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Research Article

Inhibition of protein deacetylation by trichostatin A impairs microtubule-kinetochore attachment

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Abstract. Inhibition of protein deacetylation arrests cells in mitosis, but the mechanism is unknown. To understand why inhibiting protein deacetylation causes cell cycle arrest, we treated HeLa cells beyond G1/S transition with trichostatin A (TSA), a potent protein deacetylase inhibitor, and found that the cells arrested at prometaphase with ectopic spindles and unaligned chromosomes. The hyper-acetylated cells encountered a serious microtubule (MT)-kinetochore attachment problem, although the kinetochores are intact at ultrastructural level. By immunofluorescence staining of kinetochore proteins, we found that the pericentromeric H3K9Me3-HP1 pathway was disrupted and that the CENP-A-dependent outer plate protein dynamics of kinetochores was greatly diminished by the drug treatment. The treatment also caused the loss of chromosome passenger complex (CPC), the proposed error checking system, from centromere and impaired the microtubule dynamics of the cells. Overall, we propose that deacetylation inhibition impairs MT-kinetochore attachment through disrupting the centromere function and altering the kinetochore composition and MT dynamics.

Keywords. Trichostatin A, deacetylase inhibitor, kinetochore, chromosome passenger complex, mitotic centromere-associated kinesin.

Introduction

Mitotic and meiotic spindles are the essential cellular organelles that ensure the duplicated chromosomes alignment to the metaphase plate and subsequently separation into two daughter cells. A functional spindle requires the sister chromatids attached to the bipolar arrays of microtubules (MTs) through a specialized structure called the kinetochore that links the MT plus ends to the chromosomes. The assembly of kinetochores, the dynamics of MTs, and the surveillance of MT-kinetochore connection are the three fundamental issues essential for the accuracy and fidelity of the appropriate MT-kinetochore attachment. These fundamental issues are closely regulated by various protein modifications, of which the protein acetylation/deacetylation is not well understood.

A kinetochore is a conserved laminar structure mediating MT-chromosome interaction. Electron microscopy (EM) shows that kinetochores are composed of three distinct structural plates: a conspicuous electron-opaque outer plate separated by an elec-

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tron-translucent middle plate from an inner plate associated with surface of pericentric heterochromatin [1]. The inner plate pericentric chromatin incorporates CENP-A [2], a histone H3 variant, to assemble the nucleosomes. These specialized nucleosomes and other auxiliary proteins organize the centromere chromatin and recruit inner plate proteins such as CENP-C [2, 3], CENP-H [4, 5] and CENP-I [6] during late G2 phase to form a platform for outer plate assembly. Recently, more CENP-A associated CENP proteins localized in this plate have been identified through proteomics approaches [7, 8]. The outer plate, which is composed of constitutive and dynamic proteins, is the direct interface for MT attachment [9]. Three major constitutive complexes downstream of CENP-A and CENP-C, the Ndc80(Hec1)-Nuf2-Spc24-Spc25 [10], the KNL [11–13] and the Mis12 complexes, have been identified in this plate [14]. Ndc80 complex forms a network with the other two complexes to reconstitute the core MT binding site on the kinetochore. The MT binding activity of Ndc80 complex is inhibited by Ndc80 complex phosphorylation by the protein kinase Aurora B, which corrects improper MT-kinetochore connections [13, 15]. Upon the constitutive components, many dynamic proteins, such as checkpoint proteins BUB1, MAD2, Cdc20 and motor proteins CENP-E and dynein [16–18], are recruited to the outer plate to monitor the MT-kinetochore connection as well as cell cycle progression.

The dynamic instability of MTs emanating from centrosomes enables them to search the space around and capture the kinetochores [19]. Meanwhile, MTs can also be nucleated around chromosomes and sorted by motors and MAPs [20, 21], and finally focused onto the spindle poles. The nucleation of MTs around chromatin was thought to be mediated by survivin in a mitotic centromere-associated kinesin (MCAK)-dependent manner and TPX2 in a Ran-dependent manner [22]. The search and capture pathway and self-organization pathway ensure a proper MT-kinetochore attachment, although they may also cause syntelic or merotelic attachment on the kinetochores. Aurora B has a role in the correction of the syntelic and merotelic attachment. Aurora B, with INCENP, survivin and Borealin to form a chromosome passenger complex (CPC), localizes to the inner centromere region during mitosis [23–25]. The MT depolymerizer MCAK also localizes to the centromere region and may be involved in the error correction. Kinetochore assembly, MT dynamics and error correction, ensure the proper MT-kinetochore attachment.

Histone acetylation occurs on multiple sites on the tail of histone H3 and histone H4 [26], and is thought to regulate chromatin structure and gene transcription [27, 28]. Inhibition of deacetylation by histone deacetylase (HDAC) inhibitors (HDIs) arrests cells at both G1 phase and G2 phase or prometaphase [29–33]. Although it is reported that the prometaphase arrest is possibly mediated through disrupting the pericentromeric chromatin assembly and kinetochore assembly [31], the mechanism remains largely unclear. Trichostatin A (TSA) is a potent protein deacetylase inhibitor and has been extensively used to inhibit histone deacetylase activity. In this work, with TSA treatment as an approach, we analyzed the roles of protein acetylation/deacetylation on the G2-M phase transition. We found that inhibition of protein deacetylation by TSA interferes with kinetochore assembly, centromere function and MT dynamics, resulting in MT-kinetochore attachment defect and prometaphase arrest. We suggest a model for understanding the roles of acetylation/deacetylation cycle in the MT-kinetochore attachment during the G2-M phase transition.

Materials and methods

Plasmids construction. Wild-type human HP1α (NCBI S62077), human Mis12 (NCBI NM024039) and human Borealin (NCBI NM018101) were cloned by RT-PCR from HeLa cells. HP1α was inserted into pDsRedN1 (Clonetech) at the *Eco*RI and *Sal*I restriction sites. Mis12 was inserted into pcDNA3.1HA (a kind gift from Dr. Khochbin Saadi) at the *Eco*RI and *Sal*I restriction sites. Borealin was inserted into pcDNA3.1myc (Invitrogen) at the *Eco*RI and *Sal*I restriction sites.

Cell culture and synchronization. HeLa cells were grown in DMEM medium (Gibco) containing 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ streptomycin (Sigma). The cells were synchronized to G1/S boundary with routine double-thymidine treatment. The cells were transferred into fresh medium for 1, 4 (middle S phase) or 10 h (G2 phase) and then treated with TSA (Sigma) at a final concentration of 150 ng/ml for 11, 8 and 2 h, respectively.

