Phospho-regulation of HsCdc14A By Polo-like Kinase 1 Is Essential for Mitotic Progression*

Kai Yuan1‡1, Haiying Hu1‡1, Zhen Guo1‡3, Guosheng Fu‡, Andrew P. Shaw1, Renming Hu‡2, and Xuebiao Yao1‡3

From the Division of Cellular Dynamics, Hefei National Laboratory and Chinese University of Science & Technology, Hefei 230027, China, Department of Endocrinology and Metabolism, Hua Shan Hospital, Fudan University School of Medicine, Shanghai 200040, China, and Department of Physiology, Morehouse School of Medicine, Atlanta, Georgia 30310

Chromosome segregation in mitosis is orchestrated by dynamic interactions between spindle microtubules and centromeres, which in turn are governed by protein kinase- and phosphatase-signaling cascades. Previous studies showed that overexpression of human phosphatase HsCdc14A, an antagonist of cyclin-dependent kinase 1, affects several aspects of cell division. However, the molecular mechanism underlying HsCdc14A regulation in mitosis has remained elusive. Here we show that HsCdc14A activity is regulated by an auto-inhibitory mechanism via its intra-molecular association. Our biochemical study demonstrated that Polo-like kinase 1 (PLK1) interacts with and phosphorylates HsCdc14A. This phosphorylation partially releases the auto-inhibition of HsCdc14A judged by its phosphatase activity in vitro. To examine the functional relevance of such phospho-regulation of HsCdc14A in vivo, a phospho-mimicking mutant of HsCdc14A was expressed in HeLa cells. Importantly, overexpression of the phospho-mimicking mutants caused aberrant chromosome alignment with a prometaphase delay, suggesting the temporal regulation of HsCdc14A activity is critical for orchestrating mitotic events. Given the fact that HsCdc14A forms an intra-molecular association and PLK1-mediated phospho-regulation promotes HsCdc14A phosphatase activity, we propose that PLK1-HsCdc14A interaction provides a temporal regulation of HsCdc14A in chromosome segregation during mitosis.

Mitosis is orchestrated by signaling cascades that coordinate mitotic processes and ensure accurate chromosome segregation. The key switch for the onset of mitosis is the archetypal cyclin-dependent kinase, Cdc2. Besides the master mitotic kinase Cdc2, there are three protein serine/threonine kinase families, the Polo kinases, Aurora kinases, and the NEK2 kinases. The process of mitosis is complex and involves multiple independent regulatory steps, most of which are controlled by reversible protein phosphorylation. In higher eukaryotes, mitosis involves condensation of chromosomes, disassembly of the nuclear lamina, breakdown of the nuclear envelope, and disassembly of many forms of nuclear bodies, including nucleoli. Completion of mitosis requires alignment and proper segregation of chromosomes into daughter cells followed by reassembly of nuclei and cytokinesis. These and many other events, such as centrosome separation and spindle assembly, are tightly regulated, and several critical checkpoints occur during mitosis to ensure fidelity.

In the budding yeast Saccharomyces cerevisiae, Cdc14p plays a key role in the exit from mitosis by dephosphorylating cyclin-dependent kinase targets (e.g. Refs. 1, 2). Cdc14p is primarily regulated by localization, being sequestered during interphase in the nucleolus by Cfi/Net1 and then released in two stages, coordinated by the FEAR (Cdc fourteen early anaphase release) network and MEN (mitotic exit network). Upon its initial release by FEAR, Cdc14p is found in the nucleus and at the spindle pole body and spindle. After activation of the pathway in telophase (which promotes further Cdc14p release from nucleoli) it is also found in the cytoplasm (1). Interestingly, the essential role of Cdc14 in mitotic exit control is not conserved in other species, although a common process that it does appear to control in all investigated organisms is cytokinesis (e.g. Ref. 3). A comparison of localization patterns for Cdc14 homologues in different species supports this essential role (e.g. Ref. 2).

