Phosphorylation of Microtubule-binding Protein Hec1 by Mitotic Kinase Aurora B Specifies Spindle Checkpoint Kinase Mps1 Signaling at the Kinetochore*

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Tongge Zhu†‡, Zhen Dou†‡, Bo Qin§, Changjiang Jin‡, Xinghui Wang§, Lelei Xu‡, Zhaoyang Wang‡, Lijuan Zhu‡, Fusheng Liu‡, Xinjiao Gao‡, Yuwen Ke‡, Zhiyong Wang‡, Felix Aikhionbare§, Chuanhai Fu†§, Xia Ding‡, and Xuebiao Yao†§

From the †Anhui Key Laboratory of Cellular Dynamics and Chemical Biology, University of Science and Technology of China, Hefei 230026, China, the ‡Molecular Imaging Center, Morehouse School of Medicine, Atlanta, Georgia 30310, and §Beijing University of Chinese Medicine, School of Graduate Studies, Beijing 100029, China

Background: Hec1 is a core component of outer kinetochore essential for chromosome segregation in mitosis.

Results: Hec1 interacts with mitotic checkpoint kinase Mps1, and phosphorylation of Hec1 by Aurora B recruits Mps1 to kinetochore.

Conclusion: Phosphorylation of Hec1 by Aurora B specifies Mps1 signaling at the kinetochore.

Significance: Aurora B-Hec1-Mps1 axis orchestrates chromosome dynamics and stability in mitosis.

The anaphase-promoting complex/cyclosome, activated by Cdc20 or Cdh1, acts as the key downstream target of the SAC. Multiple mitotic kinases, including Aurora B, Mps1, Bub1, and BubR1, are involved in SAC signaling to prevent premature entry into anaphase (1, 2). Biochemically, the key molecular target of SAC is Cdc20, an anaphase-promoting complex/cyclosome activator (3). Mad2, Mad3/BubR1, Bub3, and Cdc20 form the mitotic checkpoint complex, which inhibits the ubiquitin E3 ligase activity of anaphase-promoting complex/cyclosome unless the SAC is satisfied (4, 5). Although it is an essential component of SAC, accumulating evidences suggest Mad2 is not a stable component of mitotic checkpoint complex. Instead, active Mad2 catalyze the conformation change of auto-inhibited Cdc20 to a BubR1-binding capable form (6, 7).

Mps1 (mono-polar spindle 1) and also known as TTK is an evolutionary conserved SAC kinase and exhibits dynamic distribution in mitosis (8). In various model systems, cells with disrupted Mps1 function are unable to maintain full SAC activity (9–15). Mps1 activity is required for the kinetochore targeting of Mad1 and Mad2 and for the maximal localization of BubR1, suggesting that Mps1 is involved upstream in SAC signaling. Previous studies indicated the kinetochore localization of Mps1 depends on the key outer kinetochore protein Hec1 and Aurora B kinase (16–18). In addition, Nek2A kinase phosphorylates Hec1 and strengthens Hec1 interaction with kinetochore microtubule (19). However, the underlying molecular mechanism remains to be elucidated. Hec1 was originally identified as a retinoblastoma-binding protein and expressed highly in cancer cell lines (20). Later, it was demonstrated that together with NuF2, Spc24, and Spc25, Hec1 forms the conserved Ndc80 complex (21, 22). Both Hec1 and NuF2 molecules have a globular CH domain at their N terminus and a long coiled-coil C terminus. The globular head of Hec1/Nuf2 involves in binding microtubule and the C terminus of Hec1/Nuf2 involves in binding Spc24/Spc25 subcomplex (22–25). Ndc80 complex is an essential structural platform to link kinetochore with microtubule...
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...tubule attachment. More importantly, the affinity of Hec1 with microtubule is under the regulation of Aurora B kinase. Phosphorylation of Hec1 N-terminal tail by Aurora B strongly destabilizes the kinetochore-microtubule attachment, which is an important molecular pathway that Aurora B exerts its error-correction function (22, 26–28).

