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REVIEW

Polo-box domain: a versatile mediator of polo-like kinase function

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Abstract Members of the polo subfamily of protein kinases have emerged as important regulators in diverse aspects of the cell cycle and cell proliferation. A large body of evidence suggests that a highly conserved polo-box domain (PBD) present in the C-terminal non-catalytic region of polo kinases plays a pivotal role in the function of these enzymes. Recent advances in our comprehension of the mechanisms underlying mammalian polo-like kinase 1 (Plk1)-dependent protein–protein interactions revealed that the PBD serves as an essential molecular mediator that brings the kinase domain of Plk1 into proximity with its substrates, mainly through phospho-dependent interactions with its target proteins. In this review, current understanding of the structure and functions of PBD, mode of PBD-dependent interactions and substrate phosphorylation, and other phospho-independent functions of PBD are discussed.

Keywords Polo kinase · Plk1 · Polo-box domain · Mitosis · Cell proliferation

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Introduction to polo-like kinases

Discoveries of Plks Named after a defective spindle pole phenotype associated with *Drosophila* gene *polo* (which literally means "pole" in Spanish) mutations [1], the polo-like kinases (collectively, Plks) are a conserved subfamily of Ser/Thr protein kinases that play pivotal roles during the cell cycle and proliferation. In mammalian cells, four Plks that apparently exhibit differential functions and tissue distributions are expressed from distinct genetic loci (see reviews in [2–4]). They include three closely related members, Plk1 [5–8], Plk2 (also called Snk) [9, 10], and Plk3 (also called Prk or Fnk) [11, 12], and one distantly related member, Plk4 (also called Sak) [13]. Homologous to those in mammals, at least three related Plks, named Plx1/Plc1, Plx2/Plc2, and Plx3/Plc3, have been isolated from frogs and worms. On the other hand, organisms such as *D. melanogaster*, *S. cerevisiae*, and *S. pombe* appear to possess a single Plk (polo [1], Cdc5 [14], and Plo1 [15], respectively). Although it is not known whether the low eukaryotic organisms accomplish the function of all isoforms of Plk1–4 (collectively, Plks) using one homolog, it is likely that higher eukaryotic organisms have acquired functionally diversified Plks to cope with various physiological challenges for multicellular organisms. The function of Plks in various organisms has recently been extensively reviewed (see reviews in [2, 16, 17]).

One of the characteristic features of the Plks is the presence of the polo-box domain (PBD) in their respective C-terminal non-catalytic region. PBD is thought to play a critical role in targeting the catalytic activity of the enzymes to specific subcellular structures [18]. Therefore, understanding the PBD-dependent interactions and, furthermore, the spatiotemporal regulation of these events is central to better comprehend the physiological functions of

Plks. In this review, we will primarily focus on discussing our current understanding of the structure and function of PBD and the underlying mechanisms of PBD-dependent biochemical and cellular events in mammalian Plks.

Differential functions of mammalian Plks Among the four mammalian Plks, Plk1 has become the most extensively studied enzyme because of its tight association with oncogenesis. Plenty of evidence suggests that Plk1 regulates diverse cellular and biochemical events at multiple stages of M phase, including centrosome maturation, bipolar spindle formation, DNA damage adaptation, mitotic entry, activation of anaphase promoting complex, and cytokinesis (see reviews in [2, 3, 16, 17]). Consistent with the critical functions of Plk1 during M-phase progression, Plk1 is highly expressed in tumors of various origins. Consequently, Plk1 is thought to be an attractive anticancer drug target [19] that is selectively required for cancer cell viability [20, 21]. Paradoxically, however, downregulation of Plk1 also appears to promote tumorigenesis [22, 23], suggesting that the proper balance of Plk1 activity is important to ensure normal mitotic progression and cell proliferation.

Data accumulated from various studies suggest that mammalian Plks have both distinct and partially overlapped roles during the cell cycle and proliferation as evidenced by the differential expression patterns of these kinases [4]. During the cell cycle, Plk1 is highly expressed at the late G2 and M phases of the cell cycle [24, 25]. However, Plk2 has been shown to be transiently expressed in G1 [9, 26]. Plk2 appears to contribute to S phase entry [27] and plays a role in maintaining cell viability after spindle poisoning [28]. Plk3 is expressed at a constant level throughout the cell cycle [29]. Plk3 regulates mitotic entry and cytokinesis [30, 31], and appears to be activated in response to DNA damage and cellular stress [11, 32–36]. Both Plk2 and Plk3 are proposed to function as tumor suppressors [37, 38]. Interestingly, overexpression of either mammalian Plk1 or Plk3 rescues the lethality associated with the loss of the budding yeast polo kinase homolog Cdc5 [39, 40]. These observations suggest that both Plk1 and Plk3 can mediate some of the Cdc5 functions critically required for budding yeast viability, and that the critical functions of Plks from budding yeast to mammals are likely conserved throughout evolution. Distinct from the roles of Plk1–3 during the cell cycle, Plk4 appears to be a key regulator of centriole duplication [41–44]. Whether Plk1–3 directly regulates some of the processes important for centriole duplication is yet to be investigated.

Given the specific roles of these multiple Plks during the cell cycle and proliferation, it is imperative to understand how they interact specifically with their binding partners and physiological substrates and how these interactions are elaborately regulated without cross-recognition. Although temporal regulation of the expression of these kinases may serve as a mechanism for defining the function of each Plk at a distinct stage of the cell cycle, their expressions are still significantly overlapped with one another. On the other hand, since PBD plays a critical role in targeting the catalytic activity of each Plk to specific subcellular locations (see below), PBD-dependent spatial regulation of the activity of each kinase may be critical to delimit its functions. To better comprehend this spatial regulation, molecular mechanisms underlying the PBD-dependent protein–protein interactions and the physiological significance of these events are discussed below.

