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Live cell imaging of anaphase bridge formation and the subsequent cleavage furrow regression induced by the Aurora B kinase inhibitor AZD1152-HQPA

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Summary

Aurora B kinase activity is known to be required for chromosome alignment, segregation and cytokinesis. Its function *in vivo* has been characterized with small-molecule inhibitors, such as ZM447439, Hesperadin and VX-680. Since the inhibition of Aurora B kinase often results in a premature exit of mitosis without chromosome alignment, the events after chromosome segregation have yet to be fully analyzed. To monitor the typical events of mitosis and cytokinesis in the living state, we constructed two fluorescent human melanoma MDA-MB-435S cell lines, expressing mPlum-histoneH3/EGFP-survivin or mPlum-histone-H3/mOrange-actin. We examined the effect of the Aurora B kinase inhibitor AZD1152-HQPA on the entire process from the initiation of mitosis to the completion of cytokinesis. We extensively captured live cell images using a multi-point time lapse imaging system equipped with a PlanApo 60x objective. Although most of the mitotic cells showed premature mitotic exit, some populations of the cells proceeded to anaphase but failed to complete cytokinesis, resulting in binucleated cells. In these cells, we noticed that a chromosome bridge had been formed at the cleavage site during chromosome segregation and that the bridge was maintained after the ingression of the cleavage furrow, known as an "anaphase bridge" in mis-segregating cells. The cytokinesis of these cells was ultimately interrupted with the cleavage furrow regression. A multi-point time lapse imaging analysis of a nonsynchronous, randomly growing cell culture in a higher magnification would be useful for characterizing the real-time effects of other Aurora kinase-inhibitors in living cells.

Keywords

Aurora B, chromosome bridge, chromosome segregation, binucleated cell, cytokinesis, AZD1152

Introduction

In cell division, sister chromatids are equally distributed, and two daughter cells are produced by cytokinesis. These processes are properly controlled by complex networks of various proteins. Some defects in the networks result in apoptosis or aneuploidy of eukaryotic cells. Aurora kinase is one of the key regulators in these networks and plays an important role in cell division (reviewed by Vader and Lens, 2008; Carmena et al., 2009).

Aurora kinase was originally identified as a serine/threonine kinase involved in the centrosome function in *Drosophila* (Glover et al., 1995). The kinase encoded in *Saccharomyces cerevisiae*, Increased ploidy 1 (Ipl1), is highly homologous to the *Drosophila* Aurora, and the loss of the Ipl1 function results in chromosome mis-segregation (Francisco et al., 1994). There are three Aurora-Ipl1 family members in mammals: Aurora A, B and C (Giet and Prigent, 1999). Aurora B was also identified as Aurora and Ipl-like midbody-associated protein (AIM-1) and is necessary for the progression of cytokinesis in mammalian cells (Terada et al., 1998). Aurora B creates chromosome passenger complex (CPC) with INCENP, survivin and Borealin (Carmena et al., 2012). Survivin and Borealin constitute a localization module of CPC associated with the N-terminal end of INCENP, while Aurora B is responsible for its catalytic activity module associated with the C-terminal end of INCENP (Carmena et al.,

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2012). The kinase activity of Aurora B is involved in chromosome alignment, segregation and cytokinesis (Terada, 2001; Afonso et al., 2017).

Aurora B has been well studied in relation to the spindle assembly checkpoint (SAC) (Krenn and Musacchio, 2015). It is required for the recruitment of SAC components, such as BubR1 and Mad2, to the kinetochore in prometaphase (Ditchfield et al., 2003). The kinase activity of Aurora B contributes to chromosome bi-orientation by selectively destabilizing erroneous kinetochore-microtubule attachment without tension (Krenn and Musacchio, 2015). The *in vivo* function of Aurora B in mitosis has been extensively analyzed with small molecules that inhibit its kinase activity. For example, Hesperadin and ZM447439 compromised the above SAC function and caused the premature exit of mitosis without chromosome alignment and segregation, leading to endoduplication (Ditchfield et al., 2003; Hauf et al., 2003). In addition, the role of Aurora B kinase in cytokinesis is widely acknowledged, and recent papers have investigated the mechanisms by which Aurora B controls cytokinesis. When Hesperadin was added to the mitotic cells after anaphase onset, the completion of cytokinesis was inhibited, resulting in a binucleated cell (Guse et al., 2005). Steigeman et al. also reported that Hesperadin and ZM1 induced the cleavage furrow regression in mis-segregating HeLa cells that had been treated with asbestos (Steigemann et al., 2009). Furthermore, cytokinesis of sea urchin zygotes was blocked by VX-680, which was more stable than Hesperadin and ZM447439 (Argiros et al., 2012). However, the Aurora B kinase inhibition of randomly growing mammalian cells has not yet been fully characterized in the living state, as the addition of these inhibitors often results in premature mitotic exit without chromosome segregation (Ditchfiela et al., 2003; Hauf et al., 2003; Guse et al., 2005).

Recently, de Groot et al. suggested that AZD1152-HQPA is the current best choice for an Aurora B-selective inhibitor under long-term live imaging (de Groot et al., 2015). In this paper, we analyze the effects of AZD1152-HQPA on the entire process, from the initiation of mitosis to the completion of cytokinesis, in high magnification by live-cell imaging of human melanoma MDA-MB-435S cell lines.

Materials and methods

Chemicals and RNAi

AZD1152-HQPA (Mortlock et al., 2007) and cytochalasin D were obtained from ApexBio (Houston, TX, USA) and Sigma-Aldrich (St. Lous, MO, USA), respectively. Lipofectamine RNAiMAX (Life Technologies, Inc., Rockville, MD, USA) was used for an RNAi experiment. Oligonucleotides for Aurora B were obtained from Dharmacon (Lafayette, CO, USA).

Construction of stable cell lines and live-cell imaging

The pmOrange-actin plasmid was constructed by inserting an mOrange2 DNA fragment, amplified with pcDNA3-mOrange2 (a gift from Dr. Tsien, University of California at San Diego), into the NheI-BglII site and a 1.1-kb DNA fragment of actin cDNA into the BglII site of pEGFP-C1 (Clontech, Palo Alto, CA, USA). The EGFP-survivin and mPlum-histone H3 plasmids have been constructed before (Fukada et al., 2004; Nakagawa et al., 2016). Human melanoma MDA-MB-435S cell-line (HTB-129), previously described as “breast cancer” (Lacroix 2009), was obtained from ATCC (Manassas, VA, USA). MDA-MB-435S cells expressing mPlum-histone H3 and EGFP-survivin or mOrange-actin were constructed and cultured in DMEM essentially as described elsewhere (Sugimoto et al., 2002; 2008; Sugimoto and Tone, 2010). The cells were inoculated into a 35-mm glass-bottom dish (Iwaki, Chiba, Japan) and grown in an INUG2-ZILCS stage top incubator (Tokai Hit, Shizuoka, Japan).

