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Review Article

The structure and function of protein kinase C-related kinases (PRKs)

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The protein kinase C-related kinase (PRK) family of serine/threonine kinases, PRK1, PRK2 and PRK3, are effectors for the Rho family small G proteins. An array of studies have linked these kinases to multiple signalling pathways and physiological roles, but while PRK1 is relatively well-characterized, the entire PRK family remains understudied. Here, we provide a holistic overview of the structure and function of PRKs and describe the molecular events that govern activation and autoregulation of catalytic activity, including phosphorylation, protein interactions and lipid binding. We begin with a structural description of the regulatory and catalytic domains, which facilitates the understanding of their regulation in molecular detail. We then examine their diverse physiological roles in cytoskeletal reorganization, cell adhesion, chromatin remodelling, androgen receptor signalling, cell cycle regulation, the immune response, glucose metabolism and development, highlighting isoform redundancy but also isoform specificity. Finally, we consider the involvement of PRKs in pathologies, including cancer, heart disease and bacterial infections. The abundance of PRK-driven pathologies suggests that these enzymes will be good therapeutic targets and we briefly report some of the progress to date.

Introduction

The PRK serine/threonine kinase family comprises three members, PRK1 (also PKN/PKN1/PKNα), PRK2 (PKN2/PKNγ) and PRK3 (PKN3/PKNβ). PRK1 was identified from a human hippocampus library [1] and shown to have a catalytic domain related to protein kinase C. PRK2 and PRK3 were later identified, with 83% and 71% similarity to PRK1, respectively [2–4]. PRK1 and PRK2 are ubiquitously expressed [5–8], whereas PRK3, initially only detectable in metastatic cancers [4], was subsequently found at low levels in all tissues [8].

The PRKs are downstream effectors of Rho family small G proteins. These GTPases behave as molecular switches for signalling pathways and the Rho family are best known as orchestrators of actin cytoskeletal rearrangements, with diverse roles in cell migration [9,10], the cell cycle [11] and control of vesicular trafficking [12].

The PRKs have been detected in the cytosol [13–15], the nucleus [4,14,16,17] and at the plasma membrane [4,18]. PRK1 also localizes to endosomes in a RhoB-dependent manner [13,19], while PRK2 has been detected on membranes in lamellipodia [14] and in apical junctions [20], and PRK3 was found in the perinuclear Golgi [4,17].

Despite their importance in regulating key cellular processes, the PRKs are understudied kinases and our structural and functional understanding of them remains incomplete.

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Domain arrangement and structures

All PRKs have broadly the same domain structure (Figure 1).

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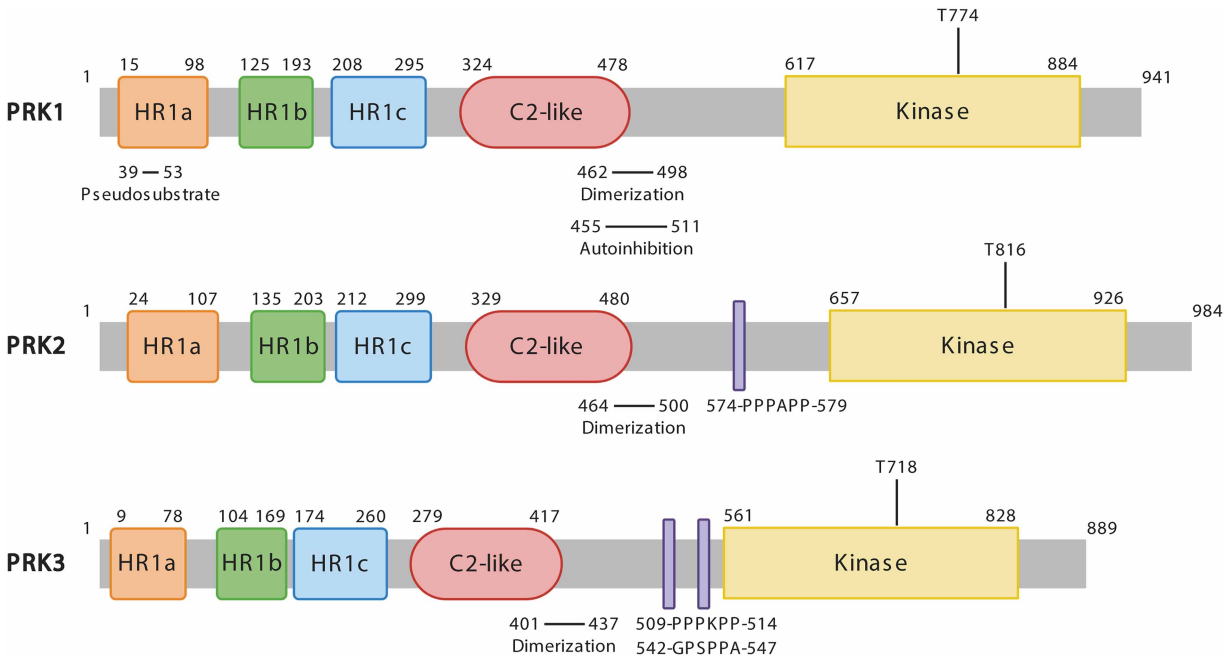

