Biochemical and Biophysical Research Communications 454 (2014) 429–435

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Regulation of the subcellular shuttling of Sgo1 between centromeres and chromosome arms by Aurora B-mediated phosphorylation

Noo Ri Lee [a], Hyun-Soo Kim [a,*], You-Sun Kim [b], Myung-Hee Kwon [c], Kyeong Sook Choi [d], Chang-Woo Lee [a,*]

[a] Department of Molecular Cell Biology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon 440-746, Republic of Korea
[b] Department of Biochemistry, Ajou University School of Medicine, Suwon 443-749, Republic of Korea
[c] Department of Microbiology, Ajou University School of Medicine, Suwon 443-749, Republic of Korea
[d] Department of Biochemistry and Biomedical Sciences, Ajou University School of Medicine, Suwon 443-749, Republic of Korea

ARTICLE INFO

Article history:
Received 15 October 2014
Available online 28 October 2014

Keywords:
Sgo1
Aurora B
Sister chromatid cohesion
Centromere
Phosphorylation
Chromosome arm

ABSTRACT

A minor fraction of cohesin complexes at chromosome arms is not removed by the prophase pathway, and maintained until metaphase and enriched at centromeres. Sgo1 localizes to chromosome arms from prophase to metaphase, and is indispensable for removing cohesin complexes from chromosome arms. However, it has not been established how the chromosome arm localization of Sgo1 leads to the establishment of cohesion on chromosomes. Here, we report that Aurora B kinase interacts with and phosphorylates Sgo1 *in vitro* and *in vivo*. Furthermore, the phosphorylation of Sgol by Aurora B kinase regulated the distribution of Sgo1 between centromeres and chromosome arms, and the expression of Aurora B kinase-dead mutants of Sgo1 caused mislocalization from centromeres to chromosome arms. These results suggest Aurora B kinase directly regulates the subcellular distribution of Sgo1 to facilitate the accurate separation of mitotic chromosomes

1. Introduction

Sister chromatid cohesion is mediated by a multi-subunit complex called cohesin, and the cohesin complex consists of four subunits, that is, two structural proteins, Smc1 and Smc3, and two regulatory proteins, Scc1/Rad21 and Scc3/SA (SA1 and SA2 in vertebrates). Recent reports suggest that cohesin forms a ring-like structure which topologically appears to embrace two sister chromatids [1–4]. In animal cells during early mitosis, most cohesin dissociates from chromosome arms in a process known as "prophase pathway" removal [5], which is partially dependent on the phosphorylation of cohesin by mitotic kinases, that is, Aurora B and Plk1 [6]. However, cohesin at centromeres is retained until metaphase to anaphase transition due to the function of shugoshin (Sgo) proteins [7,8]. In particular, Sgo1 associates with serine and threonine protein phosphatase 2A (PP2A) to protect cohesin subunit proteins from phosphorylation [7,9]. If Sgo1 is inactivated, cohesin dissociates prematurely from centromeres in the absence of separase activity. Therefore, Sgo1 is believed to play an essential role by protecting centromeric cohesion from the prophase removal pathway and to maintain cohesion until separase becomes active in metaphase. At metaphase, chromosomes are attached to spindle microtubule via their kinetochores, and this bipolar attachment is stabilized by the tension generated by the force pulling spindle microtubules and counteracting cohesive forces at centromeres. Thus, centromeric cohesion, which is largely protected by Sgo1, is essential for establishing bipolar attachment [10,11].

The recruitment of Sgo1 to centromeres depends on Bub1 mitotic checkpoint kinase and HP1a [12,13]. Loss of Bub1, like Sgo1 depletion, leads to separation of sister chromatids. Interestingly, inhibition of Aurora B kinase affects Sgo1 localization by causing its redistribution to chromosome arms during mitosis and meiosis [14–16]. Although this phenotype resulting from Aurora B inhibition might be related to the observed requirement for Aurora B in the prophase pathway, the role of Aurora B-mediated phosphorylation of Sgo1 in centromeric localization seems distinct. However, it has not been established whether the relocalization of Sgo1 to chromosome arms is also regulated by Aurora B-mediated phosphorylation. It is interesting that cohesion between chromosome arms is never completely dissolved during unperturbed mitosis [5,11]. The complete removal of arm cohesion likely appears in cells that remain in prometaphase for prolonged periods of times, and therefore, is probably due to continuous

* Corresponding authors. Fax: +82 31 299 6109.
E-mail addresses: jazz7780@skku.edu (H.-S. Kim), cwlee1234@skku.edu (C.-W. Lee).

http://dx.doi.org/10.1016/j.bbrc.2014.10.103
0006-291X/

activity of the prophase pathway [3–5]. However, it has been recently shown that cleavage of Scc1 by small amounts of separase activity is also required for cohesin removal signaling [11]. Importantly, the requirement for separase during chromosome arm opening is abolished if Sgo1 is depleted, which implies that small amounts of Sgo1 also protect cohesion on chromosome arms during the early stage of mitosis [11,17]. Nevertheless, the molecular link responsible for Sgo1 shuttling between centromeres and chromosome arms and its dependence on Aurora B remains elusive.

