Interaction and Feedback Regulation between STK15/BTAK/Aurora-A Kinase and Protein Phosphatase 1 through Mitotic Cell Division Cycle*

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STK15 is an Aurora/Ipl-1 related serine/threonine kinase that is associated with centrosomes and induces aneuploidy when overexpressed in mammalian cells. It is well known that phosphorylation and dephosphorylation of kinases are important for regulation of their activity. But mechanisms by which STK15 activity is regulated have not been elucidated. We report that STK15 contains two functional binding sites for protein phosphatase type 1 (PP1), and the binding of these proteins is cell cycle-regulated peaking at mitosis. Activated STK15 at mitosis phosphorylates PP1 and inhibits PP1 activity in vitro. In vivo, PP1 activity co-immunoprecipitated with STK15 is also reduced. These data indicate that STK15 inhibits PP1 activity during mitosis. Also, PP1 is shown to dephosphorylate active STK15 and abolish its activity in vitro. Furthermore, we show that non-binding mutants of STK15 for PP1 are superphosphorylated, but their kinase activities are markedly reduced. Cells transfected with these non-binding mutants manifest aberrant chromosome alignment during mitosis. Our results suggest that a feedback regulation through phosphorylation/dephosphorylation events between STK15 kinase and PP1 phosphatase operates through the cell cycle. Deregulation of this balance may contribute to anomalous segregation of chromosomes during mitotic progression of cancer cells.

Mitosis is morphologically the most dynamic phase in the cell cycle, and a large number of events including chromosome and microtubule dynamics are precisely coordinated at temporal and spatial levels. Reversible phosphorylation of proteins by protein kinases and protein phosphatases regulates these events, and alterations in the phosphorylated state of proteins have effects on mitotic progression (1, 2). Whereas protein kinases and protein phosphatases regulates these events, and alterations in the phosphorylated state of proteins have effects on mitotic progression (1, 2). Whereas protein kinases and protein phosphatases regulate these events, and alterations in the phosphorylated state of proteins have effects on mitotic progression (1, 2). Whereas protein kinases and protein phosphatases regulate these events, and alterations in the phosphorylated state of proteins have effects on mitotic progression (1, 2). Whereas protein kinases and protein phosphatases regulate these events, and alterations in the phosphorylated state of proteins have effects on mitotic progression (1, 2). Whereas protein kinases and protein phosphatases regulate these events, and alterations in the phosphorylated state of proteins have effects on mitotic progression (1, 2). Whereas protein kinases and protein phosphatases regulate these events, and alterations in the phosphorylated state of proteins have effects on mitotic progression (1, 2). Whereas protein kinases and protein phosphatases regulate these events, and alterations in the phosphorylated state of proteins have effects on mitotic progression (1, 2).

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‡‡ The abbreviations used are: PP1, type 1 protein phosphatase; PP2, type 2 protein phosphatase; MBP, myelin basic protein; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; WT, wild type; NB, non-binding; GFP, green fluorescent protein.
In this paper we present evidence that STK15 interacts with PP1 via functional binding domains within STK15 in vivo and in vitro, and this binding is higher at mitosis compared with interphase. These data indicate that interaction between these proteins is cell cycle-regulated. In addition, mitotic active STK15 phosphorylates PP1, and this phosphorylation reduces PP1 activity. On the other hand, PP1 dephosphorylates hyperphosphorylated active STK15 that results in loss of STK15 activity. Furthermore, we indicate that this interaction is necessary for activation of STK15 and that transient transfection of PP1 non-binding mutants of STK15 induces aberrant chromosome alignments at mitotic metaphase at higher rate compared with those of wild type STK15 and kinase-inactive STK15 mutant. Our results suggest that a feedback regulation through phosphorylation/dephosphorylation cycles exists between STK15 kinase and PP1 phosphatase and that overexpression of STK15 leads to deregulation of this phosphorylation/dephosphorylation balance causing aberration of this normal feedback regulation in cancer cells. The findings further suggest that aberrant STK15 expression affects an unknown STK15-PP1-mediated regulatory pathway in mitosis to induce mitotic anomalies in cancer cells.