Figure 1. PRK domain arrangement.

PRKs have three N-terminal HR1 domains (HR1a-c), a C2-like domain and a serine/threonine kinase domain. PRK2 and PRK3 have one and two proline-rich regions, respectively. The activation loop Thr on each PRK is labelled. The PRK1 HR1a pseudosubstrate, the PRK2 dimerization site and its PRK1/PRK3 equivalents, and the site of autoinhibition that is relieved by arachidonic acid, are indicated. The limits of the various domains are indicated above each sequence.

HR1 domains

PRKs contain three N-terminal Homology Region 1 (HR1) domains, HR1a, HR1b and HR1c. RhoA is an interacting partner of the PRK1 HR1a domain [21–23] and all three members of the Rho subfamily, RhoA, RhoB and RhoC, bind the HR1a domains of PRK1, PRK2 and PRK3 *in vitro*[24]. RhoA binds weakly to PRK1 HR1b [23,25,26] and this appears to be independent of HR1a binding [25]. In contrast, RhoB may bind cooperatively to HR1a and HR1b, implying subtle differences in the Rho subfamily interactions [24].

Rac1 interacts with PRK2 [7] and with both PRK1 HR1a and HR1b [25], but there is no evidence for cooperative binding. The interaction of Rac1 with PRK3 has not been investigated but key interacting residues are conserved in the PRK1/PRK3 HR1 domains, suggesting an interaction is likely.

The HR1c domain does not appear to bind to RhoA [23] or Rac1 (unpublished observations) and no other binding partners have been reported. Currently, its function is a puzzle, although it is as well conserved as the other HR1 domains.

HR1a is an antiparallel coiled coil that binds RhoA [27]. Although there were two potential contact sites identified in the structure (Figure 2A), the mutational analysis suggested that contact site II is used in the solution [28]. The structure of Rac1 in complex with HR1b [29] showed that HR1b interacts with Rac1 at a site equivalent to the contact II site of RhoA (Figure 2B). The Rac1 C-terminal polybasic region folds back and interacts with both the G domain of Rac1 and with HR1b (Figure 2C). There is no structure of the PRK1 HR1c domain, but our NMR studies indicate that it also forms a coiled coil with an extra short, C-terminal α-helix [30].

PRK2 and PRK3 have similar HR1 sequences so they likely form similar structures [3,4]. Whether they interact with the same set of Rho family proteins *in vivo* is not clear, although the functional differences between the PRKs suggest that at least some interactions are unique.

It is unknown whether two Rho family proteins can bind simultaneously to PRK1. This would be structurally feasible, since there are relatively long linkers between the HR1 domains. Furthermore, structures of constructs containing two or more HR1 domains are not available, leaving potential inter-domain interactions unexplored.