Recent evidence suggests that when cohesin release from chromatid arms is impaired, a substantial amount of Sgo1 is also concentrated on a discrete axis-like structure along the entire length of chromosomes where cohesin is also enriched [18]. In addition, if Sgo1 is depleted centromeric staining for Sgo1 is reduced, and Sgo1 id delocalized alienating chromosome arms [11]. These observed strongly indicate Sgo1 is essential for the regulation of sister chromatid cohesion and dissociation at centromeres and at chromosome arms. However, loss of Sgo1 from sister chromatid arms appears to be insufficient to promote cohesin release and arm resolution, and that instead Sgo1 plays an important role in stabilizing cohesion along chromosome arms [18]. Although the role of Sgo1 in the stabilization of centromeric cohesion during mitosis and meiosis has been extensively characterized, its potential contribution to stabilizing arm cohesion remains largely unrecognized. Therefore, we sought to unveil how in Aurora B depleted or inactivated cells, Sgo1 redistributes to chromosome arms from centromeres, and how Aurora B inactivation causes this redistribution to chromosome arms.

2. Materials and methods

2.1. Generations of plasmids, shRNAs, and siRNAs

Sgo1 S38A, S303A, S307A, S314A, and T319A alleles were generated by site-directed mutagenesis. cDNAs for Sgo1 WT and Sgo1 mutants were subcloned into GFP epitope-encoding vector to generate GFP-Sgo1 WT and mutants. For shRNA synthesis, the following gene-specific sequences were generated using pSuper vector (Oligoengine); Aurora B shRNA, 5′-GCAGA AGAGC TGCAC ATTT-3′; luciferase shRNA, 5′-CTACG CGGAA TACTT CGA-3′; Sgo1 shRNA, 5′-GTCTA CTGAT AATGT CTTA-3′. Gene-specific sequences for siRNA synthesis were; luciferase siRNA, 5′-CUACG CGGAA UACUU CGA-3′; and Bub1 siRNA, 5′-CCAGU GAGUU CCUAU CCAA-3′.

2.2. Constructions of inducible and stably transfected cell lines

To generate HeLa cells inducibly expressing GFP, GFP-Sgo1 WT, or GFP-Sgo1 mutant fusion proteins, HeLa Tet-on cells were transfected with pTRE2-hygro vector (BD Biosciences Clontech) containing the respective cDNAs with the GFP tag fused in-frame. Hygromycin-resistant clones were selected in culture media containing 200 mg/ml hygromycin and induced with 2 mg/ml doxycycline for 48 h.

2.3. Fractionation, and synchronization

For the fractionation of cell extracts, soluble cytosolic supernatants were prepared using PA buffer [150 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 mM DTT and a mixture of protease inhibitors]. Pellet fractions were collected by the dissolution of nuclei in XBE2 buffer [10 mM HEPES (pH 7.5), 300 mM NaCl, 1 mM EDTA, 2 mM MgCl2, 1 mM PMSF, 1 mM DTT and a mixture of protease inhibitors]. Chromatin fractions were subsequently prepared by sonication of insoluble pellet fractions in XBE2 buffer. For synchronization at the G1/S boundary, cells were grown in the presence of 1 mM thymidine (Sigma) for 14 h, washed with PBS, grown in fresh medium for 12 h, treated with thymidine for 14 h, rewashed in PBS, and placed in fresh medium. For synchronization at G2, cells were grown in the presence of 50 nM doxorubicin, and for synchronization at mitosis, cells were grown in the presence of 200 ng/ml nocodazole and collected by shake-off.

2.4. Antibodies

The antibodies used in this study were obtained as follows: anti-Sgo1, anti-INCENP (both from Abcam), anti-Aurora B, anti-PP2A (BD Biosciences, Epitomics), anti-Bub1 (MBL), anti-histone H3 pSer28 (Millipore), CREST serum (Immunovision), anti-actin (Sigma), anti-HP1 (Millipore), anti-GFP, and anti-GST (Santa Cruz Biotechnology).

2.5. Live-cell imaging and immunostaining assays

To estimate RFP and GFP emissions, HeLa-GFP-Sgo1 WT cells were transfected with an expression plasmid encoding H2B-RFP, induced with doxycycline, and then imaged. The confocal pinhole was adjusted to an optical slice thickness larger than the *z*-sampling rate and most time-lapse recordings were performed in parallel at multiple stage positions. Over the course of 24 h, 0.3-s exposures were taken every 20–60 s using an LSM500 META confocal microscope fitted with a ×20 NA0.75 objective lens (Carl Zeiss). For immunostaining, cells were cultured directly on glass coverslips, washed with PBS (for pre-extraction immunostaining, cells were pre-extracted with 0.2% Triton X-100 in PBS for 10 min and then washed with PBS), fixed in 4% paraformaldehyde, and incubated with the indicated primary and secondary antibodies.

2.6. Immunoprecipitation, immunoblotting, and in vitro binding assays

For immunoprecipitation, asynchronized HeLa cells were lysed in TNN buffer [50 mmol/L Tris–HCl (pH 7.5), 150 mmol/L NaCl, 1% NP40, 1 mmol/L EDTA, 1 mmol/L (PMSF), and 1 mmol/L DTT] containing a protease inhibitor cocktail (Roche). Cell extracts were incubated with antibodies against Sgo1 or normal IgG (control) for 1 h at 4 °C and then with protein A/G-Sepharose beads for 6 h. Beads were pelleted, washed four times with immunoprecipitation buffer, and analyzed by immunoblotting. For immunoblot assays, cells were synchronized as described above or left asynchronized, harvested by scraping, washed twice in cold PBS, and lysed in TNN buffer. For the GST-pull down assay, fusion proteins were adsorbed onto glutathione-Sepharose beads (Amersham Biosciences) and incubated with whole cell extracts (2 mg) from asynchronized HeLa cells for 4 h at 4 °C.

