



The equilibrium of ubiquitination and deubiquitination at PLK1 regulates sister chromatid separation

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Abstract PLK1 regulates almost every aspect of mitotic events, including mitotic entry, spindle assembly, chromosome alignment, sister chromatid segregation, metaphase-anaphase transition, cytokinesis, etc. In regulating the chromosome alignment and sister chromatid segregation, PLK1 has to be localized to and removed from kinetochores at the right times, and the underlying mechanism that regulates PLK1 both spatially and temporally only became clearer recently. It has been found that deubiquitination and ubiquitination of PLK1 are responsible for its localization to and dissociation from the kinetochores, respectively. The equilibrium of this ubiquitination and deubiquitination plays an important role in regulating proper chromosome alignment and timely sister chromatid segregation. Here, we summarize and discuss the recent findings in investigating the spatial and temporal regulation of PLK1 during chromosome alignment and sister chromatid segregation.

Keywords Polo-like kinase 1 · Mitosis · Usp16 · Kinetochores · Cullin 3 · E3 ligase

Abbreviations

PLK1 Polo-like kinase 1
Usp16 Ubiquitin-specific peptidase 16

CUL3 Cullin 3
SAC Spindle assembly checkpoint
KT–MT Kinetochores–microtubule
PB Polo box
PBD Polo box domain
PBIP1 Polo box domain-interacting protein 1
INCENP Inner centromere protein
APC/C Anaphase-promoting complex/cyclosome
CCAN Constitutive centromere-associated network

Introduction

Polo-like kinases are a group of evolutionarily conserved serine/threonine protein kinases. It consists of five members, and polo-like kinase 1 (PLK1) is the best-studied kinase that plays a pivotal role in mitosis [1, 2]. PLK1 regulates almost every aspect of mitotic events, including mitotic entry, spindle assembly, chromosome alignment, sister chromatid segregation, metaphase-anaphase transition, cytokinesis, etc. Downregulation of PLK1 or inhibition of its kinase activity often leads to mitotic defects that ultimately result in activation of the spindle assembly checkpoint (SAC) and apoptosis [3, 4].

One of the important functions of PLK1 is to regulate the alignment of chromosomes and the segregation of sister chromatids. It is well established that outer kinetochores phosphorylation by Aurora B decreases upon bi-orientation [5], suggesting that correction is occurring during prometaphase, but it was unclear how the initial stable kinetochores–microtubule (KT–MT) attachments are formed in prometaphase. It was shown recently that in addition to recruiting PP2A to prometaphase kinetochores leading to the dephosphorylation of Aurora B substrates [6], PLK1 specifically promotes the initial establishment of KT–MT

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attachments during prometaphase by suppressing KT–MT dynamics to balance the destabilizing activity of Aurora B [7]. Kinetochores represent a major point of contact between mitotic spindle microtubules and chromosomes [8, 9]. Numerous proteins accumulate at kinetochores to generate a “wait anaphase” signal to maintain SAC activity until all chromosomes have completed bipolar attachment.

Liu et al. showed that maintaining high level of PLK1 at kinetochores after all chromosomes were aligned at metaphase plate resulted in a failure to establish interkinetochore tension, i.e., spindle microtubules failed to exert normal pulling forces on sister kinetochores, and decreased intrakinetochore stretch [7]. Dynamic microtubules are required for the establishment of interkinetochore tension and increase of intrakinetochore stretch [10–12]. This result suggests that PLK1 activity at kinetochores regulates microtubule dynamics, and it must be removed in metaphase to maintain dynamic microtubules and allow the successful separation of sister chromatids [7]. It should be noted that constitutively active PLK1^{T201D} was used in this study [7]. It is, therefore, possible that if wild-type PLK1 was used, the removal of PLK1 from the kinetochores might have been unnecessary as dephosphorylation on PLK1 substrates might provide adequate regulation. In addition, the use of wild-type polo box domain (PBD)-containing Hec1-PLK1^{T201D} might have recruited other PBD interactors to the outer kinetochore and potentially created a non-physiologic function. Furthermore, the PLK1^{T201D} was localized by Hec1 tag to outer kinetochore, which may not necessarily be the right physiologic location for PLK1 as it is difficult to reach many of its substrates from this location.

