Molecular dynamics of PLK1 during mitosis

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Abbreviations: BUB1, budding uninhibited by benzimidazole 1 homolog; BUBR1, BUB1 β; IDL, inter domain linker; KD, kinase domain; KT–MT, kinetochore–microtubule; PLK-1, polo-like kinase 1; PBD, polo-box domain; PBIP1, polo-box interacting protein 1; PRC1, protein regulator of cytokinesis 1; SAC, spindle assembly checkpoint

Introduction

Protein kinases are essential regulatory factors that orchestrate a plethora of cellular processes in eukaryotic organisms. Their catalytic activity allows modification of specific proteins by phosphorylation, thus modulating their biochemical properties. This reversible modification can regulate substrate recognition by specific effectors, localization, conformational changes, and even protein activation or inhibition. Members of the polo sub-family of serine/threonine (Ser/Thr) protein kinases (polo-like kinases, PLKs) have emerged as important regulators of cell cycle progression. Polo-like kinase 1 (PLK1) represents the most-studied PLK family member and is crucially involved in the spatiotemporal control of mitosis.

Mitotic division allows the generation of 2 identical daughter cells from one parental cell, which is essential during organismal development and cell renewal. Mitosis can be divided into 5 stages: prophase, prometaphase, metaphase, anaphase, and telophase (Fig. 1). Entry into mitosis is a highly controlled transition during which the cell has to ensure that duplication of DNA and chromosomes has been performed properly. During prophase, the nuclear envelope breaks down and chromosomes start to condense and individualize. In prometaphase, the mitotic spindles originating from each centrosome start to attach to individual chromosomes via centromere-associated proteinaceous structures called kinetochores. In metaphase, all of the chromosomes should be correctly attached to the spindle microtubules and perfectly aligned at the metaphase plate. The metaphase to anaphase transition is under the control of the spindle assembly checkpoint (SAC) network that prevents chromosome segregation in the presence of unattached kinetochores. Only upon correct attachment of both kinetochores of all chromosomes to the spindle microtubules are the sister chromatids separated and pulled to the opposite spindle poles. During anaphase the midzone structure is built with the help of intercalating microtubules in the middle of the cell that define the future location of the cytokinetic cleavage furrow. The remnants of the midzone constitute the midbody in telophase, when final abscission and completion of cytokinesis take place to allow separation of the 2 identical daughter cells (Fig. 1).

PLK1 plays pleiotropic roles during mitosis and is required from the start until the end of the mitotic division. PLK1 controls mitotic entry, centrosome separation and maturation, chromosome arm resolution, microtubule–kinetochore attachments, SAC silencing, and cytokinesis (Fig. 1). Inhibition or depletion of PLK1 leads to arrest of cells in a prometaphase-like state, with unseparated centrosomes and monopolar spindles and defects in microtubule–kinetochore attachment and chromosome alignment. Interestingly, it was shown that bipolar spindles can still be formed if full activation of PLK1 is prevented, but localized and high PLK1 activity is absolutely required at kinetochores to promote stable attachments to spindle microtubules.

Here, we review current knowledge on the molecular mechanisms controlling PLK1 activity during mitosis. PLK1 is dynamically regulated at the level of its localization, activation, and conformation during mitotic progression. Understanding the precise mechanisms of PLK1 regulation is of great interest because dysregulation of the spatiotemporal control of mitosis is implicated in aneuploidy and carcinogenesis. Not surprisingly, the level of PLK1 expression has prognostic value for...
predicting outcomes in patients with some cancers. Thus, a number of specific PLK1 inhibitors have been developed, some of which have progressed into clinical studies in patients with locally advanced or metastatic cancers. This review discusses available data and concepts related to the dynamic regulation of human PLK1 activity during mitosis rather than how PLK1 controls mitotic progression by phosphorylation of its numerous substrates. To learn more about the latter aspects we direct the reader to several other interesting reviews.