Characterization of the kinetochore recruitment mechanism of SAC proteins is essential for a better understanding of SAC signaling in cell division control. Here, we demonstrate that phosphorylation of the Hec1 N-terminal tail by Aurora B enhances Mps1 kinetochore localization. Our biochemical analysis indicated the direct binding between the Mps1 kinetochore localization domain and the Hec1 microtubule-binding domain that was composed of CH domain and N-terminal tail. Moreover, the phosphomimetic mutant of Hec1 bypasses the requirement of Aurora B for Mps1 kinetochore targeting and induces persistent SAC signaling. Together, our data reveal Hec1 is a signaling hub coupling kinetochore-microtubule attachment to SAC signaling through its phosphorylation switch dual affinities with microtubule and Mps1.

EXPERIMENTAL PROCEDURES

Cell Culture and Drug Treatments—HeLa cells were routinely maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% FBS and penicillin-streptomycin (100 international units/ml and 100 mg/ml, respectively, Invitrogen). Thymidine was used at 2 mM, nocodazole was used at 100 ng/ml International units/ml and 100 mg/ml, respectively, Invitrogen). Thymidine was used at 2 mM, nocodazole was used at 100 ng/ml, monastrol was used at 20 μM, reversine was used at 0.3 μM, ZM447439 was used at 2.5 μM, and MG132 was used at 20 μM.

Antibodies—Usages for monoclonal anti-hMps1-N1 and anti-Mad2 antibodies have been described previously (10, 29). Anti-α-tubulin (DM1A, Sigma), anti-Aurora B (A1M-1, BD Biosciences), anti-pSer7 CENP-A (Cell Signaling Technology), and ACA tubulin (DM1A, Sigma), anti-Aurora B (AIM-1, BD Biosciences), and ACA tubulin (DM1A, Sigma) were purchased from Invitrogen.

Protein Expression, Purification, and GST Pulldown Assay—GST-Spc24/25, GST-NDC80Bonsai, and GST-NDC80BonsaiΔN fusion proteins were purified as described previously (26). GST-tagged His-Mps11–303 fusion proteins were incubated with purified His-Mps11–303 fusion proteins in PBS containing 0.2% Triton X-100 for 2 h at 4 °C. After the incubation, the beads were washed three times with PBS containing 0.2% Triton X-100 and once with PBS and boiled in SDS-PAGE sample buffer. The bound proteins were then separated on 10% SDS-PAGE.

RESULTS

The Hec1 Microtubule-binding Domain Is Required for the Kinetochore Localization of Mps1—The kinetochore localization of Mps1 and its downstream SAC components, Mad1 and Mad2, requires Hec1 (16, 22, 26, 33). These data suggest that the Ndc80–Hec1 complex is an upstream factor for Mps1 kinetochore localization. To determine whether Hec1 or other subunits of the Ndc80 complex make a direct contribution to Mps1 kinetochore targeting, a GST pulldown assay was performed. The GST-tagged Ndc80 complex, Ndc80Bonsai, pulled down His-tagged Mps11–303, the Mps1 fragment sufficient for its kinetochore localization (Fig. 1A, lane 4) (24, 34). GST-tagged Ndc80BonsaiΔN, which lacks the Hec1 N-terminal tail, also pulled down Mps11–303, but with a weaker binding affinity. This suggests that both the CH domain and N-terminal tail of Hec1 contribute to Mps1 binding. In contrast, the Spc24/Spc25 subcomplex failed to absorb Mps11–303 (Fig. 1A, lane 3). These data demonstrate that the Hec1-Nu2 complex binds Mps11–303 directly. We next determined whether the CH domain of Hec1 or Nu2 physically interacts with Mps1. To this end, GST–Hec11−196 and GST–Nu21−169 fusion proteins on glutathione-Sepharose beads were used as affinity matrices to absorb His-Mps11–303. Indeed, both Hec11−196 and Nu21−169 pulled down His-Mps11–303 (Fig. 1B, lanes 5 and 6), confirming the direct interaction between Mps1 and the CH domain of the Hec1-Nu2 subcomplex.
To examine the molecular determinants of Hec1 for localizing Mps1 to the kinetochore, we first employed siRNA-mediated knockdown strategy to suppress endogenous Hec1 and Nuf2 protein levels. Consistent with our previously report (19, 35), the siRNA-mediated knockdown produced a typical 85–90% reduction of endogenous Hec1 and Nuf2 based on Western blotting analyses. As shown in Fig. 1C, this siRNA-mediated depletion of Hec1 or Nuf2 abolished the kinetochore localization of Mps1, whereas labeling of centromere marker ACA remained unchanged, which is consistent with several previous observations (10, 18, 33).

