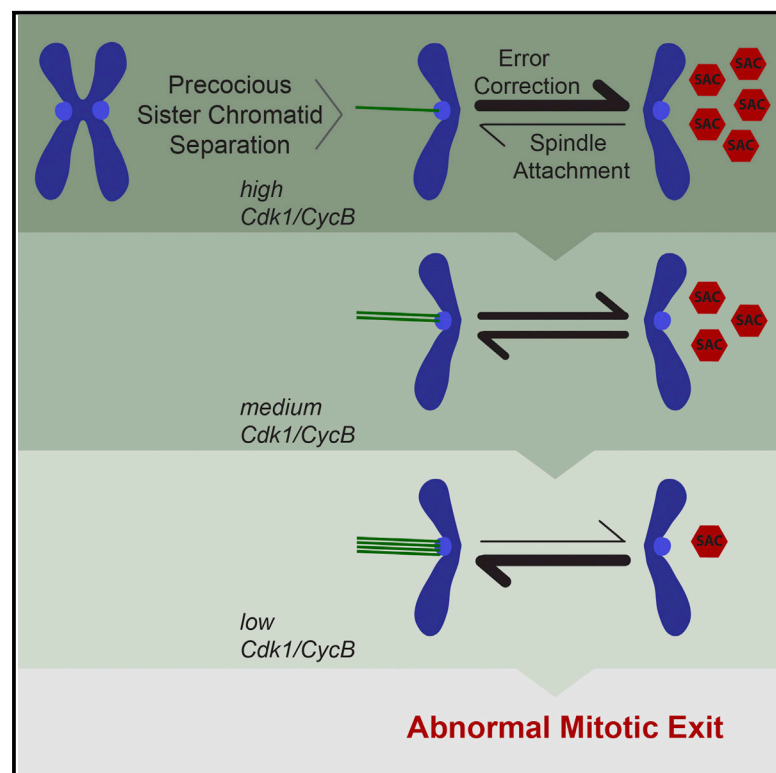


Cell Reports

Premature Sister Chromatid Separation Is Poorly Detected by the Spindle Assembly Checkpoint as a Result of System-Level Feedback

Graphical Abstract



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In Brief

Mirkovic et al. show that premature loss of sister chromatid cohesion during mitosis does not trigger a robust checkpoint response. Quantitative live-cell imaging and mathematical modeling approaches describe several feedback loops between the error-correction machinery, the spindle assembly checkpoint (SAC), and Cdk1 that compromise the efficient detection of cohesion defects.

Highlights

- Precocious sister chromatid separation does not elicit robust SAC activation
- Error-correction efficiency declines gradually upon premature cohesion loss
- Mitotic exit in the absence of cohesin is accelerated by multiple feedback loops
- Cells with premature sister chromatid separation are ultrasensitive to Cdk1 inhibition



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Premature Sister Chromatid Separation Is Poorly Detected by the Spindle Assembly Checkpoint as a Result of System-Level Feedback

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SUMMARY

Sister chromatid cohesion, mediated by the cohesin complex, is essential for faithful mitosis. Nevertheless, evidence suggests that the surveillance mechanism that governs mitotic fidelity, the spindle assembly checkpoint (SAC), is not robust enough to halt cell division when cohesion loss occurs prematurely. The mechanism behind this poor response is not properly understood. Using developing *Drosophila* brains, we show that full sister chromatid separation elicits a weak checkpoint response resulting in abnormal mitotic exit after a short delay. Quantitative live-cell imaging approaches combined with mathematical modeling indicate that weak SAC activation upon cohesion loss is caused by weak signal generation. This is further attenuated by several feedback loops in the mitotic signaling network. We propose that multiple feedback loops involving cyclin-dependent kinase 1 (Cdk1) gradually impair error-correction efficiency and accelerate mitotic exit upon premature loss of cohesion. Our findings explain how cohesion defects may escape SAC surveillance.

INTRODUCTION

Faithful chromosome segregation is governed by the spindle assembly checkpoint (SAC), a surveillance mechanism that senses spindle attachments and prevents progression through mitosis until all chromosomes are properly bioriented (Musacchio and Salmon, 2007). This checkpoint operates by generating a signal (the mitotic checkpoint complex [MCC]) that inhibits the anaphase-promoting complex/cyclosome (APC/C) and thereby anaphase onset (Musacchio and Salmon, 2007). Unattached kinetochores serve as a scaffold for the production of the MCC, but it has long been debated whether or not tension across sister chromatids (and/or intra-kinetochore tension) can also be sensed by this checkpoint (Khodjakov and Pines, 2010; Maresca and Salmon, 2010). Nevertheless, it is well accepted that tension plays a central role in SAC responsiveness, even if indirectly, by

modulating spindle attachments (Khodjakov and Pines, 2010; Maresca and Salmon, 2010; Nezi and Musacchio, 2009; Pinsky and Biggins, 2005). This regulation is achieved by error-correction (EC) mechanisms, primarily mediated by Aurora B kinase (AurB), which destabilize kinetochore-microtubule (KT-MT) interactions that are not under tension (Carmena et al., 2012; Liu et al., 2009).

Sister chromatid cohesion, mediated by the cohesin complex (Nasmyth and Haering, 2009; Peters and Nishiyama, 2012), is a major contributor to the establishment of tension, as it provides the counterforce that resists microtubule pulling forces upon spindle attachment (Oliveira et al., 2010; Tanaka et al., 2000). Cohesin is therefore essential for faithful mitosis, as it promotes biorientation and thereby prevents random genome segregation. Upon premature sister chromatid separation (PSCS), avoidance of mitotic errors relies on the SAC's ability to respond to cohesion defects and efficiently inhibit mitotic exit. However, defects in cohesion are associated with aneuploidy, including some human disorders linked to cohesin malfunction (Barbero, 2011; Brooker and Berkowitz, 2014; Losada, 2014), implying that mitotic exit takes place despite PSCS. Moreover, studies in budding yeast and mammalian cells indicate that cells with unreplicated genomes or PSCS eventually exit mitosis (Michaelis et al., 1997; O'Connell et al., 2008). This conundrum raises the possibility that cohesion loss results in weak SAC activation despite the established role for sister chromatid cohesion as a major tension contributor. The molecular mechanisms behind this poor response, however, are not fully understood. Here, we report a quantitative analysis on the robustness of SAC activation during mitosis when sister chromatid separation occurs prematurely.