An Eclipse Ti-S fluorescent microscope (Nikon, Tokyo, Japan) was equipped with a PlanApo VC 60x objective (NA 1.40; Nikon), a ProScan III H117N1 XY-axis stage controller (Prior, Cambridge, UK) and a MAC5000 controller with excitation and emission filter wheels and a Z-axis motor (Ludl Electronic Products, Hawthorne, NY, USA). A 100-W halogen lamp was used as a light source to obtain light at an appropriate wavelength (Sugimoto and Tone, 2010). The respective wavelength characteristics of excitation and emission filters were 480/17 and 517/20 nm for EGFP-survivin, 543/3 and 572/28 nm for mOrange-actin and 591/6 and 632/60 nm for mPlum-histone H3 with a dichroic mirror (Di01-R488/543/594, Semrock, Rochester, NY, USA). Time-lapsed images (10 optical sections with 2-μm distance) at 10-13 points were captured using an ORCA-AG camera (Hamamatsu Photonics, Shizuoka, Japan) through a 0.6x relay lens (Nikon), with exposure for 800 ms for EGFP-survivin and 600 ms for mOrange-actin as well as mPlum-histone H3 at 6-7 min intervals, using the Volocity software program (ver. 6.3.1; Improvision, Coventry, UK).

Fluorescence microscopy

The cells were fixed with 4% paraformaldehyde for 20 min, treated with 0.1% Triton X-100 in PBS for 5 min, and stained with DAPI. Mitotic cells were observed under an Eclipse E600 fluorescent microscope (Nikon) equipped with a PlanApo 100x objective (NA 1.40; Nikon) and an ORCA-ER camera (Hamamatsu Photonics). The images were obtained and analyzed using the LuminaVision software program for MacOS X (Mitani Corporation, Fukui, Japan).

Results

The Aurora B kinase inhibitor AZD1152-HQPA inhibits the completion of cytokinesis

To visualize the mitotic processes, we constructed a stable human melanoma cell-line expressing mPlum-histone H3 and EGFP-survivin (MDA-H3/survivin). Fig. 1A shows the representative images from the initiation of mitosis to the completion of cytokinesis. EGFP-survivin was found in the kinetochore and nuclear envelope region in late G2/prophase (0 min, Fig. 1A). It co-localized with the mitotic chromosomes in prometaphase and metaphase (28-56 min). It stayed in the midzone after anaphase onset (63 min) and was finally concentrated into the midbody (84 min), as reported previously (Skoufias et al., 2000; Fukada et al., 2004). It took 35 min from the initiation of chromosome condensation until the anaphase onset of chromosome segregation (28-63 min) and a further 20 min to complete cytokinesis, totaling 1.5 h. This is consistent with our previous findings with MDA435 cells (Sugimoto et al., 2002).

We then performed the Aurora B RNAi experiment with the MDA-H3/survivin cells. Most of the mitotic cells (75%) showed premature mitotic exit, while 25% showed chromosome alignment and segregation, as shown in Fig. 1B and C. Interestingly, most of the cells failed to complete cytokinesis, resulting in binucleated cells (18 0min, Fig. 1C). To examine whether or not the Aurora B kinase inhibitor AZD1152-HPQA also inhibits the completion of cytokinesis, we captured the live-cell images of MDA-H3/survivin cells in the presence of different concentrations of AZD1152-HQPA. Mitotic cells showed premature mitotic exit at a higher concentration (89% at 150 nM), while some populations of the mitotic cells failed to complete cytokinesis at 100 nM (will be summarized later).

Fig. 1D shows the typical images of the MDA-H3/survivin cells that entered mitosis but failed to complete cytokinesis. In the G2 phase, EGFP-survivin localized to the kinetochore and nuclear envelope region (0 min). Chromosome condensation and nuclear envelope breakdown occurred at 12 min (prophase). Most of the mitotic chromosomes were aligned at the spindle equator in metaphase, but some still did not align even up to 114 min. This is quite different from the above results in the absence of the inhibitor (Fig. 1A). Furthermore, the chromosome alignment was once disturbed at 138 min and then finally completed at 192 min. It took 3 h for this mitotic cell to initiate chromosome segregation (12-198 min). In anaphase, we noticed that some chromosomes were left behind near the cleavage furrow (204 min). They were located near both sides of the midzone where EGFP-survivin was localized, as if they had been caught by the contractile ring. The shape of the mitotic chromsomes was extremely distorted, perhaps due to the poleward tension of the spindle (210 min). After chromosome decondensation, the two daughter cells were drawn towards each other (234 min), and cytokinesis did not progress any further after 246 min. The presence of 2 nuclei in 1 cell was obvious later in interphase (372 min).

The cleavage furrow regression induced by Aurora B inhibitors has been already reported by others, although chromosome mis-segregation was induced by treatment with asbestos or occurred spontaneously during cell culture (Guse et al., 2005; Steigemann et al., 2009). Our results indicated that the Aurora B inhibitor AZD1152-HQPA was indeed able to induce cleavage furrow regression in a randomly growing cell culture.

Cleavage furrow regression depends on mitotic chromosomes trapped at the cleavage site

To examine how mitotic chromosomes were trapped at the cleavage site during cytokinesis, we constructed another stable cell-line expressing mPlum-histone H3 and mOrange-actin (MDA-H3/actin). We then used MDA-H3/actin cells to track the contractile ring formation process. As shown in Fig. 2A, mOrange-actin was observed in the cytoplasm except for the nucleus until prophase (0 min). After chromosome condensation, it localized equally to the cortex (18 min) and stayed there until metaphase (54 min). In anaphase, it concentrated to the cleavage furrow, forming a contractile ring (60 min), and gradually shrank in telophase (66 min). During chromosome decondensation, it localized to the junction of two daughter cells (96 min) and finally redistributed into the cytoplasm in early G1 (120 min), consistent with previous reports (Murthy and Wadsworth, 2005; Steigemann et al., 2009). In the presence of AZD1152-HQPA, mitotic cells showed premature mitotic exit