We next knocked down endogenous Hec1/Nuf2 and exogenously expressed various siRNA-resistant Hec1/Nuf2 truncations with a GFP tagged at their C termini and examined the Mps1 localization in HeLa cells depleted endogenous Hec1/Nuf2. As shown in Fig. 1D, exogenous expressing WT Hec1, but not the Hec1 without the N terminus (Hec1197–642, bottom panel), rescued the kinetochore localization of Mps1 (Fig. 1D, middle panel). To the contrary, exogenous Nuf2 without the N terminus (Nuf2170–464) rescued the kinetochore localization of Mps1 (Fig. 1D, upper panel).

We next determined whether the Hec11–196 fragment was sufficient for recruiting Mps1. For this purpose, the endogenous Mps1 localization was examined after knocking down Hec1 with concurrent expressing a fusion protein containing Mis12 and Hec11–196 tagged with a GFP at its C terminus. As shown in Fig. 1E, the Hec11–196-Mis12-GFP fusion protein localized to kinetochores correctly and recruits a correct localization of Mps1 to the kinetochore (bottom panel). As a control, there was no detectable Mps1 signal in the kinetochore.
expressing exogenous Mis12-GFP in transfected cells (middle panel, enlarged inset). Thus, we conclude that the N-terminal tail and CH domain of Hec1 are required and sufficient for targeting Mps1 to kinetochores.

**Aurora B Regulates the Kinetochore Localization of Mps1 via Phosphorylation of Hec1**—The kinetochore localization of Mps1 has been reported to be under the control of Aurora B (17, 36). However, how Aurora B regulates Mps1 localization is unknown. An attractive hypothesis is that Aurora B enhances Mps1 kinetochore recruitment through phosphorylating the N-terminal tail of Hec1, a process known to destabilize the kinetochore-microtubule attachment (11, 22, 26, 27, 37). To test this hypothesis, non-phosphorylatable and phosphomimicking mutants of Hec1 were generated. Specifically, nine Aurora B phosphorylation sites were mutated to Ala or Asp/Glu, respectively. Western blotting analyses show that both non-phosphorylatable and phosphomimicking mutants of Hec1 expressed at similar level in transiently transfected cells. As shown in Fig. 2A, the Mps1 level at the kinetochore decreased sharply in Hec19A-expressing cells, and the Mps1 level increased in Hec19D-expressing cells as compared with that of Hec1WT-expressing cells. Statistical analyses shown that Mps1 level on the kinetochore expressing phosphomimicking mutants of Hec1 is significantly higher than those kinetochores expressing wild type and non-phosphorylatable Hec1 (Fig. 2B, p < 0.001 and p < 0.005, respectively).

To test whether Mps1 level on the kinetochore expressing phosphomimicking mutant of Hec1 is insensitive to the Aurora B activity, aliquots of HeLa cells expressing wild type and phosphomimicking mutants of Hec1 were treated with Aurora B inhibitor ZM447439 along with MG132 to minimize potential protein degradation via anaphase-promoting complex/cyclosome. As shown in Fig. 2C and D, inhibition of Aurora B results in a significant reduction of Mps1 level on the kinetochore of wild type-expressing kinetochore (Fig. 2C, upper panel). However, the level of Mps1 on the kinetochore expressing phosphomimicking mutants of Hec1 was largely unaffected in cells expressing Hec19D (Fig. 2C, lower panel). Thus, we conclude that the phosphomimetic Hec1 mutant bypasses the requirement of Aurora B activity for localization of Mps1 to the kinetochore.

