이학석사학위논문

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2013 년 8 월

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이 논문을 이학석사 학위논문으로 제출함

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Studies on PLK1 phosphorylation of pericentrin for centriole disengagement

A dissertation submitted in partial
fulfillment of the requirement
for the degree of

MASTER OF PHILOSOPHY

to the Faculty of
School of Biological Sciences
at
Seoul National University
by
Jaeyoun Kim

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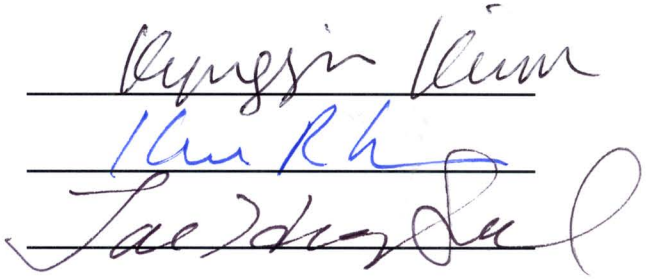

ABSTRACT

Studies on PLK1 phosphorylation of pericentrin for centriole disengagement

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Centrosome is a non-membrane bound organelle which functions as a spindle pole body during mitosis. Abnormalities in centrosome number cause multipolar spindles which result in chromosomal instability or cell death. Therefore the precise regulation of centrosome number is important for maintaining genome stability. During mitotic exit, procentrioles are disengaged from the mother centriole during mitotic exit, which is considered a licensing step for centriole duplication. It was recently reported that pericentrin cleavage is necessary and sufficient for centriole disengagement during mitosis. Here, I report that the pericentrin cleavage is regulated by PLK1. PLK1

phosphorylates S2259 and S2267 residues of pericentrin. FLAG-PCNT$^{S2259A, S2267A}$ in which these two residues are substituted to alanine is not cleaved during mitosis. Finally, centriole disengagement was inhibited in the pericentrin-depleted cells rescued with the phospho-resistant FLAG-PCNT mutants at S2259 and S2267. Based on the results, I propose that PLK1 phosphorylation is prerequisite to pericentrin cleavage and eventually to centriole disengagement during mitotic exit.

Key Words: PLK1, Pericentrin, Centriole disengagement

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CONTENTS

LIST OF FIGURES

INTRODUCTION

Centrosome is a non-membrane bound organelle which consists of centrioles and pericentriolar material (PCM). In animal cells, the centrosome is a main microtubule organizing center (MTOC) and it functions as a spindle pole body during mitosis (Nigg and Stearns, 2011). Abnormalities of centrosome number can cause multipolar spindles which result in chromosomal instability (CIN) or cell death (Ganem et al., 2009). Therefore, the precise regulation of centrosome number is important for maintaining genome stability.

The centrosome is duplicated once per cell cycle (Tsou and Stearns, 2006a, b). The centrosome duplication begins at S phase as the procentriole is orthogonally nucleated next to mother centriole. And centrosome duplication is completed when procentriole is detached from mother centriole during mitotic exit, which calls centriole disengagement (Nigg, 2007).

Centriole disengagement is regarded as a licensing mechanism to regulate the number of centrosome. Engaged centrioles cannot duplicate new procentrioles, even if it remains in S phase (Tsou and Stearns, 2006b). And it was reported that premature centriole disengagement causes centrosome overduplication (Prosser et al., 2012),

and it triggers multipolar spindles leading to chromosomal instability or cell death (Ganem et al., 2009). Therefore, the precise regulation of centriole disengagement is critical for maintaining the centrosome number and genome stability.

It has been previously proposed that centriole disengagement is dependent on separase and PLK1 activity which is a cysteine protease and a mitotic kinase, respectively (Tsou et al., 2009). And it has been recently reported that pericentrin cleavage by separase is necessary for centriole disengagement (Lee and Rhee, 2012; Matsuo et al., 2012). However, the functions of PLK1 to regulate centriole disengagement remained to be elucidated.

Here, I report that the PLK1 regulates the pericentrin cleavage. PLK1 phosphorylates pericentrin at S2259 and S2267 residues. And its phosphorylation is required for pericentrin cleavage and for centriole disengagement during mitotic exit. Thus, I propose that PLK1 phosphorylation of pericentrin at S2259 and S2267 is prerequisite to pericentrin cleavage and eventually to centriole disengagement during mitotic exit.

MATERIALS AND METHODS

DNA constructs and transfection

All Pericentrin constructs were subcloned into pLVX-IRES-PURO vector (Clonetech) and tagged with 3×FLAG and Myc at 5'- and 3'- end, respectively.

To change amino acid residues, polymerase chain reaction was conducted with mutant oligonucleotides primers and $PCNT^{6444-10008}$ fragment subcloned into pGEM-T Easy vector (Promega).

To express DNA constructs, Fugene HD (Promega, E2311) was used according to the manufactures' protocols.

RNA interference

To deplete PLK1 and Pericentrin, siRNA sequence with 5' -AAG CGG GAC TTC CTC ACA TCA-3' and 5'- UGG ACG UCA UCC AAU GAG ATT -3' (Zimmerman et al., 2004) were used, respectively. As a control, scrambled siRNA sequence with 5' - GCA AUC GAA GCU CGG CUA CTT -3' was used.

To transfect siRNAs into cells, Lipofectamin RNAiMAX (Invitrogen, 13778-075) was used according to the manufactures' protocols.

Cell culture and stable cell lines

HeLa cells were cultured in DMEM (WELGENE, LM 001-05) supplemented with 10 % fetal bovine serum and 5 μg/ml Plasmocin (Invivogen, ANT-MPT) at 37 ℃ and 5 % CO_2.

To make stable cell lines, DNA constructs were transfected into the HeLa cells. One day after transfection, 1 mg/ml puromycin (Calbiochem, 540222) was treated to select the cells which stably express the plasmid for 2-3 weeks. After then monoclonal cells were obtained by dilution cloning.

Cell cycle synchronization and drug treatments

To collect mitotic cells, thymidine-taxol block were used. Briefly, 2 mM thymidine (Sigma, T9250) was treated for 24 hours and subsequently 5 μM taxol (Sigma, T7402) was treated for 13 hours after thymidine washout. 2 μM ZM447493 (Cayman chemical company, 13601) was treated to force mitotic exit.

100 nM BI-2536 (Selleck chemicals, S1109) was treated to inhibit PLK1's activity during mitosis.

Antibodies

The antibodies specific to pericentrin and C-Nap1 were used as previously described (Kim and Rhee, 2011; Kim et al., 2008). And antibodies for FLAG (Sigma, F3165), cyclin B1 (Santa Cruz, sc-245), GAPDH (Ambion, AM4300), Centrin (Millipore, 04-1624), and α-tubulin (Abcam, ab18251) were purchased.

Immunofluorescence microscopy

HeLa cells were fixed with ice-cold methanol for 10 min and blocked with blocking solution (3% bovine serum albumin in 0.3% Triton X-100 in PBS) at room temperature for 25 min. And then Cells were incubated with primary antibodies which were diluted in blocking solution at room temperature for 1 hr. After then cells were incubated at room temperature for 30 min with secondary antibodies which were also diluted in blocking solution. To visualize the DNA, DAPI was used.