Immunofluorescence. HeLa cells were fixed with precooled methanol for 5 min and immunostained routinely with the following primary antibodies, mouse monoclonal antibodies against α-tubulin (Sigma), Aurora B (BD bioscience), HEC1 (Abcam) and INCENP (Abcam), goat antibodies against BUB1 (Santa Cruz) and CENP-E (Santa Cruz), and rabbit polyclonal antibodies against CENP-A (Upstate), HA (Sigma), myc (Sigma), survivin (Santa Cruz), MAD2

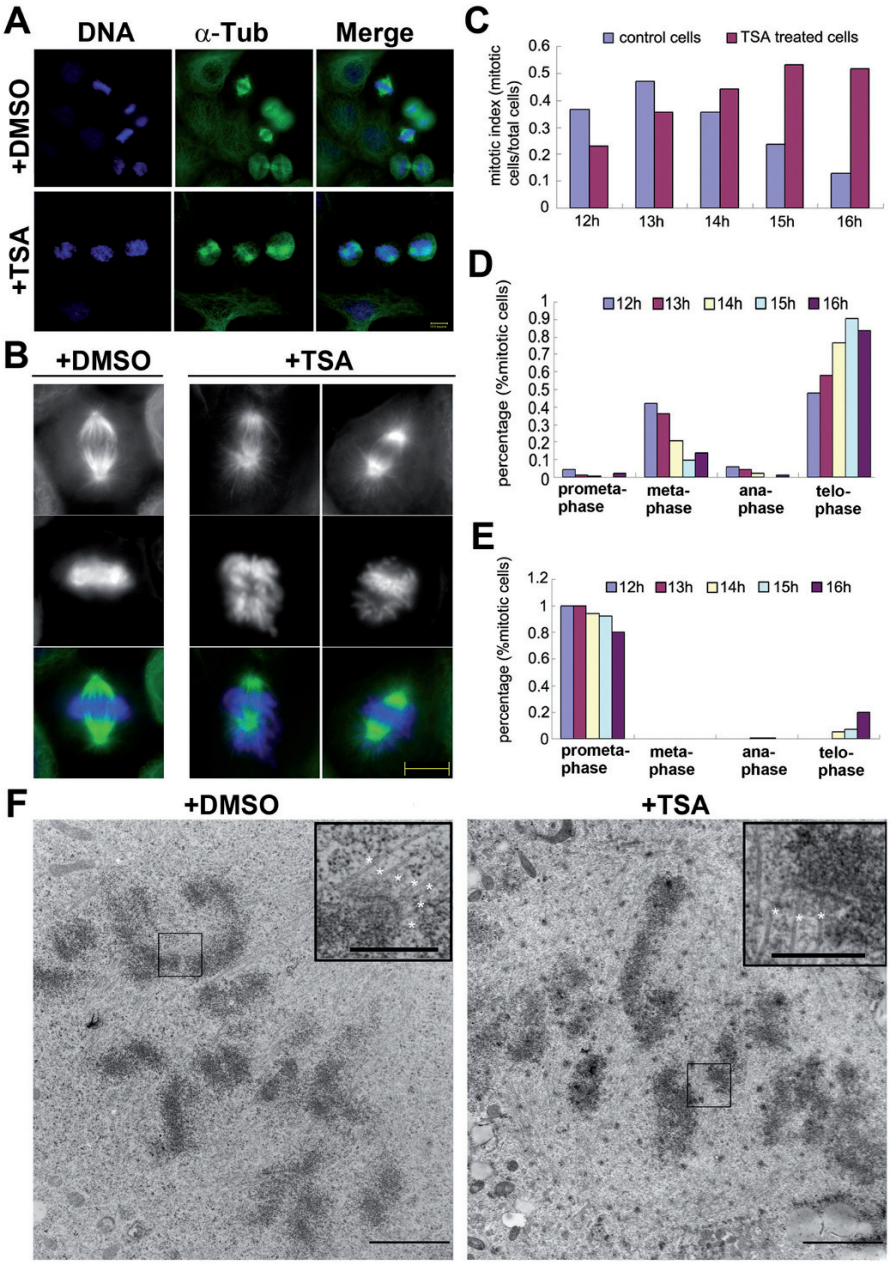
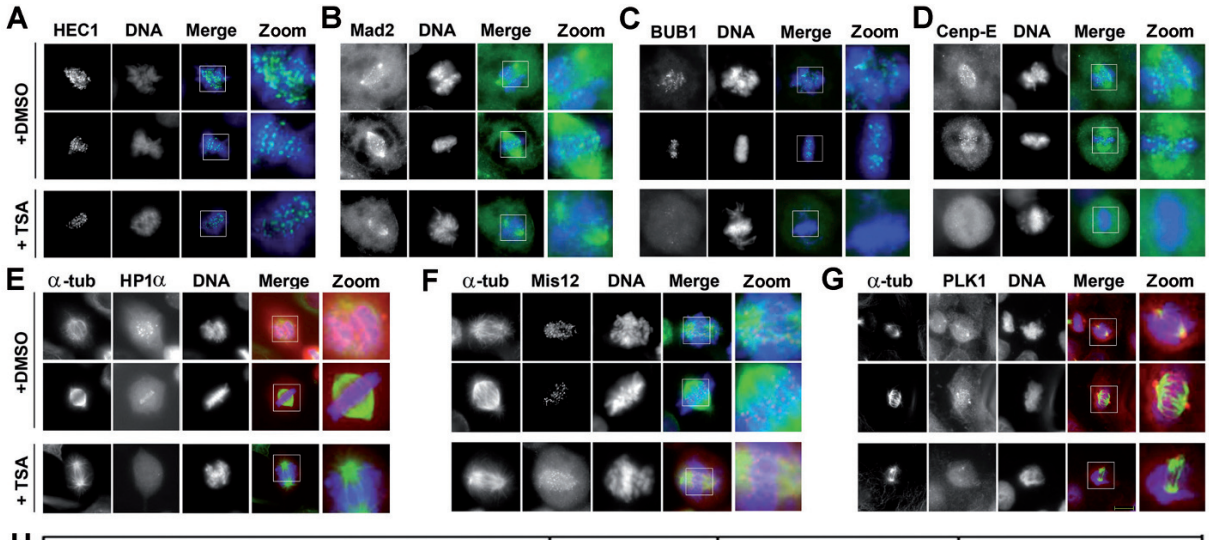


Figure 1. Trichostatin A (TSA) treatment blocks HeLa cells at prometaphase. (*A*) HeLa cells immunostain with anti-α-tubulin antibody. Note that the cells treated with TSA accumulate at prometaphase. (*B*) HeLa cells treated with TSA display completely unaligned or partially aligned chromosomes and their spindles are aster-like. (*C*) The statistics show the mitotic index of HeLa cells treated with DMSO or TSA. The mitotic index of TSA treated cells keeps increasing up to 15 h and then reaches a plateau. (*D*) HeLa cells treated with DMSO show a decrease in the number of metaphase cells and an increase of telophase cells as cell cycle progressed. (*E*) HeLa cells treated with TSA show a subtle decrease of prometaphase cells and a slight increase of telophase cells. (*F*) Ultrastructure of kinetochores of HeLa cells. Kinetochores treated with DMSO display the typical three plates with many microtubules (MTs) connected; when treated with TSA, the kinetochores still have the typical three plates but with only very few MTs. Bar in (*A*) and (*B*), 10 μm. In (*F*), bars in the low-power pictures indicate 2 μm, and 500 nm in the higher-power inset. The stars indicate the MT.