2.7. In vitro kinase assay

For the *in vitro* kinase assay, bead-bound Sgo1 WT or mutant proteins were washed twice with kinase buffer [100 mM Tris–HCl (pH 7.5), 2 mM EDTA (pH 8), 20 mM $MgCl_2$, 10 mM $MnCl_2$, 1 mM DTT and 1 mM PMSF] and reacted with 0.4 μg of recombinant Aurora B (Sigma) proteins in the presence of radio-labeled [γ^{32}P]ATP (10 mCi) at 37 °C for 1 h.

3. Results

3.1. Aurora B interacted with and phosphorylated Sgo1 in vitro and in vivo

To determine whether Sgo1 interacts functionally with Aurora B, endogenous Sgo1 was immunoprecipitated from asynchronized HeLa cells and immunoblotted with Aurora B antibody (Fig. 1A). In addition, Sgo1 was immunoprecipitated from HeLa cells inducibly expressing GFP-Sgo1 WT treated with thymidine (Thy), doxorubicin (Doxo), or nocodazole (Noco) to synchronize cells at the G1-S boundary, G2, or mitosis, respectively, and immunoblotted with Aurora B antibody (Fig. 1B). These assays revealed that Sgo1 formed a complex with Aurora B in asynchronized and synchronized cells. To define the domains responsible for the Sgo1–Aurora B interaction, a series of GST fused Sgo1 full length and deletion mutants were incubated with HeLa cell extracts (Fig. 1C and D). An *in vitro* binding assay showed that the central domain of Sgo1 protein (amino acids 301–458) interacted with Aurora B, whereas the amino-terminal (amino acids 1–300) and the carboxyl-terminal (amino acids 459–529) domains did not. Interestingly, the central domain of Sgo1 protein also interacted with SA2 cohesin subunit protein and heterochromatin protein 1 (HP1). However, the amino-terminal domain of Sgo1 was required for the interaction with protein phosphatase 2 A (PP2A), whereas the amino-terminal and the central domain of Sgo1 both interacted with INCENP, a component of CPC complex.

To determine whether the Sgo1–Aurora B interaction leads to Sgo1 phosphorylation, we incubated a series of GST-Sgo1 fusion proteins with purified recombinant Aurora B in the presence of