Immunofluorescence image were obtained from a fluorescence microscope (Olympus, IX-51) equipped with a CCD camera (Qimaging, Qicam fast 1394).

Immunoblot analysis

HeLa cells were lysed in RIPA buffer(150 mM NaCl, 1 % triton X-100, 0.5 % sodium deoxycolate, 0.1 % SDS, 50 mM Tris-Cl(pH8.0), 10 mM NaF, 1 mM Na3VO4, 1 mM EDTA, and 1 mM EGTA) with protease inhibitor cocktail (sigma, P8340) and subsequently incubated on ice for 10 min. And then lysate was centrifuged at 4 ℃, and 12,000 rpm for 10 min. Supernatant were mixed with 4×SDS sample buffer (250 mM Tris-Cl pH 6.8, 8 % SDS, 40 % glycerol, and 0.04 % bromophenol blue), and 10 mM DTT. After then samples were boiled for 5 min.

To detect pericentrin, 15~20 ㎍ of proteins was loaded in SDS-PAGE and transferred from gel to nitrocellulose membranes. The membranes were blocked with blocking solution (5 % nonfat milk in 0.1 % Tween 20 in TBS) for 1 hr. And then membranes were incubated with primary antibodies which were diluted in blocking solution at 4 ℃ for 16 hr. After then membranes were incubated with peroxidase-conjugated secondary antibodies which were also diluted in blocking solution at room temperature for 30 min. To detect the signal of secondary antibodies, ECL reagent (ABfrontier, LF-QC0101) and X-ray film (Agfa) were used.

RESULTS

The PLK1 activity is required for pericentrin cleavage during mitotic exit.

It was previously reported that PLK1 and separase are essential for centriole disengagement (Tsou et al., 2009). It has been also reported that pericentrin cleavage by separase is necessary and sufficient for centriole disengagement (Lee and Rhee, 2012; Matsuo et al., 2012). Because pericentrin is phosphorylated by PLK1 during mitosis(Lee and Rhee, 2011), we hypothesized that PLK1 phosphorylation of pericentrin is critical for its cleavage during mitosis.

To test our hypothesis, I treated mitotic cells with BI2536, a PLK1 inhibitor, and observed the pericentrin cleavage. HeLa cells were synchronized at M phase with treatment of 2 mM thymidine for 24 h and subsequently with 5 μM taxol for 13 h after thymidine washout. BI2536 (100 nM) was treated for 3 h to inhibit the PLK1 activity. The cells were subsequently treated with ZM447439 (2 μM) to force the mitotic exit. The lysates from the cells at indicated time points after the ZM447439 treatment were subjected to immunoblot analyses (Figure 1A). The immunoblot with the pericentrin antibody detected two specific bands which correspond to an intact and a cleaved pericentrin

protein (Figure 1B; Lee and Rhee, 2012). BI2536 treatment reduced the intensity of the cleaved pericentrin band, proposing the involvement of PLK1 activity in the pericentrin cleavage (Figure 1B). BI3536 did not affect mitotic exit since cyclin B1 was normally degraded 2 h after the ZM447439 treatment (Figure 1B).

I performed the pericentrin cleavage assay with the *siPLK1*-transfected cells. The cellular PLK1 levels were significantly reduced 48 h after the transfection (Figure 2A, B). As expected, the cleaved protein was significantly reduced in the PLK1-depleted cells (Figure 2B). Based on the results, I conclude that the PLK1 activity is required for the pericentrin cleavage during mitotic exit.

Specific phosphorylation of pericentrin is critical for its cleavage.

It is known that PLK1 is involved in diverse mitotic events by phosphorylating many cellular proteins during mitosis (Petronczki et al., 2008). Pericentrin is a known substrate of PLK1 and 22 potential phosphorylation sites have been previously identified (Lee and Rhee; Data not shown). Therefore, I hypothesized that the pericentrin cleavage is regulated by PLK1 phosphorylation.

To test the hypothesis, I established stable cell lines which

express FLAG-tagged wild type pericentrin (FLAG-PCNT) or pericentrin in which 22 candidate phosphorylation sites are substituted with alanines (FLAG-PCNT^{22A}). The ectopic FLAG-PCNT proteins are expressed in comparable levels to the endogenous pericentrin protein and properly localized at the centrosome (Figure 3A). I performed the pericentrin cleavage assay with the FLAG-PCNT stable cell lines. The results showed that FLAG-PCNT was effectively cleaved during mitotic exit while FLAG-PCNT^{22A} was not (Figure 3B). The expression level of ectopic protein was confirmed by immunocytochemistry and immunoblot with the FLAG antibody (Figure 3A and B). The cleaved FLAG-PCNT^{22A} band was reduced compared with FLAG-PCNT regardless of the BI2536 treatment (Figure 4). This result implies that PLK1 phosphorylation at one or more of 22 candidate phosphorylation sites are essential for the pericentrin cleavage.

Phosphorylation at S2259 and S2267 residues is important for the pericentrin cleavage.

In order to pinpoint a critical phosphorylation site for the pericentrin cleavage, I made a series of phospho-resistant pericentrin mutants in which selected serine or threonine residues were substituted

to alanine (Figure 5A, B). A group of the candidate phosphorylation sites reside near the cleavage site which is arginine 2231, but the others are located far from the cleavage sites. First, I compared FLAG-PCNT with phospho-resistant substitutions near (16A) or far (6A) from the cleavage sites. The results with stable cell lines showed that FLAG-PCNT^{16A} was not effectively cleaved but FLAG-PCNT^{6A} was (Figure 6A). I also performed the pericentrin cleavage assays with the transiently transfected cells. The results showed that FLAG-PCNT^{6A} was cleaved in comparable to FLAG-PCNT (Figure 6B). On the other hand, FLAG-PCNT1^{16A} as well as FLAG-PCNT^{22A} was hardly cleaved during mitotic exit (Figure 6B). These results indicate that the phosphorylation site for regulation of the pericentrin cleavage is located near the pericentrin cleavage sites.

To further narrow down the phosphorylation sites critical for the pericentrin cleavage, I performed the pericentrin cleavage assays with stable cell lines expressing FLAG-PCNT^{7A} or FLAG-PCNT^{9A} (Figure 5A and B). The amount of the FLAG-PNCT^{9A} cleaved band was reduced in comparison to FLAG-PCNT^{WT} and FLAG-PNCT^{7A} (Figure 7A). To confirm this result, pericentrin cleavage assay was carried out in HeLa cells transiently transfected with FLAG-PCNT^{7A} and FLAG-PCNT^{9A}. As expected, the amount of the FLAG-PCNT^{9A} cleaved band was reduced, while that of FLAG-PCNT^{7A} was not

(Figure 7B). These two results imply that the essential phosphorylation sites causing pericentrin cleavage are among the 9 residues which are nearest of the pericentrin cleavage site.

In order to identify a single phosphorylation site for the pericentrin cleavage, I prepared FLAG-PCNT point mutant constructs in which a single site among the 9 candidate sites is substituted to alanine. Each construct was transfected into HeLa cells and subjected to the pericentrin cleavage assay. Contrary to expectation, however, all FLAG-PCNT point mutants generated cleavage bands as much as FLAG-PCNT, although FLAG-PCNT^{S2259A} showed a slightly low intensity (Figure 8). These results suggest that two or more phosphorylation sites are required for regulation of the pericentrin cleavage.