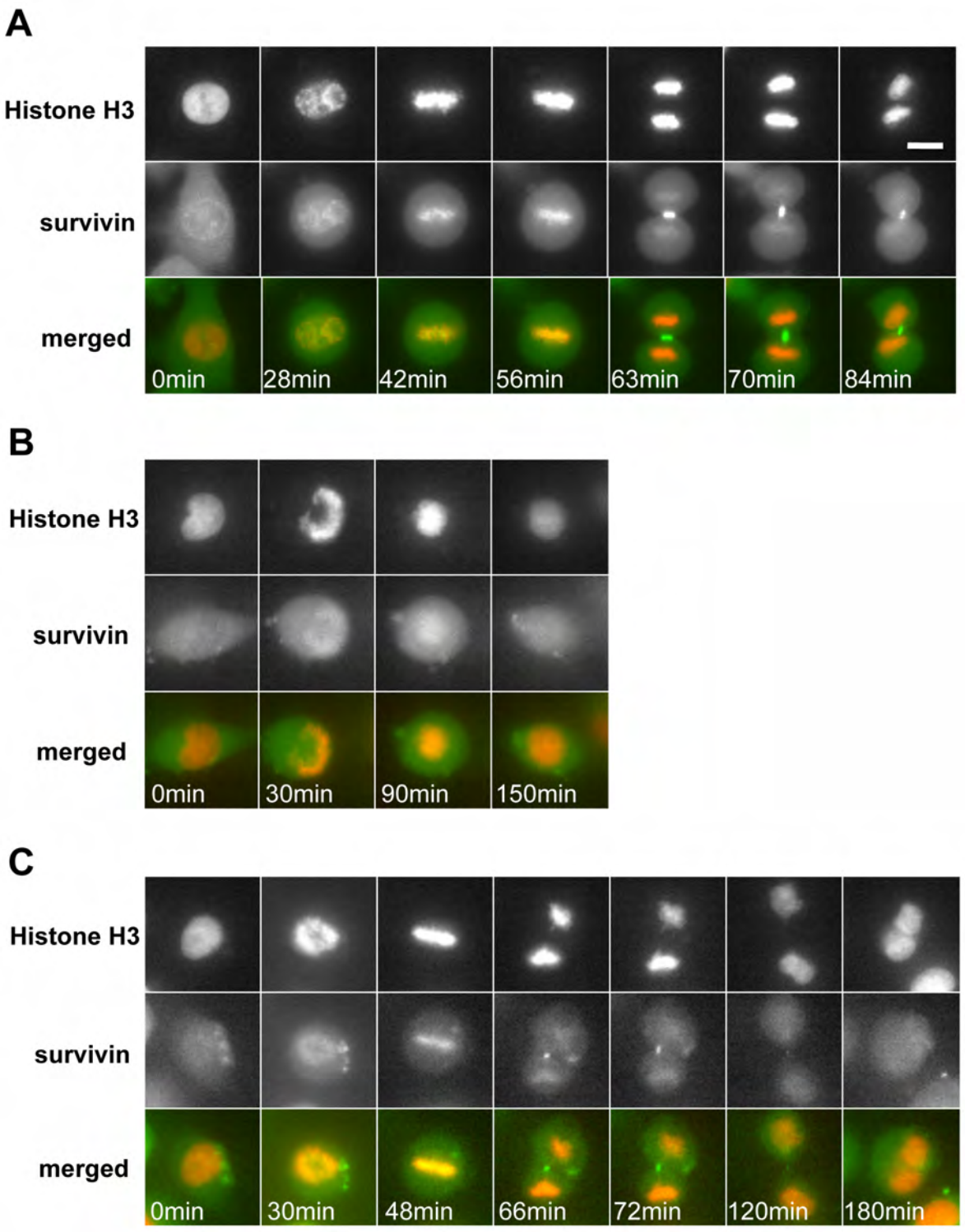
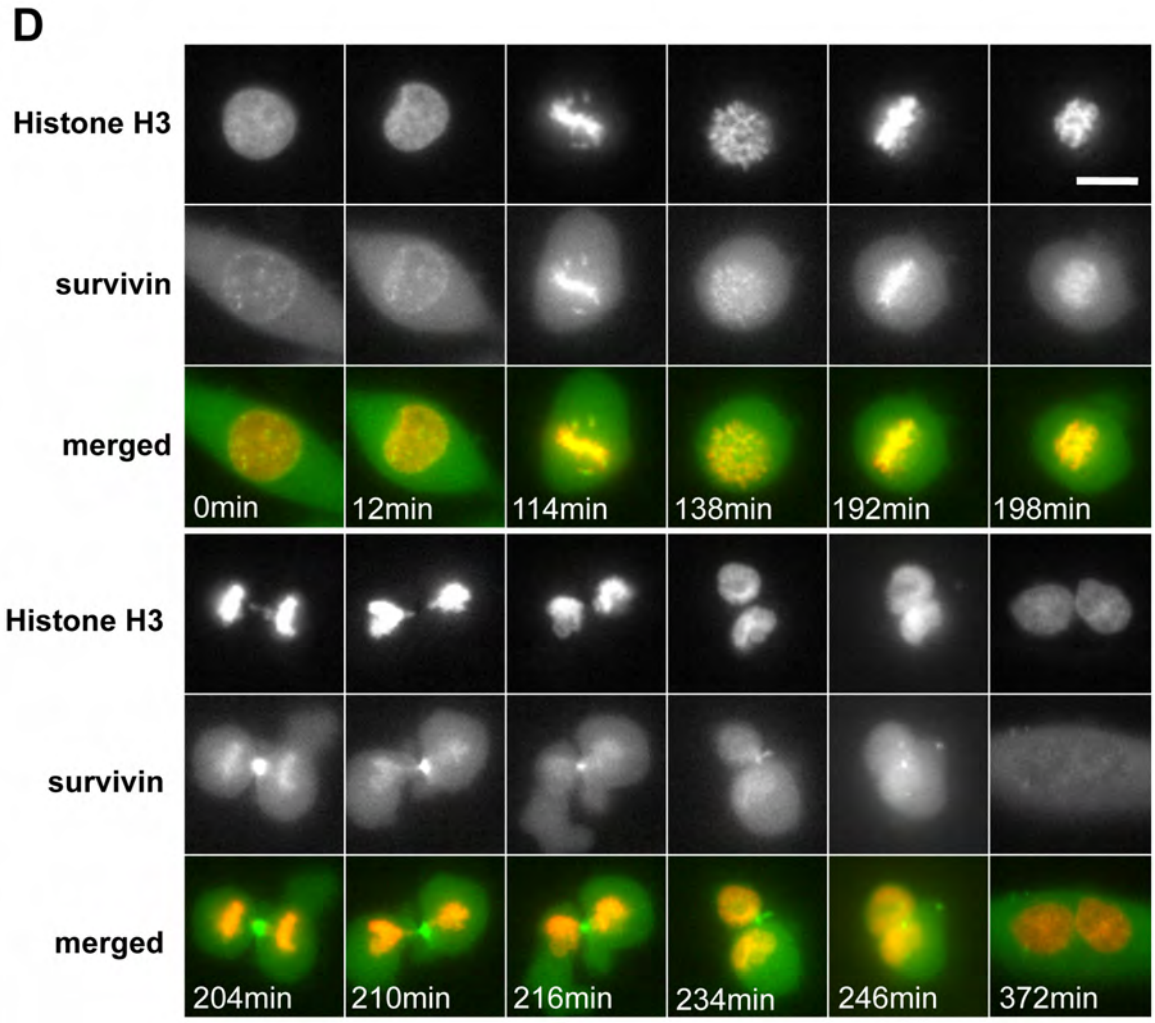