PLK1 and the Polo-like Kinase Family

In humans, 5 PLK family members (PLK1 to 5) that perform different functions during cell cycle progression have been identified to date. All PLKs have a similar architecture and share a conserved N-terminal Ser/Thr kinase catalytic domain and one or more C-terminal polo boxes that are involved in substrate recognition and are referred to as the polo-box domain (PBD; Fig. 2). The first PLK members were identified in Drosophila (polo) and in Saccharomyces cerevisiae (Cdc5) by screening for gene mutations that result in defective cell division. The human ortholog PLK1 has been a focus of numerous studies and rapidly accumulating evidence has confirmed its role in diverse cellular events during mitotic division, including centrosome maturation, kinetochore function, mitotic spindle formation, and cytokinesis. PLK2 expression and activity is mainly required during S phase for the control of centriole duplication and centrosome maturation whereas PLK3 is involved in cellular stress control and at the G1/S transition for the promotion of DNA replication. PLK4 is a critical regulator of centriole duplication. However, the different members of the PLK family may play overlapping roles in some cellular aspects during cell cycle progression. A fifth member of the PLK family was described more recently. Despite an apparent loss of catalytic activity due to the presence of a stop codon in the kinase domain of the human gene that generates a kinase-deficient PBD-containing protein, PLK5 plays an important role in neurons.

Molecular architecture of PLK1

PLK1 is the most evolutionarily conserved member of the polo-like kinase family. PLK1 is expressed in embryonic tissues during development and predominantly in proliferative tissues in adults. PLK1 expression is regulated in a cell cycle-dependent manner, with basal expression in interphase cells that starts to increase at the completion of S phase and continues during G2 and mitosis. The peak level of expression is observed during the mitotic phase and degradation occurs during mitotic exit. The expression level correlates with an increase in kinase activity during G2, which can be detected by phosphorylation status.
of the Thr210 residue in the activation loop of the kinase domain, reaching a maximum during mitosis.23

The N-terminal kinase domain (KD) of PLK1 is followed by the inter domain linker (IDL) and the C-terminal PBD domain (Fig. 2). The KD of PLK1 is similar to the corresponding domains of other Ser/Thr kinases, whereas the PBD is a characteristic and unique feature of the PLK family. The PBD plays an essential role in targeting PLK1 to different subcellular structures and thereby determines the mitotic distribution of PLK1 and its localized activities.7,24-27

Analysis of the crystal structure of the PBD of PLK1 bound to a phosphopeptide revealed that the 2 polo-boxes display the same folding (6-stranded anti-parallel β-sheet and α-helix) and form a heterodimeric phospho-binding module (also called a phospho-pincer).28,29 This module can specifically recognize a sequence motif composed of Ser-(pThr/pSer)-(Pro/ X).29 Specific residues within the PBD domain mediate binding to phosphorylated substrates and constitute the phospho-pincer region. The residues Trp414 (W414) and Leu490 (L490) located in the first polo-box are critical for substrate recognition through non-polar interactions, whereas residues His538 (H538) and Lys540 (K540) in the second polo-box motif are directly involved in the interaction with the negatively charged phosphate groups on Thr/Ser residues of the substrates (Fig. 2).29,30 Interestingly, all 4 residues are conserved in the PBDs of PLK1, 2, and 3, emphasizing the importance of this substrate recognition mechanism within the PLK family. It is now widely accepted that the PBD domain is critical for targeting the catalytic activity of PLK1 to specific subcellular locations.7,29,31

Indeed, a single W414F mutation is sufficient to disrupt localization and function of PLK1 without affecting its kinase activity.25 Moreover, overexpression of a PBD construct in human cells has a dominant-negative effect and interferes with the normal localization and function of endogenous PLK1, leading to cell death after prolonged mitotic arrest,32 similar to siRNA-mediated downregulation or inhibition of PLK1.6,9