Polo-box domain: a multitalented mediator of protein–protein interactions

PBD-dependent subcellular localization of Plks Plk1 has been shown to localize to the centrosomes and kinetochores in late interphase, and to remain at these locations until telophase. In anaphase, Plk1 localization to these sites weakens, as a fraction of Plk1 relocalizes to the spindle midzone (later it becomes midbody) [24, 25, 45, 46] (Fig. 1). These findings demonstrate that Plk1 binds to various targets at distinct subcellular locations in a temporally and spatially regulated manner. Interestingly, Plk2 also localizes to centrosomes [26, 47], while Plk3 localizes to centrosomes, mitotic spindle, midbody, nucleolus, and Golgi [48–50]. Overexpressed Plk3 was also found in midbody, cellular cortex, and perinuclear granules [31, 51]. Plk4 localizes to centrosomes and also to cleavage furrow when overexpressed [41, 52] (Table 1). Differential localization of Plks to multiple subcellular structures presages the functional complexity of these enzymes during the cell cycle.

It is now widely appreciated that PBD plays a critical role in proper subcellular localization of Plks at specific subcellular structures. The initial clue for the importance of PBD in the subcellular localization of Plks originated from the analyses of various Plk1 PBD mutants in budding yeast. Without altering the kinase activity, a single point mutation at a conserved amino acid residue (W414F) within the polo-box 1 (PB1) of Plk1 (Fig. 2) was sufficient to delocalize Plk1 and disrupt its function [18]. Later studies confirmed that PBD is required for proper localization and functions of various other Plks in their native organisms [26, 53–56]. Furthermore, inhibition of the PBD function by a dominant-negative Plk1 PBD was sufficient to interfere with the PBD-dependent subcellular localization and mitotic functions of Plk1 [46, 57]. Thus, PBD-dependent protein–protein interactions are fundamentally

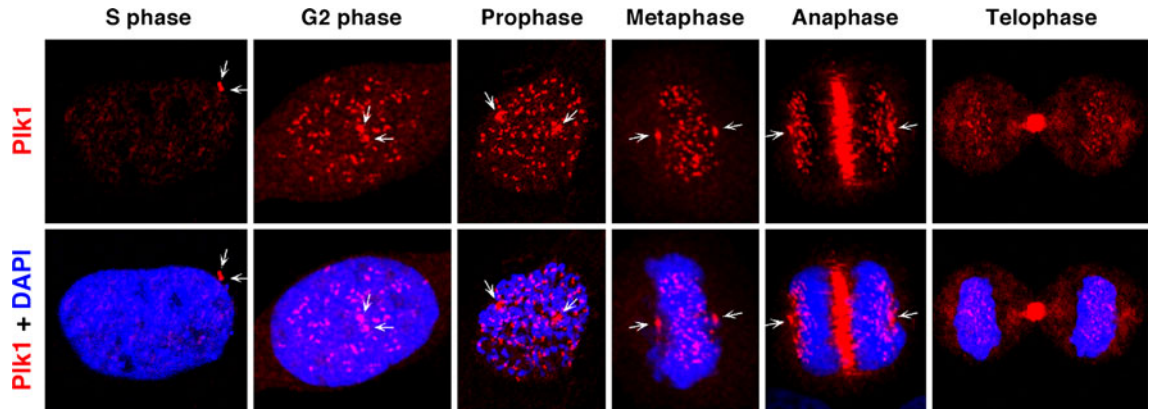

Fig. 1 Subcellular localization of Plk1 during the cell cycle. Plk1 localizes to the centrosomes as early as S phase. As Plk1 becomes more abundant during the late stages of the cell cycle, Plk1 localization to the centrosomes (*arrows*) and kinetochores (*dotted red fluorescent signals*) are manifest. In anaphase, a fraction of Plk1 relocalizes to the spindle midzone, which then condenses to a midbody in telophase

Table 1 Subcellular localization of Plks

	Endogenous	Overexpressed
Plk1	Centrosome, kinetochore, midzone, midbody	Centrosome, kinetochore, mitotic spindle, midzone, midbody
Plk2	Centrosome	Centrosome
Plk3	Centrosome, mitotic spindle, midbody, around nuclear envelope, nucleous, Golgi	Centrosome, cellular cortex, midbody, nucleous, perinuclear granules, cytosolic granules
Plk4	Centrosome	Centrosome, nucleous, around nuclear envelope, cleavage flow

required for proper functions of Plks from budding yeast to mammals.

Structures of the PBDs Alignment of the C-terminal PBDs of mammalian Plk1 to Plk4 (for simplicity, we will call them PBD1 to PBD4, respectively, hereafter) revealed that PBD1–3 exhibit a high level of homology in two distinct polo-box motifs, PB1 (aa 411-aa 489 in Plk1) and PB2 (aa 511-aa 592 in Plk1) [5, 52] (Fig. 2). However, PBD4 present in one of the two Plk4 variants, Sak-a [13], contains a greatly divergent C-terminal sequence from the other three Plks and possesses only the PB1 motif (Fig. 2), hinting that the binding mode of PBD4 is distinct from that of PBD1–3.

A breakthrough in our understanding of the PBD-dependent protein–protein interaction came from the work of Yaffe and his colleagues who have been searching for cellular proteins binding to short phospho-Thr-Pro (pThr-Pro)-containing degenerate peptides. This study led to the identification of PBD1 as a specific pSer- or pThr-binding domain with a consensus binding motif of [Pro/Phe]-[Φ/Pro]-[Φ]-[Thr/Gln/His/Met]-Ser-[pThr/pSer]-[Pro/X] (Φ represents hydrophobic residues and X means any residues) [58, 59]. Additional peptide library screening for the PBDs from *Homo sapiens* Plk2 and Plk3, *Xenopus lavis* Plx1, and *Sacchoromyces cerevisiae* Cdc5 revealed that these PBDs also bind to a pSer/pThr peptide motif