Mammalian cells express two homologues of Cdc14, termed HsCdc14A and HsCdc14B, which are both functional homologues of yeast Cdc14 (4). Although these two proteins are still poorly understood, recent evidence points to isoform-specific roles in centrosome separation/maturation and spindle stability, with the possibility of additional roles in mitotic exit and cytokinesis. Until recently, most studies focused on HsCdc14A, which was shown to interact with interphase centrosomes and to regulate the centrosome duplication cycle (5, 6). Recently, HsCdc14B, which is nucleolar at interphase and then found at the central spindle and midbody during mitosis, has been shown to play a role in spindle stability through direct binding and bundling of microtubules (7). However, it remains elusive as to how HsCdc14A/B function is integrated in mitotic regulation and whether it plays an active role in chromosome segregation in mitosis.

Here we show the intra-molecular binding of HsCdc14A and
its inhibitory role on the phosphatase activity of HsCdc14A. Moreover, PLK1 interacts with and phosphorylates HsCdc14A and partially releases HsCdc14A from self-inhibition. Furthermore, overexpression of phospho-mimicking mutant of HsCdc14A in HeLa cells caused aberrant prometaphase delay. Our study suggests that the spatiotemporal regulation of HsCdc14A is essential for faithful chromosome segregation in mitosis.

MATERIALS AND METHODS

Cell Culture and Synchronization—HeLa and 293T cells, from American Type Culture Collection (Rockville, MD), were maintained as subconfluent monolayers in Dulbecco’s modified Eagle’s medium (Invitrogen) with 10% fetal bovine serum (Hyclone, Logan, UT) and 100 units/ml penicillin plus 100 μg/ml streptomycin (Invitrogen) at 37 °C with 10% CO₂. Cells were synchronized at G₁/S with 5 μM thymidine for 16 h and then washed with phosphate-buffered saline (PBS) three times and cultured in thymidine-free medium for 12 h to release.

cDNA Construction—The cDNA of HsCdc14A (NM_033312) was kindly provided by Jiri Lukas. To generate green fluorescent protein (GFP)-tagged full-length HsCdc14A and deletions, PCR-amplified cDNA was cloned into pEGFP-C1 vector (Clontech). The bacterial expression constructs of HsCdc14A were cloned into pET-22a (Novagen) and pGEX-6P (Amersham Biosciences). FLAG-tagged HsCdc14A cDNA was cloned by inserting the PCR product into the pcDNA3-FLAG vector (Invitrogen). The bacterial expression constructs of PLK1 were cloned into pGEX-6P (Amersham Biosciences). Insect expression construct of PLK1 was a generous gift from Ray Erickson (Harvard University).

Recombinant Protein Expression—Human PLK1 was expressed in bacteria as a glutathione S-transferase (GST) fusion protein. The GST fusion protein in bacteria in the soluble fraction was purified by using glutathione-agarose as previously reported (8). Briefly, 500 ml of Luria Bertani medium was inoculated with bacteria BL21(DE3) pLysS transformed with GST-PLK1. The protein expression was induced with 0.2 mM isopropyl-D-galactopyranoside at 30 °C for 4 h. Bacteria were then collected by centrifugation and resuspended in PBS containing a protease inhibitor mixture (Sigma) followed by sonication. The lysis solution was clarified by centrifugation for 20 min at 10,000 × g.

The histidine-tagged HsCdc14A proteins were purified as previously reported (9). The expression of protein was induced by addition of 0.5 mM isopropyl-1-thio-β-D-galactopyranoside. Bacteria were harvested by centrifugation 3 h after the induction, resuspended in PBS containing protease inhibitors (leupeptin, pepstatin, and chymostatin; 5 μg/ml), and sonicated for four bursts of 10 s each by using a probe-tip sonicator. The lysis solution was clarified by centrifugation for 20 min at 10,000 × g. The soluble fraction was applied to a column packed with nickel-agarose beads, followed by extensive washes with PBS. Histagged proteins were then eluted with 200 mM imidazole and dialyzed against appropriate buffers for experimentation.