(Abcam), Plk1 (self-raised by injecting rabbits) and MCAK N terminus (self-raised by injecting rabbits), followed by relevant fluorescent-dye-conjugated secondary antibodies. These secondary antibodies were TRITC-conjugated goat anti-rabbit Ig, FITC-conjugated goat anti-rabbit Ig, FITC-conjugated goat anti-mouse Ig and TRITC-conjugated goat anti-mouse Ig from Jackson Immunoresearch and FITC-conjugated rabbit anti-goat Ig from Santa Cruz. For survivin immunostaining, the cells were fixed with 3.7% paraformaldehyde for 15 min at room temperature followed by permeabilization with 0.2% Triton X-100 for 5 min. For Plk1 immunostaining, the cells were permeabilized with 0.2% Triton X-100 for 1 min before fixation with 3.7% paraformaldehyde. The cells were then mounted in Mowiol (Sigma) containing 1 μg/ml DAPI (Sigma). Images were captured under a Zeiss fluorescence microscope 200M equipped with a 63× objective and a cooled charged-coupled device AxioCam MRm camera.

Transfection. HeLa cells were transfected using the calcium precipitation method as described previously [34].



H

		DMSO-treated cells	TSA-treated cells
Inner plate constitutive proteins	CENP-A	+	+
	CENP-C	+	+
Outer plate stable proteins	Hec1	+	+
	HP1	+	–
	Mis12	+	+
Outer plate dynamic proteins	Mad2	+	+/–
	Bub1	+	–
	CENP-E	+	–
	PLK1	+	–

Figure 2. Immunofluorescence of kinetochore components and spindle checkpoint proteins. (*A*) Immunofluorescence of Hec1. TSA treatment cannot remove Hec1 from kinetochore. (*B*) Immunofluorescence of Mad2. TSA treatment can largely reduce Mad2 signal at the kinetochores. (*C*) Immunofluorescence of BUB1. TSA treatment largely removes BUB1 from kinetochores. (*D*) Immunofluorescence of CENP-E. TSA treatment largely removes CENP-E from the kinetochores. (*E*) The localization of HP1α-RFP. TSA treatment removes HP1α-RFP from centromere. (*F*) Immunofluorescence of Mis12-HA. TSA treatment cannot change the localization of Mis12-HA. (*G*) Immunofluorescence of PLK1. After TSA treatment the kinetochore localization of PLK1 disappears, while the centrosome localization remains. (*H*) The summary table indicates the localization change of the kinetochore components. Data in the table are from 100 cells. Bars, 10 μm.

Transmission EM. Monolayer HeLa cells were fixed with 1.5 % glutaraldehyde (Sigma) in 0.1 M phosphate buffer for 1 h at room temperature followed by 1 % osmium tetroxide for 2 h. The samples were embedded in Epon 812 and sectioned with a diamond knife. Sections were double-stained with uranyl acetate and lead citrate. Images were captured on a transmission electron microscope JEOL1010 using a cooled charged-coupled device transmission EM camera AMT XR40.

Mitotic *Xenopus* egg extract preparation and spindle assembly *in vitro*. CSF *Xenopus* egg extract was prepared as described [35]. The sperm heads were added to the fresh extract to the final concentration of 100/μl and TSA (in DMSO) was added to the final concentration of 1.5 μg/ml. As control, DMSO was used instead of TSA. Reactions were incubated at RT for 60 min and fixed with 2 % formaldehyde. Images were taken under an IX71 Olympus microscope equipped with a 60× objective and a cooled charged-coupled device Spot RT camera.

Western blot. The protein samples were resolved on 10 % SDS-PAGE gels and transferred onto nitrocellulose membrane filters. The filters were probed with the antibodies against acetylated tubulin (monoclonal, Sigma), Aurora B, survivin, INCENP, myc, or GFP (self-raised by injecting rabbits), followed by horseradish peroxidase (HRP)-conjugated goat anti-rabbit or mouse secondary antibodies (Jackson Immunoresearch). The filters were developed for visualization by enhanced chemiluminescence (Sigma) and X-ray film.

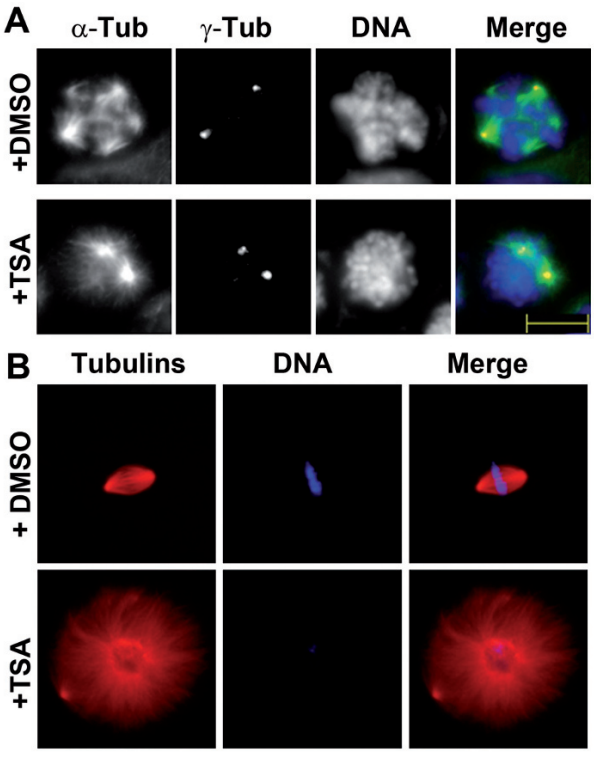


Figure 3. TSA treatment affects chromatin-driven MT nucleation and MT dynamics. (*A*) MT re-nucleation in DMSO-treated control cells or TSA-treated cells after nocodazole treatment. MT only nucleate from centrosomes in DMSO treated cells. Bar, 10 μm. (*B*) Spindle assembly in frog egg extract. The spindle assembly is completely disrupted in TSA-treated extract. Instead, huge asters with long and thick MTs form around the sperm centrioles.