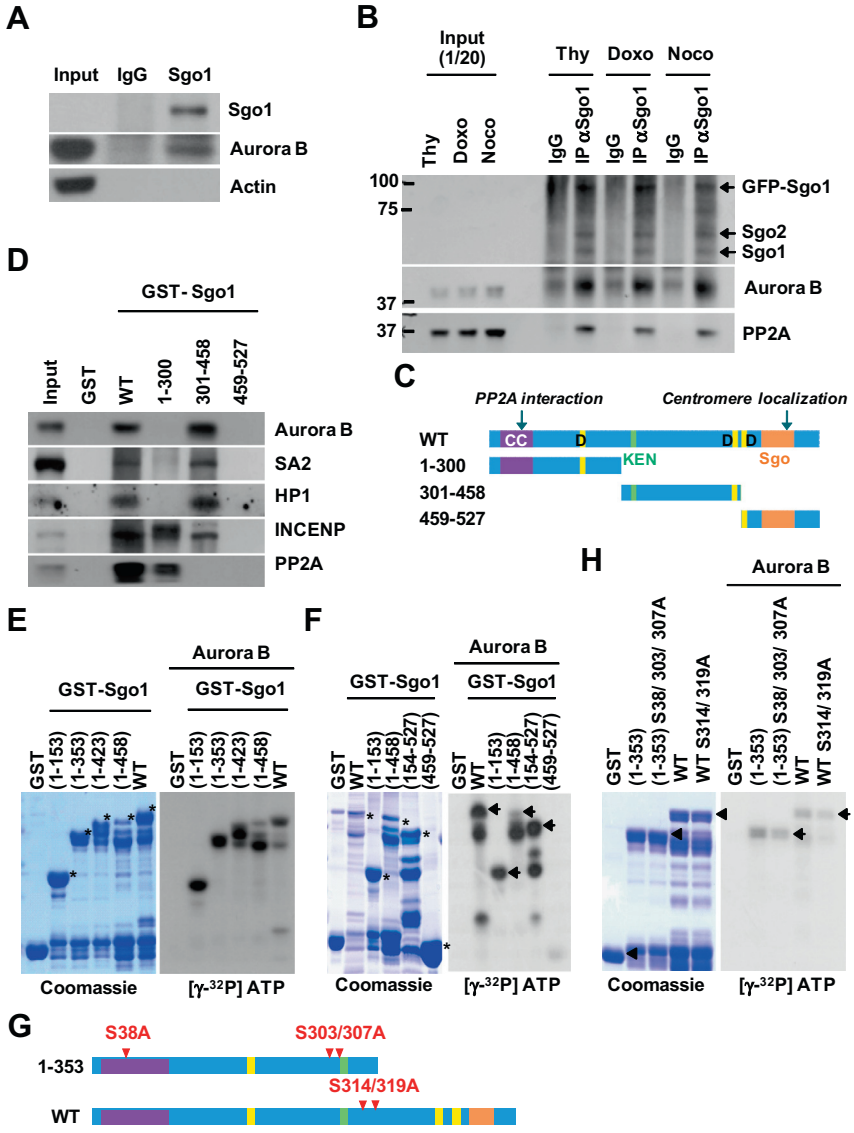

Fig. 1. Aurora B interacted with and phosphorylated Sgo1 *in vitro* and *in vivo*. (A) Asynchronized HeLa cell extracts were immunoprecipitated with normal immunoglobulin IgG or a polyclonal anti-Sgo1 antibody, and immunoprecipitates were immunoblotted with anti-Aurora B or anti-Actin (negative control) antibodies. (B) HeLa cells inducibly expressing GFP-Sgo1 WT were synchronized by using thymidine (Thy), doxorubicin (Doxo), or nocodazole (Noco). Synchronized cells were extracted and immunoprecipitated with normal IgG or anti-Sgo1 antibody, and immunoblotted with antibodies as indicated. (C) Schematic drawing of Sgo1 protein showing the conserved coiled-coil domain (purple), D-box (yellow), KEN-box (green), and Sgo motif (orange). (D) HeLa cell lysates were incubated with beads bound to GST alone (GST) or to a series of GST-fused Sgo1 deletion mutants. Bound proteins were resolved and immunoblotted with antibodies as indicated. (E and F) GST and GST-fused Sgo1 deletion mutants were expressed in and purified from *E. coli*. After incubation with recombinant Aurora B in the presence of [γ^{32}P]ATP, GST-fusion proteins were analyzed by SDS–PAGE and autoradiography. (G) Schematic representation of putative phosphorylation sites in Sgo1 protein. (H) Purified GST, GST-Sgo1 WT and MTs were incubated with purified Aurora B in the presence of [γ^{32}P]ATP, resolved by SDS–PAGE, and analyzed by autoradiography. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

[γ^{32}P]ATP (Fig. 1E and F). An *in vitro* kinase assay revealed that Sgo1 was phosphorylated in the presence of Aurora B kinase. However, somewhat unexpectedly, all of the Sgo1 deletion mutants were found to be phosphorylated by Aurora B *in vitro*, except the Sgo-motif deletion mutant (amino acids 459–527). Thus, Sgo1 includes a number of potential substrate sites which could be phosphorylated by Aurora B. In addition, the group-based prediction system 2.1 software predicts the residues at Ser 38, Ser 303, Ser 307, Ser 314, and Thr 319 of Sgo1 protein as targets for Aurora B-mediated phosphorylation (Fig. S1). Therefore, we generated the GST-fused Sgo1 point mutant proteins which are involved in the five putative sites of Aurora B-mediated phosphorylation (Fig. 1G). As expected, the replacement of S38/303/307 or S314/319 with alanine (A) in Sgo1 proteins reduced the phosphorylation by Aurora B (Fig. 1H). Together, these data show that Aurora B interacts with and phosphorylates Sgo1.

3.2. Sgo1 colocalized with Aurora B during mitosis

Next, we performed immunostaining analysis using anti-Sgo1, anti-Aurora B antibodies, and CREST serum (Fig. 2A). Consistent with previous reports, Sgo1 and Aurora B were observed to colocalize in the nucleus during interphase [19], and while cells progressed into mitosis from G2, Sgo1 and Aurora B colocalized at centromeres until metaphase. However, Sgo1 staining at centromeres/kinetochores was significantly reduced at the onset of anaphase. The attenuation of Sgo1 signals at centromeres appeared to cause anaphase-promoting complex/cyclosome activation, as has been previously reported [20]. On the other hand, in contrast to Sgo1, Aurora B was localized at mid-bodies at anaphase and cleavage furrows at telophase, indicating that the Sgo1–Aurora B interaction occurs from interphase to early mitosis. To confirm the subcellular distributions of Sgo1 during mitotic progression, we transfected the GFP-fused Sgo1 expression plasmid into HeLa cells, and monitored the localization of the GFP signal (Fig. 2B). As was expected, GFP-Sgo1 protein localized at centromeres at prophase and then moved to kinetochores, whereas endogenous Aurora B remained at centromeres until metaphase (Fig. 2B).

To examine the effect of Aurora B depletion on Sgo1 localization, we generated doxycycline inducible HeLa cells expressing GFP-Sgo1 fusion proteins. These inducible cells (HeLa-GFP-Sgo1) were cotransfected with shRNA specifically targeting Aurora B (shAur B), Bub1 (shBub1), or Plk1 (shPlk1) and with an expression plasmid encoding RFP-tagged H2B (H2B-RFP) (Fig. 2C). GFP fusion Sgo1 was induced by doxycycline, and GFP and RFP emissions were digitally monitored by time-lapse microscopy. In the control shLuc-transfected cells, Sgo1 was clearly enriched at centromeres during early mitosis. Interestingly, depletion of Aurora B caused a dramatic change in the distribution of Sgo1, which localized along whole chromosomes. The localization of Sgo1 in Bub1-depleted cells also showed differences versus control cells, as reported previously [12]. However, depletion of Plk1 did not affect to the centromere/kinetochore localization of Sgo1 protein during mitosis (Fig. 2C). Together, these results suggest Aurora B and Bub1