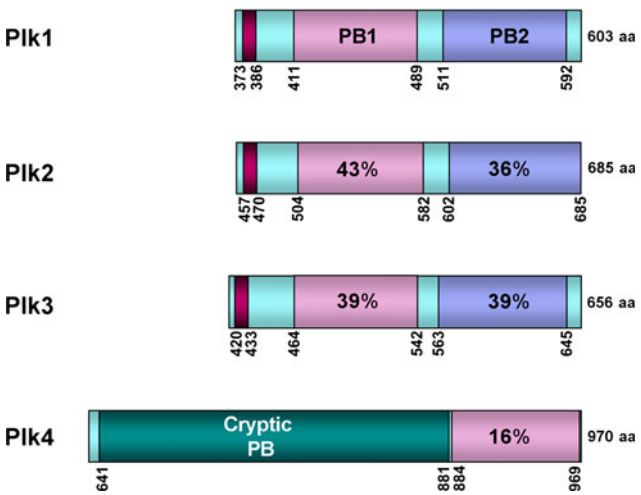

Fig. 2 Schematic diagram of the PBDs from human Plk1–4. Sequence identities among *PB1s* (*light pink*) and *PB2s* (*light blue*) are given in percentages. A short sequence (*burgundy*) upstream of PB1 represent a short alpha-helix polo-cap denoted as αA in Fig. 3. The total lengths of *Plk1–4* are indicated in amino acid residue *numbers* on the *right*. The *numbers* indicate the positions of the amino acid residues in a given gene. *Cryptic PB* indicates a region of Plk4 that is also sufficient for dimerization [2, 62]

preceded by a Ser residue at the pThr-1 position (referred to hereafter as Ser-1 to indicate its relative position from the phosphorylated Thr residue) [59].

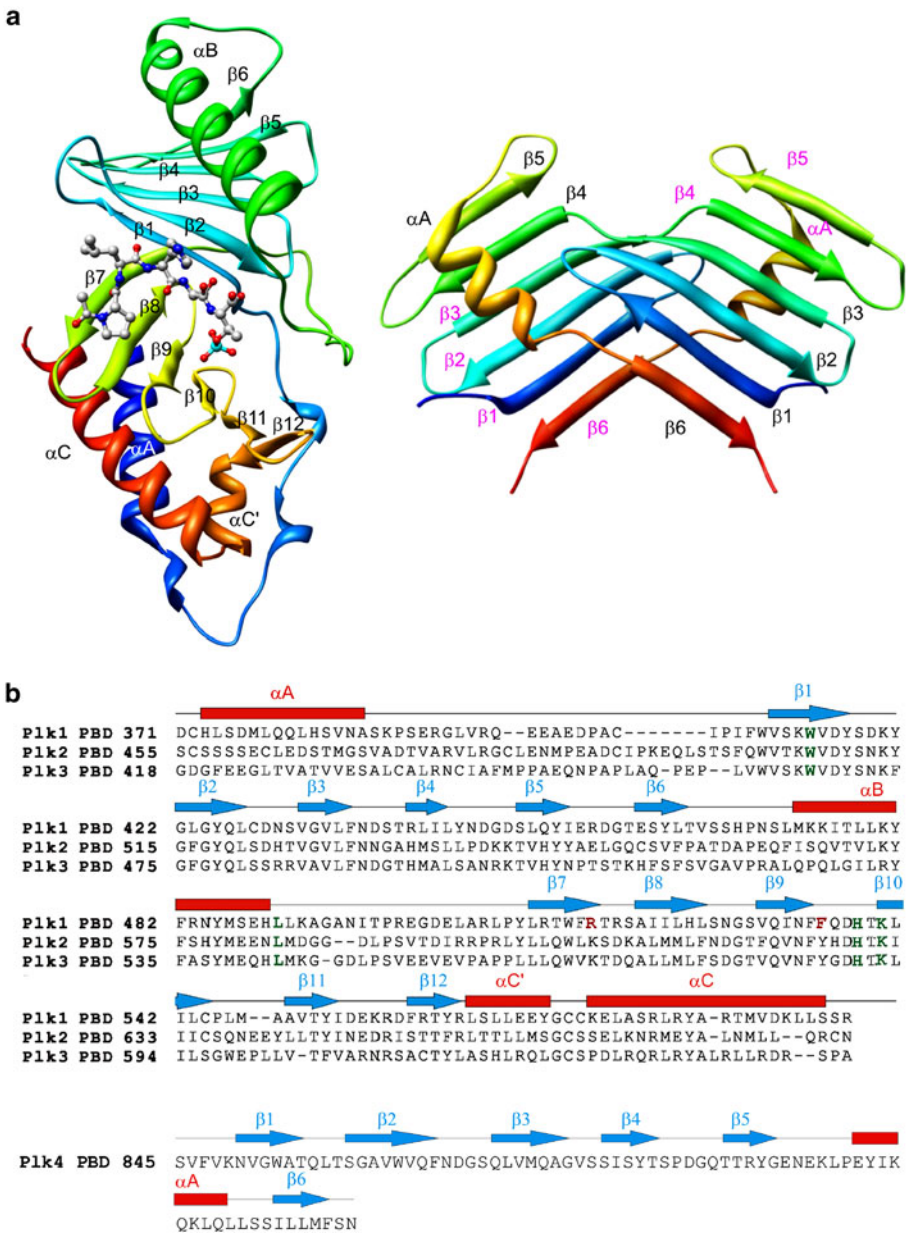

Fig. 3 Structures of the PBDs from human Plk1–4. **a** Comparison of the crystal structures of PBD1 and PBD4. The structure of PBD1 is shown in complex with PLHSpT drawn as a *ball and stick* representation [61]. The structure of PBD4 is adopted from Leung et al. [62]. Notations for α-helices and β-strands are given in order. **b** The sequence alignment of the PBDs from Plk1–3. Notations for α-helices and β-strands are done in the same manner as in (**a**). Four conserved residues (W414, L490, H538, and K540) from the PBD1 important for binding to the MQSpTPL-containing optimal peptide are highlighted in *green*. Two unique residues (R516 and F535) critical for accommodating the N-terminal Pro of PLHSpT are highlighted in *red*. PBD4 does not exhibit a significant level of homology with PBD1–3, and thus is shown separately