In Vitro Pulldown Assay—GST fusion protein-bound Sepharose beads were used as an affinity matrix to isolate proteins interacting with PLK1 by using the soluble fraction from mitotic cell lysates that ectopically expressed GFP-HsCdc14A and deletion mutants or purified histidine-tagged full-length and deletion mutants of HsCdc14A that expressed in bacteria. Briefly, the GST-PLK1 fusion protein-bound Sepharose beads were incubated with 293T cell lysates or purified proteins for 4 h at 4 °C. After the incubation, the beads were extensively washed with PBS and boiled in SDS-PAGE sample buffer, followed by fractionation of bound proteins on 10% SDS-PAGE gel. Proteins were then transferred onto a nitrocellulose membrane for Western blotting using an appropriate antibody.

Transient Transfection and Immunoprecipitation—293T cells were grown to ~50% confluency in Dulbecco’s modified Eagle’s medium and co-transfected with FLAG-PLK1 and GFP-HsCdc14A using Lipofectamine 2000 (Invitrogen). Cells were collected 24–36 h after transfection, and proteins were solubilized in lysis buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 2 mM EGTA, 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 10 g/ml leupeptin, and 10 g/ml pepstatin A). Lysates were clarified by centrifugation at 16,000 × g for 10 min at 4 °C. FLAG-tagged fusion proteins were incubated with anti-FLAG M2-linked agarose beads (Sigma). Beads were washed five times with lysis buffer and then boiled in protein sample buffer for 2 min. After SDS-PAGE, proteins were transferred onto nitrocellulose membrane. The membrane was probed with antibodies against the FLAG epitope and GFP. Immunoreactive signals were detected with an ECL kit (Pierce) and visualized by autoradiography on Kodak BioMAX film.

The target sequence for siRNA against PLK1 is 5′-AAGAT- CACCTCCTTTAATAT-3′ (10). The 21-mer oligonucleotide RNA duplexes were synthesized by Dharmacoon Research, Inc. (Boulder, CO). In the trial experiments, different concentrations of siRNA duplexes were used for different time intervals as detailed previously (e.g. Ref. 9). In brief, 293T cells were synchronized and transfected with 21-mer siRNA oligonucleotides or control scramble oligonucleotide, and the efficiency of siRNA-mediated protein suppression was judged by Western blotting analysis.

Protein Phosphatase Activity Assay—Assay of the phosphatase activity of the phosphatase-containing domain of HsCdc14A was carried out using the SensoLyte™ MFP Protein Phosphatase Assay kit (AnaSpec, San Jose, CA) using the manufacturer’s protocol. The phosphatase activities of wild type and mutant HsCdc14A proteins immuno-isolated from 293T cells were judged using p-nitrophenyl phosphate as a substrate as previously described (11).

To examine whether activity of HsCdc14A is a function of PLK1, we used an anti-HsCdc14A antibody to immuno precipitate HsCdc14A protein and its accessory proteins from lysates of 293T cells transfected with siRNA to suppress PLK1 protein level. Aliquots of immuno-isolated HsCdc14A protein were subject to phosphatase assay and immunoblotting for HsCdc14A protein and its phosphorylation as previously described (12).
Flow Cytometry—For cell cycle analysis, 293T cells were transiently transfected to express GFP and various GFP-HsCdc14 proteins. Thirty-six hours after transfection, adherent cells were trypsinized and pooled with the floating cells. Cell suspension of 1 × 10⁶ cells was washed with PBS, fixed in ice-cold 70% ethanol at −20 °C, and stained with a 20 μg/ml-propium iodide, 0.1%-Triton X-100, 200 μg/ml-RNase A solution. Stained cells were analyzed using a FACScan (BD Biosciences) and Modfit 2.0.

In Vitro Phosphorylation—GST-tagged PLK1 kinase was expressed in insect and purified by glutathione-agarose beads. The kinase reactions were performed in 20 μl 1× kinase buffer (25 mM HEPES, pH 7.2, 5 mM MgSO₄, 1 mM dithiothreitol, 50 mM NaCl, 2 mM EGTA) containing 100 ng of eluted PLK1 kinase, 2 μg of eluted His-tagged proteins, 5 μCi of [γ-32P]ATP, and 50 μM ATP. The mixtures were incubated at 30 °C for 30 min. The reactions were stopped with 2× SDS sample buffer and separated by 6–16% gradient SDS-PAGE. The gel was stained with Coomassie Brilliant Blue, dried, and quantified by a PhosphorImager (Amersham Biosciences) and Modfit 2.0.