MT regrowth assay. Mock- and TSA-treated HeLa cells were treated with nocodazole at a final concentration of 300 ng/ml for 1 h. The treated cells were washed once with PBS and then incubated in pre-warmed fresh complete medium at 37°C for 5 min before immunofluorescence staining.

Results

Inhibition of deacetylation by TSA blocks HeLa cells at prometaphase by disrupting the MT-kinetochore attachment. Previous studies have shown that the protein deacetylase inhibitor TSA causes both G1 and G2 phase arrests in multiple cell lines and distinct organisms [29–33]. To study the roles of deacetylation in G2-M phase transition, we firstly re-evaluated the effect of TSA on the cells in the S-G2-M transition by treating HeLa cells released from G1/S block with TSA. At 12 h after release from G1/S block, the control cells entered diverse mitotic stages. When treated with TSA, the cells were conspicuously blocked at prometaphase as reported in various cell lines [31] (Fig. 1A). We further observed that, in about 36% of these prometaphase-arrested cells, the chromosomes were only partially aligned (Fig. 1B, middle panel), and in 64% of these cells, the chromosomes were completely unaligned (Fig. 1B, right panel). Furthermore, the spindles in these cells are aster-like, rather than oriented.

To distinguish whether the accumulation of prometaphase cells with TSA treatment was a result of either a general cell cycle delay or a prometaphase arrest, we first measured the mitotic index at the selected time points after the release of G1/S block (Fig. 1C). In control cells, the mitotic index peaked at 13 h and gradually dropped down afterwards. In TSA-treated cells, the mitotic index kept increasing until 15 h, when it reached a plateau. The lack of further mitotic index increase subsequently was probably be due to the arrest by drug of the remaining fraction of the cells at G1 phase. We then quantified the population of cells in different stages at different time points (Fig. 1D, E). We observed that, along with the cell cycle progression, the number of cells decreased in the metaphase fraction and increased in the telophase fraction among the control cells, suggesting that these cells were going through mitosis. In contrast, the TSA-treated cells showed only a slight decrease in the number of prometaphase cells and a tiny increase of anaphase and telophase cells, suggesting these cells were blocked at prometaphase. We thus propose that the accumulation of prometaphase cells with TSA treatment was due to prometaphase blockage but not to cell cycle delay.

As it was clear that the TSA treatment disrupted chromosome alignment, we tested whether these cells encountered a serious defect in MT-kinetochore attachment. We observed that, compared with those in control cells, none of the 37 kinetochores from the 21 TSA-treated cells checked showed apparent defects at ultrastructural level, and that the three plates of the kinetochores could be clearly distinguished in the TSA-treated cells (Fig. 1F). However, 95% of the kinetochores showed an obvious reduced MT-binding activity. In the control cells, the numbers of MT connected to kinetochores ranged from six to ten, while in TSA-treated cells, the numbers of MT connected to kinetochores were usually between two and three. We assumed that the ectopic decreased MT-kinetochore attachment by TSA treatment might due to disruption of either the integrity of kinetochore and/or centromere or the MT dynamics.

Inhibition of deacetylation impairs kinetochore integrity. As the morphology of the kinetochore in the TSA-treated cells appeared normal, we investigated whether the kinetochore organization and integrity

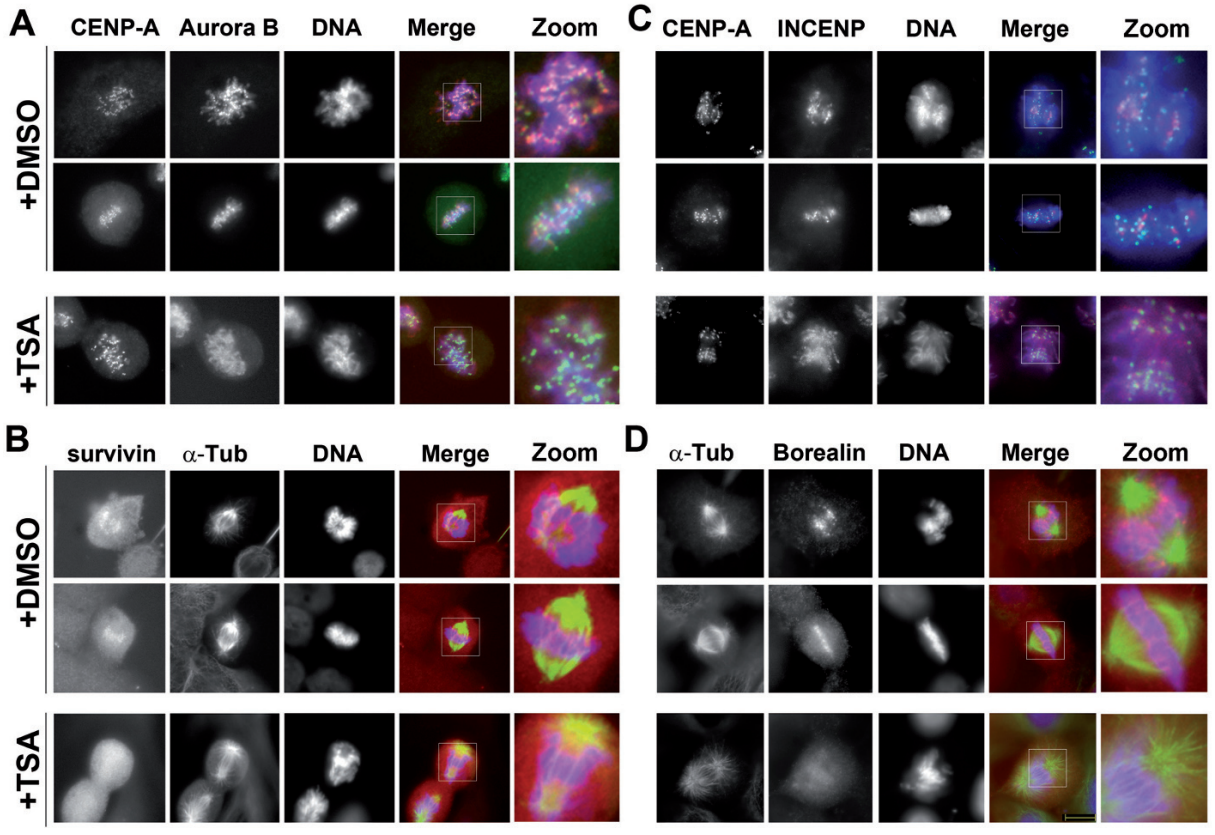


Figure 4. TSA disrupts the localization of the chromosome passenger complex (CPC) to the centromere. (*A*) Immunofluorescence of Aurora B. After TSA treatment, the centromere targeting of Aurora B disappears, while the chromosome arm localization persists. (*B*) Immunofluorescence of survivin. The centromere targeting of survivin disappears after TSA treatment. (*C*) Immunofluorescence of INCENP. The centromere targeting of Aurora B disappears, while the chromosome arm localization persists after TSA treatment. (*D*) Immunofluorescence of Borealin-myc. The centromere targeting of Borealin-myc disappears after TSA treatment. Bars in (*A*)–(*D*) and (*G*), 10 μm. This figure shows representative data from 100 cells.