Fig. 1. Effects of AZD1152-HQPA on chromosome alignment, segregation and cytokinesis of MDA-H3/surviving cells. (A) Live-cell images of mPlum-histone H3 and EGFP-survivin in MDA-H3/survivin cells. (B) Time-lapse images of premature mitotic exit without chromosome segregation treated with Aurora B siRNA. (C) Time-lapse images of MDA-H3/surviving cells treated with Aurora B siRNA, showing chromosome alignment, segregation and cytokinesis. (D) Time-lapse images of the mitotic cells that failed in the completion of cytokinesis in the presence of 100 nM of AZD1152-HQPA. Images were captured at 6- to 7-min intervals. Bar, 10 μm.



at a higher concentration (98% at 150 nM), but some population of mitotic cells failed to complete cytokinesis at 100 nM (will also be summarized later). Fig. 2B shows the typical images of MDA-H3/actin cells that initiated mitosis but failed to complete cytokinesis. In interphase, mOrange-actin was localized in the cytoplasm, except for the nucleus (0 min). After chromosome condensation and nuclear envelope breakdown, it localized to the cell cortex (12 min). However, the chromosomes did not align at the spindle equator until 102 min, although cytokinesis would have already occurred at this point in the absence of the inhibitor. The cleavage furrow ingressed in anaphase, and mOrange-actin was concentrated at the cleavage furrow (114 min). We once again observed that some chromosomes had not been properly segregated and were linking to the mass of mitotic chromosomes that had moved to opposite poles. As the contractile ring further shrank (120 min), the shape of the segregated chromosomes was extremely distorted (126 min), consistent with the above observation with MDA-H3/survivin cells (Fig. 1B). Even after chromosome decondensation, the sister chromatids, or chromatin, remained knotted, and the two daughter nuclei developed protrusions toward the cleavage site (138 min), similar to the typical appearance of “anaphase bridge”, which is well known to be caused by DNA replication stress (Acilan et al., 2007). The daughter cells were connected to each other for 2.5 h (204 min), but cytokinesis was suddenly retrograded, and the daughter nuclei were drawn closer to each other at 312 min. It took 5 h for this mitotic cell to initiate the cleavage furrow regression. This result suggested that the cleavage furrow regression depends on mitotic chromosomes trapped at the cleavage site in anaphase.

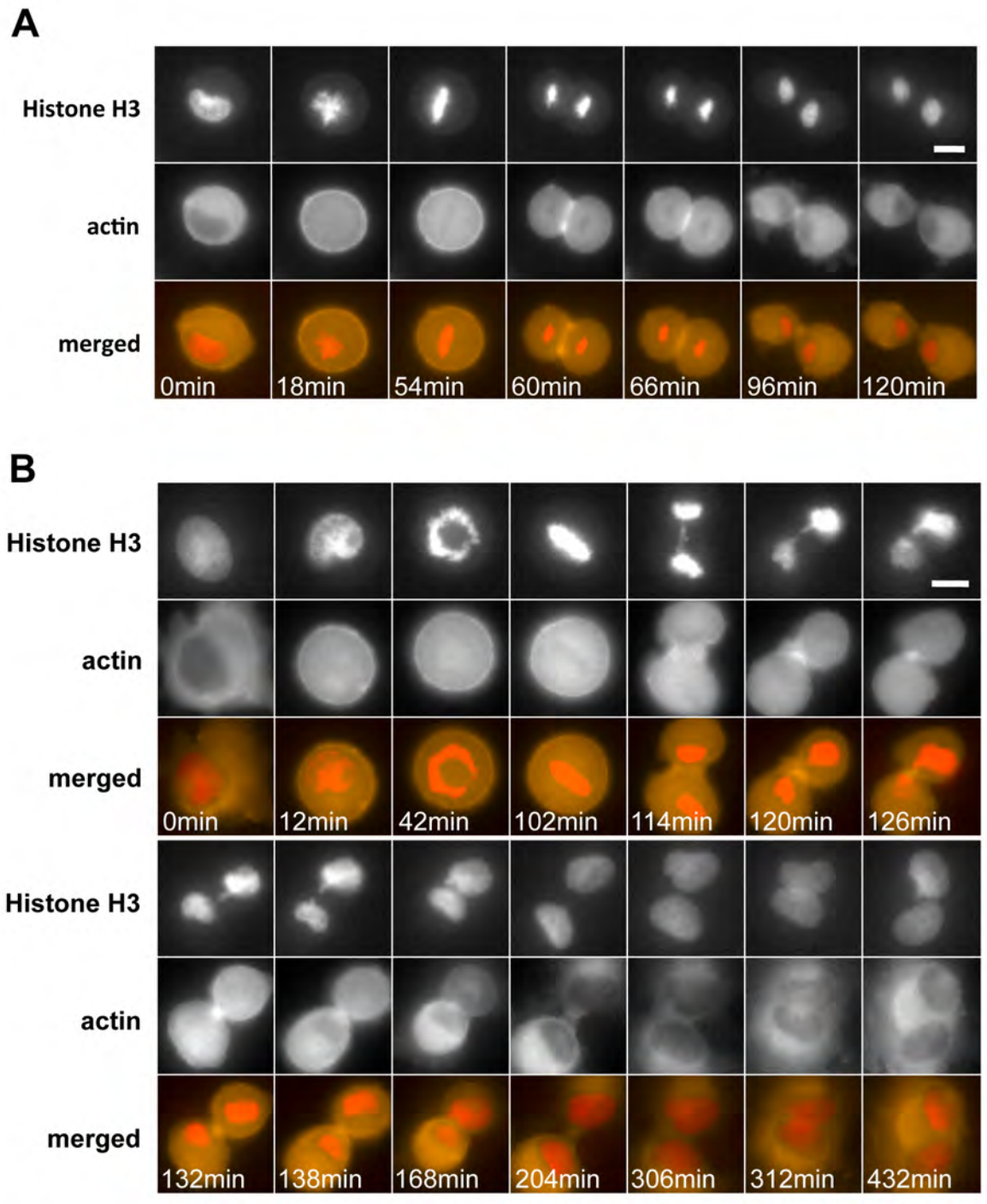


Fig. 2. Effects of AZD1152-HQPA on chromosome alignment, segregation and cytokinesis of MDA-H3/actin cells. (A) Live-cell images of mPlum-histone H3 and mOrange-actin in MDA-H3/actin cells. (B) Time-lapse images of the mitotic cells that failed to complete cytokinesis in the presence of 100 nM of AZD1152-HQPA. Bar, 10 μm.