EXPERIMENTAL PROCEDURES

Cell Culture, Synchronization, and Fluorescence-activated Cell Sorter Analysis—HeLa cells were grown in Dulbecco’s modified Eagle’s medium with 10% heat-inactivated fetal bovine serum. For synchronization at the G1/S boundary, HeLa cells were synchronized by a double thymidine block and release (31). Exponentially growing cells were blocked for 16 h with 2.5 mM thymidine, released from the block by 10 μg/ml leupeptin, 10 μM pepstatin A, 100 mM NaF, 20 mM NaCl, 20 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and released from the block by 15,000 g for 30 min on ice and followed by centrifugation at 15,000 g. The supernatant was used for immunoblotting and immunoprecipitation.

Immunoprecipitation, Immunoblotting, and in Vitro Kinase Assay—For detection of the binding of STK15 to PP1, 5 μg each of GST-PP1α, GST-PP1γ, and GST-PP1β bound to glutathione-Sepharose beads was mixed with 1 μg of cell lysate for 1 h at 4 °C. The beads were washed 5 times with lysis buffer and then subjected to SDS-PAGE. The protein was transferred to nitrocellulose membrane and probed with anti-STK15 antibody. For detecting the binding of PP1 to GST-STK15 WT, GST-STK15 Lys → Arg, and NB, the same condition was used except that 2 μg of cell lysate was used and probed with anti-PP1 antibody.

PP1 and PP2 Treatment—Immunoprecipitated STK15 from mitotic cell lysate was washed with IP washing buffer and then washed 4 times with phosphatase buffer (50 mM Tris-HCl, pH 7.5, 1 mM MgCl2, 0.1 mM EDTA, 5 mM diethiolethritol, 0.01% Brij 35 for PP1, and 50 mM Tris-HCl, pH 8.5, 20 mM MgCl2, 1 mM diethiolethritol for PP2A). The immunocomplex was incubated for 30 min at 30 °C with 0.5 units of PP1 (New England Biolabs) or 0.5 units of PP2A (Promega) and then washed 5 times with kinase buffer, and subsequently an in vitro kinase assay to dephosphorylated β-casein was performed as described above. The samples were subjected to SDS-PAGE, transferred to nitrocellulose membrane, and probed with anti-STK15 antibody, followed by autoradiography.

Protein Phosphatase Assay—Phosphatase activity of PP1 associated with anti-STK15 immunocomplex and in vitro phosphorylated GST-PP1α, -γ1, and -δ by STK15 was measured by using 32P-labeled myelin basic protein (32P-MBP) as the substrate according to the manufacturer’s protocol (New England Biolabs). After beads were washed 5 times with kinase buffer, beads were incubated for 10–30 min at 30 °C in phosphatase buffer containing 50 mM 32P-MBP. The reactions were stopped by 20% trichloroacetic acid precipitation. After removing the precipitated protein and beads by centrifugation, the relative phosphatase activity was determined by the amount of soluble 32P released.

Immunofluorescence Microscopy—HeLa cells growing on poly-l-lysine-coated glass coverslips were transiently transfected with pEGFP empty vector and pEGFP-STK15 constructs for 16 h. Immunostaining was performed as described (3). Anti-phosphohistone H3 antibody and 4,6-diamidino-2-phenylindole were used for staining phosphorylated histone H3 and DNA, respectively. Anti-phosphohistone H3 antibody was kindly provided by Dr. C. David Allis.

RESULTS

STK15 Interacts with PP1 in Vivo and in Vitro—To study the regulation of phosphorylation of STK15, we searched STK15 amino acid sequence and found two similar motifs to consensus motif for binding to PP1 (Fig. 1A). To investigate whether STK15 interacts with PP1 and if the two motifs identified are responsible for interaction with PP1, we constructed FLAG-tagged mutants in which phenylalanine 165 and phenylalanine 346 in the putative consensus motifs were substituted with alanine (NB). In nocodazole-treated cells arrested at mitosis, the interaction to PP1 of both F165A (NB1) and F346A (NB2) mutants was significantly decreased compared with wild type (WT) and kinase-inactive mutant (Lys → Arg) (Fig. 1B). In contrast, the differences in the degree of interactions between WT, Lys → Arg, and the non-binding mutants with PP1 were
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**Fig. 1.** STK15 has two functional PP1 binding domains. A, alignment of consensus PP1-binding motif and corresponding STK15 sequence. To construct non-binding mutants (NB1 and NB2), phenylalanine 165 and phenylalanine 346 indicated by an asterisk were mutated to alanine, respectively. B, transiently transfected HeLa cells with empty vector (Emp), NB1, NB2, wild type (WT), and kinase-inactive mutant (K/R) were cultured with or without nocodazole for 16 h. After cell extraction, expressed protein was immunoprecipitated (IP) with anti-FLAG antibody and then immunoblotted (IB) with anti-PP1 antibody (top panel) and anti-STK15 antibody (bottom panel). C, 5 μg of GST (1st lane), GST-NB1 (2nd lane), GST-NB2 (3rd lane), and GST-WT (4th lane) bound to Sepharose beads was incubated with 2 mg of mitotic extracts and then Sepharose beads were washed with lysis buffer. PP1 was probed with anti-PP1 antibody.