are affected by deacetylation inhibition. We probed the kinetochore localization of several constitutive and dynamic kinetochore components through immunofluorescent labeling using specific antibodies (Fig. 2). To examine the constitutive proteins of the inner plate, we probed the histone H3 variant CENP-A, the top hierarchy protein of the kinetochore assembly, and found that it localized normally in the kinetochore in the TSA-treated cells. We then probed the outer plate constitutive protein Hec1, the proposed kinetochore-MT contact interface [15, 36], and found that Hec1 also remained on the kinetochore. Mis12, which facilitates the binding between the KNL and Hec1 complex [13], was also present at the centromere with a level indistinguishable from that in control cells.

Mis12 forms a complex with HP1α and HP1γ to be recruited to the pericentromeric chromatin [37], and deacetylation inhibitors prevent HP1 from localizing to the heterochromatin [31]. In our experiments we observed that, in the presence of the deacetylase inhibitor, the pericentrimeric HP1α-RFP disappeared from the pericentromeric chromatin (Fig. 2E), while Mis12 largely remained (Fig. 2F).

We also probed several outer plate dynamic proteins and found that Bub1, CENP-E and Plk1 were completely eliminated from the kinetochore and that Mad2 was seriously reduced (about 80%) on the kinetochore by TSA treatment (Fig. 2). To investigate whether the phenotypes observed with the TSA treatment are primarily due to effects on early mitotic events, or to cumulative effects on events in S and/or G2 that then resulted in the mitotic phenotypes, we treated the synchronized cells at middle S phase with TSA (4 h after release) and found that the cells were almost totally arrested at prometaphase. When treated at G2 phase (10 h after release), a large fraction of the cells were able to pass through mitosis. We co-immunostained the cells with antibodies to the inner plate protein CENP-A and Aurora B, and observed that, unlike CENP-A, Aurora B was totally abolished at kinetochore when treated with TSA at middle S phase. When treated at the G2 phase, Aurora B remained at kinetochore (data not shown). These

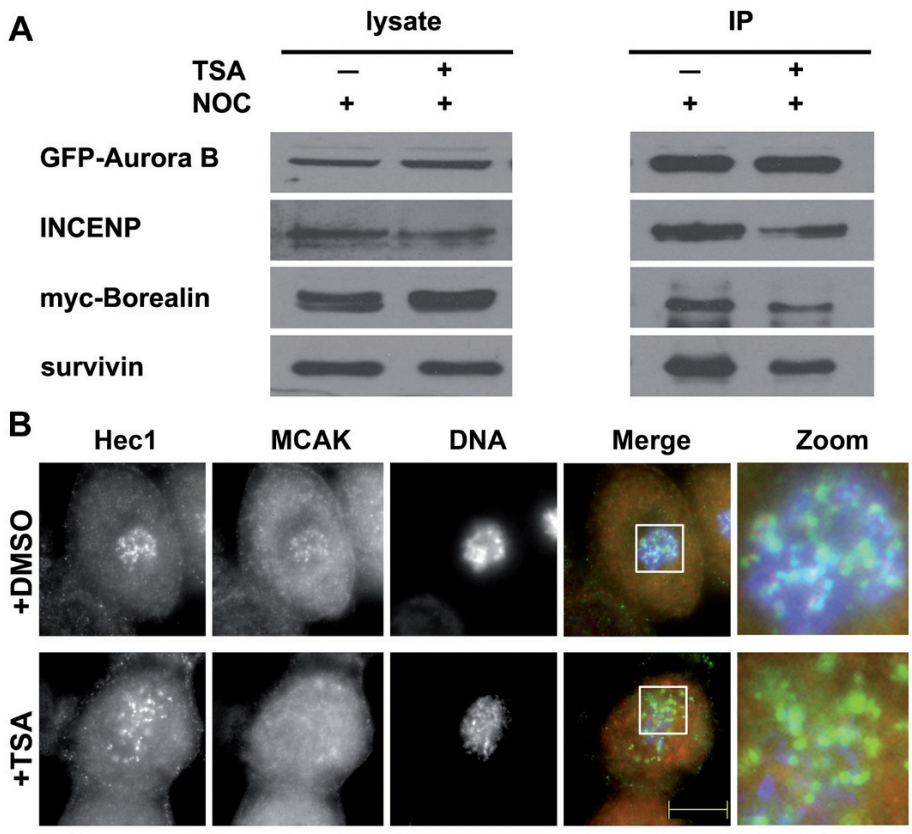


Figure 5. TSA disturbs the CPC-MCAK pathway. (*A*) Immunoprecipitation of GFP-Aurora B in HeLa cell line stably expressing GFP-Aurora B and transiently expressing Borealin-myc. Immunoprecipitation was performed with anti-GFP antibodies. Precipitates were immunoblotted using antibodies against Aurora B, c-myc, survivin and INCENP. TSA treatment causes less efficient binding of CPC constituents. (*B*) Immunofluorescence of MCAK. The centromere targeting of MCAK disappears after TSA treatment.

results suggest that TSA caused the prometaphase-arrest phenotype through inhibiting the accumulation of outer plate kinetochore proteins on the kinetochore from late S phase to G2/M phase, rather than by eliminating them from this area. Taken together, our kinetochore protein analysis findings indicate that TSA treatment somehow impairs kinetochore integrity, resulting in the loss of most of the dynamic components of kinetochores.

Inhibition of deacetylation affects chromatin-driven MT nucleation and MT dynamics. We then asked why the connection between MT and kinetochore was dramatically reduced by TSA treatment. We treated HeLa cells with nocodazole to completely depolymerize MTs and then released the cells into medium at 37°C to allow MTs to re-nucleate. We found that, in control cells, MTs could nucleate from both centrosomes and chromosomes, while in TSA-treated cells, MTs could only nucleate from centrosomes (Fig. 3A). This result suggests that acetylation/deacetylation is also involved in chromatin-driven MT nucleation.