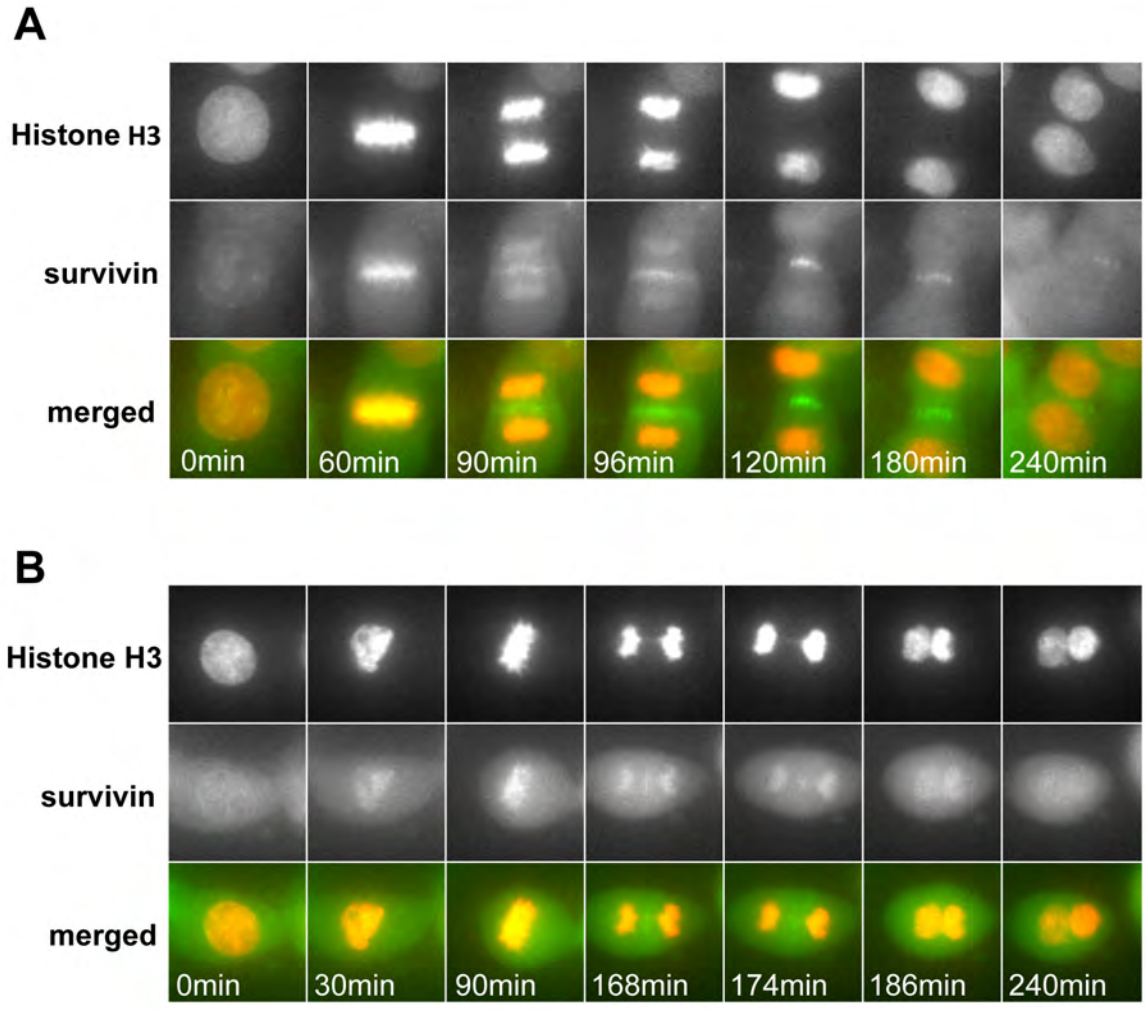


Fig. 3. Formation of a chromosome bridge without cleavage furrow ingression. Time-lapse images of the mitotic cells expressing mPlum-histone H3 and EGFP-survivin (A) Live-cell images of mitosis without cleavage furrow ingression. The cell was treated with 600 nM cytochalasin. (B) Live-cell images of chromosome bridge formation without cleavage furrow ingression. The cell was treated with 100 nM AZD1152-HQPA and 600 nM cytochalasin.

Mitotic chromosomes are trapped at the cleavage site without ingression of the cleavage furrow

We initially believed that the mitotic chromosomes were trapped at the cleavage site by the cleavage furrow ingression. We then examined the chromosome movement at the cleavage site in the presence of cytochalasin, an actin polymerization inhibitor. Fig. 3A shows the time-lapse images of MDA-H3/survivin cells. Until metaphase, EGFP-survivin displayed the same localization as the control cells (see Fig. 1A). EGFP-survivin stayed at the midzone in anaphase and telophase (90-180 min). Although the ingression of the cleavage furrow did not occur, the mitotic chromosomes were segregated properly, producing a binucleated cell at 240 min. In contrast, the mitotic chromosomes were trapped at the cleavage site in the presence of AZD1152-HQPA, without the ingression of a cleavage furrow in anaphase (168-174 min, Fig. 3B). The inhibition of cytokinesis by cytochalasin did not cancel the chromosome bridge formation of these cells. This result suggested that the sister chromatids of mitotic chromosomes, trapped at the cleavage site, had already been connected to each other before the chromosome segregation in anaphase.