Microtubules must be dynamic to allow correction of any errors in KT–MT attachment [13, 14]. Increased PLK1 level at metaphase also results in increased attachment errors [7, 15]. In metaphase, PLK1 substrates are dephosphorylated at kinetochores [16, 17], and the dephosphorylation of the PLK1 substrates is likely a result of both the recruitment of protein phosphatase 1 [18] and reduction of PLK1 levels [7]. Thus, the localization of PLK1 on the kinetochores must be regulated in a timely manner to ensure a smooth transition of mitotic events. The molecular mechanism underlying the regulation of PLK1 both spatially and temporally remained unknown until recently.

PLK1 is regulated spatially and temporally during prometaphase/metaphase transition

The multifaceted role of PLK1 in mitosis is preceded by dynamic changes of its sub-cellular localization. Recent results suggest that a fine balance of PLK1 protein levels and its kinase activity is required for chromosome

alignment and faithful chromosome segregation [7, 17, 19]. Initially, PLK1 localizes to centromeres in G1 [20] and G2 phases [21]. Later, a portion of PLK1 accumulates at the kinetochores during the prometaphase stage to promote the initial establishment of KT–MT attachments. Once the KT–MT attachments have been established, most PLK1 needs to be removed from the kinetochores in metaphase to allow for the stabilization of KT–MT interactions, SAC silencing, and anaphase onset. The spatial and temporal regulation of PLK1 from prometaphase to metaphase relies on a delicate molecular mechanism.

The initial association of PLK1 with kinetochores was thought to depend on its binding to PBD-interacting protein 1 (PBIP1), a kinetochore scaffold protein [21]. This binding appears to be self-regulated as PLK1 phosphorylation at Thr-78 of PBIP1 generates a binding motif for PBD. PBIP1 forms a stable complex with another kinetochore component, CENP-Q [22]. It is shown that the PBIP1-CENP-Q complex becomes hyperphosphorylated and rapidly delocalized from kinetochores as cells enter mitosis. PLK1 phosphorylates the CENP-Q subunit of the PBIP1-CENP-Q complex at nine sites to promote the dissociation of CENP-Q from chromatin and prevent the CENP-Q from localizing to interphase constitutive centromere-associated network (CCAN). Interestingly, both the 9A and 9D/E mutants of CENP-Q induce a defect in proper chromosome segregation, suggesting that both timely localization of the PBIP1-CENP-Q complex to CCAN and delocalization from kinetochores are critical for normal mitosis progression. Although PLK1 did not alter the level of PBIP1 and CENP-Q ubiquitination, PLK1-dependent phosphorylation and dissociation of these proteins from kinetochores appeared to indirectly regulate their degradation in the cytosol.

After PBIP1 is degraded in early mitosis, some PLK1 is retained on the kinetochores and centromere through its binding to certain kinetochore-localized proteins, possibly including BubR1, and inner centromere protein (INCENP) [23], respectively. At kinetochores, PLK1 phosphorylates BubR1 [17, 24], and this phosphorylation allows the recruitment of a phosphatase, PP2A-B56, which counteracts the function of microtubule-destabilizing kinase Aurora B [24] and, therefore, stabilizes the initial KT–MT attachments. Testis expressed 14 (Tex14) was also identified as a kinetochore-localized protein that binds to PLK1 in a CDK1-dependent manner [25]. PLK1 phosphorylates Tex14 and recruits it to the kinetochores, and this recruitment appears to be essential for the formation of stable KT–MT attachments [25]. During metaphase, the PLK1-dependent phosphorylation of Tex14 promotes anaphase-promoting complex/cyclosome (APC/C)-mediated Tex14 degradation and metaphase-anaphase transition. Inhibition of this phosphorylation event causes retention of Tex14 at