In the TSA-arrested prometaphase cells, we also noticed a defective morphology of the spindle. The spindles in the presence of TSA were quite aster-like, with a lot of hairy MTs, suggesting that MT dynamics might have encountered problems and could not take part in MT-kinetochore attachment. To confirm whether MT dynamics were affected by deacetylase inhibition, we carried out an *in vitro* spindle assembly assay using a frog egg extract with the addition of TSA. In control extracts, a bipolar spindle formed with chromosome alignment to the spindle equator. In the TSA-treated extract, the spindle assembly was completely disrupted, and huge asters with long and thick MTs formed around the nuclei, but no MT plus ends of the asters were observed to connect with the kinetochore (Fig. 3B).

Inhibition of deacetylation disrupts the localization of the error-checking system to the centromere. As mentioned above, in TSA-treated cells the chromosomes were only partially aligned, a result very similar to the CPC function inhibition [38, 39]. We tested whether TSA affected the targeting of the CPC to the inner centromere using immunofluorescent labeling (Fig. 4). In untreated HeLa cells, Aurora B was localized primarily on inner centromeres, with partial spreading onto chromosome arms. After TSA treatment, the centromere targeting of Aurora B disappeared, while the chromosome arm localization persisted. INCENP staining mirrored that of Aurora B, spreading on the chromosome arms without inner centromere targeting, whereas no signals of survivin

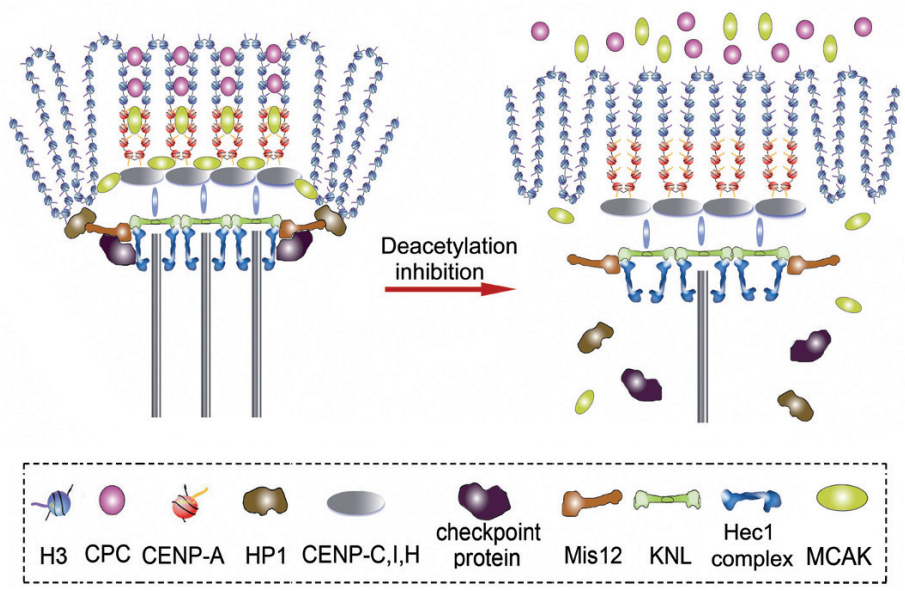


Figure 6. A model for understanding prometaphase arrest by deacetylation inhibition. Deacetylation inhibition increases histone acetylation and abolishes the normal localization of HP1, resulting in ectopic pericentromeric chromatin organization. The aberrant pericentromeric chromatin disrupts the base for outer plate dynamic proteins recruitment. Deacetylation inhibition eliminates CPC from the centromere region and reduces the ability of Hec1 complex to catch centrosomal MTs. In addition, deacetylation inhibition perturbs MCAK function and hence impairs the searching ability of centrosomal MTs for kinetochores. Finally, disruption of this multistep pathway by deacetylation inhibition results in the dramatic prometaphase blockage.

and Borealin were detectable on either the centromere or chromosome arms. It has been reported that Aurora B exists in two complexes, the complex with INCENP, survivin and Borealin and the complex with INCENP [40]. Our result suggest that only the Aurora B-INCENP complex localizes to chromosome arms.

Inhibition of deacetylation may disturb the CPC-MCAK pathway. We further tested whether deacetylation inhibition disrupted CPC assembly or CPC targeting using a cell line stably expressing GFP-Aurora B. We found that, following TSA treatment, INCENP, survivin and Borealin-myc could still be co-immunoprecipitated with GFP-Aurora B by anti-GFP antibody, while the binding strength among these components was reduced (Fig. 5A).

The CPC complex was thought to function in the MT-kinetochore attachment error correction [41–43]. The error correction role was thought to be fulfilled through recruiting the MT depolymerase MCAK to the centromere region [42]. Inhibition of CPC function causes merotelic MT-kinetochore connection. To test whether MCAK was affected by deacetylation inhibition, we stained MCAK with an antibody against its N terminus. We found that the level of MCAK was also diminished in the centromere region (Fig. 5B). This result suggests that the chromosome alignment problem under TSA treatment could also be caused by perturbation of the CPC-MCAK pathway.

Discussion

Acetylation regulates kinetochore organization. Although the kinetochore components and the assembly have been studied in different organisms for decades, how kinetochores are spatio-temporally organized still remains largely unknown. Here we have shown that acetylation is involved in the kinetochore organization.

Inhibition of deacetylation by the chemical drug TSA did not prevent the localization of either Hec1 and Mis12 to kinetochore, but the localization of HP1 was completely abolished, indicating that Mis12 localization to kinetochore might be acetylation dependent, but not HP1 dependent. We also found that the levels of the dynamic proteins of the outer plate, such as BUB1, CENP-E and PLK1, were also diminished on the kinetochores. As the recruitment of these dynamic proteins to kinetochores is CENP-A dependent, our result also suggests that the CENP-A-dependant pathway of outer plate dynamic proteins might also be regulated by protein acetylation.

Acetylation regulates the error correction system. CPC is an essential protein complex for regulating MT-kinetochore attachment, MT attachment error correction, MT nucleation and chromosome segregation, but how this complex is targeted to the centromeric region is not clear. We have shown here that the

localization of CPC to the centromere region is regulated by acetylation and that Aurora B and INCENP could localize to the chromosome arm in the presence of deacetylase inhibitors. MCAK has been proposed to prevent merotelic attachment through phosphorylation by Aurora B [42, 44]. Here we found that the centromere localization of MCAK can be disrupted by deacetylation inhibition, suggesting that protein deacetylation is involved in the Aurora B-MCAK pathway of MT-kinetochore attachment error correction.