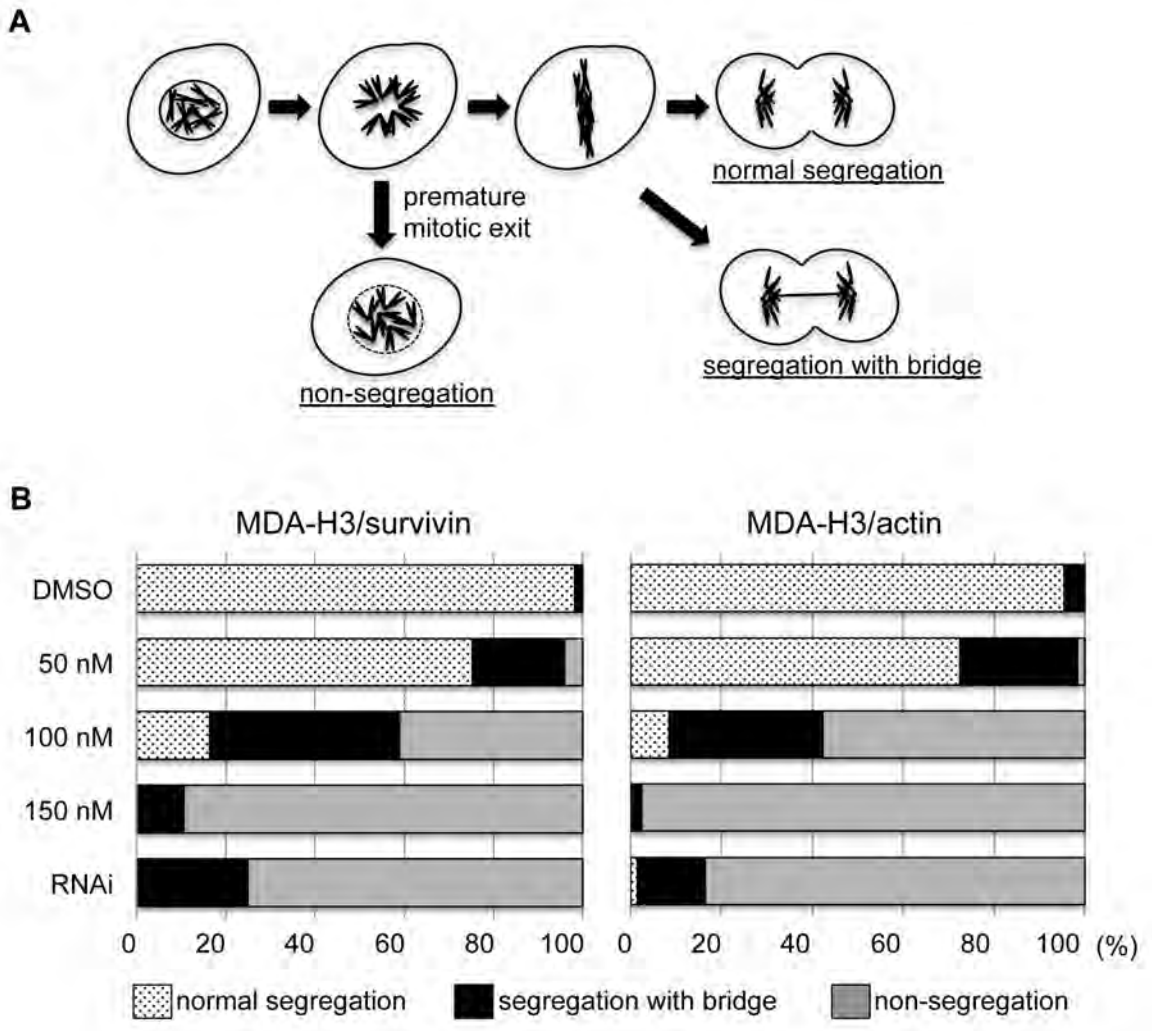


Fig. 4. Frequencies of chromosome bridge formation in mitotic cells with Aurora B inhibition. (A) Chromosome segregation phenotypes of mitotic cells with Aurora B inhibition. When Aurora B was severely inhibited, mitotic cells prematurely exited mitosis without chromosome alignment ("non-segregation"). However, when Aurora B was partially inhibited, mitotic chromosomes were aligned but segregated with a chromosome bridge ("segregation with bridge"). If the inhibition was not effective enough, mitotic chromosomes were aligned at the spindle equator and segregated to opposite poles without a chromosome bridge ("normal segregation"). (B) Relative frequency of chromosome bridge formation of mitotic cells with Aurora B inhibition. Live-cell images of randomly growing MDA-H3/survivin (left) and MDA-H3/actin cells (right) treated with RNAi and AZD1152-HQPA were captured at multiple points of the X-Y axis at 6- to 7-min intervals. AZD1152-HQPA was added at 0 (DMSO), 50, 100 and 150 nM. After imaging analyses of mitotic cells (n=64-169 for MDA-H3/survivin and n=42-109 for MDA-H3/actin), the cells were classified into three categories according to the phenotypes, as illustrated in (A). The percentage of each category was shown as a horizontal bar of "normal segregation" (dotted), "segregation with bridge" (black) and "non-segregation" (gray).

Partial inhibition of Aurora B kinase causes a chromosome bridge, but it does not always induce retrograde cytokinesis

The above results showed that mitotic cells without the bridge completed cytokinesis, while the mitotic cells that failed to complete cytokinesis retained a chromosome bridge at the cleavage site in anaphase (Fig. 1D and Fig. 2B). Although the inhibition of Aurora B kinase itself is known to induce cleavage furrow regression (Guse et al., 2005), cleavage furrow regression may have been induced by the presence

