Feedback control of chromosome separation by a midzone Aurora B gradient

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Accurate chromosome segregation during mitosis requires the physical separation of sister chromatids before nuclear envelope reassembly (NER). However, how these two processes are coordinated remains unknown. Here, we identified a conserved feedback control mechanism that delays chromosome decondensation and NER in response to incomplete chromosome separation during anaphase. A midzone-associated Aurora B gradient was found to monitor chromosome position along the division axis and to prevent premature chromosome decondensation by retaining Condensin I. PP1/PP2A phosphatases counteracted this gradient and promoted chromosome decondensation and NER. Thus, an Aurora B gradient appears to mediate a surveillance mechanism that prevents chromosome decondensation and NER until effective separation of sister chromatids is achieved. This allows the correction and reintegration of lagging chromosomes in the main nuclei before completion of NER.

The formation of a nuclear envelope that compartmentalizes genomic DNA involves the recruitment of membranes around the decondensing chromatin and insertion of nuclear pore complexes (NPCs) at the anaphase-telophase transition of mitosis (1). However, it is unknown how cells coordinate nuclear envelope reassembly (NER) with the spatial separation of chromosomes during anaphase. Here, we found that the spindle elongation velocity and the respective duration of anaphase in Drosophila S2 cells were inversely correlated, which suggested that incomplete chromosome separation in spindles that elongate more slowly is compensated by increasing anaphase duration (fig. S1, A to C). Pharmacological or RNA interference (RNAi)–based attenuation of spindle elongation velocity also correlated with an increase in anaphase duration (Fig. 1, A to D, and fig. S1, D to G). A similar response was observed

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in human cells (fig. S2, A to C and F to H, and fig. S3, A to C). NER was also delayed on lagging chromosomes and DNA bridges relative to the main nuclei (Fig. 2, A to C). In most cases (~50%), this promoted the correction and reintegration of lagging chromosomes into the main nuclei, and only a smaller fraction (~20%) formed micronuclei. Thus, the anaphase-telophase transition in metazoans cannot be explained by a "clock" model that is set at the onset of anaphase but must take into account the effective separation of sister chromatids before triggering NER. This spatial control of NER appears to be important for the fidelity of chromosome segregation.

The chromosomal passenger protein Aurora B relocates from centromeres to the spindle midzone in anaphase, which produces a phosphorylation gradient (2). Aurora B inactivation on chromatin is also required for chromosome decondensation and NER (3). We noticed that NER was inversely correlated with Aurora B activity on chromosomes and to the proximity of the spindle midzone (Fig. 2, D and D′; fig. S3, D and D′; and fig. S4, A and A′). Measurement of Aurora B activity in S2 cells using a chromatin-targeted Förster resonance energy transfer (FRET) sensor (2) revealed a lower Aurora B activity (FRET increase) as chromosomes separated during anaphase (fig. S4, B to G). Thus, chromosome position along the division axis appears to be monitored by a midzone-associated Aurora B activity gradient that spatially controls NER.

To test this idea, we performed laser microsurgery in metaphase S2 cells to generate acentric chromosome fragments (i.e., devoid of kinetochores and centromeric Aurora B) and found that NER was significantly delayed or inhibited on the lagging acentric fragments (9 out of 11 cells) (Fig. 3, A and B). Furthermore, Aurora B accumulation at the spindle midzone and the respective duration of anaphase were correlated (fig. S5, A and C). Prevention of Aurora B localization at the spindle midzone by depletion of the conserved kinesin-6 Subito (Mklp2) (4, 5) extended spindle elongation and anaphase duration (Fig. 3C and fig. S5, A, B, D, and E). Given that global Aurora B inhibition at anaphase onset with the Drosophila-specific Aurora B inhibitor Binucleine-2 (Bi-2) (6) did not perturb anaphase duration (fig. S6A and Fig. 4, A and B), we attributed the anaphase delay after Subito RNAi to Aurora B retention on chromatin (3, 4). NER occurred simultaneously on all chromosomes after Subito RNAi (7 out of 8 cells) or global Aurora B inhibition (12 out of 12 cells), independently of their position along the cell division axis (Fig. 3D and fig. S6A). Thus, Aurora B localization at the spindle midzone is required for spatial regulation of NER. Global Aurora B inhibition at anaphase onset compromised the separation of sister chromatids and led to the formation of polyploid cells with a single nucleus (Fig. 4, A to C, and fig. S7), which indicated that Aurora B activity is required to delay NER in response to incomplete chromosome separation. Similar findings were observed in human cells (fig. S2, D to H, and fig. S3, A to C).