Minimal in the exponentially growing cells, although WT indicated higher interaction to PP1 than mutants (Fig. 1B). To confirm further the role of the consensus binding motifs in the interaction, pull-down assays were performed using bacterially expressed GST fusion STK15 proteins. GST and the GST fusion proteins were incubated with mitotic extracts, and the PP1 bound to fusion proteins was immunoblotted with anti-PP1 antibody. Fig. 1C clearly shows that GST-NB1 and GST-NB2 failed to interact with PP1. These results show that STK15 interacts with PP1 through the functional binding domains, and STK15 kinase activity is not necessary for this interaction.

To assess precisely if this interaction is cell cycle-dependent, HeLa cells synchronized by a double thymidine block and release protocol were analyzed. Whereas protein expression, phosphorylation, and activity of STK15 peak at mitosis and decline during exit from mitosis through G1 (Fig. 2A), protein expression of PP1 in the cell cycle progression of HeLa cells was constant (Fig. 2B). Similar expression pattern of PP1 protein has been earlier reported in other cell types (32). Endogenous STK15 was immunoprecipitated with anti-STK15 antibody, and the immunocomplex was immunoblotted with anti-PP1 and anti-STK15 antibodies. Consistent with the data shown in Fig. 1B, PP1 binding to STK15 increased with cell cycle progression to mitosis (Fig. 2A). Interestingly, this interaction seems to be correlated with the appearance of hyperphosphorylated STK15 that has kinase activity (Fig. 2A). To investigate the role of STK15 phosphorylation in PP1 interaction, mitotic extracts were prepared in the presence or absence of phosphatase inhibitor (okadaic acid, NaF, and EDTA), and the amount of immunoprecipitated PP1 was compared. As shown in Fig. 2C, when STK15 was dephosphorylated in the absence of phosphatase inhibitor (indicated by arrow), the interaction was decreased compared with the phosphorylated form of STK15 detected in the presence of phosphatase inhibitor (indicated by arrowhead). This indicates that phosphorylated active STK15 interacts with PP1 more effectively than inactive STK15. Paradoxically, however, we detected similar quantitative binding between STK15 and PP1 at 8, 9, and 12 h post-thymidine release, despite the fact that STK15 was predominantly seen in active hyperphosphorylated form at 8 and 9 h post-release unlike the hypophosphorylated form detected at 12 h post-release (Fig. 2A). At 12 h post-release only 4% of cells are in G2/M phase of mitosis compared with 74% and 37% of cells in G2/M phase at 8 and 9 h post-release. The reason why this interaction is still high in vivo at 12 h post-thymidine release when almost 88% of the cells have progressed to G1 phase after mitosis remains to be elucidated.

There are four isoforms of PP1 (PP1α, PP1γ1, PP1γ2, and PP1β) in mammals. To examine which isoform interacts to STK15, pull-down assay was performed using bacterially expressed GST fusion PP1α, PP1γ1, and PP1β. GST and each GST fusion protein were incubated with mitotic extracts and then STK15 bound to fusion proteins was immunoblotted with anti-STK15 antibody. Fig. 3 shows STK15 is able to interact to all PP1 isoforms. Similar result was also obtained using Myc-tagged constructs of all the PP1 isoform-transfected cells (data not shown). These results taken together indicate that STK15 interacts with PP1 both in vivo and in vitro, and this interaction is regulated in cell cycle-dependent manner.

**Dephosphorylation and Inactivation of STK15 by PP1 Not PP2A**—To investigate the biochemical significance of interaction between STK15 and PP1, we first examined whether PP1 dephosphorylates STK15 and, if so, the effect on kinase activity. Immunoprecipitated STK15 from mitotic extracts was incubated with purified PP1 and PP2A, respectively, and in vitro kinase assay was subsequently performed. Interphase extract and mitotic extract were used as positive control to confirm phosphorylation status of STK15. Whereas incubation with PP2A did not affect phosphorylation status and kinase activity of STK15 similar to control reaction, PP1 caused significant dephosphorylation and inactivation of STK15 (Fig. 4).