**Regulation of Mitotic Dynamics of PLK1**

**Localization of PLK1 to mitotic structures**

One of the most striking features of PLK1 is its dynamic localization to various subcellular structures during the successive steps of mitotic division. PLK1 dynamics are mediated by specific interactions with phosphorylated substrates or receptors at various subcellular locations. During late G2 phase and in prophase PLK1 localizes to the centrosomes, whereas in prometaphase and metaphase PLK1 is enriched on kinetochores. Following chromosome segregation in anaphase, PLK1 progressively accumulates at the midzone and finally localizes to the midbody during telophase (Fig. 1). Importantly, the localization of PLK1 to mitotic structures is dynamic and nuclear PLK1 can rapidly exchange with the cytoplasmic pool.33 However, the rates of exchange differ at distinct locations with the highest dynamics at the centrosomes, a phenomenon that is crucial for maintenance of centrosome organization as well as for spindle formation and chromosome segregation.33,34 At the G2/M transition, PLK1-dependent signaling and feedback loops control CDK1/cyclin B activation and thereby timely mitotic entry.35 PLK1 was also shown to regulate nuclear envelope breakdown during prophase by phosphorylating p150 Glued dynactin.36 Centrosomal localization of PLK1 controls recruitment of the pericentriolar material (PCM) and converts centrosomes into active nucleation centers for spindle microtubules.37 CDK1-dependent phosphorylation of the receptor protein cenexin 1 recruits PLK1 to centrosomes through a non-self priming mechanism (see below)14,38 and the main centrosomal targets of PLK1 are ninein-like protein and pericentrin.3 During prometaphase PLK1 remains localized to centrosomes, thus controlling bipolar spindle assembly, and accumulates on kinetochores, playing an important role in kinetochore–microtubule (KT–MT) attachments.6,8 Early binding of PLK1 to kinetochores is mediated by its interaction with polo-box interacting protein 1 (PBP1).39 In turn, PLK1 phosphorylates PBP1 and initiates its degradation in early mitosis. To maintain its kinetochore localization, PLK1 binds to other kinetochore receptors including budding uninhibited by benzimidazole 1 homolog (BUB1), BUB1 β (BUBR1), and inner...
centromere protein (INCENP). 3,40–42 The mechanisms by which PLK1 controls KT–MT attachments are not entirely clear although PLK1-mediated phosphorylation of Ser676 of BUBR1 was demonstrated to be important for this process. 40 Binding of PLK1 to protein regulator of cytokinesis 1 (PRC1) regulates its localization to the spindle midzone during anaphase. PRC1 interacts with the intercalating antiparallel microtubules at the midzone and this interaction is negatively regulated by CDK1. PLK1 controls its own recruitment to midzone microtubules by phosphorylating PRC1 on Thr602 through a self-priming mechanism (see below). 43 During cytokinesis PLK1 accumulates at the midbody, where it regulates important factors required for abscission process such as mitotic kinesin-like protein 2 (MKLP2), nuclear distribution gene C homolog (NUDC), and centrosomal protein 55 kDa (CEP55). 3,40

Regulation of PLK1 localization by priming

The dynamic localization of PLK1 during mitosis is controlled by PBD-mediated interactions with specific phosphoepitopes present in different subcellular structures (Fig. 3). In the current model, PLK1 substrates are initially phosphorylated by other kinases to allow PLK1 recruitment in a process called non-self priming. 14,26 Available data demonstrate that PLK1 can also create its own docking site on some substrates, confirming the existence of the so-called self-priming mechanism of PLK1.

Interestingly, a high number of PLK1 targets have been identified, most of which are phosphorylated by CDK1 and to a lesser extent by MAPK. 44 Thus, activities of the upstream kinases provide an additional layer of regulation of PLK1 dynamics and ensure completion of the earlier mitotic events before the action of PLK1. One of the best-studied examples of non-self priming is recruitment of PLK1 to centromeres by cenexin 1. 38 CDK1 phosphorylates cenexin 1 on Ser796, promoting interaction with the PBD and allowing correct recruitment of PLK1 to centromeres and thereby faithful progression of mitotic division.