I pay attention on the fact that not only S2259 and S2267 reside very close to each other, but also the amino acid sequence near two sites are identical (Figure 9). Since the cleaved FLAG-PCNT^{S2259A} band was slightly reduced, it is possible that phosphorylation at both S2259 and S2267 residues is necessary for the pericentrin cleavage. To test this possibility, I transiently transfected FLAG-PCNT^{S2259A}, FLAG-PCNT^{S2267A} or FLAG-PCNT$^{S2259, S2267A}$ into HeLa cells and performed the pericentrin cleavage assay. The results showed that the cleaved FLAG-PCNT$^{S2259, S2267A}$ band was significantly diminished, in

comparison to those of a single substituted as well as wild type FLAG-PCNT proteins (Figure 10). I also confirmed that the phospho-resistant mutant at S2259 and S2263 (FLAG-PCNT$^{S2259, S2267A}$) is located at the centrosome properly (Figure 11). These results imply that PLK1 phosphorylation at both S2259 and S2267 is essential for pericentrin cleavage during mitotic exit.

Specific phosphorylation of pericentrin is prerequisite to centriole disengagement during mitotic exit.

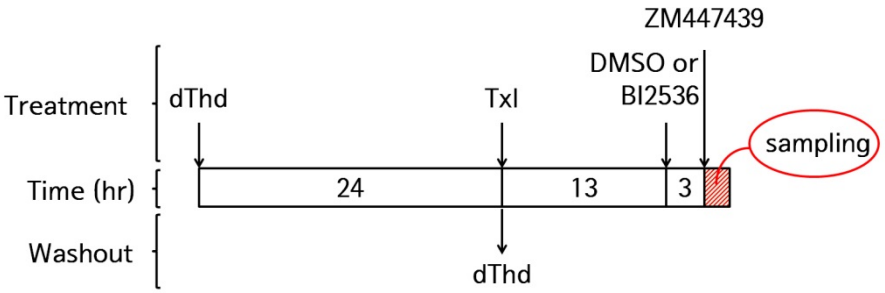
It has been previously reported that pericentrin cleavage is necessary for centriole disengagement during mitosis (Lee and Rhee, 2012; Matsuo et al., 2012). Therefore, I examined whether the phospho-resistant pericentrin prevents centriole from being disengaged or not during mitotic exit. I performed the pericentrin cleavage assays with the pericentrin-depleted cells rescued with wild type and the phospho-resistant FLAG-PCNT. Centriole disengagement can be determined with the ratio of C-Nap1 to centrin signals (Tsou and Stearns, 2006b). The results showed that the centrioles in most of pericentrin-depleted cells are disengaged (Figure 12A, B). The centrioles in the FLAG-PCNT-rescued cells also were mostly disengaged (Figure 12A, B). However, the centriole disengagement in the FLAG-PCNT^{2A}-

and FLAG-PCNT^{9A}-rescued cells was significantly inhibited (Figure 12A, B). These results suggest that PLK1 phosphorylation is prerequisite to the pericentrin cleavage and eventually for centriole disengagement during mitotic exit.

Figure 1. BI2536 blocks the pericentrin cleavage during mitotic exit.

(A) Experimental scheme. HeLa cells were synchronized at M phase with treatment of 2 mM thymidine (dThd) for 24 h and subsequently with 5 μM taxol (Txl) for 13 h after thymidine washout. BI2536 (100 nM) was treated for 3 h to inhibit the PLK1 activity. The cells were subsequently treated with ZM447439 (2 μM) to force the mitotic exit. (B) The lysates from the cells at indicated time points after the ZM447439 treatment were subjected to immunoblot analyses with antibodies specific to pericentrin (PCNT), cyclin B1, and GAPDH. The intact and cleaved pericentrin bands were indicated.

A.


ZM447439
DMSO or
BI2536
Treatment
dThd
Txl
sampling
Time (hr)
24
13
3
Washout
dThd

B.

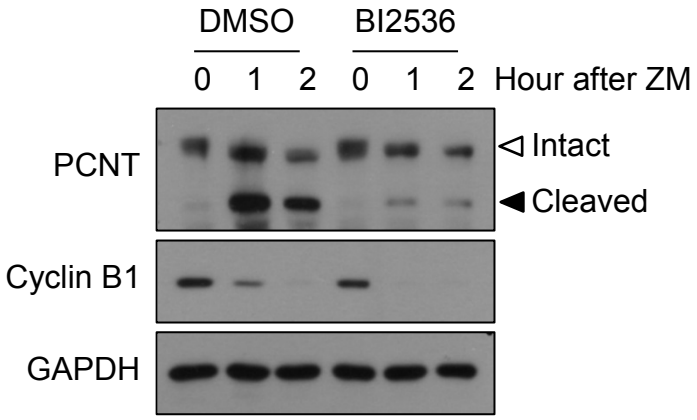
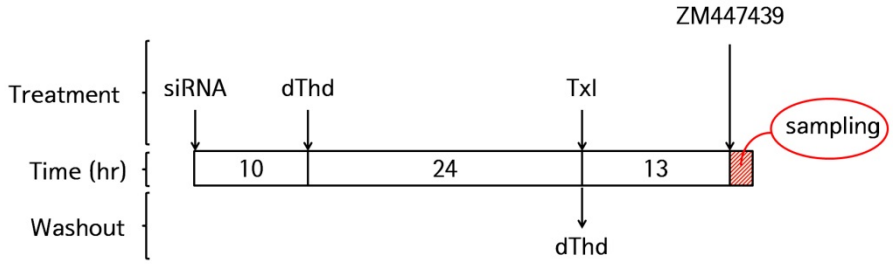

DMSO
BI2536
0 1 2 0 1 2 Hour after ZM
PCNT
Intact
Cleaved
Cyclin B1
GAPDH

Figure 2. PLK1 depletion inhibits the pericentrin cleavage during mitotic exit.

(A) Experimental scheme. HeLa cells were treated with indicated siRNA to deplete PLK1. Ten hours later, the cell cycle was synchronized at M phase with thymidine (dThd) for 24 h and subsequently with taxol (Txl) for 13 h after thymidine washout. ZM447439 (ZM) was treated to force the mitotic exit. (B) The cell lysates were subjected to immunoblot analyses with antibodies specific to pericentrin (PCNT), PLK1, cyclin B1, and GAPDH.

A.


ZM447439
Treatment
siRNA
dThd
TxI
sampling
Time (hr)
10
24
13
Washout
dThd

B.