PP1 and PP2A phosphatases are implicated in NER and counteract Aurora B activity on chromosomes (7–9). Inhibition of PP1/PP2A with 300 nM okadaic acid (OA) at anaphase onset prevented chromosome decondensation and NER and only slightly attenuated chromosome segregation velocity relative to controls (Fig. 4, A and C, and fig. S7). Specific inhibition of PP1-87B and PP2A-C by RNAi confirmed that both phosphatases were independently required for timely NER (Fig. 4, D and E, and fig. S8, A to F).

Aurora B regulates chromosome condensation by recruiting its substrate Condensin I (Barren/Cap-H in Drosophila) to chromosomes (10–14), and its localization at the spindle midzone is required for the coordination of chromosome compaction with anaphase spindle length (15). Chromosome localization of Barren fused with green fluorescent protein (Barren-GFP) correlated with chromosome condensation and disappeared as sister chromatids separated (fig. S9, A and B). Moreover, Barren-GFP was enriched on lagging chromosomes, and Aurora B inhibition at anaphase onset caused the removal of BarrenGFP from chromatin, including lagging chromosomes (fig. S9, A to C′). In contrast, PP1/PP2A inhibition with OA at

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anaphase onset prevented Barren-GFP removal from chromatin, and chromosomes remained condensed (fig. S9, D and D′). Finally, RNAi-mediated depletion of Barren was reminiscent of Aurora B inhibition, with cells completing NER before effective chromosome separation (fig. S9, E to H). Histone H3 phosphorylation on S10 and S28 did not affect the anaphase-telophase transition in S2 cells (fig. S10, A to E). Condensin I recruitment to chromosomes during anaphase is thus spatially regulated by the counteracting activities of Aurora B and PP1/PP2A to control chromosome decondensation and NER.

To investigate whether Aurora B inhibition is sufficient to trigger chromosome decondensation and NER, we kept Cdk1 activity constitutively high by the transient expression of nondegradable D90Cyclin B–GFP (fig. S11, A, B, and D), combined or not, with Aurora B inhibition by Bi-2 at anaphase onset. Cells arrested in anaphase for several hours under both conditions (fig. S11, C and G). Thus, in addition to Aurora B, Cdk1 inhibition is required for chromosome decondensation and NER, as shown previously (16–18). Expression of D90Cyclin B–GFP further impaired spindle elongation and Aurora B relocalization from centromeres to the spindle midzone during anaphase (fig. S11, E, F, H, and I), as reported in mammalian cells (18–20). In contrast, Cdk1 inhibition at anaphase onset with RO-3306 accelerated NER in the main nuclei relative to controls and Bi-2–treated cells, independently of chromosome separation, and also decreased spindle elongation velocity (fig. S6, A and B; fig. S12, A to D; and fig. S13, A to D). Thus, Cdk1 appears to work as a "clock" that temporally regulates the formation of a midzone Aurora B–based "ruler," which explains the observed minimal anaphase duration time (~5 min) (Fig. 1D and fig. S1C). Indeed, Cdk1 inhibition at anaphase onset led to a fast accumulation of Aurora B at the spindle midzone (fig. S12, E and F) and, contrary to Aurora B inhibition, NER never took place on lagging chromosomes (10 out of 10 cells) (fig. S6B). Simultaneous inhibition of Aurora B and PP1/PP2A or Cdk1 and PP1/PP2A (with or without Aurora B inhibition) at anaphase onset prevented timely chromosome decondensation (fig. S14). Thus, PP1/PP2A activities are required to dephosphorylate Aurora B and Cdk1 substrates during anaphase and for the spatiotemporal regulation of chromosome decondensation and NER.

