophase is regulated by polo-like kinase. Mol Cell 9, 515–525.

Sumner AT (1998). Induction of diplochromosomes in mammalian cells by inhibitors of topoisomerase II. Chromosoma 107, 486–490.

Uhlmann F, Lottspeich F, Nasmyth K (1999). Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. Nature 400, 37–42.

Urban E, Nagarkar-Jaiswal S, Lehner CF, Heidmann SK (2014). The cohesin subunit Rad21 is required for synaptonemal complex maintenance, but not sister chromatic cohesion, during Drosophila female meiosis. PLoS Genet 10, e1004540.

Vidwans SJ, DiGregorio PJ, Shermoan AW, Foat B, Iwasa J, Yakubovich N, O’Farrell PH (2002). Sister chromatids fail to separate during an induced endoreplication cycle in Drosophila embryos. Curr Biol 12, 829–833.

Watanabe Y (2005). Shugoshin: guardian spirit at the centromere. Curr Opin Cell Biol 17, 590–595.

Yeh E, Haase J, Palulis IW, Joglekar A, Bond L, Bouck D, Salmon ED, Bloom K (2008). Pericentric chromatin is organized into an intramolecular loop in mitosis. Curr Biol 18, 81–90.