We next determined whether phosphorylation regulates the direct binding between Hec1 and Mps1. To this end, GST-tagged Hec11–196(WT), Hec11–196(9A), and Hec11–196(9D) were used as affinity matrices to pull down Mps11–303 more efficiently, and Hec11–196(9A) pull down Mps11–303 less efficiently (Fig. 2E, lane 8). To test whether Aurora B-mediated phosphorylation regulates Hec1-Mps1 interaction, we performed an immunoprecipitation assay using 293T cells transiently transfected to express FLAG-Mps1 and GFP-Hec1 (wild type and mutants). As shown in Fig. 2F, FLAG-Mps1 WT effectively immunoprecipitated Hec19D-GFP protein (lane 9), but it pulled down a much lower amount of Hec19A-GFP protein (lane 8). The Mps1-Hec1 complex isolation is independent of Mps1 kinase activity as immunoprecipitation using kinase-dead Mps1 exhibited same profile as FLAG-Mps1 KD failed to bring down Hec11–196(9A) (Fig. 2G, lane 7). As a negative control, FLAG-Mps1 did not to pull down Hec1197–642 (Fig. 2F, lane 10), demonstrating the specificity of Mps1-Hec1 interaction.

Several lines of evidence demonstrate that Hec1 N-terminal tail phosphorylation is a key mechanism to regulate kinetochore-microtubule attachments to allow for error correction (26, 27, 37). To rule out the possibility that the enhanced Mps1 signal in Hec19D-expressing cells is due to robust SAC signaling induced by unstable microtubule attachments, we examined the Mps1 kinetochore signal in HeLa cells expressing wild type and mutant Hec1 proteins. In those experiments, transfected cells were treated with nocodazole or taxol to elicit robust SAC signaling. As shown in Fig. 2H, an elevated level of Mps1 kinetochore was evident in Hec19D-expressing cells exposing to nocodazole while a greatly reduced level of Mps1 was noticed in kinetochore expressing Hec19A of nocodazole-treated cells (Fig. 2I, p < 0.001), demonstrating that nocodazole-elicited spindle checkpoint activation is not sufficient for localizing Mps1 to the kinetochore. Similar profile of Mps1 localization to the kinetochore was also noted in HeLa cells exposed to taxol treatment as Hec19A failed to recruit Mps1 in the persistently active SAC (Fig. 2, I and K, p < 0.001). These data indicate that the elevated Mps1 kinetochore signal is a direct effect of phospho- phosphorylation of the Hec1 N-terminal tail, but not a side effect or direct outcome from SAC activation.

**Aurora B Activity Is Not Altered by Persistent Expression of Phosphomimicking Hec1**—Given the complexity of multiple kinase cascades cross-talk in SAC, one may argue that elevated Mps1 at kinetochores is due to enhanced Aurora B activity in the Hec19D-expressing cells. To rule out this possibility, the localization and activity of Aurora B was examined. As shown in Fig. 3A and quantified in Fig. 3B, the intensity of centromere Aurora B was unaffected in Hec19A- and Hec19D-expressing cells, relative to the signal in cells expressing Hec1WT. In addition, we also examined and quantified the level of phospho-Ser-7-CENP-A, a cognate substrate of Aurora B, as readout of Aurora B activity on the kinetochore. As shown in Fig. 3C and quantified in Fig. 3D, the intensity of phospho-Ser-7-CENP-A at the centromere was comparable in Hec19A- and Hec19D-expressing cells, relative to the signal in cells expressing Hec1WT. Thus, we conclude that elevated level of Mps1 at the kinetochore of Hec19D-expressing cells is not directly related to Aurora B kinase activity or protein level at the kinetochore.