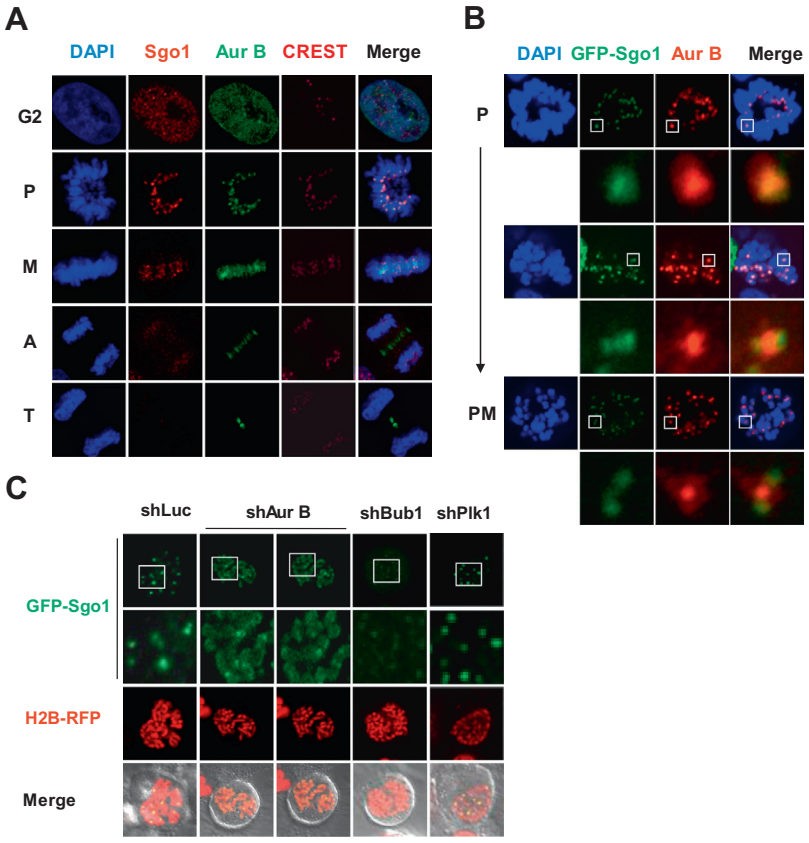

Fig. 2. Sgo1 colocalized with Aurora B during mitosis. (A) HeLa cells were fixed and stained with anti-Sgo1 antibody (red), anti-Aurora B antibody (green), and CREST serum (purple). DNA was visualized by DAPI staining. 'P', 'M', 'A', and 'T' indicate prophase, metaphase, anaphase, and telophase, respectively. (B) HeLa cells were co-transfected with expressing plasmid encoding GFP-fused Sgo1 (GFP-Sgo1). At 48 h post-transfection, cells were stained with anti-Aurora B antibody (red) and DAPI (blue). (C) Inducible HeLa cells expressing GFP-Sgo1 were cotransfected with expressing plasmids encoding RFP-tagged H2B (H2B-RFP) and control luciferase (shLuc), Aurora B (shAur B), Bub1 (shBub1) or Plk1 (shPlk1) knock-down constructs. At 12 h post-transfection, cells were induced with 2 μg/ml doxycycline and cultured for a further 48 h. Time-lapse images taken at 3 min intervals showing GFP (GFP-Sgo1) and RFP (H2B-RFP) emissions. Nuclear envelop break down (NEBD) occurred at 0 min.

participate in the regulation of the localization of Sgo1, but that they act via different pathways.

3.3. Aurora B regulated the shuttling of Sgo1 distribution between centromeres and chromosomes

Inducible HeLa-GFP-Sgo1 cells were cotransfected with shAur B or H2B-RFP expression plasmids, and GFP and RFP emissions were digitally monitored by time-lapse microscopy (Fig. 3A). Nuclear envelope break-down (NEBD), which occurs as a cell enters mitosis, was determined by the appearance of sister chromatid disorganization, as indicated by H2B-RFP. Consistently, Sgo1 was enriched in the centromere regions of control cells, but the depletion of Aurora B led to the redistribution of Sgo1 and its localization along whole chromosome regions.

To characterize the correlation between Aurora B and the subcellular localization of Sgo1, inducible HeLa-GFP-Sgo1 cells were treated with control DMSO or a selective Aurora B kinase inhibitor (ZM447439) (Fig. 3B). Interestingly, treatment with ZM447439 caused chromosome arm localization of Sgo1, which resembled the phenotype observed in Aurora B-depleted cells. These results suggest that the centromeric localization of Sgo1 depends on Aurora B kinase.

3.4. Phosphorylation of Sgo1 by Aurora B regulated the subcellular distribution of Sgo1 between centromeres and chromosome arms

Next, we examined Sgo1 phosphorylation patterns throughout cell cycle progression. HeLa cells were synchronized by double-thymidine block (G1-S boundary), doxorubicin treatment (G2), or nocodazole treatment (mitosis), and then lysed and immunoblotted with anti-Sgo1 and anti-actin (loading control) antibodies. Interestingly, slower migrating bands of Sgo1 were observed in the G2 and mitotic phase fractions. Furthermore, these bands migrated more rapidly after λ-phosphatase treatment, indicating that Sgo1 is phosphorylated during the G2 and early mitotic phases (data not shown). To explore the functional meaning of these phosphorylations, we generated plasmids encoding GFP-fused Sgo1 mutants, in which the five putative phosphorylation sites were mutated singly or in combination (Fig. 4A and B). Interestingly, replacing Ser 38 with Ala (A) significantly reduced the centromere localized Sgo1, but greatly increased chromosome arm localized Sgo1 (Fig. 4B and C). Furthermore, mutations of four putative phosphorylation sites (Ser 303, Ser 307, Ser 314, and Thr 319) sharply changed the subcellular distribution of Sgo1, whereas mutations of Ser 303 and Ser 307 with Ala did not affect the subcellular distribution of Sgo1 (Fig. 4B and C). Next, we monitored the subcellular localization of GFP-fused Sgo1 mutants (S38A and 4A) following the depletion of endogenous Aurora B in HeLa cells by transfecting shAurora B. The transfection of Aurora B shRNA clearly abolished the expression of endogenous Aurora B and the level of phosphorylated histone H3 at Ser 28, a marker substrate for Aurora B-mediated phosphorylation during mitosis (Fig. S2A). Interestingly, the localization of chromosome arm in cells expressing GFP-Sgo1 mutants was significantly increased by Aurora B-depletion compared to those in cells expressing GFP-Sgo1 WT (Fig. S2B and S2C). Taken together, these results suggest that the phosphorylation of Sgo1 by Aurora B regulates the subcellular distribution of Sgo1 between centromeres and chromosome arms.