Analyses of the crystal structure of PBD1 in complex with its optimal phosphopeptide containing MQSpTPL revealed that PBD1 forms a single modular, pSer/pThr-binding, zipper-like structure with residues contributed alternatively from each PB (Fig. 3a) [59, 60]. The PB1 and PB2 contain identical folds of β6α (a six-stranded anti-parallel β-sheet and an α-helix) and form a hetero-dimeric phosphopeptide-binding module that bears a short alpha-helix polo-cap (αA) region (aa 373-aa 386 in Plk1) upstream of the PB1 (Fig. 3b). This cap region is thought to stabilize the fold [59, 60] (see Fig. 3a). Interestingly, the PBD1-binding phosphopeptide is located at one end of a shallow cleft between the two β-sheets from each PB. Consistent with the requirement of both PB1 and PB2 for the subcellular localization of Plk1 [46], the bound phospho-peptide interacts with key amino acid residues from both PBs. The Trp414 and Leu490 residues from PB1 are critical for the recognition of the phospho-peptide through non-polar interactions, while the His538 and Lys540 residues from PB2 are central for the interactions with the negatively charged phosphate of the pThr residue [59, 60]. In addition, a strict selectivity for the Ser-1 residue could be attributable to its engagement in two hydrogen bonding interactions and van der Waals interactions with the critical Trp414 residue [59]. As expected if the four residues—namely, Trp414, Lue490, His538, and Lys540—were crucial for the interactions with phosphopeptide, they are commonly found in the PBD1–3 (Fig. 3b).

Comparative analyses of the crystal structures of PBD1 in complex with various phosphopeptide ligands or no peptide

revealed that the binding of a phosphopeptide does not induce any detectable level of conformational change [61], suggesting that the interaction between PBD1 and its binding peptide follows a classical lock-and-key model. Although no structure is yet available to grasp the binding modes of PBD2 and PBD3, the observation that PBD2 and PBD3 also efficiently bind to Pro-directed phosphopeptides [59] suggests that phosphoepitope is universally required for the binding of all three PBDs. Determination of the crystal structures of PBD2 and PBD3 will be essential to provide new insights into the mechanism of how specific interactions between PBDs and their binding targets are achieved.

Although PBD4 appears to be crucially required for the function of Plk4, whether it mediates Plk4 function by binding to a phosphorylated target is not known. Analyses of the crystal structure of the single PB1 motif of PBD4 from murine Plk4/Sak-a revealed that, unlike the PB1 and PB2 motifs of PBD1, the PB1 motif of PBD4 generates an intermolecular dimer with another PB1 motif of the PBD4 molecule [62]. The dimerized PB1s of PBD4 contain two α-helices and two six-stranded anti-parallel β-sheets, and generates an interfacial, semi-enclosed, pocket (Fig. 3a), suggestive of a functionally important surface for binding. Each β-sheet is made of four strands from one molecule of PB1 and the other two strands from the other molecule of PB1, thus providing the molecular basis for intermolecular dimerization. Unexpectedly, however, a region N-terminal to the PB1 motif (from aa 641 to aa 881; also called a cryptic PB [2]) (Fig. 2) also appears to be sufficient for dimerization, suggesting the presence of more than one dimerization motif. Consistent with this observation, proper subcellular localization of Plk4/Sak-a requires both the PB1 and the cryptic PB motif [62].

A phospho-recognition domain with a myriad of interacting proteins The dynamic nature of Plk1 localization to multiple subcellular structures suggests that PBD1 binds to a large number of proteins in a temporally and spatially regulated manner. Numerous PBD1-binding proteins have been isolated and characterized over the years (Table 2). To comprehensively identify additional PBD1-binding proteins, Lowery et al. [63] employed a combined biochemical and proteomic approach and identified more than 600 mitosis-specific proteins that bind to PBD1 in a phosphorylation-dependent manner. Given that some of the PBD1-binding proteins could be expressed under specific stages of the cell cycle or culture conditions, the number of PBD1-binding proteins may continue to increase. Interestingly, these binding targets include not only proteins involved in mitosis but also proteins required for proper translational control, RNA processing, and vesicle transport [63], suggesting that PBD1-dependent interactions are involved in a wide variety of cellular processes. The currently characterized physiological Plk1 substrates and both phospho-dependent and phospho-independent PBD1-binding proteins are listed in Table 2.

Unlike a large number of PBD1-binding proteins, however, only a handful of potential PBD2- and PBD3-binding proteins have been isolated (Table 2). This is likely due to the limited number of studies on these two kinases compare to Plk1. Thus, isolation and characterization of PBD2- and PBD3-binding proteins are much needed to better comprehend both common and distinct characters of these PBDs in comparison to those of PBD1. Since the subcellular localizations of Plk1–3 appear to be significantly different (Table 1), the proteins that interact with the PBDs of these three enzymes are likely distinctive. In support of this view, in vitro pull-down assays revealed that PBD1 and PBD2 can bind to not only common but also distinct sets of proteins [64].

Then how is the specificity of PBD-dependent interaction achieved? Although largely unknown, a glimpse on attaining PBD1-binding specificity can be gained from the recently characterized interaction between the PBD1 and its specific phosphopeptide ligand derived from the T78 region of a kinetochores protein, PBIP1/MLF1IP/KLIP1/CENP-50/CENP-U (for simplicity, PBIP1 hereafter) [61]. Close examination of the binding mode of a minimal phospho-T78 (p-T78) peptide, PLHSpT, to the PBD1 revealed that the N-terminal residues are crucial for providing specificity to the interaction, while the C-terminal SpT dipeptide functions as a high-affinity anchor. The N-terminal Pro residue appears to play an important role in conferring the specificity by docking its side chain into a hydrophobic core surrounded by the Trp414, Phe535, and Arg516 residues, while concomitantly participating in polar contact (hydrogen bonding) between its carbonyl oxygen and the guanidinium moiety of Arg516 of the PBD1 [61]. The PBD2 and PBD3 possess Lys and Tyr residues at positions corresponding to the Plk1 Arg516 and Phe535 residues, respectively (Fig. 3b). As a result, they fail to generate as favorable an environment to interact with the N-terminal Pro residue, thus providing a molecular basis for the specificity of PBD1 binding.

Close analyses of the crystal structures of PBD1 in complex with various phosphopeptides revealed that many amino acid residues directly participate in the formation of the PBD1-binding cleft. Theoretically, any combination of these residues may potentially generate a binding pocket to stably interact with a given target. Thus, the mode of PBD1-dependent interactions may differ from one binding target to another. Further investigations on diverse PBD1-dependent protein–protein interactions will be necessary to gain additional insight into the mechanism of achieving PBD1-binding specificity.