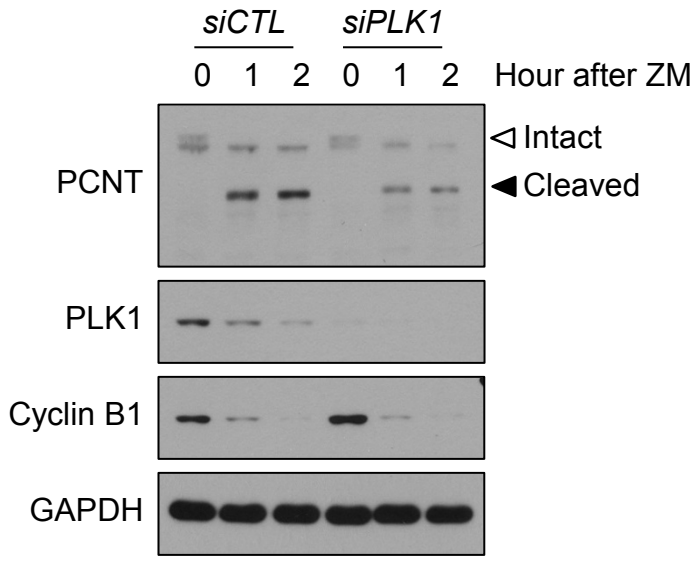
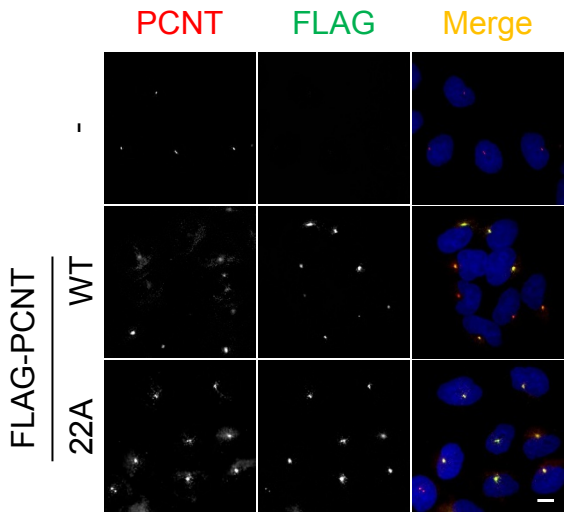

siCTL
siPLK1
0 1 2 0 1 2 Hour after ZM
PCNT
◁ Intact
◀ Cleaved
PLK1
Cyclin B1
GAPDH

Figure 3. Stable expression of the wild type or 22A mutant of FLAG-PCNT in HeLa cells.

(A) The stable cell lines were immunostained with antibodies specific to pericentrin (PCNT, red) and FLAG (green). DNA was visualized with DAPI (blue). Bar, 10 μm. (B) Lysates from each stable cell line were subjected to immunoblot analysis with antibodies specific to pericentrin, FLAG, and α-tubulin.

A.


PCNT
FLAG
Merge
-
FLAG-PCNT
WT
22A

B.

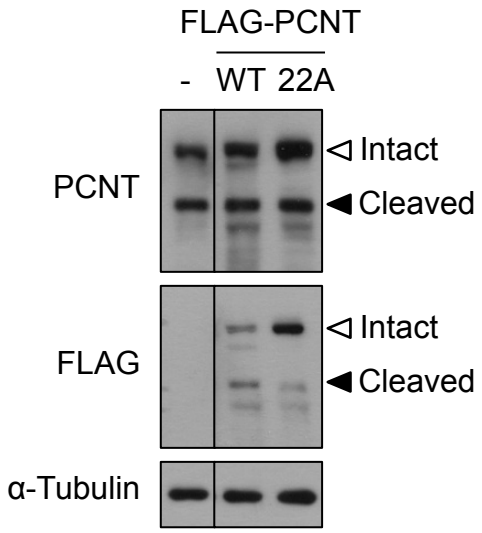

FLAG-PCNT
-
WT
22A
PCNT
Intact
Cleaved
FLAG
Intact
Cleaved
α-Tubulin

Figure 4. $PCNT^{22A}$ is not efficiently cleaved during mitotic exit.

We performed the pericentrin cleavage assays as described in Figure 1A with stable HeLa cell lines in which the wild type or 22A mutant of pericentrin is ectopically expressed. Immunoblot was performed with antibodies specific to pericentrin (PCNT), cyclin B1, and GAPDH.

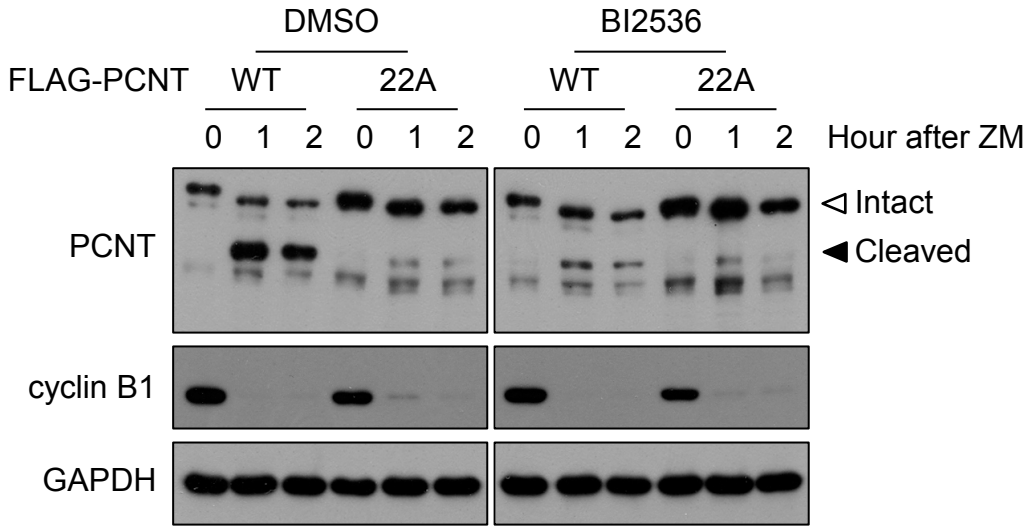

DMSO
BI2536
FLAG-PCNT
WT
22A
WT
22A
0 1 2 0 1 2 0 1 2 0 1 2
Hour after ZM
PCNT
cyclin B1
GAPDH
◁ Intact
◀ Cleaved

Figure 5. PLK1 phosphorylation sites within the pericentrin protein.

(A) Selected PLK1 phosphorylation sites within pericentrin were substituted with alanines. The constructs were named based on the number of substitution mutations (black lines). The red line indicates the cleavage site during mitotic exit. The green and blue boxes indicate the FLAG and Myc tags, respectively. (B) The mutation sites of the constructs are described.

A.

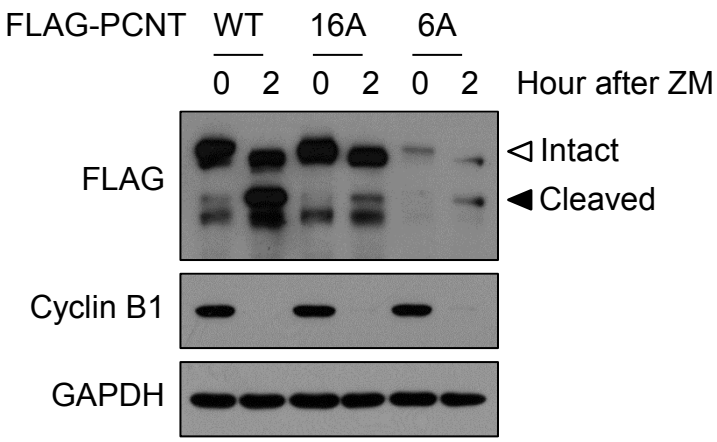
B.