During the preparation of this manuscript, Nijenhuis and co-workers (38) reported that the Hec1 microtubule binding domain is important for Mps1 localization to the kinetochore without detection of Aurora B-elicited phospho-regulation of Hec1. We noticed that Nijenhuis and colleagues (38) had expressed the N-terminal GFP fusion Hec1 protein in their experiment. Because the Hec1 N-terminal tail is critical for Mps1 recruitment, it is likely that the N-terminal GFP tag may have interfered the Mps1-Hec1N-tail interaction due to steric hindrance. To test this possibility, we sought to examine Mps1 localization in cells expressing various Hec1 constructs with a N-terminal GFP tag and compared with the Mps1 kinetochore localization in cells expressing Hec1 with a C-terminal GFP tag. Our preliminary characterization showed that the transient transfection produced exogenously expressed Hec1 proteins at similar level regardless the location of GFP tag. To test whether the local-
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We then sought to examine the influence of GFP tag to the Hec1 in Mps1 localization to the kinetochore. As shown in Fig. 3E, the level of Mps1 at the kinetochore Hec19A-GFP-expressing cells is much lower than that of Hec1WT-GFP-expressing cells, even in the presence of reversine. Consistent with the
Aurora B-mediated phosphorylation of Hec1 in Mps1 localization to the kinetochore, the level of Mps1 at the kinetochore Hec19A-GFP-expressing cells is significantly higher than that of Hec1WT-GFP-expressing cells (Fig. 3G, p < 0.05). Interestingly, the level of Mps1 in GFP-Hec19A-expressing cells was unaltered as compared with that of GFP-Hec1WT-expressing cells, despite the fact that the level of Mps1 localization at the kinetochore is significantly increased in GFP-Hec19D-expressing cells (Fig. 3G and H). Therefore, the epitope-tagging strategy used by Nijenhuis and colleagues (38) may account for their conclusion regarding the role of Hec1 phosphorylation in Mps1 localization.
Aurora B-elicited Hec1 Phosphorylation Links Kinetochore-microtubule Attachment to SAC

Several lines of early investigation have suggested that Aurora B phosphorylation of the Hec1 N-terminal tail destabilizes aberrant kinetochore-microtubule attachments (26, 27, 37). As demonstrated above, phosphorylation of the Hec1 N-terminal tail by Aurora B enhances the kinetochore recruitment of Mps1. Given the fact that accurate localization of Mps1 is essential for SAC signaling, we sought to test whether Hec1 phosphorylation by Aurora B serves as kinetochore-microtubule attachment/tension sensor for the SAC signaling. To this end, the kinetochore localization of SAC activity reporter Mad2 was assessed in cells expressing Hec1WT, Hec19A, and Hec19D. HeLa cells were co-transfected with Hec1 siRNA plus various siRNA-resistant Hec1 constructs with a C-terminal GFP tag. At 36 h after transfection, cell was treated with dimethyl sulfoxide (A) alone, ZM447439 plus MG132 (C) or reversine (Rev.; E) plus MG132 for 1 h, respectively. Afterward, cells were fixed and co-stained for Mad2 (red), ACA (shown as a black-and-white image), and DNA (blue). The boxed areas are shown magnified in the right panels. Scale bar represents 10 μm. B, bar graph showing quantification of the Mad2 kinetochore signal in cells treated as described in A. Bars indicate mean ± S.E. from analyses of >100 kinetochore pairs from five cells. Student’s t test was used to calculate p values for comparison of Hec1WT and Hec1 mutants. D and F, quantitative analyses of the Mad2 kinetochore signal in cells treated as in C and E. Bars indicate mean ± S.E. from analyses of >100 kinetochore pairs from five cells. Student’s t test was used to calculate p values for comparison of drug-treated groups and untreated Hec1WT group. G, quantitative analyses of mitotic index of different groups of cells treated as indicated. HeLa cells were co-transfected with Hec1 siRNA plus various siRNA-resistant Hec1 constructs with a C-terminal GFP tag. At 24 h after transfection, cells were treated with thymidine for 12 h. 6 h after thymidine release, cells were treated with nocodazole (Noc.) alone, nocodazole plus ZM447439 (Noc. + ZM), or nocodazole plus reversine (Noc. + Rev.) for 6 h, respectively. Cells then were fixed, and mitotic index was calculated (at least 200 cells were counted for each group from two separate experiments).
Mad2 localization on Hec1\textsuperscript{9D}-expressing cells is sensitive to Aurora B inhibition. As shown in Fig. 4C, the Mad2 kinetochore signal was diminished in Hec1\textsuperscript{WT} expressing cells upon addition of selective Aurora B inhibitor ZM447439. In contrast, the Mad2 signal was unaffected in Hec1\textsuperscript{9D}-expressing cells (Fig. 4C, bottom panel), demonstrating that the Hec1\textsuperscript{9D}-promoted recruitment of Mad2 is insensitive to Aurora B activity. However, the Mad2 signal disappeared in cells expressing Hec1\textsuperscript{9D} and expressing Hec1\textsuperscript{9D} in response to Mps1 inhibitor reversine, suggesting that persistent phosphorylation of Hec1 by Aurora B prevents SAC silencing (Fig. 4, E and F).