In the self-priming model, PLK1 creates its own docking sites by phosphorylating specific substrates, which is predicted to enhance their interaction with PLK1. It was initially found that chemical inhibition of PLK1 leads to its dissociation from centromeres, kinetochores, and midzone/midbodies, 6 confirming that PLK1 somehow controls its own localization during mitosis. The best-studied example of the self-priming mechanism comes from the interplay between PLK1 and one of its kinetochore-binding partners, PBIP1. 39 Phosphorylation of Thr78 of PBIP1 on kinetochores by PLK1 promotes its own recruitment, which consequently triggers phosphorylation of more kinetochore-associated molecules of PBIP1 and further recruitment of PLK1 in a product-activated feed-forward mechanism. 45

Removal of PLK1 from mitotic structures

The localized activity of PLK1 at kinetochores is required for establishment of stable KT–MT attachments prior to metaphase. 40 During metaphase, the kinetochore levels of PLK1 decrease dramatically, allowing silencing of the spindle assembly checkpoint (SAC) and chromosome segregation. If kinetochore dissociation of active PLK1 is prevented by its fusion to a constitutive kinetochore component, the mitotic spindle is disorganized and correct attachments are inhibited. 46 Likewise, persistent tethering of PLK1 to the centrosomes results in defective spindle formation and activation of the SAC. 33 These results emphasize the importance of PLK1 dynamics and its timely removal from the mitotic structures for anaphase onset.

Our own findings suggest a model in which removal of PLK1 from kinetochores is mediated by a non-proteolytic ubiquitylation of PLK1 by the E3-ligase complex composed of cullin3 (CUL3) and the adaptor protein kelch-like protein 22 (KLHL22). 47 We demonstrated that KLHL22 interacts directly with PLK1 through its kelch domain and that the CUL3/KLHL22 complex is able to ubiquitylate PLK1 on Lys492. Interestingly, this residue is located within the PBD domain of PLK1 in the region between the 2 polo-box motifs near the phospho-binding region (Fig. 2). Our results suggested that this modification could disrupt the PBD-dependent phospho-binding capacity of PLK1 or possibly cause a conformational change in the folding of the PBD domain. Indeed, BUBR1 is phosphorylated on Ser676 by PLK1 and regulates the stability of MT–KT attachments, thus controlling SAC signaling that prevents premature onset of anaphase. 40 The self-priming phosphorylation of BUBR1 by PLK1 enhances its own recruitment to kinetochores via the PBD domain. Our data strongly suggest that ubiquitylation of PLK1 on Lys492 prevents interactions with the kinetochore receptors including BUBR1, leading to dissociation of PLK1 from kinetochores, SAC silencing, and anaphase onset. 47 Interestingly, it was also reported that kinetochore recruitment of phosphatases such as PP1 inhibits PLK1 association, most likely by dephosphorylating potential binding sites for the PBD domain. 46 Since CUL3/KLHL22 does not regulate the centrosomal localization of PLK1, 47 it will be interesting to determine the precise molecular mechanisms underlying the centrosomal dynamics of PLK1.

Regulation of PLK1 Activity During Mitosis

PLK1 expression and activity increase at the G2/M transition and peak during mitosis. 4 Phosphopeptide mapping and mass spectrometry analysis identified phosphorylation of Thr210, located in the T-loop inside the KD (Fig. 2), as the main post-translational modification on PLK1 during mitotic division. 48,49 Substitution of Thr210 with alanine leads to partial inactivation of PLK1 activity and its replacement with aspartic acid results in constitutive activation. 24,50 Therefore, phosphorylation of Thr210 is considered the first step triggering PLK1 activation during mitosis (Fig. 3). Several upstream kinases were proposed to activate PLK1 during mitosis. Protein kinase A and Ste-like kinase (SLK) were shown to phosphorylate Thr210 of PLK1 or the corresponding residue of the Xenopus Plx1 homolog, in vitro. 49,51,52 However, involvement of these kinases in the activation of PLK1 in vivo remains controversial. Several laboratories reported that aurora A is the initial kinase responsible for activation of PLK1 during G2 and transition to mitosis. 53,54 Indeed, initial activation of PLK1 seems to occur at centrosomes, where
aurora A is localized before centrosome duplication. Both chemical inhibition and RNA interference of aurora A prevent phosphorylation of Thr210 of PLK1 on centrosomes during prophase. Moreover, Thr210 phosphorylation correlates with an increase in aurora A kinase activity.\(^{53}\) Activated PLK1 is also observed on kinetochores at a low level during prophase and increasing until the later stages of mitosis (peaking at the prometaphase to metaphase transition), but the mechanism of kinetochore-associated PLK1 activation remains unclear. It would be interesting to use advanced microscopy techniques to analyze the fate of the centrosome-activated PLK1 to determine whether it relocates to kinetochores or whether there is a need for kinetochore-associated activating kinases. However, recent data suggest that once PLK1 is activated at centrosomes minimal amounts of aurora A kinase activity are required to sustain full PLK1 activity until the end of mitosis, thus confirming aurora A as the major PLK1 activator.\(^{55,56}\)