Mutant	Mutated sites
22A	T1209A, S1211A, S1229A,S1235A, S1241A, S1688A, T1690A, S1693A, S2108A, S2109A, S2111A, S2114A, T2154A, T2160A, S2183A, S2189A, S2222A, S2259A, S2267A, S2318A, T2324A, T2584A
16A	S1688A, T1690A, S1693A, S2108A, S2109A, S2111A, S2114A, T2154A, T2160A, S2183A, S2189A, S2222A, S2259A, S2267A, S2318A, T2324A
6A	T1209A, S1211A, S1229A,S1235A, S1241A, T2584A
7A	S1688A, T1690A, S1693A, S2108A, S2109A, S2111A, S2114A
9A	T2154A, T2160A, S2183A, S2189A, S2222A, S2259A, S2267A, S2318A, T2324A

Figure 6. Determination of PLK1 phosphorylation sites critical for the pericentrin cleavage.

(A) Stable cell lines with FLAG-PCNT^{16A} or FLAG-PCNT^{6A} were subjected to the pericentrin cleavage assay. (B) The wild type or phosphor-resistant mutants of FLAG-PCNT were transiently transfected into HeLa cells and subjected to the pericentrin cleavage assay.

A.


FLAG-PCNT
WT
16A
6A
0 2 0 2 0 2
Hour after ZM
FLAG
Intact
Cleaved
Cyclin B1
GAPDH

B.

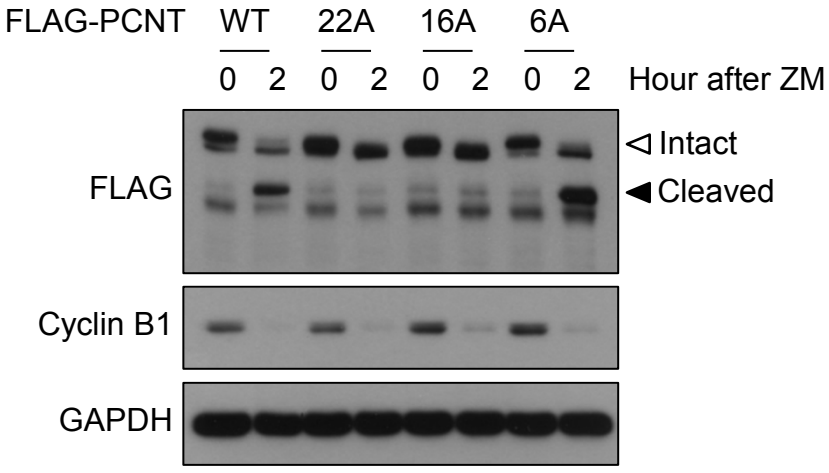
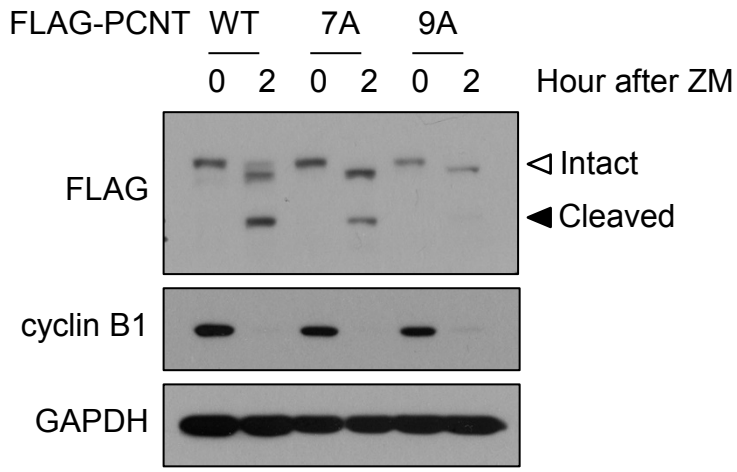

FLAG-PCNT
WT
22A
16A
6A
0 2 0 2 0 2 0 2
Hour after ZM
FLAG
Intact
Cleaved
Cyclin B1
GAPDH

Figure 7. Pericentrin cleavage assays with the phosphor-resistant pericentrin proteins.

(A) Stable cell lines with FLAG-PCNT^{7A} or FLAG-PCNT^{9A} were subjected to the pericentrin cleavage assay. (B) The wild type or phospho-resistant mutants of FLAG-PCNT were transiently transfected into HeLa cells and subjected to the pericentrin cleavage assay.

A.


FLAG-PCNT
WT
7A
9A
0 2 0 2 0 2
Hour after ZM
FLAG
Intact
Cleaved
cyclin B1
GAPDH

B.

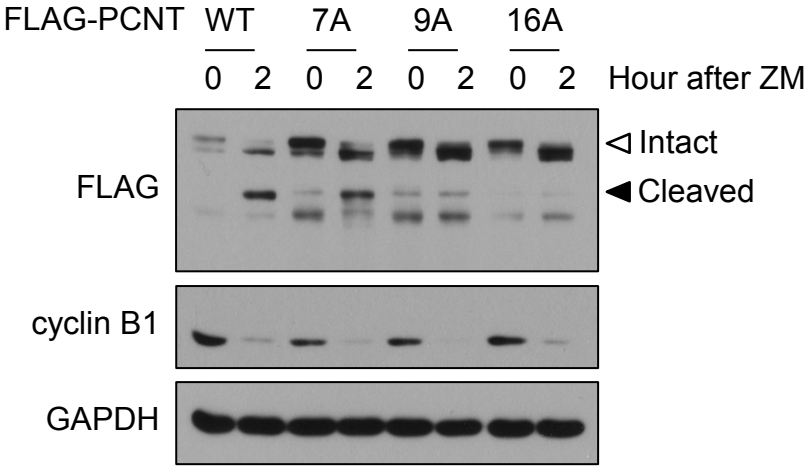
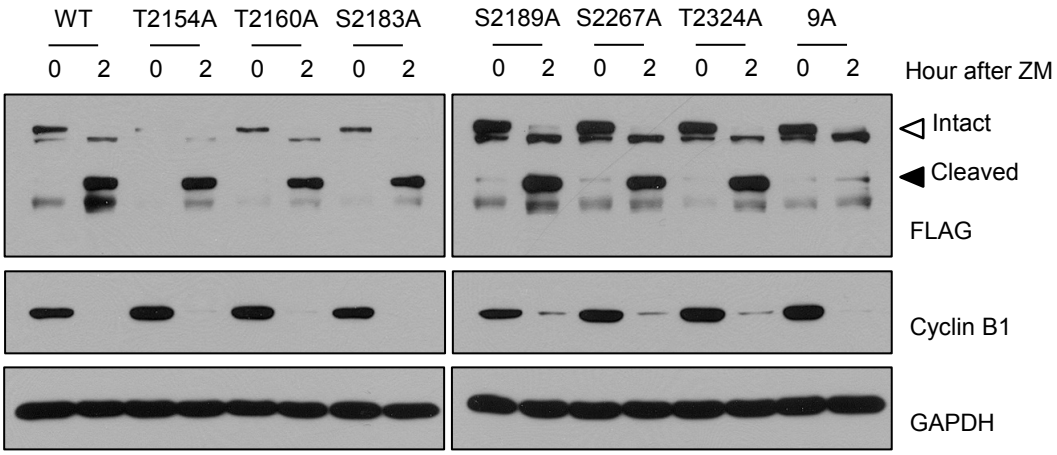

FLAG-PCNT
WT
7A
9A
16A
0 2 0 2 0 2 0 2
Hour after ZM
FLAG
Intact
Cleaved
cyclin B1
GAPDH

Figure 8. Examination of the pericentrin cleavage bands.