Immunofluorescence Microscopy—For immunofluorescence, cells were seeded onto sterile, acid-treated 12-mm coverslips in 24-well plates (Corning Glass Works, Corning, NY). Double thymidine-blocked and released HeLa cells were transfected with 1 μl of Lipofectamine 2000 pre-mixed with 1 μg of various plasmids as described above. In general, 36–48 h after transfection, HeLa cells were rinsed for 1 min with PHEM buffer (100 mM PIPES, 20 mM HEPES, pH 6.9, 5 mM EGTA, 2 mM MgCl₂, and 4 M glycerol) and were permeabilized for 1 min with PHEM plus 0.1% Triton X-100 as previously described (13).

RESULTS

Overexpression of the HsCdc14A Phosphatase Domain Prevents Cells from Entry into Mitosis—Recent studies have pointed to the role of mammalian Cdc14A/B in centrosome separation/maturaton, spindle stability, and cytokinesis (5, 6, 7). Because overexpression of HsCdc14A gave no obvious phenotype in mitotic progression, we speculated that HsCdc14A phosphatase activity may be suppressed by either intra-molecular or inter-molecular association. To validate this hypothesis, we generated a series of deletion mutants and asked whether up-regulation of HsCdc14A phosphatase activity would override mitotic regulation. To this end, 293T cells transiently transfected to express full-length GFP-HsCdc14A, GFP-HsCdc14A₁–348, GFP-HsCdc14A₃⁴⁸–₆₂₃, or GFP alone were harvested 24 h after the transfection for flow cytometric analysis of cell cycle distribution. Typically, expression level of GFP-HsCdc14A in positively transfected cells is 4-fold higher than that of endogenous protein. As shown in Fig. 1B, overexpression of the phosphatase domain led to an increase in cells arrested in G₂/M phase. Approximately 20–21% of cells were in G₂/M when transfected with GFP or GFP-HsCdc14A C-ter-
minal, whereas 29.1% of cells transfected with GFP-full-length HsCdc14A and 36.4% of GFP-HsCdc14A1–348-transfected cells were in G2/M (Fig. 1B). To examine the exact phase of such G2/M block, transfected cells were double-stained with 4',6-diamidino-2-phenylindole and scored for mitotic index (13). Whereas a typical 17 ± 2% mitotic index was seen in mock-transfected and full-length HsCdc14A-transfected cells, the mitotic index in GFP-HsCdc14A1–348-expressing cells was 2 ± 1% (Fig. 1C). The majority of the cells overexpressing GFP-HsCdc14A1–348 failed to enter mitosis, suggesting that the C-terminal HsCdc14A may mask the phosphatase activity of HsCdc14A.

**HsCdc14A Phosphatase Activity Is Regulated by an Auto-inhibition Mechanism**—To examine whether the N-terminal phosphatase domain binds directly to its C-terminal half, we expressed the GST-tagged phosphatase domain of HsCdc14A and used it as an affinity matrix to isolate histidine-tagged HsCdc14A1–348. As shown in Fig. 2A, purified histidine-tagged HsCdc14A1–348 binds to the HsCdc14A phosphatase domain (Cdc14A1–348) in a dose-dependent and saturable manner judged by Western blotting analysis using an anti-HsCdc14A antibody (lower panel), indicating that the HsCdc14A indeed forms a self-association.

To test whether this self-association of HsCdc14A is responsible for the inhibition of HsCdc14A enzymatic activity, we measured the phosphatase activity of recombinant HsCdc141–348 using the Sensolyte™ MFP Protein Phosphatase Assay kit. As shown in Fig. 2B, right panel, addition of C-terminal HsCdc14A resulted in inhibition of HsCdc14A phosphatase activity in a dose-dependent manner. However, addition of an equal amount of albumin into the assay did not alter the phosphatase activity of HsCdc14A, indicating that the C-terminal HsCdc14A indeed inhibits its enzymatic activity via an intra-molecular association.