**Phosphorylation and Inhibition of PP1 by STK15**—We did in
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Interaction with PP1 Is Essential for STK15 Kinase Activation—Based on the results from Fig. 4, we speculated that both NB mutants might be more phosphorylated compared with WT at mitosis and display higher kinase activity. To address the effect of reduced interaction for PP1 on the kinase activity and phosphorylation status of STK15, we performed immunoblotting and in vitro kinase assay of immunoprecipitated NB mutants from mitotic extract. As expected, both NB mutants were more phosphorylated compared with WT and Lys → Arg (Fig. 6, lanes 2 and 3). Surprisingly, both hyperphosphorylated (indicated by arrow) and super-phosphorylated (the slowest migrating band indicated by arrowhead) NB mutants together had much less kinase activity than that detected for the WT form, which displayed strong kinase activity, as expected (Fig. 6). This result provides evidence that inhibitory phosphorylation sites exist in STK15 and that dephosphorylation of these sites by PP1 is required for activation of STK15.

Loss of Interaction to PP1 Induces Aberrant Chromosome Alignment during Mitosis—Next, to study whether overexpression of NB mutants affects mitotic progression; indirect immunofluorescence microscopy of HeLa cells transiently transfected with pEGFP-NB mutants was performed. Interestingly, NB mutant-transfected cells frequently demonstrated misalignment of chromosomes at metaphase plate compared with control empty vector and WT-transfected cells (Fig. 7A, panels B, F, J, and N). GFP-NB mutants showed similar centrosome localization as in WT-transfected cells (Fig. 7A, panels E, I, and M) with no apparent influence on phosphorylation of histone H3 (Fig. 7A, panel C, G, K, and O). The proportions of cells showing abnormally aligned chromosomes in the NB mutant-transfected cells were, however, significantly increased compared with those of WT and Lys → Arg-transfected cells (Fig. 7B). These results indicate that interaction between STK15 and PP1 is involved in regulation of chromosome alignment/segregation at metaphase.

DISCUSSION

STK15 and its related kinases have been implicated in regulating equal segregation of chromosomes during mitotic cell division cycle. The mechanisms underlying activation of STK15 through phosphorylation and regulation of centrosome as well as mitotic microtubule functions by activated STK15, however, remain to be elucidated. In this paper, we present the first evidence in human cells that (i) STK15 interacts with PP1 via consensus binding domains, and this interaction is necessary for STK15 activation; (ii) activated STK15 at mitosis phosphorylates PP1, which leads to reduction in PP1 phosphatase activity; (iii) dephosphorylation of STK15 by PP1 causes inhibition of its kinase activity; and (iv) interaction between STK15 and PP1 is involved in regulating chromosome alignment/segregation at metaphase.

We demonstrated that non-binding mutant STK15 is highly phosphorylated at mitosis, resulting in significant inhibition of its kinase activity. This result implies two points. First, single or multiple inhibitory phosphorylation sites as well as activation site(s) exist on STK15. Second, these sites phosphorylated by unknown inhibitory kinase(s) are dephosphorylated by PP1 at mitosis, leading to STK15 activation. This idea is supported by a report by Walter et al. (33) which shows that threonine 288 residue on STK15, only phosphorylated in interphase but not in mitosis, can be dephosphorylated by PP1 in vitro (33). Although Thr-288 appears to be at least one candidate responsible for preventing STK15 activation in interphase, we cannot exclude the possibility of the existence of other inhibitory phosphorylation sites as well. Further studies are needed to identify both activation and inhibitory phosphorylation sites and to elucidate their functional significance during cell cycle progression. It is

Fig. 3. STK15 interacts with three PP1 isoforms. 5 μg of GST (lane 1), GST-PP1α (lane 2), GST-PP1γ (lane 3), and GST-PP1P (lane 4) bound to Sepharose beads were incubated with 1 mg of mitotic extracts, and Sepharose beads were washed with lysis buffer. STK15 was probed with anti-STK15 antibody. Lane 5 shows direct immunoblotting of mitotic extract as control.