RESULTS

Premature Loss of Sister Chromatid Cohesion Does Not Elicit a Robust SAC Response

To determine the strength of SAC response to PSCS, we used a tool for acute removal of cohesin in *Drosophila melanogaster*, based on artificial cleavage of the cohesin protein Rad21 by an exogenous protease (tobacco etch virus [TEV]) (Oliveira et al., 2010; Pauli et al., 2008). We focused our analysis on developing larval brain neuroblasts (NBs), stem cells that give rise to the

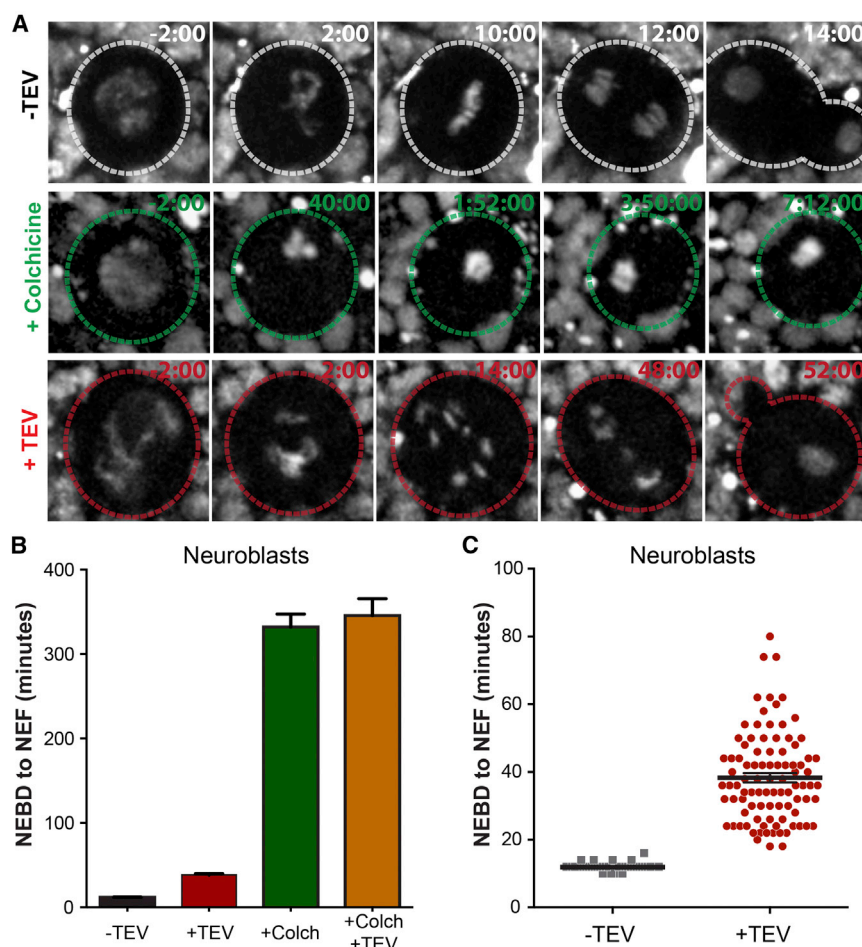


Figure 1. Premature Loss of Sister Chromatid Cohesion Induces a Short Mitotic Delay

(A) Images of dividing *Drosophila* neuroblasts from heat-shocked control strains (top), wild-type brains in 100 μ M colchicine (middle), and strains surviving solely on Rad21^{TEV} after TEV expression (bottom); strains express HisH2Av-mRFP1. Times (min:s) are relative to NEBD; scale bar, 5 μ m.

(B) Average mitosis duration (NEBD to NEF) in heat-shocked control (n = 41, N = 4), TEV-mediated cohesin cleavage (n = 93, N = 8), colchicine-treated (n = 57, N = 6) and colchicine-treated after cohesin cleavage (n = 15, N = 2) larval neuroblasts (mean \pm SEM).

(C) Mitosis duration (NEBD to NEF) in wild-type (heat-shock control) and TEV-mediated cohesin cleavage larval neuroblasts.

n, number of cells; N, number of brains. See also Figure S1 and Movies S1 and S2.

CNS. These cells arrest for many hours in mitosis when incubated with spindle poisons such as colchicine (Figures 1A and 1B). To induce cohesin cleavage, we used strains that contain solely TEV-sensitive cohesin complexes and express TEV protease under the heat-shock promoter (Pauli et al., 2008). Heat shock delays mitotic entry and nuclear division is resumed 148 \pm 75 min (n = 113 cells, N = 14 brains analyzed) after heat shock, enabling analysis of cohesin loss within a single cell cycle (Figure S1). To evaluate the robustness of the SAC in the presence of PSCS, we quantified the time cells spend in mitosis (from nuclear envelope breakdown [NEBD] to nuclear envelope formation [NEF]). Whereas mitosis in control cells lasts \sim 12 min (with or without heat shock), TEV-mediated cohesin cleavage results in longer mitosis (38.3 \pm 13.1 min) (Figures 1 and S1D; Movies S1 and S2). NBs from larvae not subjected to heat shock do not show any mitotic delay (Figure S1D). These results indicate that NBs elicit a SAC response that delays mitotic exit in response to PSCS. However, this arrest is relatively modest when compared to colchicine-induced arrest (Figure 1B). A similar response was observed in ganglion mother cells (GMCs), secondary precursor cells that derive from NBs (Figures S1E and S1F). Importantly, cohesin cleavage does not shorten the mitotic arrest in colchicine (Figure 1B), implying that cohesin

depletion alone has no major effect on the SAC signaling capacity.