Pericentrin cleavage assays were performed with the FLAG-PCNT mutants at indicated phosphorylation sites. FLAG-PCNT (WT) and FLAG-PCNT^{9A} (9A) were used as cleavage-sensitive and -resistant controls, respectively.


WT
T2154A
T2160A
S2183A
S2189A
S2267A
T2324A
9A
0 2 0 2 0 2 0 2 0 2 0 2 0 2 0 2
Hour after ZM
Intact
Cleaved
FLAG
Cyclin B1
GAPDH

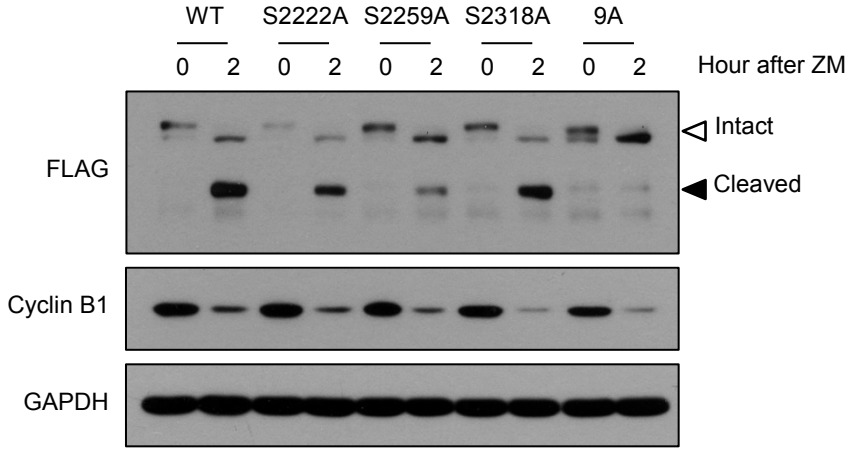

WT
S2222A
S2259A
S2318A
9A
0 2 0 2 0 2 0 2 0 2
Hour after ZM
Intact
Cleaved
FLAG
Cyclin B1
GAPDH

Figure 9. Analysis of the amino acid sequence at 2215-2273 of pericentrin.

The underlined amino acid sequences around S2259 and S2267 are identical.

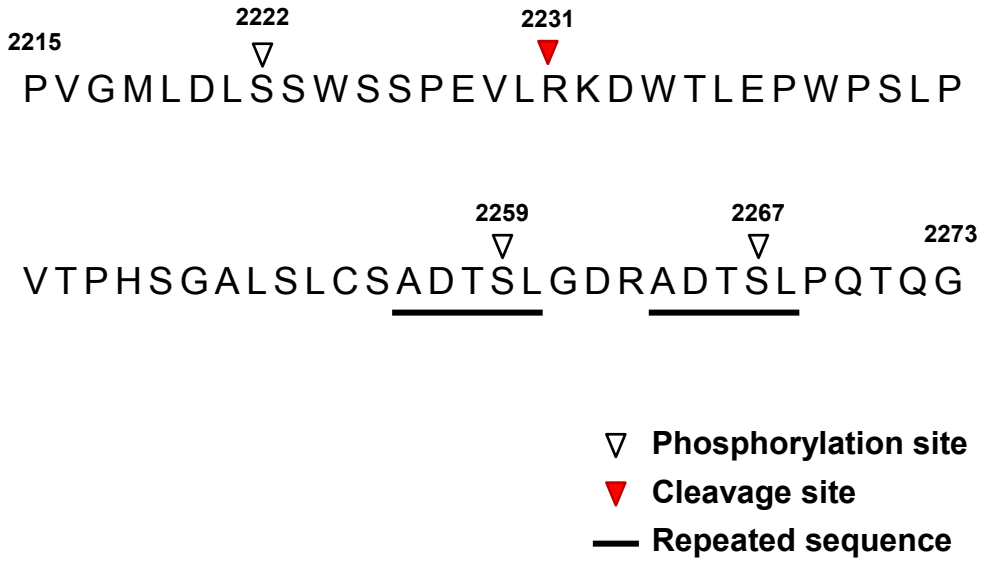

2215
2222
2231
PVGMLDLSSWSSPEVLRKDWTLEPWPSLP
2259
2267
2273
VTPHSGALSLCSADTSLGDRADTSLPQTQG
Phosphorylation site
Cleavage site
Repeated sequence

Figure 10. The phosphorylation at S2269 and S2267 residues is critical for the pericentrin cleavage.

HeLa cells were transiently transfected with FLAG-PCNT^{S2259A} (S2259A), FLAG-PCNT^{S2267A} (S2267A), or FLAG-PCNT$^{S2259, 2267A}$ (2A) and were subjected to the pericentrin cleavage assays. FLAG-PCNT (WT) and FLAG-PCNT^{9A} (9A) was used as cleavage-sensitive and -resistant controls, respectively.

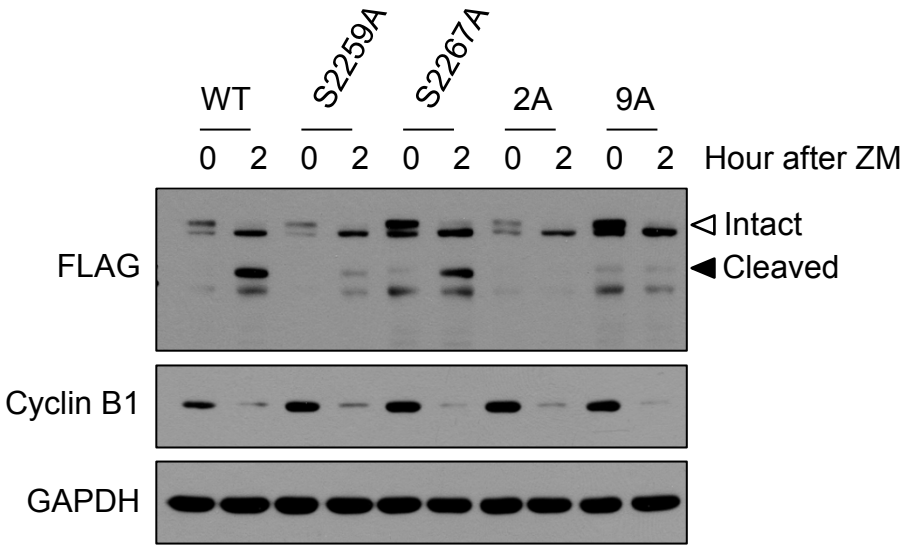

WT
S2259A
S2267A
2A
9A
0 2 0 2 0 2 0 2 0 2
Hour after ZM
FLAG
Cyclin B1
GAPDH
◁ Intact
◀ Cleaved

Figure 11. Stable expression of indicated stable cell lines.

Stable cell lines with FLAG-PCNT, FLAG-PCNT^{S2259A}, FLAG-PCNT^{S2267A}, and PCNT^{2A}, and FLAG-PCNT9A were coimmunostained with antibodies specific to FLAG (red) and Myc (green). DNA was stained with DAPI. Bar, 5 μm.