Another factor that contributes to PLK1 activation is the co-activator of aurora A, BORA. Interestingly, expression of BORA protein follows the same profile as that of aurora A, peaking during late G2 with a drastic decrease during mitosis.\(^{54,57}\) This correlation was the first argument for a role of BORA in the activation of PLK1. Importantly, BORA was also shown to form a complex with PLK1 in G2 phase.\(^{53,54}\) This interaction is enhanced by CDK1-dependent non-self priming of BORA and

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**Figure 3. Dynamic regulation of PLK1 in mitosis.** In the auto-inhibited state (upper diagram) the kinase domain (KD, green) and polo-box domain (PBD, orange) of PLK1 fold onto and reciprocally inhibit each other. The initial activation of PLK1 is triggered either by phosphorylation of T210 and/or S137 in the KD by the aurora A/BORA complex or other kinases, or by binding of the PBD to a phospho-substrate in a self or non-self priming manner (see text for details). These modifications trigger a conformational change that separates the KD from the PBD and converts PLK1 to a DFG-in, active state (activated PLK1; middle diagram). Inhibition of total or localized kinase activity of PLK1 can be achieved in many different ways (inactivated PLK1; lower diagram). Dephosphorylation of substrates by PP1C/MYPT1 or other phosphatases can reduce interactions mediated by the PBD. Mono-ubiquitylation of K492 by CUL3/KLHL22 can trigger removal of the active PLK1 specifically from kinetochores, whereas poly-ubiquitylation by APC/C or other E3-ligases can lead to proteasomal degradation of PLK1 thereby attenuating its total kinase activity. The modifications that control the orientation of the activation loop of PLK1 (DFG motif) and can lead to conformational changes that inactivate the kinase (DFG-out) are currently unclear. DFG motif, Asp194, Phe195, Gly196.
by the presence of BORA in vitro.\textsuperscript{54,57} \hspace{1em} In agreement with the crucial role of Thr210 phosphorylation in the activation of PLK1, depletion of aurora A or BORA delays mitotic entry, reminiscent of PLK1 knockdown.\textsuperscript{9} However, one study showed that depletion of BORA does not lead to the formation of monopolar spindles,\textsuperscript{57} as commonly observed after depletion of PLK1.\textsuperscript{9} These results can be explained by the fact that PLK1 inactivation leads to accumulation of BORA, which can induce aurora A mis-localization leading to monopolar spindle formation.\textsuperscript{57} Indeed, PLK1 phosphorylates BORA to create a phospho-degron that is recognized by the βTrCP subunit of the SCF ubiquitin ligase that targets BORA for proteasomal degradation.\textsuperscript{54,57} Co-depletion of BORA and PLK1 partially restores the localization of aurora A and thus bipolar spindle formation, indicating that the phenotype of PLK1 depletion is at least in part caused by accumulation of BORA. However, another recent study showed that reduced expression of BORA in human cells does lead to monopolar spindles, similar to aurora A depletion.\textsuperscript{55} Moreover, co-depletion of aurora A and BORA triggered monopolar spindle formation to the same level as reduced expression of PLK1, suggesting that minimal levels of aurora A and BORA are sufficient for sustained activity of PLK1. Nevertheless, it would be interesting to know whether BORA-independent mechanisms of PLK1 activation exist at distinct locations during mitotic progression.