Table 2 The substrates of Plks

Protein	Plks phosphorylation site(s)	Plk binding[a]	PBD binding	Determined PBD-binding site(s)	Cdk priming	References
Plk1 substrates						
Bcl-xl	GYS^{23}WSQ, several others	ND	ND	ND	ND	[87]
β-Catenin	YRS^{718}FHS	Yes	ND	ND	ND	[88]
Bora	DMS^{49}7GYN, YNT^{501}QNC	Yes	Yes*	ND	ND	[89]
Brca2	DMS^{193}WSS, DES^{239}LKK	ND	ND	ND	ND	[90]
B23	EDS^{4}MDM	Yes	ND	ND	ND	[91]
Bub1	ND	Yes	Yes	ST^{609}P	Yes	[92]
BubR1	EDS^{676}REA	Yes	Yes	ST^{620}P	Yes	[93]
Cdc25C	EFS^{198}LKD	Yes	Yes	ST^{130}P	Yes	[58, 94]
Cep55	NES^{436}LVE	Yes	ND	ND	ND	[95]
CEP170	ND	Yes	ND	ND	ND	[96]
Cyclin B	ETS^{133}GCA, AFS^{147}DVI	Yes	ND	ND	ND	[97, 98]
Emil	EDS^{145}GYS, YSS^{149}FSL	ND	ND	ND	ND	[99]
FoxM1	NDS^{715}LSK, DIS^{724}FPG	Yes	Yes	ST^{596}P, ST^{678}P	Yes	[100]
Grasp65	ND	Yes	Yes	SS^{217}P, several others	Yes	[101, 102]
HBO1	DSS^{57}PVR	Yes	Yes	PT^{85}P, VT^{88}P	Yes	[103]
HsCYK-4	DES^{149}GSI, DIS^{157}FDK, DES^{164}LDW, DSS^{170}LVK, NES^{214}IVA, DST^{260}LNS	Yes	Yes	SS^{170}L, ST^{260}L, several others	No	[70, 71]
HSF1	NDS^{216} GSA, LFS^{419}PSV	Yes	ND	ND	ND	[104, 105]
IKKβ	DQS^{733}FTA, DWS^{740}WLQ, EHS^{750}CLE	ND	ND	ND	ND	[106]
Kif2A	ND	Yes	ND	ND	ND	[107]
Kiz	DLT^{379}ISI	Yes	Yes	ND	ND	[108]
MKLP1	RRS^{904}STV, RSS^{905}TVA	Yes	Yes**	ND	ND	[109]
MKLP2	EHS^{528}LQV	Yes	Yes	HS^{528}L	No	[65]
Myt1	DSS^{426}LSS, DDS^{435}LGP, DLS^{469}DIN, EDT^{495}LDP	ND	ND	ND	ND	[110]
MyoGEF	EDT^{574}DED	Yes	Yes**	ND	ND	[111]
Nedd1	TDT^{382}LSK, FSS^{397}FDD, DES^{426}IGK, RYS^{637}VNE	Yes	Yes	ST^{550}P	Yes	[112]
Nlp	EDS^{87}S^{88}SLE, ST^{161}KEA, EKS^{686}QEV	Yes	ND	ND	ND	[113]
NudC	ENS^{274}KLS, DFS^{326}KAK	Yes	Yes**	ND	ND	[114]
PBIP1	HST^{78}AIY	Yes	Yes	ST^{78}A	No	[66]
PICH	ND	Yes	Yes	ST^{1063}P	Yes	[115]
PIN1	KHS^{65}QSV	Yes	ND	ND	ND	[116]
PRC1	AST^{578}YSE, HST^{602}NIQ	Yes	Yes	ST^{602}N	No	[69]
Ran	AKS^{135}IVF	Yes	ND	ND	ND	[117]
Rock2	DAT^{967}IAS, EES^{1099}QIR, DSS^{1133}SIG, NQS^{1374}IRR	Yes	Yes	ND	ND	[63]
TAp73	DST^{27}YFD	Yes	ND	ND	ND	[118]
TCTP	DDS^{46}LIG, TES^{64}TVI	Yes	Yes**	ND	ND	[119]
Topors	YES^{718}SYR	Yes	ND	ND	ND	[120]
TopoIIα	DFS^{1337}DFD, EES^{1524}DED	Yes	ND	ND	ND	[121]
TRF1	ISS^{435}DSE	Yes	Yes	GT^{344}P, VT^{371}P	Yes	[122]
Vimentin	QDS^{82}VDF	Yes	Yes	SS^{55}P	Yes	[123]
Wee1A	EDS^{53}AFQ	Yes	Yes	SS^{123}P	Yes	[124]
Plk2 substrates						
α-Synuclein	MPS^{129}EEG	ND	ND	ND	ND	[125]
SPAR	ND	Yes	Yes	SS^{1328}P	Yes	[126]
Plk3 substrates						
CDC25C	EIS^{191}DEL, EFS^{198}LK	Yes	ND	ND	ND	[127]
Chk2	LSS^{62}LET, LYS^{73}IPE	ND	ND	ND	ND	[128]

Table 2 continued

Protein	Plks phosphorylation site(s)	Plk binding[a]	PBD binding	Determined PBD-binding site(s)	Cdk priming	References
p73	ND	Yes	ND	ND	ND	[129]
TopoIIα	EKT^{1342}DDE	Yes	ND	ND	ND	[130]
VRK1	DLS^{342}VVE	Yes	ND	ND	ND	[51]
Plk4 substrates						
ND						

Yes Phospho-dependent PBD binding, *Yes** phospho-independent PBD binding, *Yes*** phosphorylation-dependency remains unknown, *ND* not determined

[a] Based on co-immunoprecipitation and/or pull-down assays

Two distinct modes of PBD1 binding

Nonself-priming and binding Screening of Ser-pThr-containing peptide libraries with PBD1 and systematic mutational analyses of an optimal PBD1-binding peptide unveiled that Pro is one of a few favored residues for the pThr + 1 position [58]. These observations suggest that PBD1-binding targets are commonly generated by Cdc2 or other Pro-directed kinases in vivo. Consistent with this notion, a large fraction of the currently characterized PBD1-dependent interactions involve Cdc2-dependent priming events at a Pro-directed site (Table 2). This finding is in good agreement with the observation that various mitotic processes frequently require a concerted action of Cdc2 and Plk1.