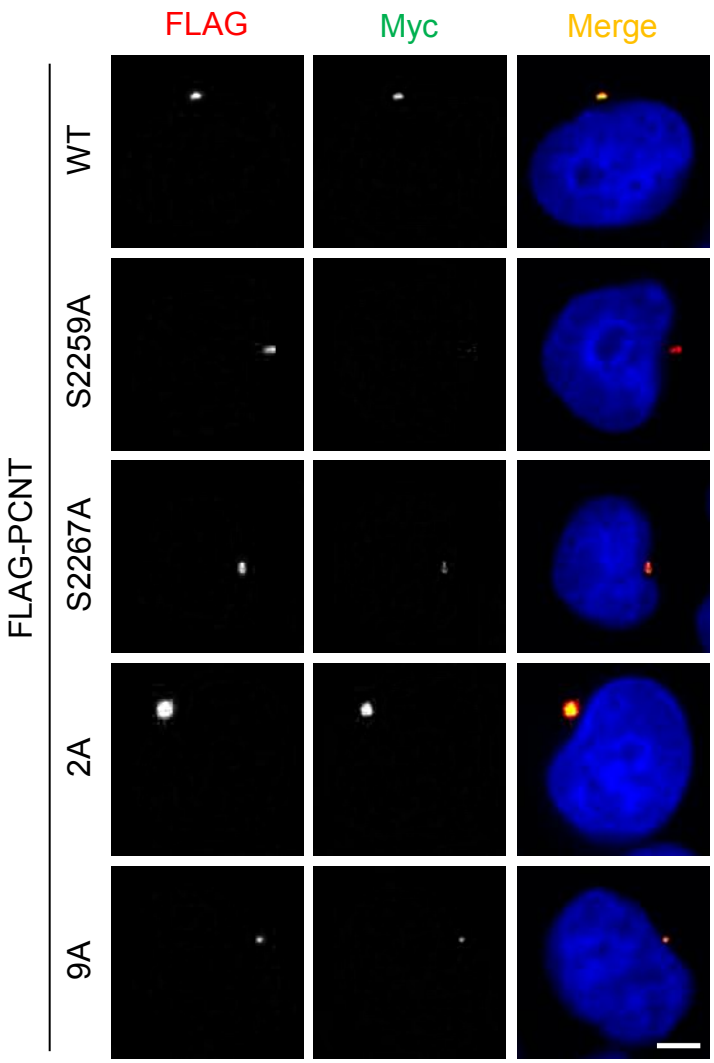
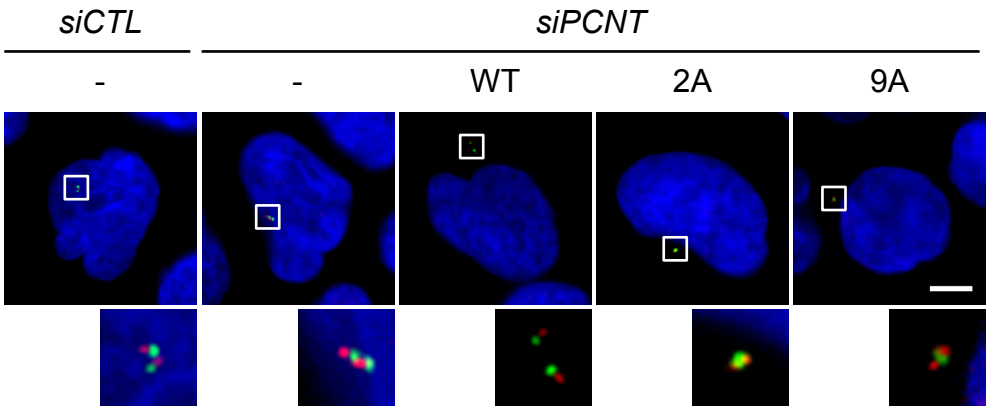

FLAG
Myc
Merge
FLAG-PCNT
WT
S2259A
S2267A
2A
9A

Figure 12. The phosphorylation of S2269 and S2267 residues is essential for centriole disengagement during mitotic exit.

(A) The pericentrin-depleted cells were rescued with the wild type or phospho-resistant FLAG-PCNT mutants. The cells were treated with RO3306 for 16 h and immunostained with antibodies specific to centrin (red) and C-Nap1 (green). DNA was stained with DAPI (blue). Insets are magnified images of the centrosomes. Bar, 15 μm. (B) The number of cells with engaged centrioles were determined at each experimental group. Two green dots with two red dots were determined as a disengaged centriole while a single green dot with two red dots were determined as an engaged centriole

A.


siCTL
siPCNT
-
-
WT
2A
9A

B.