4. Discussion

Since Sgo1 protects cohesin complexes, the localization and regulation of Sgo1 are important aspects of faithful chromosome segregation. Despite the importance of chromosome segregation, little is known of the mechanisms that regulate the localization of Sgo1 in vertebrate cells. In this study, Sgo1 was found to interact directly with Aurora B and act as a key substrate. In addition, it was observed that the phosphorylation of Sgo1 is required for dissociating Sgo1 from chromosome arms, thereby enabling Sgo1 to be recruited by centromeres in a Bub1 dependent manner [12].

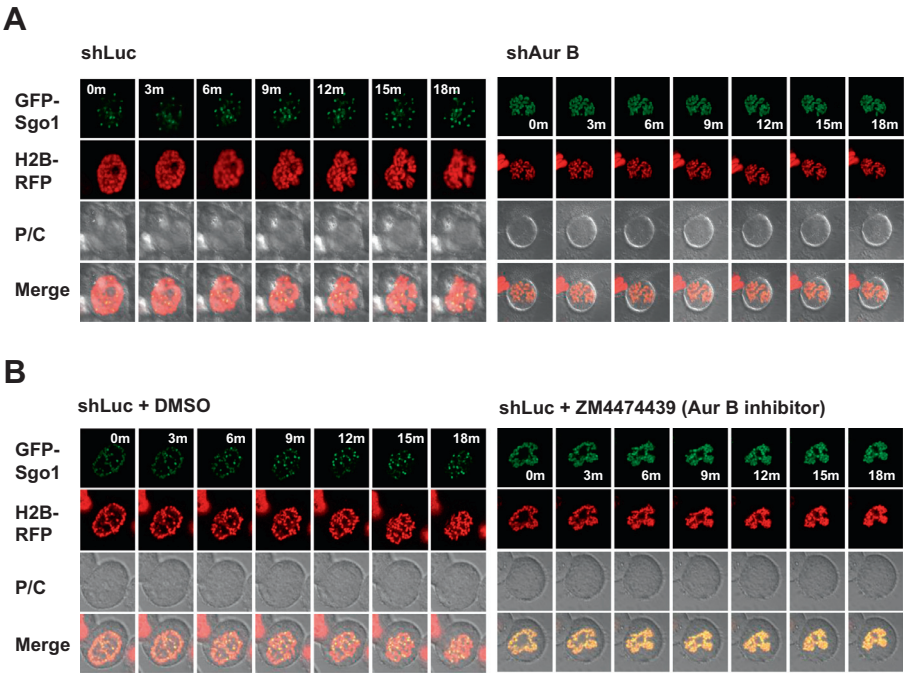

Fig. 3. Aurora B regulated the shuttling of Sgo1 distribution between centromeres and chromosomes. (A and B) Inducible HeLa cells expressing GFP-Sgo1 (GFP-Sgo1) were transfected with expressing plasmids encoding RFP-tagged H2B (H2B-RFP). At 12 h post-transfection, cells were induced with 2 μg/ml doxycycline and cultured for a further 48 h (A). Inducible HeLa cells were treated with nocodazole to enrich cells in the prometaphase and then treated with DMSO (control) or ZM447439 (a selective Aurora B kinase inhibitor) (B). Time-lapse images taken at 3 min intervals showing GFP (GFP-Sgo1) and RFP (H2B-RFP) emissions. Nuclear envelop break down (NEBD) occurred at 0 min.