In the Pro-directed PBD1-binding model (referred to as "nonself-priming and binding" as opposed to "self-priming and binding", described below), phosphorylation and generation of a PBD1-binding site by a priming kinase such as Cdc2 is a prerequisite step for subsequent PBD1 binding and PBD1-dependent Plk1 function (Fig. 4). Thus, the mechanism of nonself-priming imposes an additional layer of regulation in PBD1-dependent Plk1 functions. This mechanism also ensures that prior cell cycle events mediated by a priming kinase are completed before PBD1-dependent interaction allows Plk1 to carry out its own function. Currently, a large number of PBD1-binding proteins that appear to follow the nonself-priming and binding model have been isolated (Table 3), suggesting that this mechanism is commonly employed to mediate PBD1-dependent Plk1 function.

Self-priming and binding Besides the prevalent nonself-priming and binding mechanism described above, evidence accumulated in recent years suggests that Plk1 also generates PBD1-binding sites to promote its own localization to specific subcellular structures. The initial hint that led to the existence of this type of PBD1-binding mode came from the observation that Plk1 phosphorylates a mitotic kinesin-like protein Mklp2 at S528, and that this event is important for PBD1 binding in vitro [65]. The S528A mutant fails to recruit Plk1 to the central spindle [65], suggesting that Plk1-dependent S528 phosphorylation is likely required for proper Plk1 localization to the central spindle. Later, close analyses of the interaction between Plk1 and PBIP1 demonstrated that Plk1-dependent PBIP1 phosphorylation at T78 is critical for the Plk1–PBIP1 interaction both in vitro and in vivo [66]. Subsequent detailed investigations on the Plk1–PBIP1 interaction unequivocally demonstrated that Plk1 is the only enzyme that generates the p-T78 epitope and binds to it [67, 68]. Thus, to distinguish this alternative PBD1-binding mechanism from the above mechanism that involves another kinase, this new mechanism is termed "self-priming and binding" [67] (Fig. 4). Although not as frequently observed as the nonself-priming and binding, the number of PBD1-binding proteins that follow this alternative mechanism appears to grow. Recently, Neef et al. [69] showed that a microtubule-associating protein, PRC1, is an anaphase-specific PBD1-binding target that requires Plk1-dependent self-priming and binding. Similarly, Plk1-dependent phosphorylation of a subunit of the centralspindlin complex, HsCYK-4, promotes PBD1 binding in vitro, although the PBD1-docking site has not been determined [70, 71]. Since Cdc2 activity is low at the mitotic kinetochores or is already downregulated in anaphase, self-priming and binding of PBIP1, Mklp2, PRC1, and HsCYK-4 by Plk1 could be a means of ensuring proper regulation of these proteins during the intricate process of M-phase progression (Table 3).

Physiological significance Whereas the nonself-priming and binding mechanism requires an action of another kinase to elicit PBD1 binding, the self-priming and binding mechanism necessitates an action of Plk1 itself. These differences seem to offer distinct physiological implications. Because of the requirement of another kinase for priming the PBD1-binding site, the nonself-priming and

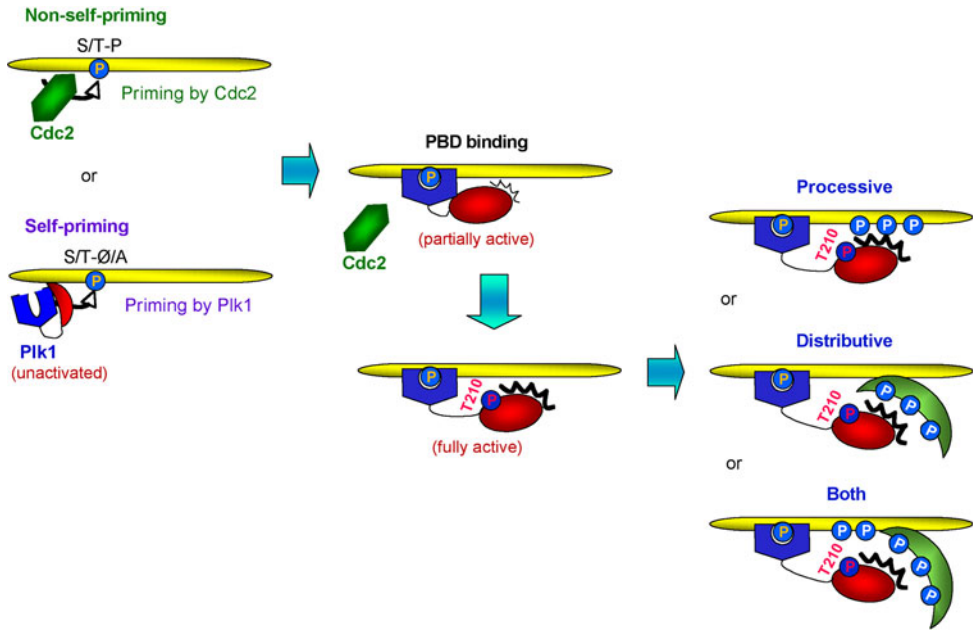

Fig. 4 Schematic diagram for Plk1 activation, PBD1-dependent binding, and substrate phosphorylation. Generation of PBD1-binding targets is achieved either by a Pro-directed kinase such as Cdc2 (*nonself-priming*) or by Plk1 itself (*self-priming*). Upon binding to a phosphorylated target through PBD1, Plk1 becomes partially active via physical dissociation of its kinase domain from the PBD1. Phosphorylation of Plk1 at T210 by an upstream kinase such as Aurora A/Bora [80, 86] fully activates the enzyme prior to G2/M transition. Once Plk1 binds to a phosphorylated target, Plk1 phosphorylates either the same protein binding to its PBD1 (*processive* phosphorylation) or a third protein associating with the PBD1-binding target (*distributive* phosphorylation). These two phosphorylation models are not mutually exclusive and may operate in a concerted manner (*both*)