Here, we have described a conserved surveillance mechanism that coordinates chromosome separation with chromosome decondensation and NER involving three essential components: (i) a sensor (Aurora B gradient) that monitors incompletely separated chromosomes, (ii) an effector (PP1/PP2A) that promotes decondensation and NER on fully separated chromosomes, and (iii) a target (Condensin I) that maintains chromosome condensation in response to incomplete chromosome separation (fig. S15). This surveillance mechanism operates after spindle assembly checkpoint (SAC) satisfaction and fulfills the criteria to qualify as a "chromosome separation checkpoint." Accordingly, "checkpoints" delay or arrest a late cell-cycle event until completion of an early event and, typically, are only detected in the event of errors (21). One of the strongest pieces of evidence for a checkpoint is the "relief of dependence" when one finds conditions that permit a late event to occur even when an early, normally a prerequisite, event is prevented (21). Here, we showed that chromosome decondensation and NER depend on the previous separation of chromosomes during anaphase. This is monitored by a midzone-associated Aurora B phosphorylation gradient, perturbation of which (either by inactivating global or localized kinase activity) permits chromosome decondensation and NER without completing separation. Thus, the dependence of chromosome decondensation and NER on chromosome separation can be relieved by inactivating global or localized Aurora B kinase activity. This could give rise to polyploidy or micronuclei associated with chromosome aberrations (22, 23). Because NER starts at the distal regions of chromatin during late anaphase (1), a chromosome separation checkpoint would provide an opportunity to correct errors "invisible" to the SAC (e.g., merotelic attachments or catenated DNA) and allow reintegration of lagging chromosomes in the main nuclei before completion of NER. Lagging chromosomes

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sustain Aurora B activity to inhibit abscission during cytokinesis—the so-called NoCut checkpoint that monitors clearance of chromatin from the spindle midzone (24–26). The critical differences between these potential checkpoints are that the spatial regulation of NER involves a default mechanism mediated by a midzone Aurora B gradient that is active during a normal mitosis, whereas NoCut is activated only in the presence of chromatin at the cleavage furrow for successful completion of cytokinesis.

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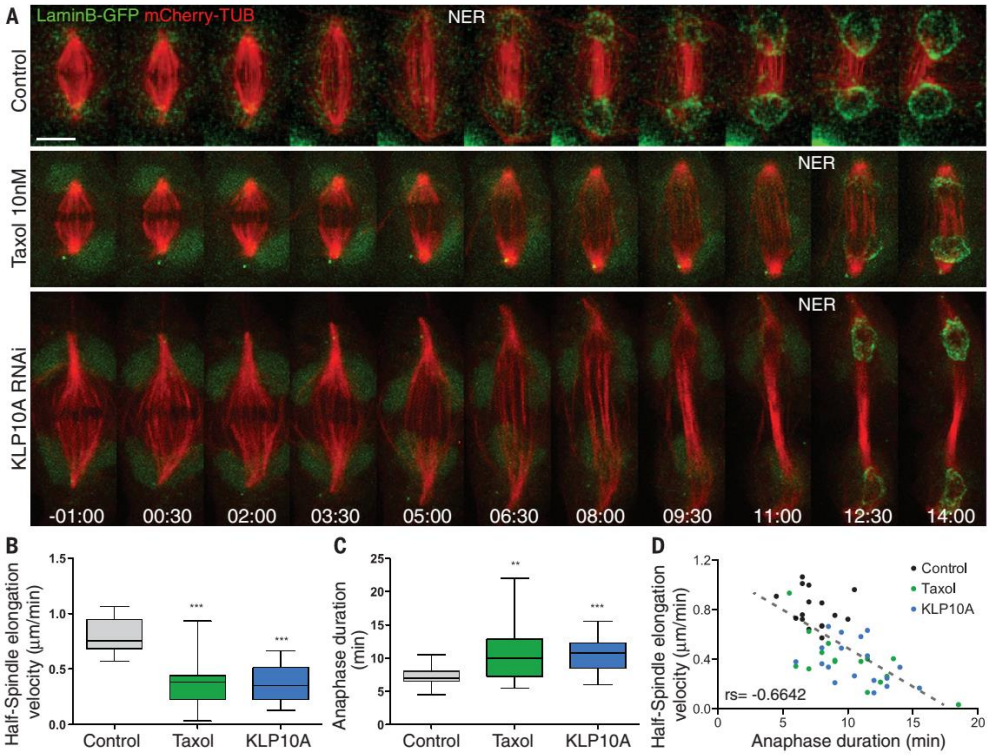