To further characterize SAC, we next examined the checkpoint strength using the mitotic index as readout. Consistent with the Mad2 intensity measured above, Hec1\textsuperscript{WT} and Hec1\textsuperscript{9D}-expressing cells exhibit a comparable mitotic index around 30% in response to SAC activators taxol and nocodazole (Fig. 4G). However, Hec1\textsuperscript{9A}-expressing cells exhibit a much reduced response to taxol and nocodazole, suggesting that kinetochore delocalization of Mps1 in Hec1\textsuperscript{9A}-expressing cell may have compromised SAC function (Fig. 4G). Consistent with this notion, the mitotic index of Hec1\textsuperscript{WT} and Hec1\textsuperscript{9A}-expressing cells decreased greatly in response to Aurora B inhibition (Fig. 4G). However, Hec1\textsuperscript{9D}-expressing cell maintains a high mitotic index even in the presence of ZM447439, again confirming the fact that persistent phosphorylation of Hec1 activates SAC beyond Aurora B activity. As expected, all three groups failed to exhibit functional SAC in response to nocodazole in the presence of reversine (Fig. 4G). Together, these data indicate that Aurora B phosphorylation of the Hec1 N-terminal tail is sufficient to orchestrate an accurate SAC signaling due to its ability to recruit SAC components to the kinetochore. Although the phosphomimetic Hec1 mutant bypasses the requirement of Aurora B activity for Mad2 kinetochore localization, Mps1 kinase activity is essential for faithful SAC signaling in this situation.

Persistent Phosphorylation of the Hec1 Results in a Chronic Activation of SAC—To examine the potential impact of persistent phosphorylation of Hec1 in mitotic progression, we carried out real-time imaging of chromosome segregation in HeLa cells expressing mCherry-H2B and GFP-tagged Hec1 mutants. As shown in Fig. 6A, exogenous expression of Hec1\textsuperscript{WT} (at a moderate level) did not perturb normal mitotic progression. However, exogenous expression of Hec1\textsuperscript{9A} caused a brief mitotic delay and chromosome segregation defects (Fig. 6B). On the contrary, Hec1\textsuperscript{9D}-expressing cells achieved a pseudo-metaphase alignment in a normal fashion but could not enter anaphase, even after 2 h (Fig. 6C). However, Hec1\textsuperscript{9D}-expressing cells entered anaphase promptly when reversine was added into
culture medium (Fig. 6D), consistent with a role of persistent expression of Hec1N9D in SAC activation. Quantitative analyses show that the average time from nuclear envelope breakdown to anaphase was 50 min in cells expressing Hec1WT. However, the average time from nuclear envelope breakdown to anaphase was extended to 100 min for Hec19A-expressing cells (Fig. 6E). Importantly, >70% Hec19A-expressing cells entered anaphase with lagging or misaligned chromosome, suggesting a compromised SAC activity in Hec19A-expressing cells (Fig. 6F). Together, these results indicate that mitotic arrest of Hec19D-expressing cells was due to persistent SAC activation.

**DISCUSSION**

The Ndc80 complex is composed of Hec1, Nuf2, Spc24, and Spc25. This complex is an essential kinetochore core component highly conserved across species, with a crucial role in...
kinetochore assembly and proper chromosome segregation during mitosis (20–22). In addition, Ndc80 complex governs the lateral to end-on conversion of chromosome-microtubule attachment via its interaction with mitotic kinesin CENP-E (35, 41, 43). In this study, we have characterized that the phosphorylation of Hec1 by Aurora B specifies Mps1 signaling at the kinetochore. Our studies demonstrate that Aurora B phosphorylation of Hec1 serve as a rheostat for accurate mitotic progression by governing accurate kinetochore-microtubule attachments and SAC signaling.