kinetochores and defects in chromosome segregation and delayed metaphase-anaphase transition. However, Tex14 is unlikely generally required for the formation of stable KT–MT attachments, because Tex14 is not ubiquitously expressed [26], and the defects in Tex14 knockout mice are restricted to germ cells [27]. Recently, chromatin remodeler RSF1 has been identified as an essential protein for the recruitment of PLK1 to kinetochores [28]. CDK1 phosphorylates kinetochore-localized RSF1 at S1375 and generates a PBD-binding motif. PLK1, in turn, further phosphorylates RSF1 and stabilizes the localization of PLK1 on kinetochores, and hence, promotes the initiation of KT–MT attachment.

Dynactin is a protein that forms a complex with dynein. During mitosis, they are required for spindle pole focusing, helping chromosomes engage with and move on spindle microtubules, and removing SAC proteins from kinetochores to facilitate the silence of SAC. Dynactin also helps recruiting PLK1 to kinetochores. CDK1 phosphorylates dynactin at Thr186 of its p27 subunit and generates a binding motif for PBD at kinetochores. Removal of p27 from dynactin results in reduced levels of PLK1 and its phosphorylated substrates at kinetochores in prometaphase, leading to aberrant KT–MT interactions, improper chromosome alignment, and abbreviated mitosis.

It has become very clear that PBD plays an important role in the localization of PLK1 to kinetochores. Recent studies have shown that deubiquitination and ubiquitination of residue(s) in PBD play a major role in regulating the recruitment of PLK1 to the kinetochores in prometaphase and the dissociation of PLK1 from kinetochores in metaphase, respectively.

PBD and its function

There are five PLK members (from PLK1 to PLK5) in humans, all share a closely related catalytic domain at the amino terminus and a characteristic sequence motif, the PBD, in the carboxy-terminal region [29]. The PBD of PLK1–3 is composed of two structurally similar polo box (PB) motifs, PB1 and PB2. The two PBs form a module binding to phospho-Ser/Thr motifs [30]. PLK4 only has one PB that exhibits a lower level of homology with PB1 or PB2. The PB of PLK4 homodimerizes to form a stable dimer [31, 32], and the dimerized PB binds to a target in a way that does not require a phosphorylated motif [33]. It is unclear whether the PBD of PLK5 also binds to a phospho-Ser/Thr motif at this stage.

PLK1 is the most evolutionarily conserved and the best-studied member of the PLKs [34]. The PBD of PLK1 serves as an essential molecular mediator that brings the kinase domain of PLK1 into proximity with its substrates,

and targets PLK1 to specific sub-cellular locations. The binding motif on the substrates usually needs to be pre-phosphorylated [35], and the motif contains [Pro/Phe]-[Φ/Pro]-[Φ]-[Thr/Gln/His/Met]-Ser-[pThr/pSer]-[Pro/X], where Φ represents hydrophobic residues and X means any residues [30, 36]. The phosphorylated motif can be generated by PLK1 itself (self-priming), but in most of the cases, by a kinase other than PLK1, such as CDK1 and CaMKII [37, 38] (non-self-priming). As an example of self-priming, PLK1 phosphorylates the T78 residue of PBIP1 and binds to the resulting phosphorylated motif to recruit PLK1 itself to kinetochores [21], whereas in the case of non-self-priming, CaMK II phosphorylates Emi2 to generate a binding motif for PLK1 during the release of cytostatic factor-induced meiotic cell cycle arrest [37]. Regardless of the mode of priming, by binding to these phosphorylated motifs, PLK1 is targeted to various sub-cellular locations [33].