Fig. 1. Incomplete chromosome separation delays the anaphase-telophase transition. (A) Control, Taxol-treated, and kinesin-like protein KLP10A RNAi S2 cells stably expressing Lamin B–GFP and mCherry–α-tubulin (mCherry-TUB).Time shown as minutes: seconds. (B and C) Quantification (box plots) of anaphase duration in response to experimental attenuation of half-spindle elongation velocity. Whiskers define the maximum and the minimum value. Statistics: t test or Mann-Whitney U test depending on the normality of the samples (***P < 0.001; ** P < 0.01). (D) Linear regression and distribution of anaphase duration versus half-spindle elongation velocity in the different conditions. rs, Spearman correlation coefficient, P < 0.001. Note that there is a minimal anaphase duration time (~5 min).

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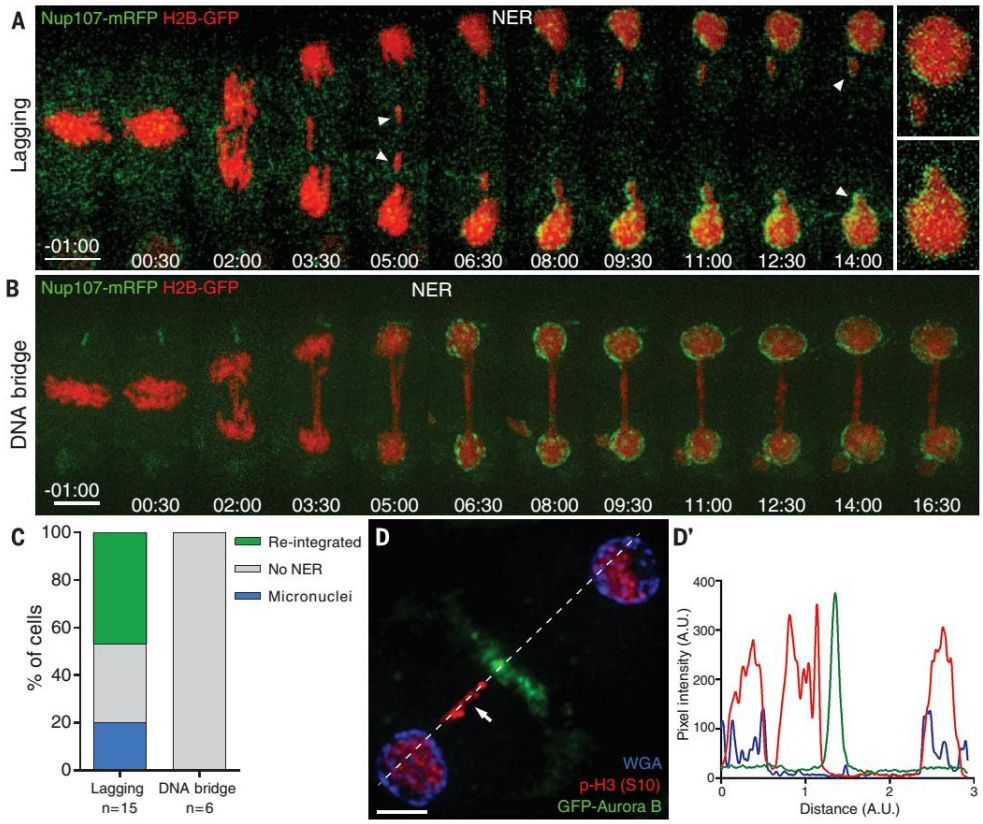