Table 3 Self-priming or nonself-priming PBD1-binding targets

Nonself-priming	Bub1, BubR1, Cdc25C, FoxM1, Grasp65, HBO1, Nedd1, PICH, TRF1, Vimentin, Wee1A
Self-priming	HsCYK-4, MKLP2, PBIP1, PRC1

binding mechanism functions as a means of keeping an order of complexly organized cell cycle events. On the other hand, since the self-priming and binding mechanism is not contingent upon the completion of an earlier cell cycle event and is self-regulatory, it creates a condition that permits auto-amplification of Plk1-dependent events. For instance, newly recruited Plk1 molecules can, in turn, generate additional p-T78 epitopes for subsequent recruitment of other Plk1 molecules to the PBIP1-loaded kinetochores, thus inducing a rapid accumulation of a high level of active Plk1 population at this location. This mechanism would also guarantee a fast elimination of deactivating Plk1 population from a specific subcellular location, thus allowing an autonomous regulation of the level of Plk1 activity at this site. The self-priming and binding mechanism is functionally analogous to a positive amplification loop frequently observed in various signal transduction pathways.

It is noteworthy that, unlike Plk1-dependent priming of Mklp2, PRC1, and HsCYK-4, which happens when Plk1 is already activated, Plk1-dependent PBIP1 priming and binding occurs in early interphase sufficiently ahead of Plk1 activation in late G2. This raises a question of how Plk1 initially generates the p-T78 epitope on PBIP1 during early interphase. One plausible scenario is that unactivated Plk1 binds to PBIP1 in a low affinity as it is being expressed in S or early G2, and phosphorylates the T78 motif with its basal activity to generate a high affinity-binding site. Subsequent PBD1 binding to the p-T78 epitope partially activates Plk1, which then generates additional p-T78 motifs on other PBIP1 molecules more efficiently (Fig. 4). The early steps leading to a partial activation of Plk1 through the initial low-affinity PBD1 binding could be viewed as a prelude to the auto-amplification process observed in the self-priming and binding mechanism.

Mechanism of PBD1-dependent substrate phosphorylation; processive phosphorylation versus distributive phosphorylation

Although the interactions between PBD1 and its cognate binding targets are vitally important for proper Plk1 functions, a recent report clearly demonstrates that Plk1 binds to the p-S796 motif of a centrosomal protein

hCenexin1, but does not phosphorylate the latter [72]. This finding led to the speculation that Plk1 not only phosphorylates the same protein bound to its PBD1 but also phosphorylates a distinct protein(s) that associates with its PBD1-binding target. To account for potentially diverse modes of PBD1-dependent Plk1 phosphorylation onto its substrates, Lowery et al. [73] originally proposed two mutually non-exclusive models (Fig. 4). In the first model, termed "processive phosphorylation", the PBD1 binds to a primed site on a binding target and positions its catalytic activity to readily phosphorylate other sites on the same protein. A large number of PBD1-binding proteins have turned out to be physiological Plk1 substrates, thus following this mode of regulation (Table 4). A similar type of processive phosphorylation has been previously described for an SH2 domain-containing Tyr kinase Src [74–76] and a Ser/Thr kinase GSK-3 [77, 78].

In the second "distributive phosphorylation" model, a PBD1-dependent interaction with its binding target allows the catalytic activity of Plk1 to phosphorylate a third protein that is either directly interacting with the PBD1-binding protein or indirectly through another protein within a complex. At present, no solid example of a PBD1-binding target that links to subsequent Plk1-dependent phosphorylation onto another protein has been identified. However, at least one bona fide PBD1-binding protein, hCenexin1, does not appear to be phosphorylated by Plk1 [72] (Table 4), suggesting that the existence of a distributive phosphorylation mechanism is likely.

Other types of interactions

Phospho-independent or PBD1-independent interactions It is now well appreciated that PBD1 is sufficient for proper subcellular localization of Plk1. In a further extension of this finding, Hanish et al. [57] have shown that a phosphopincer PBD1(H538A, K540M) mutant fails to localize to specific subcellular structures, suggesting that the phospho-binding module of the PBD1 is critically required for proper localization. However, additional evidence suggests that Plk1 may also localize to specific subcellular structures in a phospho-independent manner. The initial suggestion for this possibility arises from the work of Garcia-Alvarez et al. [79] which shows that the full-length Plk1 bearing the two phospho-pincer mutations can localize to the centrosomes efficiently. Close investigation of the localization pattern of EGFP-fused full-length Plk1 revealed that these mutations substantially cripple, but do not eliminate, the capacity of Plk1 to localize to the centrosomes, kinetochores, and midbody (Fig. 5). In line with these findings, the *Drosophila* polo localizes to interphase microtubules by interacting with a microtubule-associating protein, Map205, in a manner that requires both the PBD and the kinase domain, but not the priming phosphorylation [54]. It has also been shown that the interaction between Plk1 and Bora does not involve priming phosphorylation [80]. In this case, either the PBD1 or the kinase domain of Plk1 appears to be sufficient for the interaction [80]. These findings suggest that Plk1 can also interact with some of its binding targets through a phospho-independent or PBD1-independent binding mechanism, hinting that the modes of PBD1-dependent interactions are likely a lot more divergent than originally conjectured. Identification and characterization of additional PBD-binding proteins is necessary to better comprehend the various modes of interactions between Plks and their binding proteins.