Previous studies have established that the localization of the Ndc80 complex is exterior to the inner kinetochore proteins such as the Mis12 complex (44, 45), and the Ndc80 complex localizes to the interior of CENP-E (35, 46). Thus, the Ndc80 complex is postulated to link microtubule-binding proteins and chromatin-bound centromere core proteins via forming a multi-subunit complex containing KNL1 and Mis12 subcomplexes. It has been reported that the main function of the Ndc80 complex is to stabilize the microtubule-kinetochore attachment as cells lacking Nu2 or Hec1 often carry unstable spindle microtubules. Our recent finding of CENP-E-Nu2 interaction provides a novel link between spindle microtubules and the kinetochore core complex via mitotic kinesin CENP-E (35). Our recent study showed that phosphorylation of Mis13 by Aurora B governs accurate kinetochore-microtubule attachment by recruiting outer kinetochore proteins and correcting aberrant kinetochore-microtubule attachment errors (45). In addition to error correction, Aurora B is implicated in SAC signaling (47). An important advance in understanding SAC signaling is the finding that Aurora B kinase corrects kinetochore-microtubule attachments by spatial separation of itself with its kinetochore substrates (48, 49). However, the key substrate(s) of Aurora B for initializing SAC signaling remains unknown. In the present study, we demonstrated that Hec1 is a key target of Aurora B to activate SAC signaling. Phosphoinertic Hec1 recruits Mps1 and Mad2 and bypasses the requirement for Aurora B activity. We favor a model showing that, after cells enter mitosis, phosphorylated Hec1 recruits Mps1 to enable SAC signaling (Fig. 6G). Before the kinetochores achieve correct microtubule attachments, Aurora B phosphorylates the Hec1 N-terminal tail. This phosphorylation destabilizes incorrect microtubule attachments and recruits Mps1 to maintain robust SAC signaling. Thus, the phosphorylation of Hec1 by Aurora B integrates correct microtubule attachment with tension-dependent SAC signaling. Upon establishment of bi-orientation, Aurora B activity does not reach the Hec1 N-terminal tail due to spatial separation. As a result of reduced Aurora B substrates phosphorylation, more protein phosphatase 1γ was recruited on kinetochores (50). Therefore, the Hec1 was dephosphorylated quickly and Mps1/Mad1/Mad2 left kinetochores to allow the satisfaction of SAC. Although we favor that the hyperactivation of SAC in Hec19D-expressing cells is due to persistent recruitment of Mps1, we note it is also possible that the mitotic delay phenotype of Hec19D-expressing cells is caused by the reduced microtubule-binding activity of Hec19D.

Nijenhuis and colleagues (38) have suggested a model in which the microtubule binding activity and Mps1 binding activity of Hec1 are mutually exclusive. In this model, microtubule binding prevents the localization of Mps1. However, this model does not explain how Mps1 kinetochore localization and SAC activity are maintained in a syntelic kinetochore-microtubule attachment situation. Our finding of Hec1 phosphorylation-dependent recruitment of Mps1 can fit the “Aurora B senses chromosome bi-orientation” model and explain the checkpoint activation in syntelic attachment situation (48). Nevertheless, it would be of great interest to visualize how Aurora B-elicited phosphorylation releases the lateral attachment to microtubule to promote the temporal association with Mps1 in vitro and in vivo using super-resolution imaging analyses.

Taken together, we have demonstrated that the Hec1 microtubule-binding domain physically interacts with Mps1 and directs the kinetochore localization of Mps1. The Aurora B-elicited phosphorylation of Hec1 orchestrates SAC signaling by recruiting Mps1 to the kinetochore. Our findings illustrate a unique molecular mechanism underlying Aurora B-dependent kinetochore localization of Mps1 and define a novel role for Aurora B-Hec1-Mps1 signaling axis in governing accurate chromosome segregation in mitosis.

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