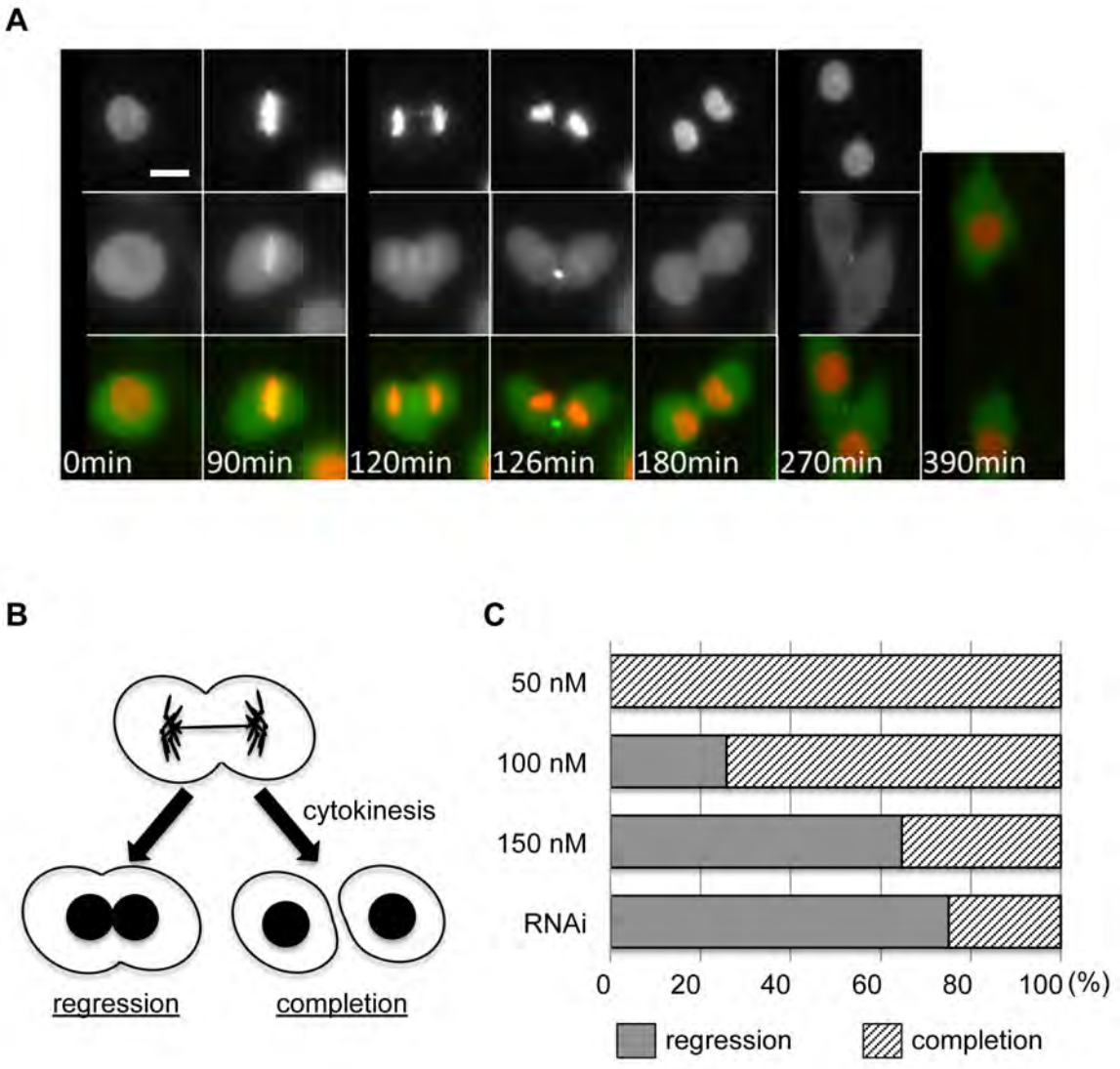


Fig. 5. Frequencies of cleavage furrow regression in mitotic cells with anaphase bridges. (A) Time-lapse images of the mitotic cells expressing mPlum-histone H3 and EGFP-survivin in the presence of 100 nM AZD1152-HQPA. Live-cell images of mitosis with an anaphase bridge but without cleavage furrow regression, resulting in two daughter cells. (B) Cytokinesis phenotypes of mitotic cells with chromosome bridges. Mitotic cells were grouped into two categories: "regression", which showed the cleavage furrow regression, resulting in binucleated cells; and "completion", which resulted in two daughter cells. Bar, 10 μm. (C) Relative frequency of cleavage furrow regression of mitotic cells with chromosome bridges. Mitotic MDA-H3/survivin cells with Aurora B inhibition were further divided into two groups: "regression" (gray) and "completion" (hatched). The total numbers of cells analyzed here are n=15 (50 nM), n=31 (100 nM), n=17 (150 nM) and n=16 (RNAi).

of mitotic chromosomes trapped at the cleavage site by the inhibition of Aurora B kinase. To accurately measure the frequency of the bridge formation, we added AZD1152-HQPA to a randomly growing cell culture of MDA-H3/survivin and MDA-H3/actin at different concentrations and analyzed as many mitotic cells as possible using a multi-point time lapse imaging system. Although most of the mitotic cells showed premature mitotic exit at a higher concentration, we still observed mitotic cells with a bridge. Fig. 4A illustrates chromosome segregation phenotypes of the mitotic cells with Aurora B inhibition, categorized as "normal segregation", "non-segregation" and "segregation with bridge". The relative frequency

of these phenotypes apparently depends on the concentration of AZD1152-HQPA. With MDA-H3/survivin cells, the frequency of the cells with a bridge increased from 21% at 50 nM to 43% at 100 nM but decreased to 12% at 150 nM (Fig. 4B, left). With MDA-H3/actin cells, the frequency increased from 26% at 50 nM to 34% at 100 nM but decreased to just 2% at 150 nM (Fig. 4B, right). These results indicated that chromosome bridges were formed in a narrow range of the inhibitor concentration of AZD1152-HQPA.

We monitored these mitotic cells with bridges in the subsequent stage of cytokinesis and found that they did not always show retrogression of cytokinesis. As shown in Fig. 5A, the mitotic cells with the anaphase bridge entered cytokinesis, and the cleavage furrow ingressed at the equator (120 min). EGFP-survivin, localized at the midzone, was concentrated to the midbody (126 min). After decondensation of mitotic chromosomes, daughter cells were separated from each other (270 min) and finally split in half (390 min). We then examined the correlation between the cleavage furrow regression and the inhibitor concentration. The mitotic cells with chromosome bridges were further grouped into two categories: “regression”, which did not complete cytokinesis, resulting in binucleated cells; and “completion”, which completed cytokinesis, resulting in two daughter cells (Fig. 5B). Fig. 5C shows the relative frequency of “regression” and “completion” of mitotic cells in the next stage of cytokinesis. Interestingly, all of the mitotic cells completed cytokinesis at 50 nM, but 65% of the cells did not complete cytokinesis at 150 nM. In contrast, in the RNAi experiment, more than two-thirds (75%) of mitotic cells with bridges showed cleavage furrow regression. This may suggest that not only the kinase activity but also the presence of Aurora B protein is important for the completion of cytokinesis.

The chromosome bridge is caught by microtubule bundles at the cleavage site and maintained in interphase as a chromatin bridge

We further investigated the effect of the kinase inhibition on the central spindle with MDA-H3/imp/tub cells that we had constructed previously (Fukada et al., 2007, Sugimoto et al., 2008). In anaphase, the chromosomes were partially covered with a nuclear envelope (importin α), and the central spindle (α-tubulin) was formed at the cleavage site (Fig. 6A). EGFP-tubulin was localized to the intercellular bridge and concentrated to the midbody in telophase (Fig. 6C), consistent with the observation by electron microscopy (Mullins and Biesele, 1977). In AZD1152-HQPA-treated cells, the chromosome bridge was located parallel to the central spindle and colocalized with a thick microtubule of the spindle in anaphase (Fig. 6B), as observed with TAMRA-tubulin in NRK cells (Wheatly et al., 1998). In telophase, the chromosome bridge was caught by microtubule bundles at the cleavage site (Fig. 6D). In the binucleated cell, two daughter nuclei were connected by the chromatin bridge that was also covered with a nuclear envelope (Fig. 6E). These results suggest that the bridge formed in anaphase is maintained as a chromatin bridge in interphase.

Discussion

In this study, we examined the effects of the Aurora B inhibitor AZD1152-HQPA from the initiation of mitosis to the completion of cytokinesis of melanoma cell-lines MDA-MB-435S, in which histone H3 and survivin or actin were visualized with two different fluorescent proteins. Although most of the mitotic cells prematurely exited mitosis without chromosome segregation, we observed that some populations of the cells proceeded to anaphase but often resulted in a binucleated cell. The cleavage furrow regression in mis-segregating cells with chromosome bridges, called an “anaphase bridge”, has already been reported in detail by others with light and electron microscopy (Mullins and Biesele, 1977) as well as with flow cytometry and live-cell imaging (Pampalona et al., 2012). In addition, Gues et al. reported that another Aurora B inhibitor (Hesperadin) inhibited cytokinesis when added after the onset of anaphase and indicated that the completion of cytokinesis was regulated by the phosphorylation of MKLP1 (Guse et al., 2005). Our live-cell imaging analysis in a higher resolution showed that AZD1152-HQPA itself was able to induce the formation of a chromosome bridge in anaphase at the first round of the cell cycle. When the concentration of this inhibitor was increased (150 nM), the percentage of premature exit of mitosis increased to 89%–98%, but we still observed the cleavage furrow regression in cytokinesis. RNAi treatment also induced the formation of anaphase bridges in some mitotic cells, and these cells often did not complete subsequent cytokinesis. We suspect that other Aurora B inhibitors may also induce chromosome bridges in anaphase during cell division.