Fig. 2. NER is delayed on lagging chromosomes and DNA bridges. (A and B) S2 cells stably expressing Nup107-mRFP and H2B-GFP showing a delay in NER on lagging chromosomes [white arrowheads in (A)] and (B) DNA bridges. Time shown as minutes: seconds. (C) Many lagging chromosomes are reintegrated into the main nuclei or form micronuclei. Those cases in which the envelope did not form 30 min after anaphase onset were classified as "No NER." (D and D′) NER correlates with lower Aurora B activity [p-H3 (S10)] and chromosome separation. Wheat germ agglutinin (WGA) staining was used to reveal the nuclear envelope. A.U., arbitrary units. Scale bars, 5 μm.

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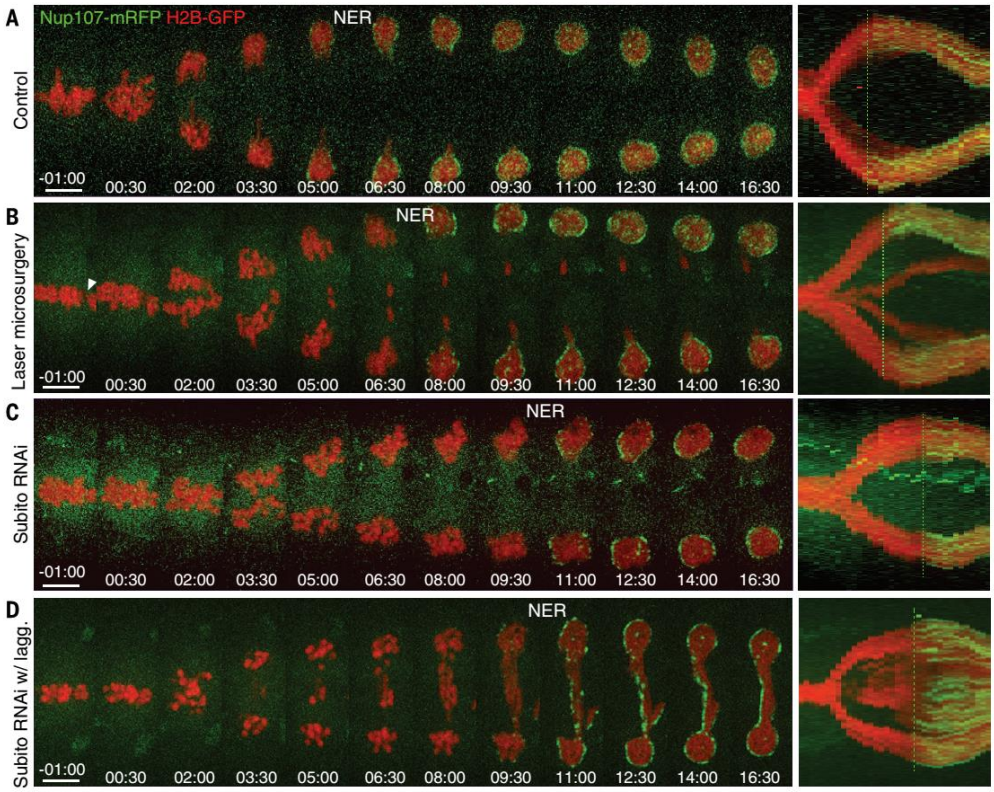