Usp16 is a novel substrate of PLK1

In an effort to identify novel PLK1 substrates, ubiquitin-specific peptidase 16 (Usp16) was identified as a PLK1 interacting protein in a co-immunoprecipitation (co-IP) assay in a PBD-dependent manner [15]. Usp16 is a deubiquitinase in the USP family, and is able to deubiquitinate mono-ubiquitinated histone H2A at K119 at the execution phase of apoptosis [39]. Its inactivation blocks progression in cell cycle [40]. Primary structure analysis shows that Usp16 contains a BUZ domain at its N-terminus and a catalytic domain at its C-terminus.

PLK1 is able to phosphorylate Usp16 at S330, S386, and S486 *in vitro*, and this phosphorylation has been verified by mass spectrometry analysis of peptides derived from endogenous Usp16 isolated from HeLa cells. Meanwhile, Usp16 is also a substrate of CDK1 that phosphorylates Usp16 at S552, which is within the PBD-interacting region. The phosphorylation of Usp16 by CDK1 creates a binding motif for PBD, and hence, promotes further phosphorylation of Usp16 by PLK1. Importantly, the phosphorylation by PLK1 activates Usp16, as the sequential phosphorylation of Usp16 by CDK1 and PLK1 significantly increases the deubiquitination activity of Usp16 *in vitro*, and decreases the ubiquitination level of histone H2A in mitotic cells [15].

Deubiquitination and cell cycle

Like phosphorylation, ubiquitination is a reversible process of protein modification, and the reverse process is catalyzed by a group of enzymes call deubiquitinase

(DUB). There are five subfamilies of DUBs: (1) the ubiquitin C-terminal hydrolases (UCHs), (2) the ubiquitin-specific proteases/ubiquitin-specific processing proteases (USPs/UBPs), (3) the ovarian tumor proteases (OTUs), (4) the Josephin or Machado–Joseph disease protein domain proteases (MJDs), and (5) the Jab1/MPN domain-associated metalloisopeptidase (JAMM) domain proteins. So far, more than 100 DUBs have been identified in human genome [41]. Ubiquitin ligases and DUBs participate in cell cycle control at almost every level [42]. A main function of the DUBs is to maintain the intracellular ubiquitin level by cleaving ubiquitin off substrates before the tagged substrates being translocated into the proteasome, so the ubiquitin can be recycled for the next ubiquitination. Failure to do so will lead to depletion of intracellular ubiquitin and could cause delay in cell cycle progression [43, 44]. In addition, failure to cleave ubiquitin off the substrates that are already engaged with the proteasome and targeted for degradation would impair proteasome function and, consequently, cell cycle progression [45].

Interestingly, DUBs are often found in the same complex with ubiquitin ligase, such as Usp7 and Mdm2 [46–48]; and Brca1/Bard1 and two DUBs: UCH Bap1 and the JAMM-domain DUB Brcc36 [49, 50]. By regulating the stability or activity of the E3 ligases, DUBs control cell cycle. In the case of Usp7 and Mdm2, depletion of Usp7 results in premature degradation of Mdm2 mediated by Mdm2 itself, and causes the accumulation of p53, a target of Mdm2, leading to cell cycle arrest in G1 or G2 phase.

DUBs also control cell cycle by regulating the stability or activity of some key cell cycle regulators, including transcription factors and cyclins. For example, Usp28 protects c-Myc from SCF^{Fbw7 α} -dependent ubiquitination and degradation and, therefore, promotes c-Myc-induced cell proliferation [51]. Cell cycle progression also depends on the accessibility of chromatin by transcription factors, and the accessibility is directly related to modification of histones. One of these modifications is the transient ubiquitination of histones. The progression through mitosis requires the deubiquitination of histone H2A, and suppression of Usp16 causes elevated level of histone H2A ubiquitination in mitosis and accumulation of mitotic cells [52]. DUBs also regulate cell cycle checkpoints. In mitosis, the SAC suppresses the activity of the APC/C to allow the attachment of microtubules to kinetochores. Once chromosomes are aligned properly, the APC/C is activated, which leads to the ubiquitination of Cdc20 and the dissociation of its inhibitor Mad2 [53]. Usp44, however, counteracts the activity of the APC/C by deubiquitinating Cdc20, thereby preventing premature Mad2-dissociation and SAC silencing [54]. Thus, Usp44 enhances the SAC by directly counteracting APC/C-dependent ubiquitination.