Loss of Sister Chromatid Cohesion Activates EC Mechanisms during Early Mitosis

Drosophila neuronal cells are therefore highly SAC competent in response to spindle poisons but fail to respond robustly to cohesion loss. Prematurely separated single chromatids can form transient attachments to the spindle, yet these attachments lack forces significant

enough to oppose microtubule pulling forces and likely have a reduced ability to generate tension (both inter- and intrakinetochores tension). Transient attachments should therefore be destabilized by the EC machinery (Carmena et al., 2012; Liu et al., 2009), creating unattached kinetochores that provide a SAC signal sufficiently strong enough to prevent mitotic exit. In contrast, our findings imply that the EC machinery and the SAC respond inefficiently to PSCS. Recent evidence suggests that, upon depletion of cohesin subunits, AurB is not properly localized and shows reduced activity toward its targets (Carretero et al., 2013; Kleyman et al., 2014; Yamagishi et al., 2010). In accordance, AurB, as well as the related haspin-mediated chromatin mark (histone-H3T3 phosphorylation), is delocalized and specifically reduced in the centromere vicinity upon cohesin cleavage (Figure S2). However, two critical observations indicate that a malfunctioning error correction cannot fully explain the reduced SAC response. First, during the initial stages of the arrest, we observe high levels of chromosome motion with oscillatory movements between the poles (Figures 2 and S3; Movies S2 and S3). Quantitative analysis of chromosome movement, estimated from the displacement of centromere positions (see details in Supplemental Experimental Procedures), reveals a high degree of chromatid motion, as evidenced by the high frequency

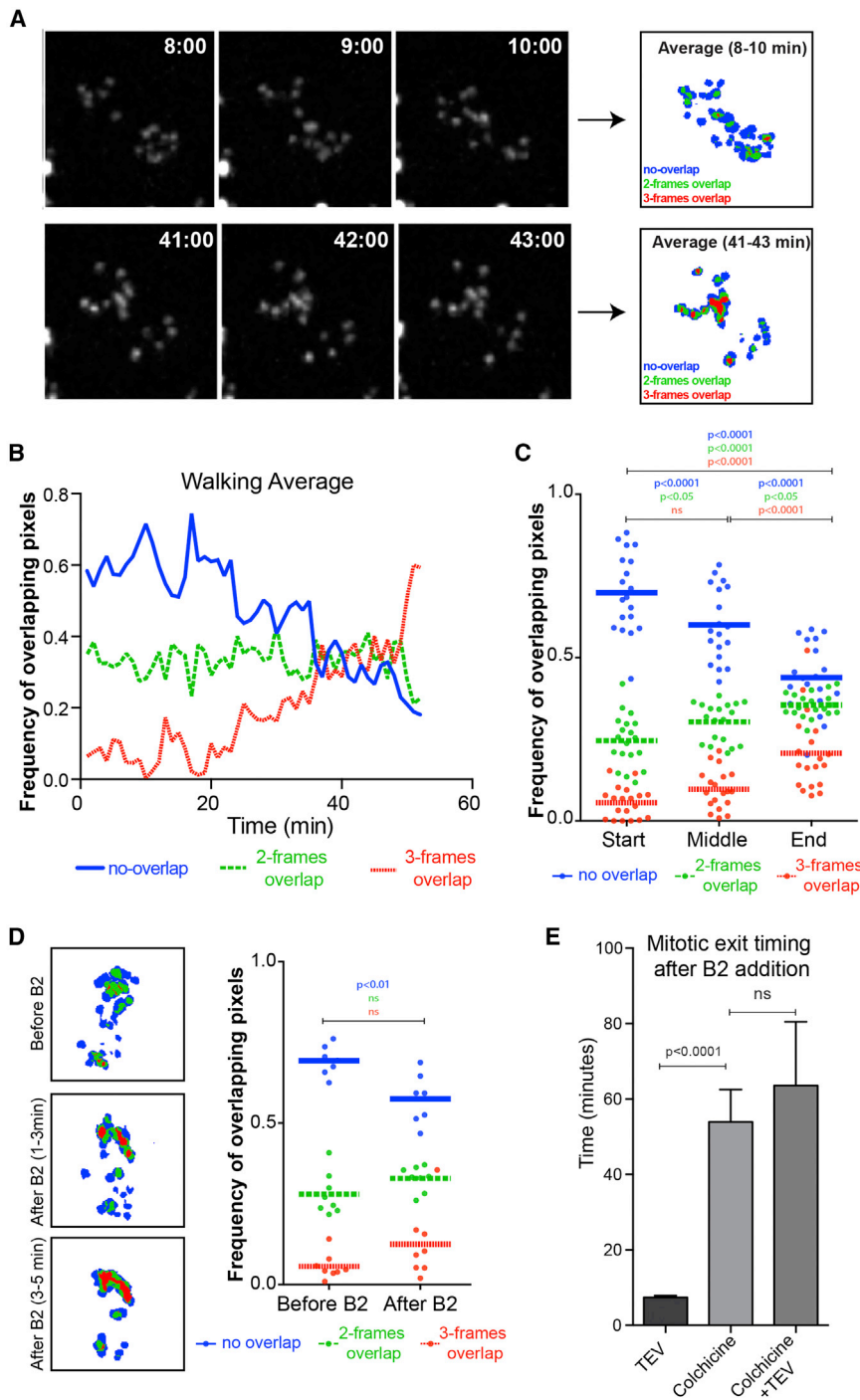


Figure 2. Single Chromatids Display a Highly Mobile Behavior that Gradually Declines during Cohesin Cleavage-Induced Mitotic Delay

(A) Stills from live-cell imaging of CID-EGFP-expressing neuroblasts upon cohesin cleavage (t_0 = NEBD); left panel represents average of the binary images of three consecutive frames, used to estimate centromere displacements: blue, non-overlapping pixels; green, two- out of three-frame overlap; red, three-frame overlap.

(B) Frequency of overlapping pixels to estimate centromere displacement (as in A), throughout mitosis with PSCS.

(C) Centromere displacement at different times of arrest upon TEV-mediated cohesin cleavage: start, 6–10 min after NEBD; end, 6–10 min before anaphase onset; middle, 5 min at the midpoint of the arrest ($n = 23$, $N = 3$); p , adjusted p value by two-way ANOVA.

(D) Centromere displacement before and after addition of the AurB inhibitor binucleine-2 (final concentration, 25 μ M); binucleine-2 was added 6–10 min after NEBD and centromere displacement was measured immediately after until anaphase onset ($n = 8$, $N = 3$); p , adjusted p value by two-way ANOVA.