**HsCdc14A Binds to PLK1 via its C Terminus**—In budding yeast, Cdc5, homologue of mammalian PLK1, interacts with and regulates Cdc14 distribution (14). To examine whether PLK1 forms a complex with HsCdc14 in mammalian cells, we used anti-FLAG antibody-coupled beads to incubate mitotic lysates from 293T cells transiently co-transfected to express FLAG-PLK1 and GFP-HsCdc14A. As shown in Fig. 3A, purified histidine-tagged HsCdc14A was pulled down by FLAG immunoprecipitation of PLK (lane 5). No GFP was precipitated with FLAG IgG (lane 4); we therefore conclude that the interaction between PLK1 and HsCdc14A is specific.

We also examined whether PLK1 kinase activity is required for such an association. To this end, we used anti-FLAG antibody-coupled beads to incubate mitotic lysates from 293T cells transiently co-transfected to express kinase-death PLK1 tagged
with FLAG and GFP-HsCdc14A. As shown in Fig. 3A, Western blot using GFP antibody confirmed that HsCdc14A was pulled down by FLAG kinase-death PLK1 as it was by wild type kinase (lane 6), indicating kinase activity is not required for PLK1-HsCdc14A interaction.

To map the domain involving the HsCdc14A-PLK1 association, we used recombinant GST-PLK1 on glutathione-agarose beads as an affinity matrix to incubate lysates of 293T cells transiently transfected to express GFP-HsCdc14A and its deletion mutants. As shown in Fig. 3B, GST-PLK1 pulled down both full-length and C-terminal HsCdc14A (lanes 9 and 11), but not the N-terminal phosphatase domain (lane 10). Because PLK1 contains two Polo boxes located to its C terminus that involve binding PLK1 accessory proteins, we asked whether the Polo boxes involve PLK1-HsCdc14A association. To this end, we generated recombinant PLK1 and its deletion mutants tagged with GST as illustrated in Fig. 3C and then used them as affinity matrixes to isolate histidine-tagged HsCdc14A 345–623. As shown in Fig. 3D, both full-length PLK1 and its kinase domain bind to HsCdc14A and this binding is mediated by the kinase domain of PLK1 and HsCdc14A 345–623, raising the possibility that HsCdc14A may be a substrate of PLK1.

PLK1 Phosphorylates HsCdc14A—To explore whether HsCdc14A is a substrate for PLK1, we first conducted computational analyses using GPS (Group-based phosphorytion Scoring method) (15), which predicts that HsCdc14A is a good substrate for PLK1. To evaluate whether HsCdc14A is indeed a substrate of PLK1, bacterially expressed full-length HsCdc14A and its deletion mutants were phosphorylated in vitro using recombinant PLK1 purified from insect cells in the presence of [32P]ATP. Although the full-length N- and C-terminals of HsCdc14A proteins can be phosphorylated by PLK1 in vitro judged by the 32P incorporation, the full-length HsCdc14A contained the strongest labeling (Fig. 4A, lanes 2, 3, 5). Based on phosphorylation site prediction using GPS and on-line computing, the Ser-351 and Ser-363 of HsCdc14A were identified as potential substrates of PLK1, which is consistent with our 32P labeling experiment in which HsCdc14A 300–400 was phosphorylated by PLK1 in vitro (Fig. 4A, lane 4). To confirm whether both Ser-351 and Ser-363 are PLK1 substrates, we generated...
non-phosphorylatable HsCdc14A by mutating either or both serines to alanine and repeated the in vitro phosphorylation experiment. As expected, $^{32}$P failed to incorporate into the HsCdc14A mutant proteins in which either or both Ser-351 and Ser-363 were replaced with alanine (Fig. 4A, lanes 6 and 7). Thus, we conclude that Ser-351 and Ser-363 of HsCdc14A are PLK1 substrates.