Usp16 deubiquitinates PLK1 and promotes the recruitment of PLK1 to the kinetochores

In the presence of mitotic stress, PLK1 is targeted by Chfr E3 ligase for degradation during G2-M transition, which results in a delay in CDK1 activation, representing a novel checkpoint pathway [55]. PLK1 is also ubiquitinated by cullin 3 (CUL3)-based E3 ubiquitin ligase in mitosis, but this ubiquitination does not lead to PLK1 proteolysis [15, 56]. Surprisingly, it was discovered that while Usp16 is a substrate of PLK1, it can, in turn, deubiquitinate PLK1. Importantly, the deubiquitination of PLK1 by Usp16 promotes the localization of PLK1 to the kinetochores and the proper alignment of chromosome on metaphase plate. It is thought that the localization of PLK1 on the kinetochores depends on its binding to kinetochore-localized proteins, and the deubiquitination of PLK1 most likely facilitates its binding to these proteins. BubR1 is a kinetochore-localized protein, though its interaction with PLK1 seems to not relate to the recruitment of PLK1 to kinetochores, it was found that deubiquitination of PLK1 enhanced its binding towards BubR1, suggesting an increased binding towards other kinetochore-localized PLK1 substrates. Knockdown of Usp16 results in a decrease of PLK1 level on the kinetochores and ~40% chromosomal misalignment [15]. Though the direct connection between the loss of PLK1 on the kinetochores and misalignment of chromosomes has not been firmly established, and the possibility that Usp16 deubiquitinates other substrate(s) on the kinetochores to regulate chromosome alignment in mitosis is not excluded, this result is consistent with the previous report that PLK1 is required for the initial establishment of KT–MT attachment [7].

CUL3-based E3 ligase ubiquitinates PLK1 and promotes the removal of PLK1 from the kinetochores

The E3 ubiquitin ligases can be divided into three major families on the basis of their assembly and mechanism of action: the HECT (homologous with E6-associated protein C-terminus) domain E3s, the RING finger E3s, and RBR (RING-between RING–RING) E3s. HECT E3s accept ubiquitin from E2~ubiquitin to form a covalent thioester intermediate via a conserved cysteine residue of the E3 itself before transferring the ubiquitin on to the substrate. In contrast, RING E3s directly transfer ubiquitin to the substrate by bringing both E2~ubiquitin and the substrate in close proximity to each other. The RBR ligases represent an additional family of E3s that combine characteristics of both HECT and RING families, as they recruit E2~ubiquitin conjugates by an N-terminal RING domain and then

transfer ubiquitin on to a HECT-type C-terminal catalytic cysteine residue of the E3 before final transfer on to the substrate [57].

Cullin-based E3 ligases are a group of RING finger E3s. Cullins are proteins that play a role in post-translational modification of proteins including ubiquitination. The cullin family is highly conserved among species [58]; seven different cullins have been identified in mammals (Cul1, 2, 3, 4A, 4B, 5 and 7) [59, 60]. Each cullin forms a distinct class of cullin-based E3 ligase complex consisting of different adapters and/or substrate recognition subunits [59, 61]. This diversity of functions is given by each of the adapters present in the complex.

Compared to other cullin-based complexes, the cullin complex 3 (CUL3) does not require different adapters to recognize its target protein, but only requires a protein with a bric-a-brac/tramtrack/broad-complex (BTB) domain to recognize it. BTB domains were originally found in transcription factors of *Drosophila melanogaster*, but it is now known that all eukaryotic species express a variety of BTB domain proteins [62]. In recent years, the complex based on CUL3 has been implicated in processes, such as the cell cycle regulation.