(E) Mitotic exit time after binucleine-2 addition in TEV-cleavage ($n = 33$, $N = 3$), colchicine treatment ($n = 26$, $N = 5$), and colchicine + TEV ($n = 20$, $N = 3$) experiments (mean \pm SEM).

n , number of cells; N , number of brains. See also [Figures S2](#) and [S3](#) and [Movies S2](#) and [S3](#).

of non-overlapping centromere positions between consecutive frames ([Figures 2A–2C](#)). Such movements likely result from consecutive cycles of chromosome attachment, which are subsequently detached due to their low-tension state. Accordingly, movements are strongly reduced when AurB is inhibited by a specific inhibitor (binucleine-2) ([Figure 2D](#)). Second, the short but noticeable SAC response observed after cohesin cleavage depends on AurB activity. The addition of binucleine-2 to cells

that have just entered mitosis, and would thus be expected to delay mitotic exit for ~ 40 min, leads to abrupt mitotic exit in $\sim 7.5 \pm 0.5$ min ([Figure 2E](#)). This sharp mitotic exit could be attributed to the impairment of AurB activity in the destabilization of tension-less KT-MT attachments or, alternatively (or additionally), to the known role of this kinase in the SAC signaling ([Hauf et al., 2003](#); [Maldonado and Kapoor, 2011](#); [Santaguida et al., 2011](#); [Saurin et al., 2011](#)). If AurB activity contributes primarily to SAC activity, its inhibition should abrogate the SAC abruptly even when the checkpoint is activated by the absence of spindle attachments. To test this, we monitored the time of mitotic exit upon binucleine-2 addition to colchicine-arrested cells, revealing that NBs eventually exit mitosis but take longer to do so, regardless of whether cohesin has been cleaved or not ([Figure 2E](#)). These results suggest that reversion of AurB-mediated phosphorylation events required for SAC maintenance is kinetically slow. We therefore favor that the sudden mitotic exit observed upon AurB inhibition in cohesin cleavage experiments results primarily from the inhibition of EC activity.

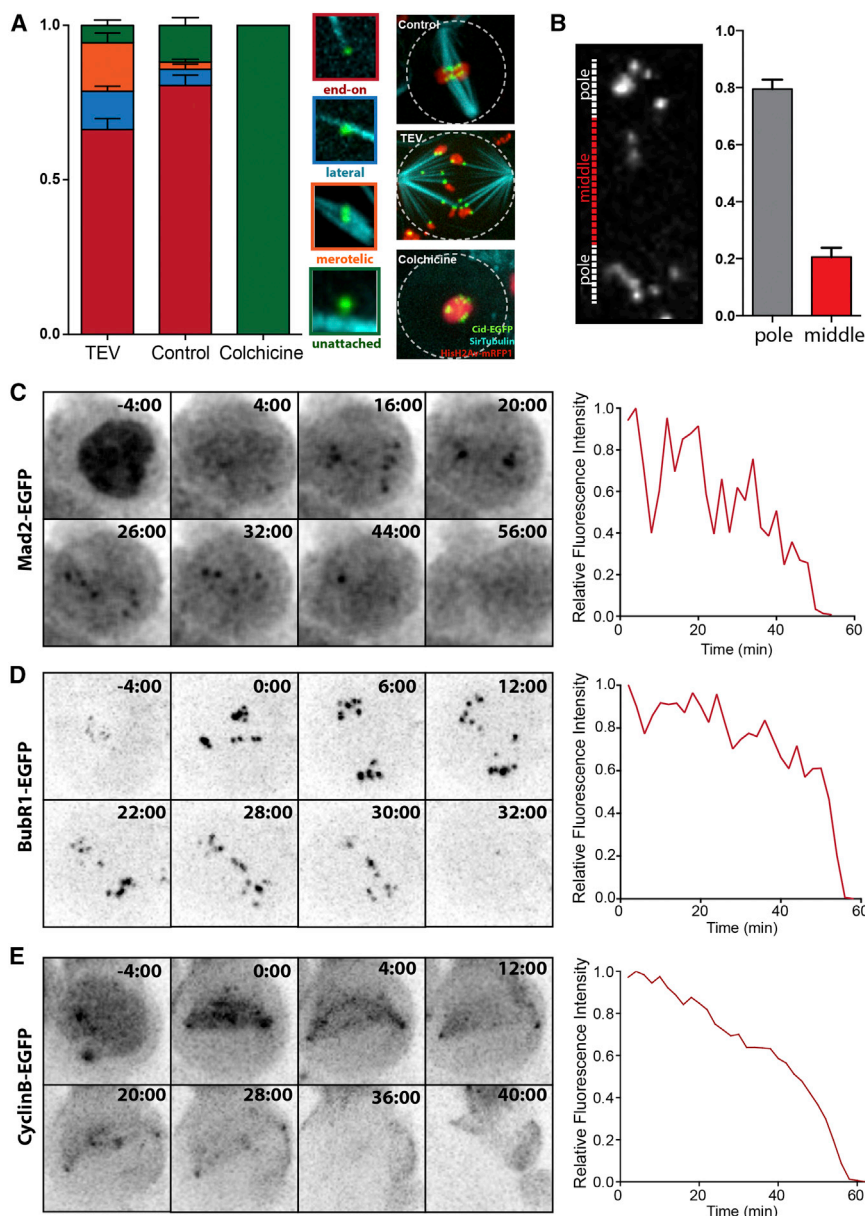


Figure 3. High Frequency of End-on Attachments Leads to Low Production of MCC and Premature Decay of Cyclin B

(A) Frequency of kinetochore attachment observed upon after cohesin cleavage; brains expressing HisH2Av-mRFP1 (red) and Cid-EGFP (green) were shortly incubated with 1:10,000 Sir-Tub probes (cyan) before brain squash. Graph shows average attachment profile for control (prometaphase and metaphase cells), cohesin-cleaved, and colchicine-treated NBs ($n > 25$ NBs, $N = 3$; mean \pm SEM).

(B) Centromere distribution at the time of mitotic exit in cohesin cleaved NBs. For each image, the segregation plane, determined based on the two most distal centromeres, was divided into two equally sized regions as exemplified ($n = 20$, $N = 4$; mean \pm SEM).