Interfacial interactions between PBD1 and the kinase domain In addition to the role of PBD1 in interacting with other cellular proteins, a large body of evidence suggests that PBD1 also regulates the function of its own kinase activity through interfacial interactions between the PBD1 and the kinase domain. Earlier studies report that the full-length Plk1 binds to a phosphorylated peptide significantly worse than the PBD1 alone [58], whereas the provision of PBD1 inhibits the Plk1 kinase activity [56]. In addition, deletion of the C-terminal PBD1 substantially increased the kinase activity of Plk1 [39, 81]. These observations suggest that the PBD1 and the kinase domain may interact with each other in a mutually inhibitory fashion. This interaction may induce conformational alterations for both the PBD1 and the kinase domain in such a way that reciprocally hinders their interactions with PBD1-binding targets and kinase substrates, respectively. A similar mechanism has been observed with the SHP family of phosphatases that show that the interaction between the phospho-binding SH2 domain and the C-terminal phosphatase domain not only occludes the catalytic cleft but also distorts the

Table 4 Processive versus distributive phosphorylated target proteins

	Target proteins
Processive	Bub1, BubR1, Cdc25C, FoxM1, Grasp65, HBO1, HsCYK-4, Kizuna, MKLP2, Nedd1, PBIP1, PICH, PRC1, Rock2, TRF1, Vimentin
	Potential target proteins: MKLP1, MyoGEF, NudC, TCTP
Distributive	ND
Binding only	hCenexin1

ND Not determined

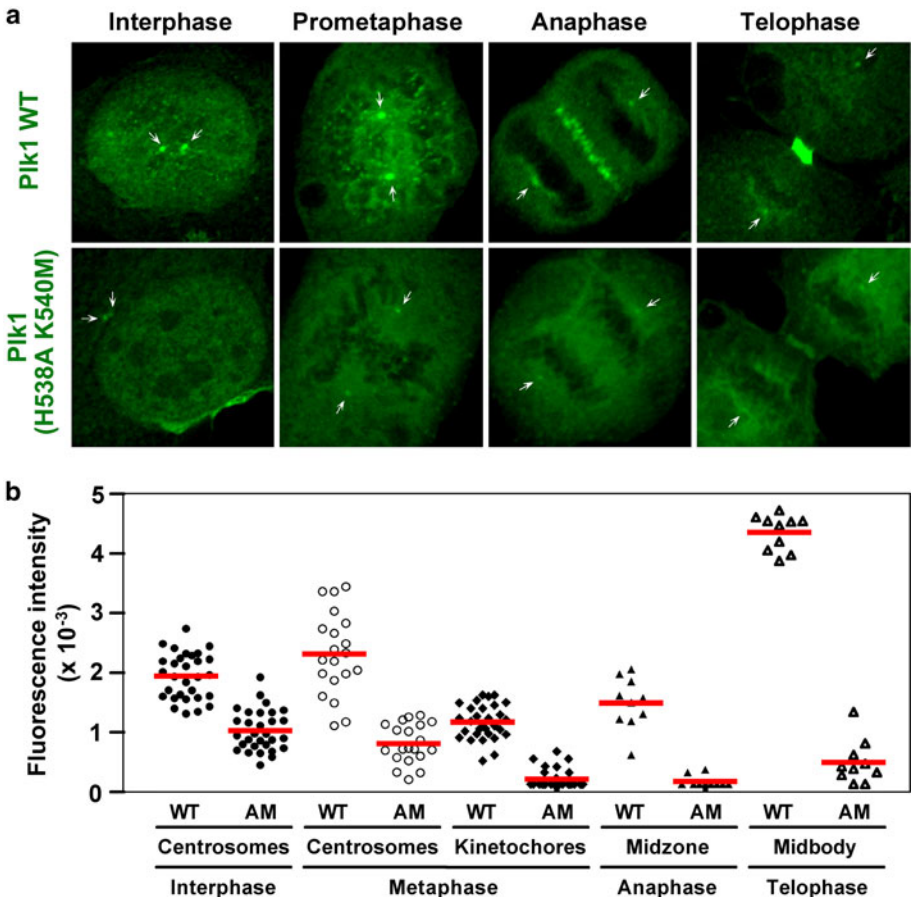

Fig. 5 Phospho-dependent interactions between PBD1 and its binding targets are critical but not essential for the subcellular localization of Plk1. HeLa cells infected with adenoviruses expressing either full-length EGFP-Plk1 (WT) or EGFP-Plk1 (H538A K540M) (AM) were fixed and imaged by confocal microscopy (**a**). The fluorescent intensities of Plk1 signals at the centrosomes, kinetochores, midzone, and midbody were analyzed (**b**)

conformation of the SH2 domain [82]. Interestingly, provision of an optimal PBD1-binding phosphopeptide enhances the activity of the full-length Plk1 [59]. Further, activational phosphorylation of the T210 residue of Plk1 present within the kinase domain renders Plk1 insensitive to PBD1-dependent inhibition [56]. These observations led to the hypothesis that the PBD1-dependent interaction with its binding target and the T210 phosphorylation within the kinase activation loop [83] may cooperatively induce physical dissociation between the kinase domain and the PBD1, thus allowing Plk1 to adopt a fully activated conformation (see Fig. 4).

Currently, the molecular basis of how PBD1 specifically interferes with the function of its own kinase domain is not known. Since the kinase domain does not appear to possess any potential pseudo-PBD1-binding motif, the site of the inhibitory interaction between the PBD1 and the kinase domain is likely distinct from that of the interaction between the PBD1 and its binding target. Determination of the crystal structure of either an inactive full-length Plk1 or the kinase domain in complex with the PBD1 may shed new light on the nature of the interfacial interactions between the two domains.

Perspective

Since the characterization of PBD as a phospho-epitope-binding module, PBD has become a member of an expanding class of phosphopeptide-binding domains that play central roles in the assembly of diverse regulatory complexes [84, 85]. Over the years, a large number of PBD1-binding proteins have been identified and the physiological significance of these interactions has been characterized. In contrast, PBD2- and PBD3-binding proteins are only beginning to emerge. Thus, to determine the underlying mechanism of PBD-binding specificity and to better understand the physiological significance of these interactions in various cellular processes, isolation and characterization of additional proteins that specifically bind to each PBD will be necessary.

Clearly, the modes of PBD-mediated interactions are far more complicated than we initially thought. Given the diversity of Plks functions, different modes of PBD-dependent interactions undoubtedly provide additional finesse to various Plk-dependent events. Further investigation on the mechanism of how each Plk mediates various biochemical and cellular events will be imperative to better comprehend the distinct functions of Plks in regulating

these processes. Future studies aimed at exploring the intricate nature of the spatial and temporal regulation of Plks may prove to be a worthwhile challenge for years to come.

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