We found that anaphase bridges were efficiently formed at a narrow concentration range of the inhibitor AZD1152-HQPA. Nevertheless, the completion of cytokinesis was not always inhibited in the cells in which chromosomes had been trapped at the cleavage site, since all of the cells with the bridge completed

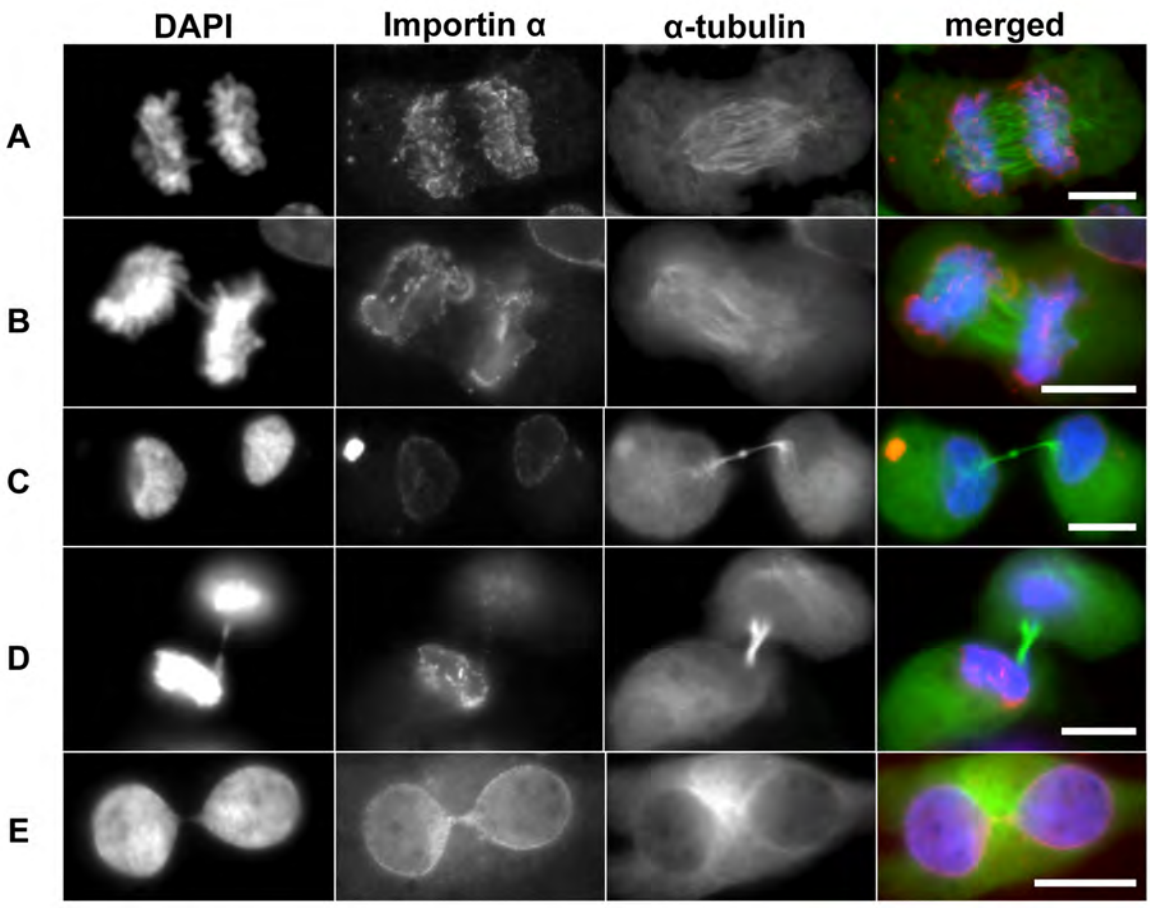


Fig. 6. Morphology of central spindles in mitotic cells with chromosome bridges. MDA435S cells stably expressing ECFP-histone H3 (not shown), DsRed-importin α (red) and EGFP-α-tubulin (green) were cultured in the presence of DMSO (control, A, C), 100 nM AZD1152-HQPA (B, D) or 100 nM AZD1152-HQPA plus 600 nM cytochalasin (E) for 20 h, fixed, and stained with DAPI (blue). Representative images of anaphase (A, B), telophase (C, D) and interphase (E) are shown. Bar, 10 μm.

cytokinesis at 50 nM. Steigemann et al. described an Aurora B-mediated abscission checkpoint (Steigemann et al., 2009). It is therefore possible that the bridge-like structure in these cells is cut or separated somehow during cytokinesis at this lower concentration, probably because of the remaining activity of Aurora B kinase. However, there seemed to be a lower limit of inhibitor concentration for inducing cleavage furrow regression in cytokinesis, even if the anaphase bridge could still be formed at a lower concentration, such as 50 nM.

Anaphase bridges are well known to be caused by DNA replication stress (Acilan et al., 2007; Burrell et al., 2013) or carcinogens, such as bleomycin (Pampalona et al., 2012), and topoisomerase inhibitors, such as ICRF-193 (Clarke et al., 1993). There are two possible explanations for how an anaphase bridge can be induced by the partial inhibition of Aurora B kinase activity. Substrates of Aurora B, such as histone H3 and condensin I, are known to be necessary for chromosome condensation (Giet and Glover, 2001; Lipp et al., 2007). The mitotic chromosomes are likely not completely condensed or are partially decondensed by limiting the kinase activity of Aurora B. Indeed, Mora-Bermúdez et al. reported that the axial shortening of chromatids was impaired by inhibiting the Aurora B activity in late anaphase (Mora-Bermúdez et al., 2007). Therefore, some mitotic chromosomes might become entangled at the cleavage furrow, although most of them are segregated to opposite poles in anaphase. Alternatively, the inhibition of Aurora B might trigger certain DNA damage, since it is well known that chromatin bridges are caused by DNA damage. If DNA cutting and joining enzymes, like

topoisomerases, were affected by Aurora B kinase, the partial inhibition of their enzymatic activity might result in some DNA damage during chromosome condensation or segregation. However, Clarke et al. reported that topoisomerase II inhibition prevented anaphase chromatid segregation in mammalian cells independently of the generation of DNA strand breaks (Clarke et al., 1993). Decatenation of sister chromatids by topoisomerase II might be affected by the presence of AZD1152-HQPA. Further studies are required to clarify how Aurora B inhibition causes the formation of chromosome bridges in anaphase.

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