PLK1 Phosphorylation Attenuates the Auto-inhibition due to the N-C Association of HsCdc14A—Having demonstrated that HsCdc14A is a bona fide substrate of PLK1 in vitro, we wanted to address whether this phosphorylation alters the self-association of HsCdc14A. To this end, His-tagged HsCdc14A$^{345-623}$ protein was incubated with GST-PLK1 beads with or without ATP, prior to its incubation with GST-tagged HsCdc14A$^{1-348}$ on agarose beads. After a 30-min incubation, bound proteins were resolved by SDS-PAGE and quantified using Western blotting analysis. As shown in Fig. 4, B and C, pretreatment of HsCdc14A$^{345-623}$ with PLK1 in the presence of ATP attenuated the ability of HsCdc14A$^{345-623}$ to retain the HsCdc14A$^{1-348}$ affinity matrix, suggesting that PLK1 phosphorylation releases the binding and inhibition of HsCdc14A$^{345-623}$ to its phosphatase domain.

To evaluate how PLK1 phosphorylation regulates HsCdc14A activity in vivo, we constructed FLAG-tagged phospho-mimicking HsCdc14A$^{S351/363D}$ and non-phosphorylatable HsCdc14A$^{S351/363A}$ mutants and transfected them into 293T cells. After immunoprecipitation, we measured the phosphatase activity using p-nitrophenyl phosphate as a substrate as liberation of p-nitrophenyl can be easily monitored by spectrometry (11). Results shown in Fig. 5, A and B, indicate that the phosphorylation-mimicking mutant has the highest phosphatase activity, as expected. Western blot with an anti-FLAG anti-
body confirmed that an equal amount of protein was used in this assay (Fig. 5C). If activity of HsCdc14A is a function of PLK1-mediated phosphorylation, suppression of PLK1 protein level would alter endogenous HsCdc14A phosphorylation and its phosphatase activity. Indeed, suppression of PLK1 using siRNA reduced serine phosphorylation of HsCdc14A but not HsCdc14 protein level (Fig. 5D). Importantly, HsCdc14A isolated from PLK1-depleted cells has a lower phosphatase activity compared with that from scramble-treated control cells although an equal amount of HsCdc14A protein was applied (Fig. 5E). Thus, we conclude that PLK1 interacts with and phosphorylates HsCdc14A and such phosphorylation stimulates HsCdc14A phosphatase activity.

HsCdc14A Primarily Co-distributes with PLK1 to the Centrosomes—To explore the spatiotemporal dynamics of HsCdc14A and PLK1 interaction, the respective distributions of both proteins were examined in HeLa cells transiently transfected to express GFP-HsCdc14A and FLAG-PLK1. Fig. 6A
shows the distribution of HsCdc14A and PLK1 at interphase and various stages of mitosis. Both HsCdc14A and PLK1 are localized to the centrosome from prophase to metaphase; by anaphase PLK1 has relocated from the centrosome to the central spindle while HsCdc14A is still at centrosome, and at telophase, PLK1 localization is seen only at the mid-body where HsCdc14A is also apparent (Fig. 6A). To examine whether the region of HsCdc14A specifies its centrosomal localization, we assessed the location of GFP-HsCdc14A1–348 and GFP-HsCdc14A345–623 in mitotic cells. As shown in Fig. 6B, GFP-HsCdc14A345–623 was found primarily located to the centrosome in a similar pattern to that of full-length HsCdc14A. However, GFP-HsCdc14A1–348 displayed diffused cytoplasmic staining, suggesting that the C-terminal HsCdc14A specifies its localization to the centrosome. Interestingly, a minority of GFP-HsCdc14A345–623 also displayed centromere-like distribution. Because PLK1 localizes to both centrosome and centromere of mitotic cells, we propose that the interaction between PLK1 and HsCdc14A345–623 specifies the localization of HsCdc14A to centromere and centrosome in mitotic cells.