Fig. 4. PP1 not PP2 dephosphorylates STK15 and abolishes kinase activity. Immunoprecipitated STK15 from mitotic extracts with anti-STK15 antibody was assayed for kinase activity either directly (lane 3) or after incubation with purified PP1 (lane 4) and PP2 (lane 5). After extensively washing the immunocomplex, in vitro kinase assay was performed using β-casein as substrate. Immunoblotting (IB) was performed with anti-STK15 antibody (top panel), and autophosphorylated STK15 (middle panel) and phosphorylated β-casein (bottom panel) were detected by autoradiography. Lane 1 and lane 2 show direct immunoblotting of interphase extracts and mitotic extracts, respectively.

vitro kinase assay to test if active hyperphosphorylated STK15 from mitotic cells can phosphorylate PP1 using GST-PP1 proteins as substrates. To detect phosphorylation, assay was performed in the presence of sodium vanadate or okadaic acid to prevent GST-PP1 from dephosphorylating hyperphosphorylated STK15. Active STK15 from mitotic cells phosphorylates all the isoforms of GST-PP1 in vitro in the presence of phosphatase inhibitors, but we could not detect phosphorylation of PP1 in the absence of these inhibitors (Fig. 5A). These data indicate that STK15 may phosphorylate common serine and/or threonine residues in all the isoforms. Next, to examine the effect of STK15-mediated phosphorylation on PP1 activity, PP1 assay was performed using 32P-labeled MBP as substrate. For this objective, we used interphase extract as negative control because interphase extracts have less STK15 kinase activity than mitotic extracts. STK15 immunoprecipitated from both extracts were reacted with GST-PP1α in the presence of okadaic acid, immediately followed by extensive washing to remove okadaic acid, and then GST-PP1α activity was assayed. As shown in Fig. 5B, activity of GST-PP1α reacted with mitotic STK15 was decreased about 25% in comparison with interphase STK15. Furthermore, to examine if this decrement was directly because of phosphorylation by STK15, we transfected WT and Lys → Arg constructs to HeLa cells, respectively. PP1 activity present in immunocomplex that was immunoprecipitated with anti-FLAG antibody was measured in the presence of 2 mM okadaic acid to inhibit PP2A activity. PP1 activity bound to WT was decreased about 25% compared with that of Lys → Arg mutant (Fig. 5C). We next measured PP1 activity bound to endogenous STK15 derived from interphase and mitotic extracts. PP1 activity bound to mitotic STK15 was decreased compared with that of interphase STK15 (Fig. 5D). Because it is known that Cdk1-phosphorylated PP1 at threonine 320 results in inhibition of PP1 activity, we examined whether STK15 phosphorylates the same site as Cdk1. GST-PP1α substituted at position 320 from threonine to alanine. We detected similar phosphorylation signals from both normal and mutant fusion proteins in the presence of active mitotic STK15 (data not shown). These results suggest that STK15 phosphorylates PP1 at different site(s) from Cdk1, and its phosphorylation leads to inhibition of PP1 activity at mitosis.
Figure 5. Inhibition of PP1 activity by STK15 phosphorylation. A, STK15 was immunoprecipitated from mitotic extracts with anti-STK15 antibody, and in vitro kinase assay was performed using GST (lane 1), GST-PP1α (lane 2), GST-PP1γ (lane 3), and GST-PP1α (lane 4) as substrate in the presence of okadaic acid. Phosphorylated proteins were detected by autoradiography. B, STK15 were immunoprecipitated from interphase extracts (black bar) and mitotic extracts (gray bar) with anti-STK15 antibody, and then in vitro kinase assay was performed using GST-PP1α as substrate in the presence of okadaic acid. After extensively washing, phosphatase assay of GST-PP1α was carried out using 32P-labeled MBP as substrate. Protein phosphatase activity was plotted as a percentage of relative activity. The error bars indicate the S.D. (n = 3). C, transiently transfected HeLa cells with FLAG-tagged WT (black bar) and Lys → Arg mutant (K/R) (gray bar) were cultured in the presence of nocodazole for 16 h. Immunocomplexes of FLAG-tagged protein and PP1 were immunoprecipitated with anti-FLAG antibody, and phosphatase assay was performed. Protein phosphatase activity was plotted as a percentage of relative activity. The error bars indicate the S.D. (n = 3). D, immunocomplexes of STK15 and PP1 from interphase extracts (black bar) and mitotic extracts (gray bar) were immunoprecipitated with anti-STK15 antibody, and then phosphatase assay was performed. Protein phosphatase activity was plotted as a percentage of relative activity. The error bars indicate the S.D. (n = 2).