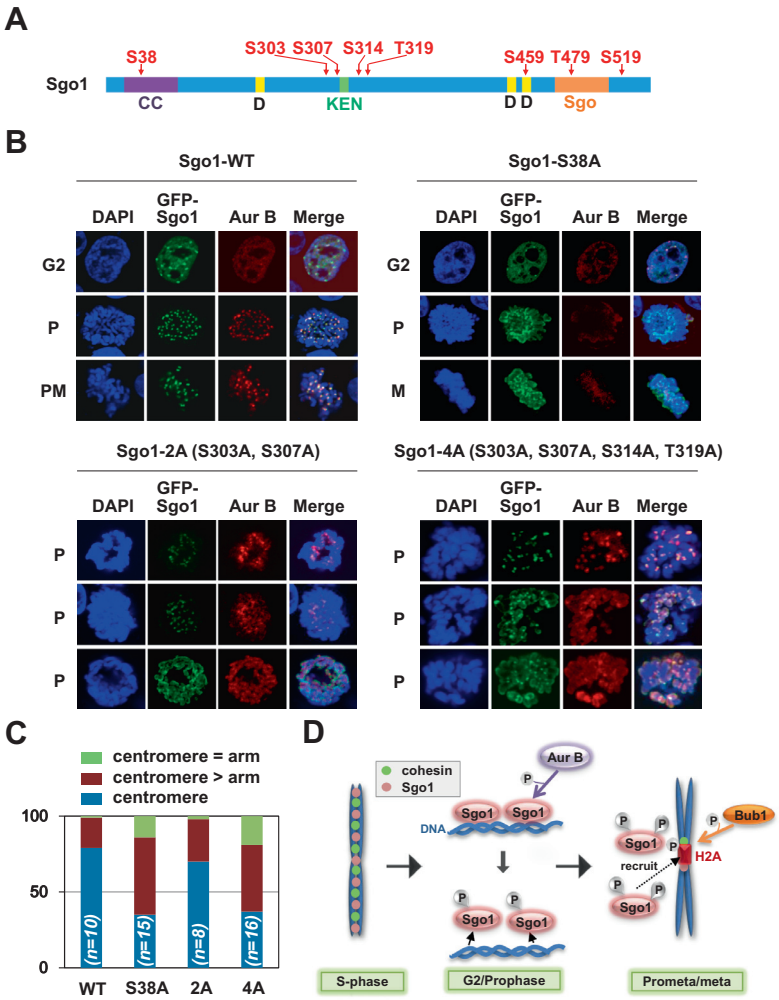

Fig. 4. Phosphorylation of Sgo1 by Aurora B regulated the subcellular distribution of Sgo1 between centromeres and chromosome arms. (A) Schematic diagram of functional motives and putative Aurora B kinase phosphorylation sites in Sgo1 polypeptide. (B and C) HeLa cells were transfected with expression plasmid encoding GFP-Sgo1 wild type (Sgo1 WT), GFP-Sgo1 S38A mutant (Sgo1 S38A), GFP-Sgo1 S303A and S307A mutant (Sgo1-2A), or GFP-Sgo1 S303A, S307A, S314A and T319A mutant (Sgo1-4A). Transfected cells were stained with anti-Aurora B antibody (red) and DAPI (blue). 'P', 'PM', and 'P' indicate prophase, prometaphase and metaphase, respectively. HeLa cells expressing GFP-Sgo1–2A or GFP-Sgo1–4A showed three different subcellular Sgo1 distribution patterns that were dependent on their expression intensities (B). The relative staining intensities of Sgo1 WT and MTs in centromeres and chromosome arms (C). (D) A schematic model of functional interplay between Sgo1 and Aurora B kinase during the regulation of sister chromatid cohesion. In mammals, the phosphorylation of Sgo1 is required during G2 or the early mitotic phase to dissociate Sgo1 from chromosome arms. This process may contribute to the delocalization of Sgo1, faithful prophase removal of cohesin complex, and centromeric protection of cohesin complex.

One of the challenging questions that remains to be answered is "does endogenous Sgo1 localize at chromosome arms after chromosome duplication?" Indeed, although a recent paper mentioned that exogenous Myc-tagged Sgo1 localized at interphase centromeres [21], no clear evidence was provided of the subcellular distribution of endogenous Sgo1 in unperturbed cells. Interestingly, we observed that GFP-tagged non-phosphorylatable Sgo1 mutant localized at chromosome arms, and this localization of Sgo1 appeared to be regulated by Aurora B-mediated phosphorylation. Furthermore, given that Sgo1 functions as a cohesion maintenance protein, we found that the ability of Sgo1 to bind to DNA was also regulated by Aurora B-mediated phosphorylation. These results suggest that the Aurora B-mediated phosphorylation of Sgo1 is an important molecular mechanism underlying the regulation of sister chromatid cohesion and separation.

It is known that Sgo1 recruits protein phosphatase to counteract the kinase activity of mitotic kinases, such as, Plk1. PP2A could be a critical mitotic phosphatase in the regulation of centromeric cohesion, and PP1 and Ssu72 [22] act as mediators of centromeric cohesion. In particular, Ssu72 becomes active during early mitosis and loses its activity in association with the Aurora B-dependent phosphorylation during mid-mitosis. This evidence strongly supports the notion that Sgo1 recruits protein phosphatases to chromosome arms to promote the cohesion of the duplicated arms of sister chromatids.

A recent study identified the phosphorylation of Sgo2 by Aurora B in its N-terminal coiled-coil region and in its mid region [10]. Based on the phosphorylation site prediction program used in the present study, there are consensus sites that could be phosphorylated by Aurora B in addition to Sgo1 Ser 38. However, further research is required to elucidate the role of Sgo1 phosphorylation in living organisms.

In summary, this study provides mechanistic insights as to how the phosphorylation of Sgo1 by Aurora B contributes to centromeric cohesin protection and chromosome alignment in human cells. Based on these results, we propose a model for the regulation of Sgo1 localization by Aurora B mediated phosphorylation (Fig. 4D). The model suggests that this regulation of Sgo1 by Aurora B is an important regulatory mechanism of the prophase pathway,

and we believe contributes to our understanding of the cohesion and separation of mitotic chromosomes.

Acknowledgments

We would like to thank the Samsung Biomedical Research Institute for supplying the equipment, technical assistance, and financial support. This study was supported by a National Research Foundation grant funded by the Korean government (MEST) (2011-0030833 and 2012-R1A1A2041656).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.10.103.