Acetylation regulates MT dynamics. Tubulin can be acetylated at its N-terminal lysine residue and this acetylation is thought to contribute to the stability of MTs [45] but not to cell cycle progression [46]. In this study, we have shown that deacetylation inhibition increases the stability of MTs, a result very similar to the inhibition of MCAK in frog egg extract [47]. We have also shown that MCAK detaches from the centromere and loses its effect on the dynamics of MTs at the vicinity of chromatin in the presence of deacetylase inhibitors.

A model for understanding prometaphase arrest by deacetylation inhibition. We propose that deacetylation inhibition blocks a multistep pathway that is essential for effective MT-kinetochore attachment (Fig. 6). We suggest that deacetylation inhibition increases histone acetylation, which abolishes the normal localization of HP1, resulting in the ectopic pericentromeric chromatin organization. The aberrant pericentromeric chromatin disrupts the base for outer plate dynamic protein recruitment and affects MT nucleation around chromatin. In addition, deacetylation inhibition eliminates CPC from the centromere region and reduces the ability of the Hec1 complex to catch centrosomal MTs. Moreover, MCAK function is perturbed and hence the searching ability of centrosomal MTs for the kinetochore is greatly impaired. This disruption of the multistep pathway of MT-kinetochore attachment by deacetylation inhibition finally results in the dramatic prometaphase block.

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1 McEwen, B. F., Hsieh, C. E., Mattheyses, A. L. and Rieder, C. L. (1998) A new look at kinetochore structure in vertebrate somatic cells using high-pressure freezing and freeze substitution. Chromosoma 107, 366 – 375.
2 Earnshaw, W., Bordwell, B., Marino, C. and Rothfield, N. (1986) Three human chromosomal autoantigens are recognized by sera from patients with anti-centromere antibodies. J. Clin. Invest. 77, 426 – 430.
3 Saitoh, S. and Takahashi, K. (2004) [Molecular basis of kinetochore structure and kinetochore-spindle interaction]. Tanpakushitsu Kakusan Koso 49, 1982 – 1989.
4 Sugata, N., Munekata, E. and Todokoro, K. (1999) Characterization of a novel kinetochore protein, CENP-H. J. Biol. Chem. 274, 27343 – 27346.
5 Sugata, N., Li, S., Earnshaw, W. C., Yen, T. J., Yoda, K. Masumoto, H., Munekata, E., Warburton, P. E. and Todokoro, K. (2000) Human CENP-H multimers colocalize with CENP-A and CENP-C at active centromere–kinetochore complexes. Hum. Mol. Genet. 9, 2919 – 2926.
6 Nishihashi, A., Haraguchi, T., Hiraoka, Y., Ikemura, T., Regnier, V., Dodson, H., Earnshaw, W. C. and Fukagawa, T. (2002) CENP-I is essential for centromere function in vertebrate cells. Dev. Cell 2, 463 – 476.
7 Foltz, D. R., Jansen, L. E., Black, B. E., Bailey, A. O., Yates, J. R. 3rd and Cleveland, D. W. (2006) The human CENP-A centromeric nucleosome-associated complex. Nat. Cell. Biol. 8, 458 – 469.
8 Yokoyama, T., Goto, H., Izawa, I., Mizutani, H. and Inagaki, M. (2005) Aurora-B and Rho-kinase/ROCK, the two cleavage furrow kinases, independently regulate the progression of cytokinesis: Possible existence of a novel cleavage furrow kinase phosphorylates ezrin/radixin/moesin (ERM). Genes Cells 10, 127 – 137.
9 Adams, R. R., Maiato, H., Earnshaw, W. C. and Carmena, M. (2001) Essential roles of *Drosophila* inner centromere protein (INCENP) and aurora B in histone H3 phosphorylation, metaphase chromosome alignment, kinetochore disjunction, and chromosome segregation. J. Cell Biol. 153, 865 – 880.
10 Wigge, P. A. and Kilmartin, J. V. (2001) The Ndc80p complex from *Saccharomyces cerevisiae* contains conserved centromere components and has a function in chromosome segregation. J. Cell Biol. 152, 349 – 360.
11 Desai, A., Rybina, S., Muller-Reichert, T., Shevchenko, A., Shevchenko, A., Hyman, A. and Oegema, K. (2003) KNL-1 directs assembly of the microtubule-binding interface of the kinetochore in *C. elegans*. Genes Dev. 17, 2421 – 2435.
12 Cheeseman, I. M. and Desai, A. (2004) Cell division: Feeling tense enough? Nature 428, 32 – 33.
13 Cheeseman, I. M., Chappie, J. S., Wilson-Kubalek, E. M. and Desai, A. (2006) The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. Cell 127, 983 – 997.
14 Kliner, D. J. (2006) Improve your ICD-9 coding with voice-recognition macros. Fam. Pract. Manag. 13, 39 – 40.
15 DeLuca, J. G., Gall, W. E., Ciferri, C., Cimini, D., Musacchio, A. and Salmon, E. D. (2006) Kinetochore microtubule dynamics and attachment stability are regulated by Hec1. Cell 127, 969 – 982.
16 Hoffman, D. B., Pearson, C. G., Yen, T. J., Howell, B. J. and Salmon, E. D. (2001) Microtubule-dependent changes in assembly of microtubule motor proteins and mitotic spindle checkpoint proteins at PtK1 kinetochores. Mol. Biol. Cell 12, 1995 – 2009.
17 Aitken, R. J., Buckingham, D., Richardson, D., Gardiner, J. C. and Irvine, D. S. (2000) Impact of a deep saturation dive on semen quality. Int. J. Androl. 23, 116 – 120.
18 Howell, B. J., Moree, B., Farrar, E. M., Stewart, S., Fang, G. and Salmon, E. D. (2004) Spindle checkpoint protein dynamics at kinetochores in living cells. Curr. Biol. 14, 953 – 964.
19 Kristofferson, D., Mitchison, T. and Kirschner, M. (1986) Direct observation of steady-state microtubule dynamics. J. Cell Biol. 102, 1007 – 1019.