The status of phosphorylated Thr210 is also under the control of specific phosphatases, suggesting the importance of fine spatio-temporal regulation of PLK1 activity during mitotic progression (Fig. 3). It was shown that PP1C is able to remove phosphorylation on Thr210, a mechanism that is initiated by priming of the regulatory PP1C subunit MYPT1, which promotes the interaction with PLK1.\textsuperscript{58} In Drosophila the mitotic kinase Greatwall was shown to counteract aurora A activation on PLK1,\textsuperscript{59} but these findings remain to be confirmed in human cells. Interestingly, depletion of the B56-PP2A phosphatase, which is inhibited by Greatwall in mitosis,\textsuperscript{60} increases the phosphorylation level of PLK1 and its recruitment to kinetochores.\textsuperscript{61}

In addition to Thr210, another important site of PLK1, Ser137, was found to be modified during mitosis and important for PLK1 activation (Fig. 2). Substitution of Ser137 with aspartic acid is also reported to increase PLK1 kinase activity.\textsuperscript{24} However, in contrast to Thr210, phosphorylation of Ser137 is not detected in G2 cells\textsuperscript{62} and is not required for initial activation of PLK1 in G2.\textsuperscript{53} Thus, the exact role of this phosphorylation event in PLK1 activation and the upstream kinase that modifies this residue during mitosis await further investigation.

PLK1 activity is maintained until telophase, when it plays an important function in completion of cytokinesis (Fig. 1).\textsuperscript{63} However, a dramatic decrease in PLK1 levels is observed after anaphase onset. This is a consequence of proteasomal degradation of PLK1 orchestrated by the anaphase promoting complex/cyclosome (APC/C) E3-ubiquitin ligase, one of the main regulators of the metaphase to anaphase transition and mitotic exit in human cells.\textsuperscript{64} Interestingly, the IDL region of PLK1 contains a canonical D-box motif RKPLTVLNK (aa 337 to 345, of which residues in bold are essential for recognition by APC/C; Fig. 2). Mutagenesis of R337 or L340 residues renders PLK1 non-degradable and leads to a delay in mitotic exit.\textsuperscript{64}

**Autoinhibition and Conformational Dynamics of PLK1**

More recent biochemical and structural data indicate that the PBD also functions as a negative regulator of the KD in PLK1, possibly through intramolecular interactions between the 2 domains.\textsuperscript{24,65} Indeed, the kinase activities of PLK1 C-terminal truncation mutants are higher than those of the full-length protein, indicating that the PBD domain may fold back on the kinase domain.\textsuperscript{24,65,66} Reciprocal inhibition of the interaction of the PBD with phosphopeptides by the KD was also proposed.\textsuperscript{29} To date, crystallographic structural data have been obtained only for the isolated PBD or KD,\textsuperscript{28,29,67} and lack of a crystal structure of the full-length PLK1 protein currently hampers studies on the autoinhibitory interactions between KD and PBD.