CUL3 overexpression promotes the ubiquitination of Aurora-A both in vivo and in vitro. Thus, CUL3 is able to regulate the entrance to mitosis in an Aurora-A-dependent manner by interacting with KLHL18 protein, therefore, mediating the activation of Aurora-A in centrosome. PLK1 has been proven to be a target for CUL3-based E3 ligase complex, and it is also recognized by a BTB protein called KLHL22 that functions as an adapter for the ligase. In the absence of KLHL22, PLK1 is accumulated at kinetochores, promoting the activation of the SAC to ensure the KT–MT attachment and proper chromosome alignment [56, 63].

CUL3/KLHL22 was reported to directly bind PLK1 and ubiquitinates it at Lys 492 and Lys 19. While the function of Lys 19 ubiquitination is unclear, the ubiquitination of Lys 492 located within the PBD leads to the dissociation of PLK1 from kinetochore-localized PBD-interacting proteins [56]. However, a recent report shows that the ubiquitination of Lys 492 may not be so crucial for cell cycle progression [64]. Though the difference in result could be attributed to different cell lines used in the experiments, the exact function of Lys 492 ubiquitination remains to be further investigated. Thus, it is possible that ubiquitination of PLK1 at other site(s) is also important for the dissociation of PLK1 from the kinetochores. KLHL22 associates with the mitotic spindle and its interaction with PLK1 increases as cells achieve proper chromosome alignment. Together, this data suggest that CUL3/KLHL22-mediated ubiquitination signals that are degradation-independent remove PLK1 from kinetochores to satisfy SAC, a process required for faithful progression through mitosis.

The ubiquitination status of PLK1 regulates its kinetochore localization, and hence, the proper chromosome alignment and timely sister chromatid segregation

CUL3-based E3 ligase and Usp16 seem to have antagonized functions on the kinetochore localization of PLK1, as Usp16 knockdown results in the reduction of kinetochore-localized PLK1 level, and KLHL22 knockdown leads to increased level of kinetochore-localized PLK1. Since Lys 492 is located within PBD, it is, therefore, possible that the equilibrium of Usp16-mediated deubiquitination and Cul3-based E3 ligase-mediated ubiquitination at this site regulates the interaction between PBD and its kinetochore-localized binding partners, and, hence, the localization of PLK1 on kinetochores. As the localization of PLK1 on the kinetochores in early mitosis is important for the establishment of the initial KT–MT attachments and proper chromosome alignment, and the removal of PLK1 from the kinetochores at metaphase is required for sister chromatid segregation, the deubiquitination/ubiquitination equilibrium, therefore, appears to be a key mechanism regulating proper chromosome alignment and timely sister chromatid segregation.

Studies from Zhou et al. and Beck et al. have deciphered the molecular mechanism underlying the regulation of this deubiquitination/ubiquitination equilibrium. As illustrated in the model (Fig. 1), in early mitosis when PLK1 activity increases, Usp 16, mainly in cytosol, is phosphorylated and activated, which, in turn, deubiquitinates a portion of PLK1 and promotes the binding of PLK1 to kinetochore-localized proteins. Though the identity of these kinetochore-localized PLK1 binding partners has not been revealed, Usp16 and BubR1 are two probable candidates. The deubiquitination of PLK1 by Usp16 not only promotes the recruitment of PLK1 to kinetochores, but also retains PLK1 on the kinetochores to ensure the establishment of KT–MT attachment. Once chromosomes are properly aligned and SAC is satisfied, this portion of PLK1 is then removed from the kinetochores to allow the timely segregation of sister chromatids. This is achieved by CUL3-based E3 ligase that ubiquitinates PLK1 at Lys 492 and possibly other sites as well, which is likely to disrupt the interaction between PBD and its binding partners, resulting in the dissociation of PLK1 from the kinetochores. How exactly the CUL3-based E3 ligase is recruited to the kinetochores at metaphase is unclear at moment, but it was shown that the association of KLHL22 with the mitotic spindle and kinetochore-localized PLK1 increases as cells achieve chromosome bi-orientation [56], suggesting that KLHL22 may be involved in the recruitment. It was reported recently that a portion of mitotic PLK1 localizes close to the inner kinetochore, internal to the histone variant CENPA [65], and