(C–E) Stills from live-cell imaging of Mad2-GFP (C), BubR1-GFP (D), and CycB-GFP (E) during the mitotic delay induced by cohesin cleavage. Times (min:s) are relative to NEBD; scale bars, 5 μ m. Graphs represent the relative fluorescence intensity in cohesin cleavage, normalized to the maximum value within each dataset.

n, number of cells; N, number of brains. See also Figure S4 and Movie S4.

stabilization in the presence of single sisters. First, stable attachments could arise from the accumulation of merotelic attachments, as previously reported in mitosis with unreplicated genomes (MUGs) (O’Connell et al., 2008). Second, attachments could be stabilized by tension in the absence of sister chromatid cohesion (e.g., due to cytoplasmic drag). Lastly, attachments may be abnormally stabilized even in the absence of maximal tension.

To distinguish among these possibilities, we analyzed KT-MT attachments in more detail (Figures 3A and S4A). This analysis revealed that cells with PSCS show high microtubule occupancy at kinetochores. The most prevalent form of

Attachments of Single Chromatids to the Mitotic Spindle Are Progressively Stabilized

Taken together, these observations imply that AurB is at least partly functional in the absence of cohesin. If so, why does PSCS not elicit a robust mitotic arrest? Given that the SAC response in the absence of cohesion depends on the ability to generate unattached kinetochores, we have monitored KT-MT interactions throughout mitosis. We first analyzed the degree of chromosome movement at different times of the arrest, as mentioned above. While chromosomes are highly dynamic in the initial stages of the arrest, their movement becomes gradually reduced, suggesting KT-MT interactions are progressively stabilized over time (Figures 2A, 2B, and S3). We envisioned three different possibilities that could account for KT-MT attachment

attachment (66%) displays kinetochores at the end of a well-defined kinetochore bundle (end-on attachment). Very few chromatids appear totally unattached (6%). These findings suggest that even in the absence of cohesion, attachments to the spindle are relatively frequent (Figure 3A). Importantly, the low proportion of merotelic attachments (16%; Figure 3A) suggests that accumulation of these abnormal attachments is not the major cause for the observed decrease in motion. To confirm that this is also the case at mitotic exit, we measured centromere positions at this stage, as merotelic attachments should place centromeres in the middle of the segregation plane. In fact, in some TEV-cleaved cells, we do find centromeres that lag behind the major chromatin mass (on average $\sim 20\%$; Figure 3B) and display obvious stretching once mitotic exit takes place, consistent with being bound to

both poles. However, most kinetochores were found to be placed facing the poles and did not stretch during poleward movement, supporting end-on attachment (Figure 3B). These results indicate that unlike the previous results in MUG cells (O'Connell et al., 2008), cohesion depletion in *Drosophila* NBs leads to mitotic exit without major accumulation of merotelic attachments.

To confirm that KT-MT attachments are indeed stabilized, we monitored the levels of Mad2-EGFP, which labels unattached kinetochores (Buffin et al., 2005), in live cells. We observe that upon cohesin cleavage, kinetochores show significant levels of Mad2 after NEBD (maximal amount approximately one-third of the levels in colchicine; data not shown) but with highly variable amounts during the initial stages of the arrest (Figures 3C, S4B, and S4D). These fluctuations in Mad2 signal are consistent with individual kinetochores undergoing repetitive cycles of Mad2 accumulation (detachment) and removal (re-attachment), as also suggested by their highly dynamic behavior (Figure 2). In addition to these fluctuations, the Mad2-EGFP signal decreases over time and cells exit mitosis once (and only when) all chromosomes are devoid of Mad2. Additionally, quantitative analysis of BubR1, a MCC component that leaves the kinetochores only when sisters are under tension (Buffin et al., 2005; Logarinho et al., 2004), reveals that its levels are reduced (one-third of the levels in colchicine cells) but relatively constant throughout the arrest (Figures 3D, S4C, and S4E; data not shown). We therefore favor that the mostly end-on spindle attachments of single sisters are progressively stabilized, even without maximal tension.

Cyclin B Is Gradually Degraded during Cohesin Cleavage-Mediated Mitotic Arrest

The results above suggest that throughout the mitotic delay, there is a gradual transition between different stages: at first, KT-MT interactions are highly unstable, resulting in a SAC signal strong enough to prevent mitotic exit; subsequently, single chromatids display more stable attachments to the spindle and thus decreased inhibitory signal production. To understand the basis of this transition, we considered the possible dynamic changes across the mitotic network. In contrast to the classical “all or nothing” view of the SAC (Rieder et al., 1995), recent evidence supports a graded SAC activity (Collin et al., 2013; Dick and Gerlich, 2013), arguing that its inhibitory activity is proportional to signal strength. It is therefore conceivable that an initial weak SAC signaling (caused by a high residence time of unstable attachments) leads to a partial APC/C activation and consequent Cyclin B (CycB) decay. To test this hypothesis, we monitored CycB-GFP levels in different experimental conditions. In the presence of colchicine, CycB levels remain high over the period of 1.5 hr (Figure S4F). In contrast, mitosis after PSCS leads to a significant decay in CycB levels (Figures 3E and S4F). This is consistent with a graded SAC response predicting that low MCC levels result in weak APC/C inhibition, leading to partial CycB degradation.