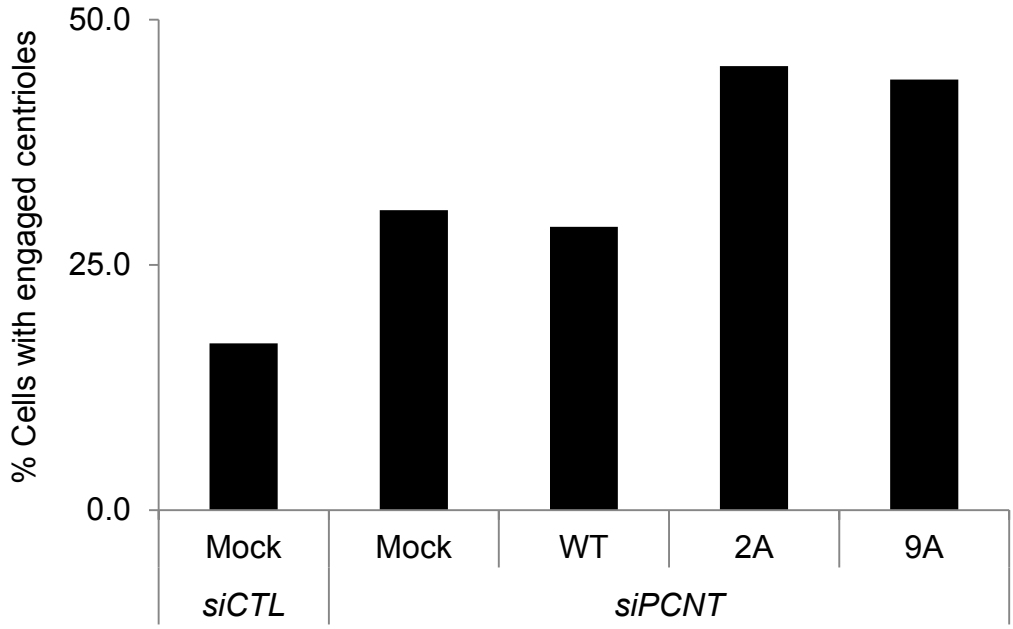
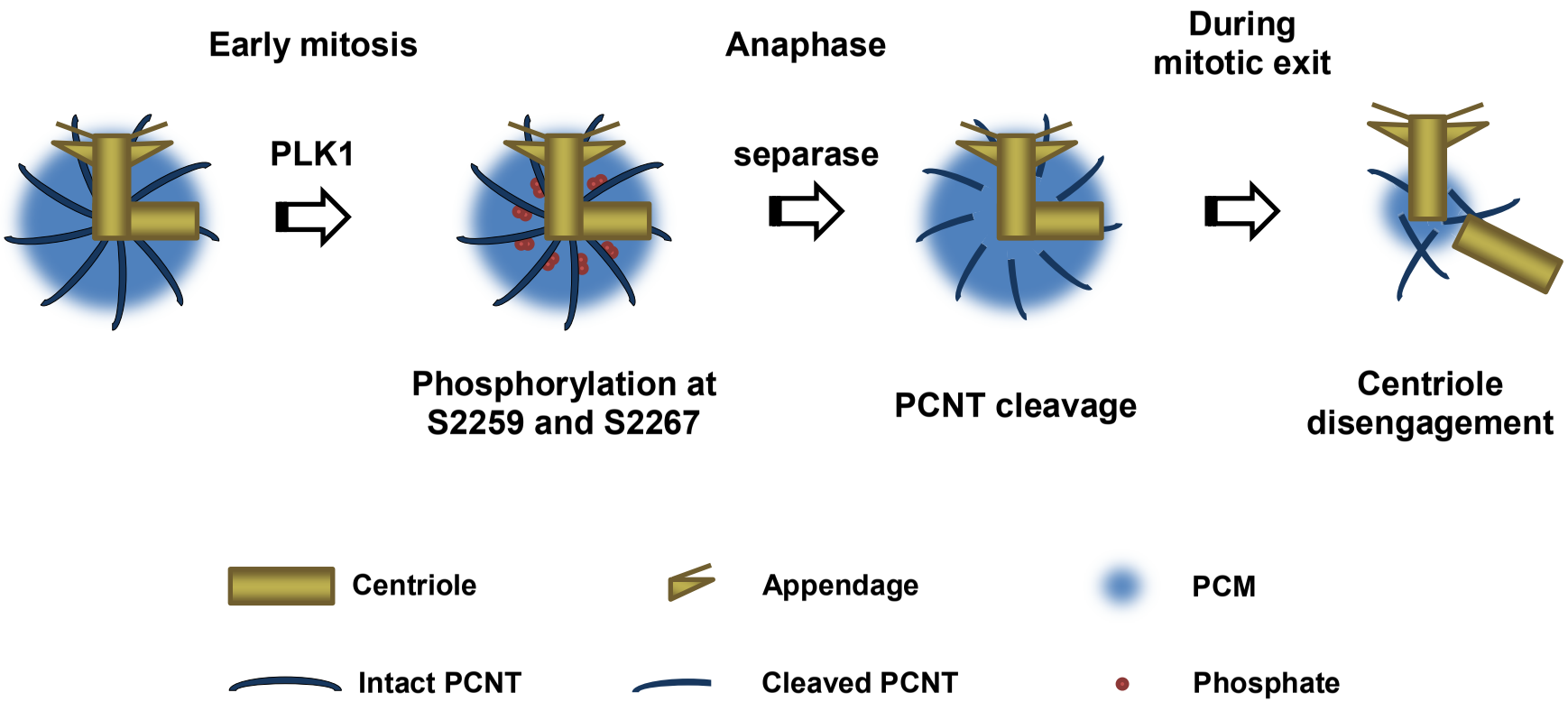

% Cells with engaged centrioles
50.0
25.0
0.0
Mock
Mock
WT
2A
9A
siCTL
siPCNT

Figure 13. A model that PLK1 phosphorylation of pericentrin is prerequisite for centriole disengagement.

During mitosis, PLK1 specifically phosphorylates S2259 and S2267 residues of pericentrin. This phosphorylation makes pericentrin susceptible to the separase attack. The pericentrin cleavage is necessary and sufficient for centriole disengagement during mitotic exit.


Early mitosis
PLK1
Phosphorylation at
S2259 and S2267
Anaphase
separase
PCNT cleavage
During
mitotic exit
Centriole
disengagement
Centriole
Appendage
PCM
Intact PCNT
Cleaved PCNT
Phosphate

DISCUSSION

It was known that PLK1 and separase are required for centriole disengagement (Tsou et al., 2009). And it has been recently reported that separase regulates centriole disengagement by cleaving pericentrin during mitotic exit (Lee and Rhee, 2012; Matsuo et al., 2012). However, the function of PLK1 to regulate centriole disengagement remained unknown. In this study, I report that the pericentrin mutant in which S2259 and S2267 residues are substituted to alanine is not cleaved and blocks centriole disengagement during mitotic exit. Therefore, I think that the PLK1's function to regulate centriole disengagement is the phosphorylation of pericentrin.

The localization of PLK1 is very dynamic in mitosis (Archambault and Glover, 2009; Barr et al., 2004; Petronczki et al., 2008). According to recent report, PLK1 briefly localizes at centrosome in anaphase although its biological meaning is not understood (Beck et al., 2013). I guess that this localization of PLK1 is important for pericentrin phosphorylation of S2259 and S2267 residues because centriole disengagement is the event after anaphase onset. So it should be investigated whether PLK1 localization during anaphase is important for phosphorylation of pericentrin and for centriole disengagement.

Currently, it is known that centriole disengagement is regulated by not only pericentrin cleavage but also SCC1 cleavage (Schockel et al., 2011). And it seems to be necessary for centriole disengagement in both cases. I guess that centriole engagement is maintained by two complementary mechanisms. One is direct linkage between mother and daughter centrioles perhaps by hSAS-6 or SCC1. The other is robust PCM lattice because it seems that mother and daughter centrioles are grabbed by PCM in super resolution microscopy studies (Lawo et al., 2012; Mennella et al., 2012). Thus, I think that centriole disengagement takes place when not only direct linkage but also PCM are disrupted during mitotic exit. Therefore, I guess that centriole disengagement inhibits when one of two mechanisms is blocked. And it might be explained that both pericentrin cleavage and SCC1 cleavage are necessary for centriole disengagement.

On the basis of my results, I propose a model that PLK1 phosphorylation of pericentrin is prerequisite for centriole disengagement (Figure 13). In early mitosis, PLk1 phosphorylates pericentrin at S2259 and S2267 residues and it makes pericentrin susceptible to separase attack. Therefore separase cleaves pericentrin and centrioles are disengaged during mitotic exit.

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국문초록

중심체는 막이 없는 대표적인 세포소기관으로서, 세포분열기에 방추극으로 작용하여 방추사를 형성한다. 염색체 수 이상에 의해 발생되는 다극 방추체는 염색체불안정성 혹은 세포사멸을 일으킨다. 따라서, 정교한 중심체 수 조절기작은 세포가 자신의 유전물질을 안정적으로 딸세포에 전달해주는데 중요한 역할을 한다.

중심립 유리현상은 중심체의 수를 조절하는 핵심 기작으로 딸중심립이 모중심립에서 떨어지는 현상을 말한다. 또한 최근 연구결과에 따르면 separase에 의한 pericentrin의 절단이 중심립 유리현상을 일으키는 주요한 원인으로 밝혀졌다. 본 연구는 PLK1에 의해 조절되는 pericentrin 절단 기작에 대해 연구하였다. Pericentrin은 PLK1에 의해 2259번 및 2267번 아미노산이 인산화되며, 이 두 아미노산이 알라닌으로 치환된 돌연변이에서는 pericentrin의 절단 및 중심립 유리현상이 줄어듦을 관찰하였다. 이 결과는 PLK1이 중심립 유리현상을 pericentrin의 인산화를 통해 조절할 수 있음을 제안한다.

주요어: PLK1, Pericentrin, 중심립 유리현상

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