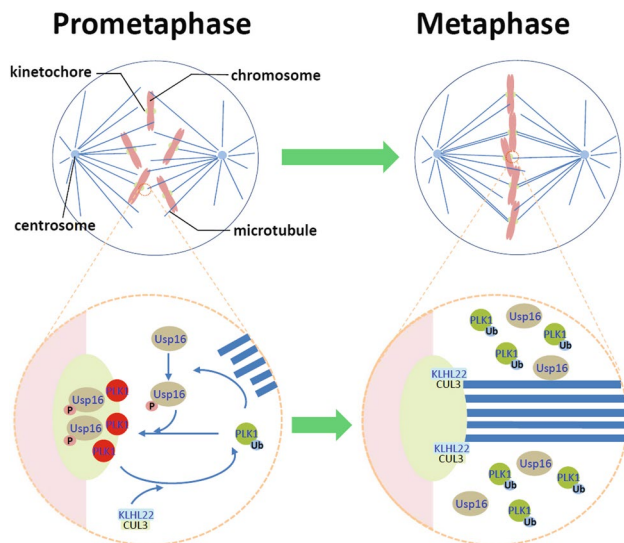


Fig. 1 Equilibrium of ubiquitination and deubiquitination at PLK1 regulates its localization on the kinetochores and KT–MT attachment. In prometaphase, activated PLK1 phosphorylates and activates Usp16, which, in turn, deubiquitinates PLK1, leading to the localization of PLK1 to the kinetochores. The kinetochore-localized PLK1 promotes the establishment of initial KT–MT attachment and proper chromosome alignment. Once the SAC is satisfied at metaphase, CUL3-based E3 ligase is enriched on the kinetochores and ubiquitinates PLK1, resulting in the dissociation of PLK1 from the kinetochores. The dissociation of PLK1 stabilizes the KT–MT attachment and allows the segregation of sister chromatids. As detailed in the text, it should be noted that a portion of PLK1 localized at inner kinetochore and centromere may not subject to this regulation

also binds to inner centromere-localized proteins INCENP and MCAK [23, 66]. As a portion of PLK1 remains at the kinetochores during anaphase [20], it is possible that this portion of inner kinetochore and/or inner centromere-localized PLK1 may not subject to ubiquitination mediated by CUL3-based E3 ligase.

Concluding remarks

Regulating proper chromosome alignment and timely sister chromatid segregation are two important functions of PLK1. It requires PLK1 being recruited to and removed from kinetochores at particular stages of mitosis. It is now evident that the recruitment and retention of PLK1 on kinetochores is regulated by Usp16-mediated deubiquitination, and the removal of PLK1 from kinetochores is promoted by CUL3-based E3 ligase that ubiquitinates PLK1 at Lys 492 located in PBD. Usp16 is phosphorylated and activated by PLK1 in early mitosis, but this phosphorylation does not regulate the localization of Usp16 on kinetochores. When and how Usp16 is recruited to and removed from kinetochores remain to be investigated. On the other hand,

CUL3-based E3 ligase seems to be present throughout the cell cycle, but only becomes enriched at kinetochores when the chromosomes are properly aligned. What is the mechanism that senses the timing and recruits the E3 ligase complex to kinetochores? These are some questions need to be addressed to completely understand the spatial and temporal regulation of PLK1, and hence, the mechanism underlying the regulation of chromosome alignment and sister chromatid segregation.

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