Fig. 3. Aurora B at the spindle midzone is required for spatial control of NER. (A) S2 cell stably expressing Nup107-mRFP and H2B-GFP.Time shown as minutes:seconds. Scale bars, 5 μm. (B) Induction of a centric chromosomes with laser microsurgery (arrowhead) generates lagging fragments that delay NER. (C) Subito/Mklp2 RNAi delays NER. (D) Simultaneous NER on lagging chromosomes and main nuclei after Subito/Mklp2 depletion.

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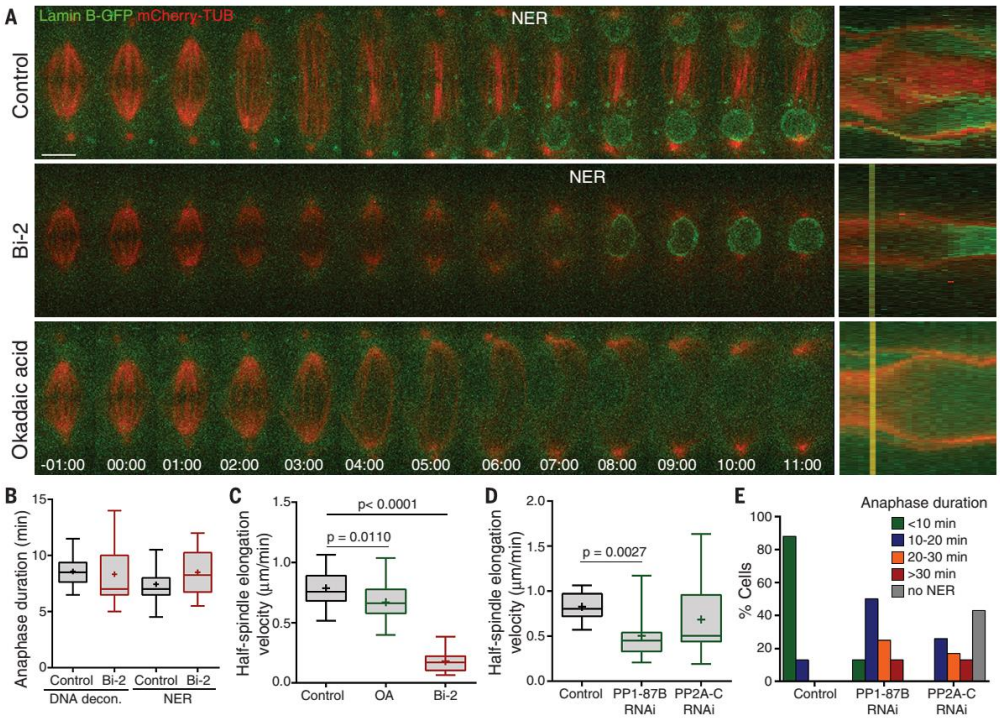

Fig. 4. PP1/PP2A phosphatases counteract Aurora B and are required to promote chromosome decondensation and NER. (A) Acute Aurora B (Bi-2) or PP1/PP2A inhibition (OA) at anaphase onset in S2 cells stably expressing Lamin B–GFP and mCherry–α-tubulin. (B) Quantification of anaphase duration until DNA decondensation or NER in control cells (n = 16 cells per condition) and Bi-2–treated cells (n = 10 cells per condition). Time shown as minutes:seconds. (C) Quantification of half-spindle elongation velocity. (D) RNAi against specific PP1 or PP2A subunits (n = 8 cells per condition) in the Lamin B–GFP/mCherry–α-tubulin cell line (Mann-Whitney U test).The whiskers define the maximum and minimum values. (E) Distribution of anaphase duration in control (n=16cells), PP1-87B (n=8cells), and PP2A-C RNAi (n = 23 cells). Scale bars, 5 µm.

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