Mathematical Modeling of Multiple Feedback across the Mitotic Network

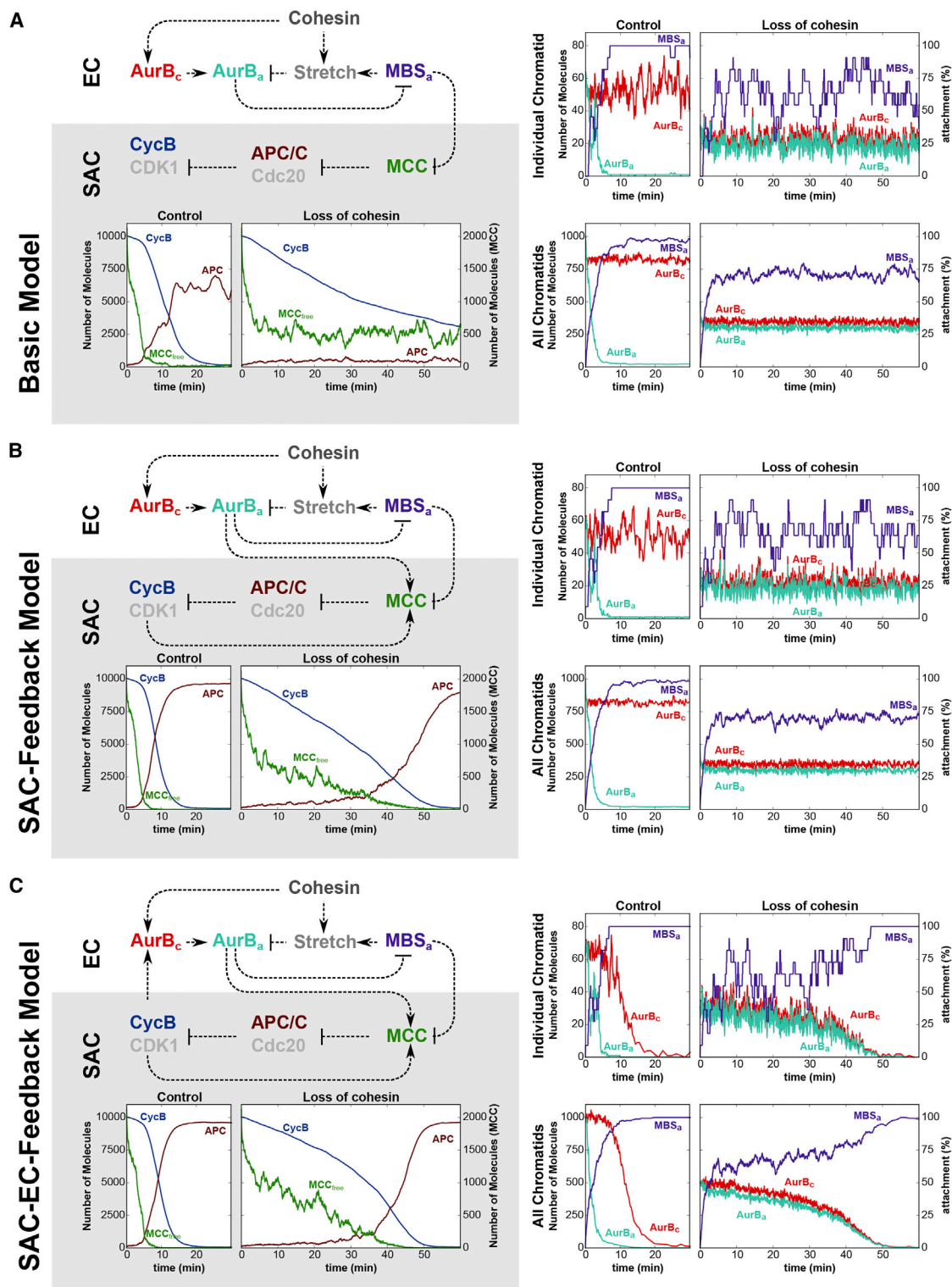
Because Cdk1 and CycB are required for almost all aspects of mitosis, a decay in CycB levels is likely the major drive for mitotic

exit. To distinguish between different possible dynamic networks, we adopted a mathematical modeling approach, which provides a quantitative framework for the description of accelerated mitotic exit observed upon PSCS (Figure 4). We centered this analysis on the EC module, characterized by the role of centromeric AurB complexes in destabilizing attached microtubule binding sites (MBS_a) at KTs (AurB —| MBS_a). AurB action is attenuated by KT stretching (stretch —| AurB), which, in turn, is enhanced by sister chromatid cohesion upon amphitelic attachment. We characterize KT tension by a stretch constant (S), which is set to 1 during normal progression and to a small value (0.2) when cohesin cleavage is induced. The choice for a small but non-zero stretch value was based on recent findings that intrakinetochores stretch contributes to SAC silencing (Maresca and Salmon, 2009, 2010; Nannas and Murray, 2014; Uchida et al., 2009), together with the fact that single sisters were often found attached to the spindle (Figure 3).

Cohesin plays a seemingly paradox role on the action and level of AurB at centromeres (Figures 4 and S5A). The increased stretch caused by sister chromatid cohesion reduces AurB activity toward its targets (MBS_a → stretch —| AurB —| MBS_a), creating a double-negative feedback loop at the heart of the EC module. On the other hand, cohesion potentiates EC by stabilization of AurB molecules at centromeres (Figure S2, (Carretero et al., 2013; Kleyman et al., 2014), captured by reduced dissociation constant of AurB in the model. The net products of the EC module are unattached kinetochores (MBS_u), which through the SAC module catalyze the assembly of the inhibitory signal (MCC) that prevents mitotic exit by inhibiting APC/C-dependent CycB degradation (Figures 4 and S5A). All of these reactions are shared by the three models presented below in order to capture the dynamics of our experimental observations upon cohesin cleavage.

In our basic model, SAC signaling is strictly downstream of the EC module by assuming a constitutive rate for the localization of AurB to the centromere (Figure 4A). The behavior of control cells is nicely recapitulated by carefully chosen set of parameters (see details in Supplemental Experimental Procedures), as in the presence of cohesin, tension lowers AurB activity to stabilize attachments and allow mitotic exit. However, the mitotic timing observed in cohesin cleavage experiments cannot be captured with a small stretch constant, a likely scenario in the absence of cohesin (Figure S5B provides an overview of the stretch parameter effect in all our models). The basic model predicts a stable mitotic arrest in the absence of sufficient tension, because the EC module remains active and generates unattached kinetochores, which produce MCC and block mitotic exit (note the persistent MCC levels and absence of APC/C activation in Figure 4A). For these reasons, we assumed that additional feedback loops accelerate mitotic exit in the presence of single sisters.

In the SAC-feedback model, we considered the role of Cdk1-CycB (D'Angiolella et al., 2003; Rattani et al., 2014; Vázquez-Novelle et al., 2014) and AurB (Hauf et al., 2003; Maldonado and Kapoor, 2011; Santaguida et al., 2011; Saurin et al., 2011) in MCC assembly (Figure 4B). Introduction of these feedback loops accelerates mitotic exit, allowing us to establish kinetic



(legend continued on next page)

parameters that can fit the mitotic timing observed in both control and TEV-cleavage scenarios (Figure 4B and S5). However, this model predicts persistent stochastic fluctuations for the microtubule attachment profile (Figure 4B; note that MBS_{as} do not increase over time), which is inconsistent with our experimental observations (Figures 2 and 3). Additionally, this model postulates a slowing down in CycB degradation toward the later stages of the arrest (Figure 4B). In contrast, we observe that CycB degradation occurs in two stages: an initial linear decay followed by sharp degradation at the mitotic exit (Figure 3E; see rates of CycB degradation in Figure S5C).