A Phospho-mimicking Mutant of HsCdc14A Causes a Prolonged Delay in Chromosome Alignment—Given the interaction between PLK1 and HsCdc14A and their co-distribution to mitotic apparatus, we sought to probe the functional relevance of PLK1-HsCDC14A interaction in mitotic progression. To this end, GFP-H2B were transiently co-transfected with wild type and its phospho-mimicking mutant HsCdc14A S351/363D, followed by time-lapse imaging of chromosome movements upon the nuclear envelope breakdown. As shown in Fig. 7A, chromosomes achieved metaphase alignment ~20 min after nuclear envelope breakdown and initiated anaphase onset 20 min after the last chromosome aligned at the equator in wild type HsCdc14A-expressing cells. However, the chromosome alignment did not achieve metaphase alignment until 78 min after the nuclear envelope breakdown in HsCdc14A S351/363D-expressing cells (Fig. 7B), suggesting temporal control of HsCdc14A activity is essential for faithful chromosome congression. In addition, the anaphase onset was delayed for ~15 min. The chromosome movements in HsCdc14A S351/363A-expressing cells displayed a similar profile as that of wild type
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We surveyed approximately three cells from each group and summarized their profiles in Fig. 7D. Taking together the essence of time-lapse imaging with regulation of HsCdc14A by PLK1 revealed in this study, we conclude that temporal control of HsCdc14A phosphatase activity by PLK1 is essential for chromosome segregation in mitosis.

DISCUSSION

We have identified and characterized HsCdc14A as a substrate of PLK1 and established their inter-relationship. The temporal dynamics of PLK1-HsCdc14A interaction orchestrates faithful chromosome segregation in mitosis.

We found that HsCdc14 exhibits an inhibitory self-association in which the C-terminal domain binds and inhibits the phosphatase domain located to the N terminus. This self-association is disrupted by PLK1-mediated phosphorylation in vitro and in vivo. Our studies also revealed the novel interaction between HsCdc14A and PLK1 and their co-distribution to centrosome. Given the dynamic pattern of their distribution, it would be of great interest to illustrate the precise time and location in which PLK1-mediated phosphorylation turns on the phosphatase activity of HsCdc14A.

In budding yeast, Cdc14p and cyclin-dependent kinase 1 antagonistically orchestrate the chromosome segregation via governing the localization of Aurora B-INCENP, and perturbation of Cdc14p and cyclin-dependent kinase 1 interaction resulted in chromosome segregation defects (16). Previous studies show that down-regulation of human Cdc14A phosphatase disrupts centrosome separation and chromosome segregation (5). However, it was unclear how centrosome-localized HsCdc14A governs chromosome segregation. Our study shows that C-terminal HsCdc14A contains a centromere localization activity (e.g. Fig. 6B), raising the possibility that PLK1-mediated phosphorylation unfolds HsCdc14A, which exposes the centromere-targeting domain of HsCdc14A. However, our attempt to validate this hypothesis was compromised by the fact that overexpression of phospho-mimicking GFP-HsCdc14A S351/363D gave high cytoplasmic signal with no distinct kinetochore localization. Future experiments will be needed to design a better optical sensor to enable our visualization of HsCdc14A molecular dynamics in mitosis and illustrate its activation cascade related to chromosome segregation.

It has been shown that human HsCdc14A can dephosphorylate products of cyclin-dependent kinases, such as hCdh1 (17), cyclin E (18), and p53 (19). Because none of the aforementioned substrates is involved in chromosome segregation, it would be of great importance to understand how HsCdc14A orchestrates chromosome segregation by identifying its substrates locating to the mitotic apparatus.

Taken together, our finding of a novel interrelationship between PLK1 and HsCdc14A demonstrates a critical role for HsCdc14A in chromosome segregation in addition to its function for centrosome regulation. The fact that overexpression of constitutively active Cdc14A S351/363D causes defects in chromosome segregation and delay in anaphase onset demonstrates the importance of spatiotemporal control of the PLK1-HsCdc14A interaction in faithful chromosome segregation.

Acknowledgments—We thank Dr. Jiri Lucas (Institute of Cancer Biology, Denmark) for the gift of HsCdc14A cDNA and Dr. Ray Erickson (Harvard University) for GST-PLK1 expression constructs. We thank members of our groups for insightful discussion and technical assistance.
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