Nevertheless, a recent study provides new hints into the autoinhibitory regulation of PLK1 in zebrafish.\textsuperscript{66} The authors report a crystal structure of the complex of KD and PBD (produced independently) together with the PBD-binding motif of the microtubule-associated protein 205 (Map205) from Drosophila and propose a model in which PBD binds and rigidifies the IDL of the KD in a distinct conformation compared with that of the phosphopeptide-bound PBD. Thus, the autoinhibitory state can be achieved either by reduction of the flexibility of the hinge region or sequestration of the activation loop. During mitosis, phosphorylation of Thr210 or Ser137 by aurora A/BORA or other PLK activating kinases would prevent sequestration of the activation loop and ultimately disrupt intramolecular interactions between the IDL, PBD, and the kinase domains. In this model, binding of phosphorylated targets to the PBD domain would also help to disrupt intramolecular interactions, leading to full activation of PLK1 (Fig. 3).\textsuperscript{23,66} In future studies it would be interesting to test the importance of the conformation of the flexible activation loop of the KD for PLK1 binding to its substrates. Indeed, the beginning of the activation loop contains the characteristic DFG motif (Asp\textsuperscript{194}, Phe\textsuperscript{195}, Gly\textsuperscript{196}), a sequence determinant shared by many other protein kinases.\textsuperscript{68} As for other kinases, PLK1 is predicted to adopt either a DFG-in (active) or a DFG-out (inactive) conformation.\textsuperscript{69,70} Although crystal structures of the inactive conformation states are available only for a handful of kinases, they serve as templates for molecular simulations of the conserved kinase domains. In the DFG-in active conformation, the aspartate side chain is proposed to face the active site in order to facilitate catalysis and is also involved in the binding of the magnesium ions required for kinase activity. In addition, the neighboring phenylalanine residue occupies a hydrophobic pocket near the ATP-binding site. In the DFG-out conformation, these 2 residues are translocated away from the active site and the hydrophobic pocket. It would be important to understand which factors and which post-translational modifications orchestrate these conformational changes and how they affect mitotic functions of PLK1 (Fig. 3). Interestingly, new type
II inhibitors of PLK1 that freeze the enzyme in the DFG-out conformation were recently reported and will be useful tools for future studies. Furthermore, it would be very interesting to study the requirement for the KD in the recruitment of some PLK1 partners such as BORA or KLHL22 that were shown to bind both to the KD and to PBD in contrast to most PLK1 substrates, which exclusively contact the PBD domain. One could speculate that BORA and/or KLHL22 are involved in the regulation of conformational changes of PLK1.

Additionally, intramolecular interactions between the 2 polo-boxes within the PBD domain were shown to be crucial for recognition of substrates and phospho-substrates by PLK1. Interestingly, the CUL3/KLHL22-ubiquitylated lysine residue that promotes removal of PLK1 from kinetochores in metaphase is located between the 2 polo-boxes (Fig. 2). Therefore it would be interesting to determine whether this modification disrupts intramolecular interactions inside the PBD domain to trigger PLK1 dissociation from kinetochores and its kinetochore anchor proteins.

Conclusions and Future Directions

PLK1 is a key player orchestrating many essential steps during mitotic division, and several layers of regulation control the dynamic behavior of PLK1 during different mitotic stages. The regulatory PBD domain is a specific feature of the PLK family and is now firmly established as a substrate-bindng interface. Thus, PBD-mediated interactions are the basis for the dynamic mitotic localization of PLK1. In addition, post-translational modifications, such as phosphorylation and ubiquitylation, within the PBD and KD crucially modulate PLK1 activity and might be correlated with conformational changes of the PLK1 protein. Studying the relationship between localized PLK1 activity and its structural changes during mitotic progression will be a major future challenge and will enhance our understanding of the dynamic regulation of this kinase in time and space. Newly developed fluorescent probe sensors for PLK1 activity have already uncovered the dynamics of PLK1 at the centrosomes and kinetochores and will be important for following the conformational changes of PLK1 during mitotic progression at distinct locations, similar to sensors previously used for protein kinase C. The recent development of new type II inhibitors for PLK1 that do not target the ATP-binding pocket but modulate the activation loop dynamics (DFG conformation) may also facilitate future studies on PLK1 dynamics. In addition, there is an urgent need for structural data on full-length mammalian PLK1 in a complex with different substrates and regulatory factors. Recent findings suggest that PLK1 also plays some critical roles beyond mitosis. Indeed, PLK1 has emerged as a key regulator of the maintenance of genomic stability during DNA replication and the DNA damage response. We believe that future studies are needed to understand the precise roles of mammalian PLK1 in vivo using relevant animal models. These studies will very likely benefit from the recent development of genome editing technologies, such as the CRISPR-Cas9 system. Undoubtedly, the complex regulation of PLK1 dynamics will continue to fascinate researchers in the future.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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