For these reasons, an additional feedback loop was introduced by a positive effect of Cdk1-CycB on the EC machinery (SAC-EC-feedback model). Since Cdk1-CycB may affect EC by several mechanisms (e.g., AurB kinase activity/localization or microtubule dynamics), we simply described this effect by Cdk1-CycB dependence on centromeric AurB localization, as Cdk1 inactivation removes centromeric AurB at the metaphase-to-anaphase transition (Hümmer and Mayer, 2009; Mirchenko and Uhlmann, 2010; Pereira and Schiebel, 2003; Vázquez-Novelle and Petronczki, 2010). With the SAC-EC feedback in place, *in silico* simulations of the model fully recapitulate the mitotic progression observed upon PSCS (Figure 4C). In particular, inclusion of a positive feedback between SAC-EC makes the EC module sensitive to the levels of CycB. Consequently, simulations predict a gradual stabilization of KT-MT attachments, as seen experimentally (Figure 2). Additionally, this model postulates that CycB degradation occurs slowly during early stages of the arrest, followed by higher degradation rates at mitotic exit (Figure 3E; see also CycB-degradation rates in Figure S5C).

Cells with Premature Loss of Sister Chromatid Cohesion Are Ultrasensitive to Cdk1 Inhibition

Our experimental data are therefore best described by the SAC-EC-feedback model. Importantly, this model makes a critical testable prediction: mitosis duration upon cohesion depletion is ultrasensitive to mild Cdk1 inhibition. In contrast to a graded sensitivity scenario, in which mitotic timing would be proportional to the level of residual Cdk1 activity, our model postulates that the described feedback loops (SAC and EC feedback) will further accelerate mitotic exit in cells undergoing mitosis with PSCS. Consequently, mild Cdk1 inhibition is predicted to have a strong effect on mitosis duration in these cells (Figure 5A). Colchicine arrest also displays sensitivity to Cdk1 inhibition, although in this case to a lesser extent (note that in the absence of MT attachment, there is only one feedback [SAC feedback] potentiating sensitivity).

To test this prediction, we have first investigated the efficiency of different doses of Cdk inhibitor roscovitine in promoting mitotic exit in colchicine-arrested cells (Figures 5B and 5C). While addition of 100 μ M roscovitine is sufficient to abolish the colchicine arrest, a tenth of this inhibitor dose (10 μ M) does not promote significant mitotic exit within the tested time frame (2 hr) (Figures 5B and 5C). Importantly, control NBs incubated with 10 μ M roscovitine are able to enter and progress through mitosis with normal timing (Figure 5D). In contrast, such mild inhibition caused a significant reduction in the mitotic timing of cells undergoing mitosis with PSCS (Figure 5D).

The shorter mitotic delay observed upon mild Cdk1 inhibition is postulated to arise from an increased accumulation of KT-MT attachments with concomitant SAC signaling decrease (lower MCC production rate) (Figure 5E). Accordingly, the number of Mad2 signals at kinetochores in TEV-cleaved NBs, upon mild Cdk1 inhibition (10 μ M roscovitine), was drastically reduced when compared to DMSO controls (Figure 5F). These results indicate that mild Cdk inhibition is sufficient to stabilize KT-MT interactions and decrease SAC signaling, despite having no effect on either mitotic progression of control cells or reverting colchicine-induced arrest. Thus, cells with premature loss of sister chromatid cohesion are ultrasensitive to Cdk inhibition due to the multiple feedback loops across the mitotic network. This further suggests that among the many aspects of mitosis controlled by Cdk1, KT-MT attachment stability and SAC response are among the most sensitive ones.

DISCUSSION

Our analysis reveals that removal of a major tension contributor, such as sister chromatid cohesion, is insufficient for robust SAC activation. Such poor response can be attributed to two major findings. First, single chromatids attach to the spindle with a high residence time. This may be attributed to slow kinetics of the EC mechanisms suboptimal efficiency of the EC machinery (Figure S2; Carretero et al., 2013; Kleyman et al., 2014; Yamagishi et al., 2010), and/or the existence of additional forces (e.g. polar ejection forces) that stabilize KT-MT attachments of single chromatids (Drpic et al., 2015 [this issue of *Cell Reports*]). This, in turn, results in low MCC production. Second, low MCC levels lead to partial CycB degradation, which feeds back on EC and MCC generation, promoting further stabilization of KT-MT attachments and a decrease in MCC production.

The feedback loops described in the SAC-EC-feedback model depict an amplification (positive feedback) loop between

(A) Basic model. The EC module uses AurB activity (AurBa) to destabilize KT-MT attachments and thereby increases the frequency of attached microtubule-binding sites (MBS_{as}); MBS_{as} become stretched and reduce the action of AurBa; cohesin influences both the activity of AurB and the stretch; MBS_{as} input into the SAC module and suppress the formation of mitotic checkpoint complexes (MCCs); MCC inhibition of APC/C-dependent CycB degradation regulates Cdk1 activity, which is the output of the SAC module.

(B) The SAC-feedback model is an extension of the basic model. An additional internal positive feedback loop within the SAC module via Cdk1 and AurB promotes the production of MCCs.

(C) The SAC-EC-feedback model is a further extension of the SAC-feedback model, where Cdk1 activity not only promotes MCC assembly but also promotes centromeric AurB localization. The mutual input-output relationship between EC and SAC creates a positive feedback (amplification) loop (EC \rightarrow SAC \rightarrow EC). See also Figure S5.