Figure 6. Super-phosphorylation and loss of kinase activity of non-binding STK15 mutants. Immunoprecipitated FLAG-tagged STK15 NB1 mutant (lane 2), NB2 mutant (lane 3), WT (lane 4), and Lys → Arg mutant (lane 5) from mitotic extracts with anti-FLAG antibody were assayed for kinase activity using β-casein as substrate. Immuno-blotting (IB) was performed with anti-STK15 antibody (top panel), and autophosphorylated STK15 (middle panel) and phosphorylated β-casein (bottom panel) were detected by autoradiography. Empty vector served as a control (lane 1). Super-phosphorylated STK15 and hyper-phosphorylated STK15 are indicated by arrowhead and arrow, respectively.

Known that PP1 is phosphorylated at threonine 320 by Cdk1 and is inactivated through G2/M transition during mitosis (28, 30). It has also been reported that this inactivation is necessary for maintaining the microtubule dynamics through inactivation of unidentified proteins. In this study we showed that phosphorylation of PP1 by STK15 at still unidentified site(s) can inhibit PP1 activity. This implies that STK15 kinase is involved in negative regulation of PP1 in addition to Cdk1 kinase. Taken together, our observations suggest that a feedback regulation through phosphorylation/dephosphorylation cycles exists between STK15 kinase and PP1 phosphatase.

Genetic studies in yeast have shown that Ipl1 kinase acts in opposition to Glc-7/type 1 phosphatase. Recently, Ndc10 and histone H3 have been identified as the common target proteins for Ipl1 kinase and Glc-7 phosphatase (11–13). Ndc10 is a component of CBF3 kinetochore protein complex, and reversible phosphorylation of Ndc10 by Ipl1 and Glc-7 plays an important role in the attachment of kinetochores to mitotic spindles (11, 12). We observed abnormal chromosome alignment at metaphase in non-binding mutant-transfected cells. This indicates the possibility that STK15 and PP1 regulatory feedback mechanism is involved in regulating kinetochore/microtubule attachment in human cells. Because we did not investigate the phosphorylation status of kinetochore proteins in this study, further studies are required to elucidate this possibility. On the other hand, reversible phosphorylation of histone H3 at serine 10 by Ipl1 and Glc-7 is considered to be crucial for chromosome condensation/decondensation (13). In C. elegans it was reported that AIR-2, an orthologue of human AIM-1/Aurora-B kinase that regulates cytokinesis (34), but not AIR-1, an orthologue of human STK15/Aurora-A kinase, is involved in the phosphorylation of histone H3 at serine 10 (13). Recently, it has shown (35) that Xenopus Aurora-B kinase regulates phosphorylation of histone H3 in concert with chromatin-associated PP1. These findings indicate that chromosome condensation through phosphorylation of histone H3 by Aurora-B kinase but not Aurora-A kinase is evolutionarily conserved. Immunofluorescence detection of histone H3 phosphorylation on misaligned chromosomes of NB mutant-transfected cells, as reported in this study, reinforces the idea that STK15/Aurora A is not the primary kinase involved in phosphorylating histone H3 during G2/M phase.

There are four PP1 isoforms in mammalian cells, and their subcellular localizations are different at mitosis, PP1α is localized to centrosome; PP1γ is associated with mitotic spindles, and PP1δ associates with chromosomes (16). Such different subcellular localization of various isoforms of PP1 might explain why mutants in PP1 and inhibition of PP1 by chemical drugs show complex phenotypes with condensed chromosomes, formation of abnormal spindles, chromosome separation malfunction, and defect of cytokinesis (19–24). STK15 binds with all the isoforms examined in vitro. Our previous study has shown that STK15 is localized to the centrosome (3). This suggests that STK15 could indeed associate with PP1α in vivo. In addition, it is possible that STK15 may also interact with PP1δ as STK15 might be involved in kinetochore-microtubule
feedback regulation between STK15 and PP1 is necessary for STK15 kinase activation and PP1 phosphatase activities. It is also likely that still unidentified additional proteins help keep STK15 inactive during early interphase. Overexpression of NB mutants.

A.

GFP

DAP1

Phospho H3

Merged

Empty

WT

NB1

NB2

B.

Aberrant mitotic cells (%)

40

35

30

25

20

15

10

5

0

Emp

NB1

WT

K/R

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