20 Kenney, R. D. and Heald, R. (2006) Essential roles for cohesin in kinetochore and spindle function in *Xenopus* egg extracts. J. Cell Sci. 119, 5057 – 5066.
21 Walczak, C. E., Vernos, I., Mitchison, T. J., Karsenti, E. and Heald, R. (1998) A model for the proposed roles of different microtubule-based motor proteins in establishing spindle bipolarity. Curr. Biol. 8, 903 – 913.
22 Tulu, U. S., Fagerstrom, C., Ferenz, N. P. and Wadsworth, P. (2006) Molecular requirements for kinetochore-associated microtubule formation in mammalian cells. Curr. Biol. 16, 536 – 541.
23 Vader, G., Kauw, J. J., Medema, R. H. and Lens, S. M. (2006) Survivin mediates targeting of the chromosomal passenger complex to the centromere and midbody. EMBO Rep. 7, 85 – 92.
24 Klein, U. R., Nigg, E. A. and Gruneberg, U. (2006) Centromere targeting of the chromosomal passenger complex requires a ternary subcomplex of Borealin, Survivin, and the N-terminal domain of INCENP. Mol. Biol. Cell 17, 2547 – 2558.
25 Vong, Q. P., Cao, K., Li, H. Y., Iglesias, P. A. and Zheng, Y. (2005) Chromosome alignment and segregation regulated by ubiquitination of survivin. Science 310, 1499 – 504.
26 Davie, J. R. and Spencer, V. A. (1999) Control of histone modifications. J. Cell Biochem. Suppl. 32–33, 141 – 148.
27 Verdone, L., Caserta, M. and Di Mauro, E. (2005) Role of histone acetylation in the control of gene expression. Biochem. Cell Biol. 83, 344 – 353.
28 Prall, F. R., Drack, A., Taylor, M., Ku, L., Olson, J. L., Gregory, D., Mestroni, L. and Mandava, N. (2006) Ophthalmic manifestations of Danon disease. Ophthalmology 113, 1010 – 1013.
29 Yoshida, M. and Beppu, T. (1988) Reversible arrest of proliferation of rat 3Y1 fibroblasts in both the G1 and G2 phases by trichostatin A. Exp. Cell Res. 177, 122 – 131.
30 Strait, K. A., Warnick, C. T., Ford, C. D., Dabbas, B., Hammond, E. H. and Ilstrup, S. J. (2005) Histone deacetylase inhibitors induce G2-checkpoint arrest and apoptosis in cisplatinum-resistant ovarian cancer cells associated with overexpression of the Bcl-2-related protein Bad. Mol. Cancer Ther. 4, 603 – 611.
31 Robbins, A. R., Jablonski, S. A., Yen, T. J., Yoda, K., Robey, R., Bates, S. E. and Sackett, D. L. (2005) Inhibitors of histone deacetylases alter kinetochore assembly by disrupting pericentromeric heterochromatin. Cell Cycle 4, 717 – 726.
32 Ryu, J. K., Lee, W. J., Lee, K. H., Hwang, J. H., Kim, Y. T., Yoon, Y. B. and Kim, C. Y. (2006) SK-7041, a new histone deacetylase inhibitor, induces G2-M cell cycle arrest and apoptosis in pancreatic cancer cell lines. Cancer Lett. 237, 143 – 154.
33 Yoshida, M., Hoshikawa, Y., Koseki, K., Mori, K. and Beppu, T. (1990) Structural specificity for biological activity of trichostatin A, a specific inhibitor of mammalian cell cycle with potent differentiation-inducing activity in Friend leukemia cells. J. Antibiot. (Tokyo) 43, 1101 – 1106.
34 Sambrook, J. and Russell, D. W. (2001) Molecular Cloning: A Laboratory Manual, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
35 Murray, A. W. (1991) Cell cycle extracts. Methods Cell Biol. 36, 581 – 605.
36 Wei, R. R., Al-Bassam, J. and Harrison, S. C. (2007) The Ndc80/HEC1 complex is a contact point for kinetochore-microtubule attachment. Nat. Struct. Mol. Biol. 14, 54 – 59.
37 Obuse, C., Iwasaki, O., Kiyomitsu, T., Goshima, G., Toyoda, Y. and Yanagida, M. (2004) A conserved Mis12 centromere complex is linked to heterochromatic HP1 and outer kinetochore protein Zwint-1. Nat. Cell Biol. 6, 1135 – 1141.
38 Hauf, S., Cole, R. W., Laterra, S., Zimmer, C., Schnapp, G., Walter, R., Heckel, A., Van Meel, J., Rieder, C. L. and Peters, G. M. (2003) The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint. J. Cell Biol. 161, 281 – 294.
39 Sessa, F., Mapelli, M., Ciferri, C., Tarricone, C., Areces, L. B., Schneider, T. R., Stukenberg, P. T. and Musacchio, A. (2005) Mechanism of Aurora B activation by INCENP and inhibition by hesperadin. Mol. Cell 18, 379 – 391.
40 Gassmann, R., Carvalho, A., Henzing, A. J., Ruchaud, S., Hudson, D. F., Honda, R., Nigg, E. A., Gerloff, D. L., and Earnshaw, W. C. (2004) Borealin: A novel chromosomal passenger required for stability of the bipolar mitotic spindle. J. Cell Biol. 166, 179 – 191.
41 Dewar, H., Tanaka, K., Nasmyth, K. and Tanaka, T. U. (2004) Tension between two kinetochores suffices for their bi-orientation on the mitotic spindle. Nature 428, 93 – 97.
42 Knowlton, A. L., Lan, W. and Stukenberg, P. T. (2006) Aurora B is enriched at merotelic attachment sites, where it regulates MCAK. Curr. Biol. 16, 1705 – 1710.
43 Cimini, D., Wan, X., Hirel, C. B. and Salmon, E. D. (2006) Aurora kinase promotes turnover of kinetochore microtubules to reduce chromosome segregation errors. Curr. Biol. 16, 1711 – 1718.
44 Lan, W., Zhang, X., Kline-Smith, S. L., Rosasco, S. E., Barrett-Wilt, G. A., Shabanowitz, J., Hunt, D. F., Walczak, C. E., Stukenberg, P. T. (2004) Aurora B phosphorylates centromeric MCAK and regulates its localization and microtubule depolymerization activity. Curr. Biol. 14, 273 – 286.
45 Matsuyama, A., Shimazu, T., Sumida, Y., Saito, A., Yoshimatsu, Y., Seigneurin-Berny, D., Osada, H., Komatsu, Y., Nishino, N., Khochbin, S., Horinouchi, S. and Yoshida, M. (2002) *In vivo* destabilization of dynamic microtubules by HDAC6-mediated deacetylation. EMBO J. 21, 6820 – 6831.
46 Haggarty, S. J., Koeller, K. M., Wong, J. C., Grozinger, C. M. and Schreiber, S. L. (2003) Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation. Proc. Natl. Acad. Sci. USA 100, 4389 – 4394.
47 Walczak, C. E., Mitchison, T. J. and Desai, A. (1996) XKCM1: A *Xenopus* kinesin-related protein that regulates microtubule dynamics during mitotic spindle assembly. Cell 84, 37 – 47.