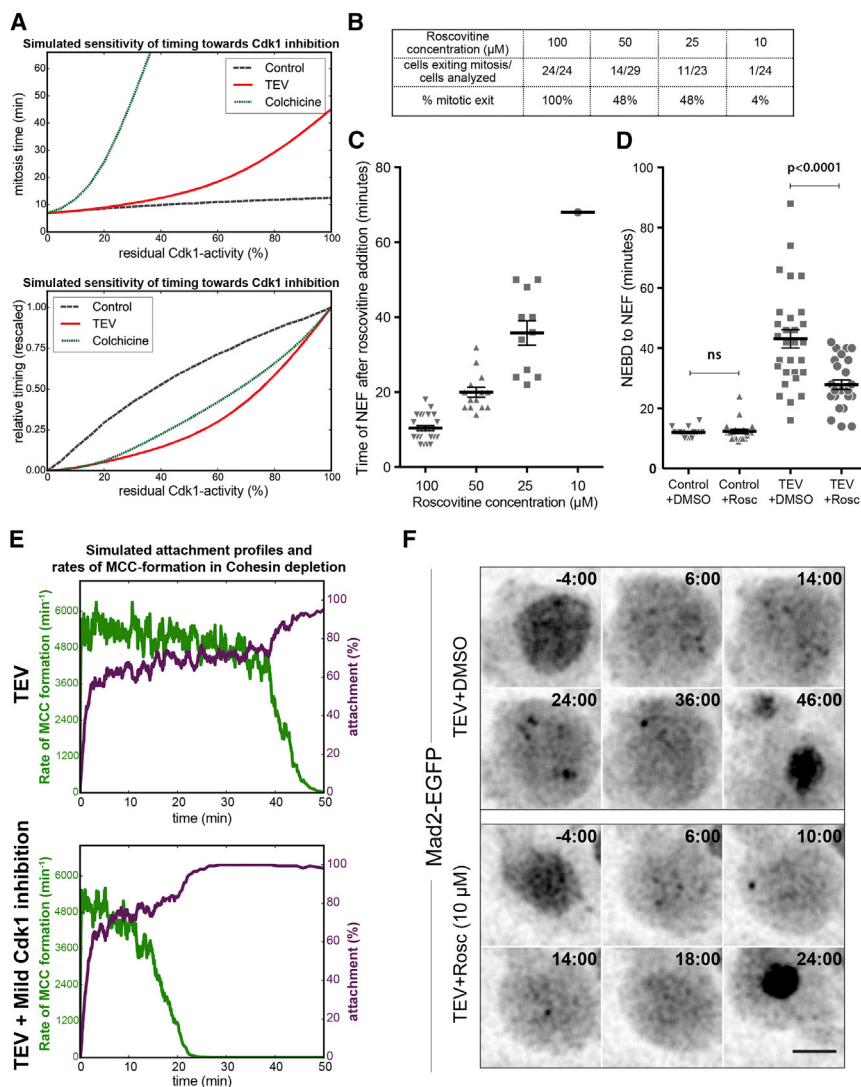


Figure 5. Mitosis with Precociously Separated Sister Chromatids Is Ultrasensitive to Cdk1 Inhibition

(A) Predicted sensitivity of control, TEV-, and colchicine-treated cells to Cdk1-inhibition. Mitotic exit timing was determined by the time when the CycB level is reduced to 10% of its initial value. Bottom panel shows relative sensitivity of the different treatments; mitotic durations were rescaled between 0 (mitotic duration at 0% Cdk1-activity) and 1 (mitotic duration at 100% Cdk1 activity).

(B and C) Frequency (B) and time (C) of mitotic exit observed upon the addition of different doses of roscovitine to colchicine-arrested brains within 2 hr.

(D) Mitosis duration in wild-type and TEV-mediated cohesin cleavage larval neuroblasts, with and without prior incubation with 10 μM roscovitine; p, adjusted p value by one-way ANOVA.

(E) Comparison of simulated attachment profiles and rates of MCC formation for cohesin cleaved cells with full Cdk1 activity (top) and subjected to $\sim 30\%$ Cdk1 inhibition (bottom).

(F) Stills from live-cell imaging of Mad2-GFP during the mitotic delay induced by TEV-mediated cohesin cleavage with and without incubation with 10 μM roscovitine. Times are relative to NEBD; scale bar, 5 μm .

the EC and SAC modules (EC \rightarrow SAC \rightarrow EC) that in control cells stabilizes the high-Cdk1-activity mitotic state until biorientation is achieved. However, these feedback loops render premature cohesin loss almost insensitive to SAC surveillance. Additionally, the high sensitivity of EC and SAC to Cdk1 inhibition described here may facilitate their rapid inactivation during anaphase, where stable KT-MT attachments have to be maintained despite the sudden loss of cohesion (Kops, 2014; Oliveira and Nasmyth, 2010). The caveat of such sensitivity is that it compromises how PSCS is sensed by the mitotic checkpoint. The frail SAC response upon PSCS may result from a weak contribution of cohesion defects as a selective pressure throughout evolution.

Drosophila has a low number of chromosomes (eight), making it more prone to silence the SAC upon cohesin cleavage within a testable time frame. As such, loss of cohesion in mammalian cells may lead to a more prolonged SAC response, due to the higher number of signaling kinetochores (e.g., mouse embryos arrest for over 17 hr upon cohesin cleavage in mitosis; Tachi-

lems associated with human disease (e.g., Cornelia de Lange, Roberts, chronic atrial, and intestinal dysrhythmia [CIAD] syndromes) are indeed characterized by relatively mild levels of sister chromatid separation (Brooker and Berkowitz, 2014; Chetaille et al., 2014).

EXPERIMENTAL PROCEDURES

To destroy cohesin by TEV protease cleavage, *Drosophila* strains were used with TEV-cleavable Rad21 (Rad21^{TEV}) in a rad21-null background (rad21^{ex15}, rad21^{550-3TEV-myc}) (Pauli et al., 2008). TEV expression was induced by heat-shocking third-instar larvae at 37°C for 45 min. Brains from third-instar larvae were dissected and prepared for immunofluorescence or live-cell imaging as previously described (Oliveira et al., 2014). The mathematical models were first devised as systems of ordinary differential equations and simulated by Gillespie's stochastic simulation algorithm (SSA) after converting the rate of elementary reactions into propensity functions. Further details on experimental procedures can be found in Supplemental Experimental Procedures, including a complete list of genotypes used and details on tissue preparation, immunofluorescence, imaging acquisition, quantitative imaging analysis, model design, equations, and parameters.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, five figures, two tables, and four movies and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2015.09.020>.

AUTHOR CONTRIBUTIONS

M.M. and R.A.O. designed the experiments; M.M. carried out the experiments. L.H.H. and B.N. performed the mathematical modelling. All authors wrote the paper.

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