References

[1] D.E. Anderson, A. Losada, H.P. Erickson, T. Hirano, Condensin and cohesin display different arm conformations with characteristic hinge angles, J. Cell Biol. 156 (2002) 419–424.
[2] C.H. Haering, J. Lowe, A. Hochwagen, K. Nasmyth, Molecular architecture of SMC proteins and the yeast cohesin complex, Mol. Cell 9 (2002) 773–788.
[3] C.H. Haering, A.M. Farcas, P. Arumugam, J. Metson, K. Nasmyth, The cohesin ring concatenates sister DNA molecules, Nature 454 (2008) 297–301.
[4] S. Gruber, C.H. Haering, K. Nasmyth, Chromosomal cohesin forms a ring, Cell 112 (2003) 765–777.
[5] J.M. Peters, A. Tedeschi, J. Schmitz, The cohesin complex and its roles in chromosome biology, Genes Dev. 22 (2008) 3089–3114.
[6] S. Hauf, E. Roitinger, B. Koch, C.M. Dittrich, K. Mechtler, J.M. Peters, Dissociation of cohesin from chromosome arms and loss of arm cohesion during early mitosis depends on phosphorylation of SA2, PLoS Biol. 3 (2005) e69.
[7] T.S. Kitajima, T. Sakuno, K. Ishiguro, S. Iemura, T. Natsume, S.A. Kawashima, Y. Watanabe, Shugoshin collaborates with protein phosphatase 2A to protect cohesin, Nature 441 (2006) 46–52.
[8] B.E. McGuinness, T. Hirota, N.R. Kudo, J.M. Peters, K. Nasmyth, Shugoshin prevents dissociation of cohesin from centromeres during mitosis in vertebrate cells, PLoS Biol. 3 (2005) e86.
[9] H. Liu, L. Jia, H. Yu, Phospho-H2A and cohesin specify distinct tension-regulated Sgo1 pools at kinetochores and inner centromeres, Curr. Biol. 23 (2013) 1927–1933.
[10] Y. Tanno, T.S. Kitajima, T. Honda, Y. Ando, K. Ishiguro, Y. Watanabe, Phosphorylation of mammalian Sgo2 by Aurora B recruits PP2A and MCAK to centromeres, Genes Dev. 24 (2010) 2169–2179.
[11] C. Gutierrez-Caballero, L.R. Cebollero, A.M. Pendas, Shugoshins: from protectors of cohesion to versatile adaptors at the centromere, Trends Genet. 28 (2012) 351–360.
[12] S.A. Kawashima, Y. Yamagishi, T. Honda, K. Ishiguro, Y. Watanabe, Phosphorylation of H2A by Bub1 prevents chromosomal instability through localizing shugoshin, Science 327 (2010) 172–177.
[13] Y. Yamagishi, T. Sakuno, M. Shimura, Y. Watanabe, Heterochromatin links to centromeric protection by recruiting shugoshin, Nature 455 (2008) 251–255.
[14] J. Dai, B.A. Sullivan, J.M. Higgins, Regulation of mitotic chromosome cohesion by Haspin and Aurora B, Dev. Cell 11 (2006) 741–750.
[15] S. Kueng, B. Hegemann, B.H. Peters, J.J. Lipp, A. Schleiffer, K. Mechtler, J.M. Peters, Wapl controls the dynamic association of cohesin with chromatin, Cell 127 (2006) 955–967.
[16] T.D. Resnick, D.L. Satinover, F. MacIsaac, P.T. Stukenberg, W.C. Earnshaw, T.L. Orr-Weaver, M. Carmena, INCENP and Aurora B promote meiotic sister chromatid cohesion through localization of the Shugoshin MEI-S332 in Drosophila, Dev. Cell 11 (2006) 57–68.
[17] M. Nakajima, K. Kumada, K. Hatakeyama, T. Noda, J.M. Peters, T. Hirota, The complete removal of cohesin from chromosome arms depends on separase, J. Cell Sci. 120 (2007) 4188–4196.
[18] K. Shintomi, T. Hirano, Releasing cohesin from chromosome arms in early mitosis: opposing actions of Wapl-Pds5 and Sgo1, Genes Dev. 23 (2009) 2224–2236.
[19] X. Wang, Y. Yang, Q. Duan, N. Jiang, Y. Huang, Z. Darzynkiewicz, W. Dai, SSgo1, a major splice variant of Sgo1, functions in centriole cohesion where it is regulated by Plk1, Dev. Cell 14 (2008) 331–341.
[20] G. Fu, X. Ding, K. Yuan, F. Aikhionbare, J. Yao, X. Cai, K. Jiang, X. Yao, Phosphorylation of human Sgo1 by NEK2A is essential for chromosome congression in mitosis, Cell Res. 17 (2007) 608–618.
[21] J. Kang, J. Chaudhary, H. Dong, S. Kim, C.A. Brautigam, H. Yu, Mitotic centromeric targeting of HP1 and its binding to Sgo1 are dispensable for sister-chromatid cohesion in human cells, Mol. Biol. Cell 22 (2011) 1181–1190.
[22] H.S. Kim, S.H. Kim, H.Y. Park, J. Lee, J.H. Yoon, S. Choi, S.H. Ryu, H. Lee, H.S. Cho, C.W. Lee, Functional interplay between Aurora B kinase and Ssu72 phosphatase regulates sister chromatid cohesion, Nat. Commun. 4 (2013) 2631.