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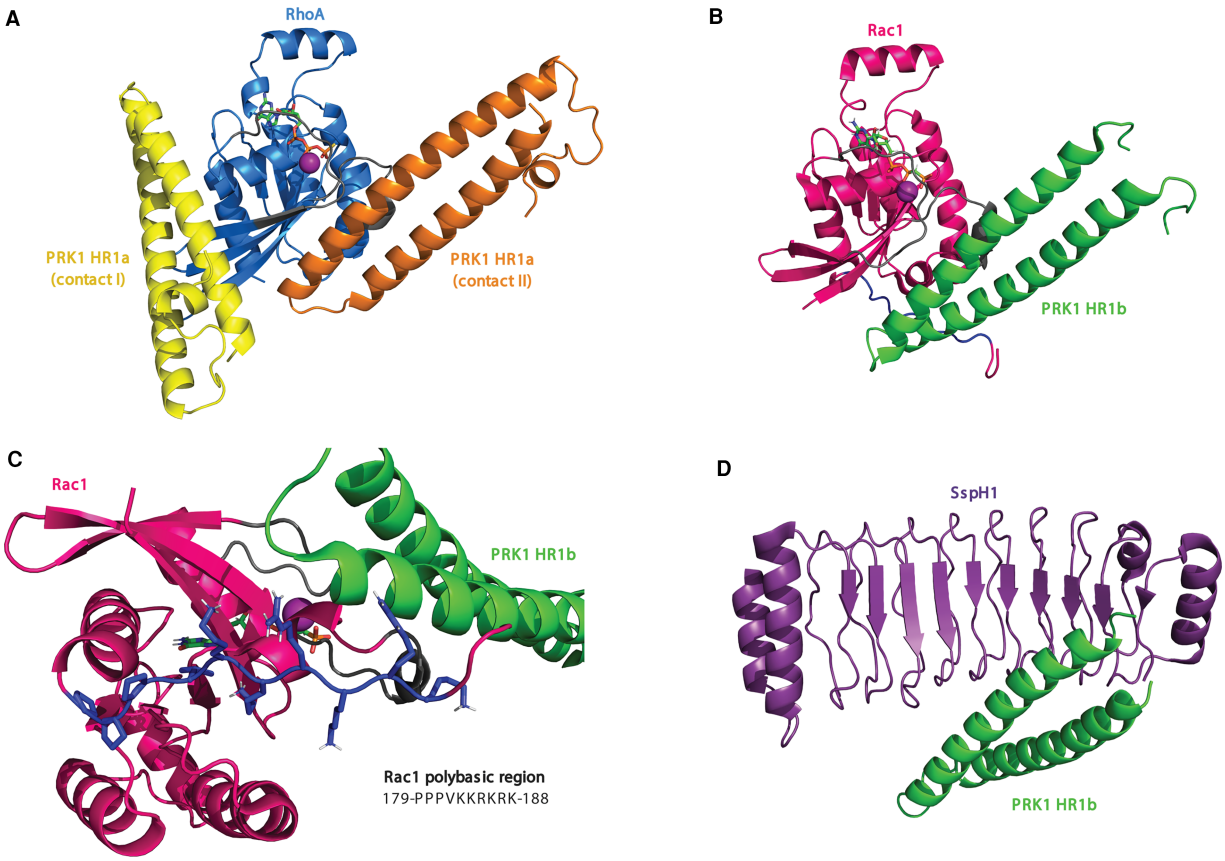

Figure 2. Structures of HR1 domain complexes.

The Mg^{2+} on GTPases is shown as a magenta sphere and the nucleotide in a stick representation. The GTPase switch regions are coloured grey. (**A**) Crystal structure of RhoA interacting with the HR1a domain of PRK1, which forms an antiparallel coiled coil [27] (PDB:1CXZ). Two different contacts, I and II, were identified in the crystals. (**B**) Solution structure of Rac1 interacting with the HR1b domain of PRK1 [29] (PDB:2RMK). The polybasic region in the C-terminal tail of Rac1 is coloured blue. (**C**) Close-up of the Rac1 polybasic region in complex with PRK1 HR1b. The polyproline helix (179-PPP-181) provides the C-terminal tail with an extended conformation, allowing the polybasic region (182-VKKRKRK-188) to contact the HR1b domain. (**D**) Crystal structure of PRK1 HR1b interacting with leucine-rich repeats of the *Salmonella* protein SspH1 [134] (PDB:4NKG).

The C2-like domain

PRKs include a C2-like domain with weak homology to the C2 domains of PKC proteins. These comprise 8 antiparallel β-sheets folded into a β-sandwich, which in classical PKCs bind to membrane phospholipids in a Ca^{2+}-dependent manner [31]. In contrast, C2 domains of novel and atypical PKCs are calcium-insensitive and bind acidic phospholipids. They are, therefore, critical for the recruitment of proteins to the plasma membrane [31]. No structural data is available for the C2-like domains of PRKs, however, they are likely to be insensitive to Ca^{2+} but bind lipids to activate the PRKs [32–34].

Proline-rich regions

PRK2 has a proline-rich region with a class II motif (PPPAPPR) between the C2 and kinase domains, which interacts with SH3 domains in the NCK/NCK2 adaptor proteins and more weakly with the SH3 domain of phospholipase Cγ [6,35]. PRK3 has a similar motif (PPPKPPR) and also a class I motif (RRGPSPP). These are involved in protein–protein interactions with SH3 domains of the RhoGAP Graf and Graf2 proteins [4,36].

Kinase domain

The PRKs are members of the AGC kinase superfamily and the PRK1 kinase domain structure has been solved alone and with inhibitors [37] (Figure 3A). A Thr in the activation loop is phosphorylated by PDK1

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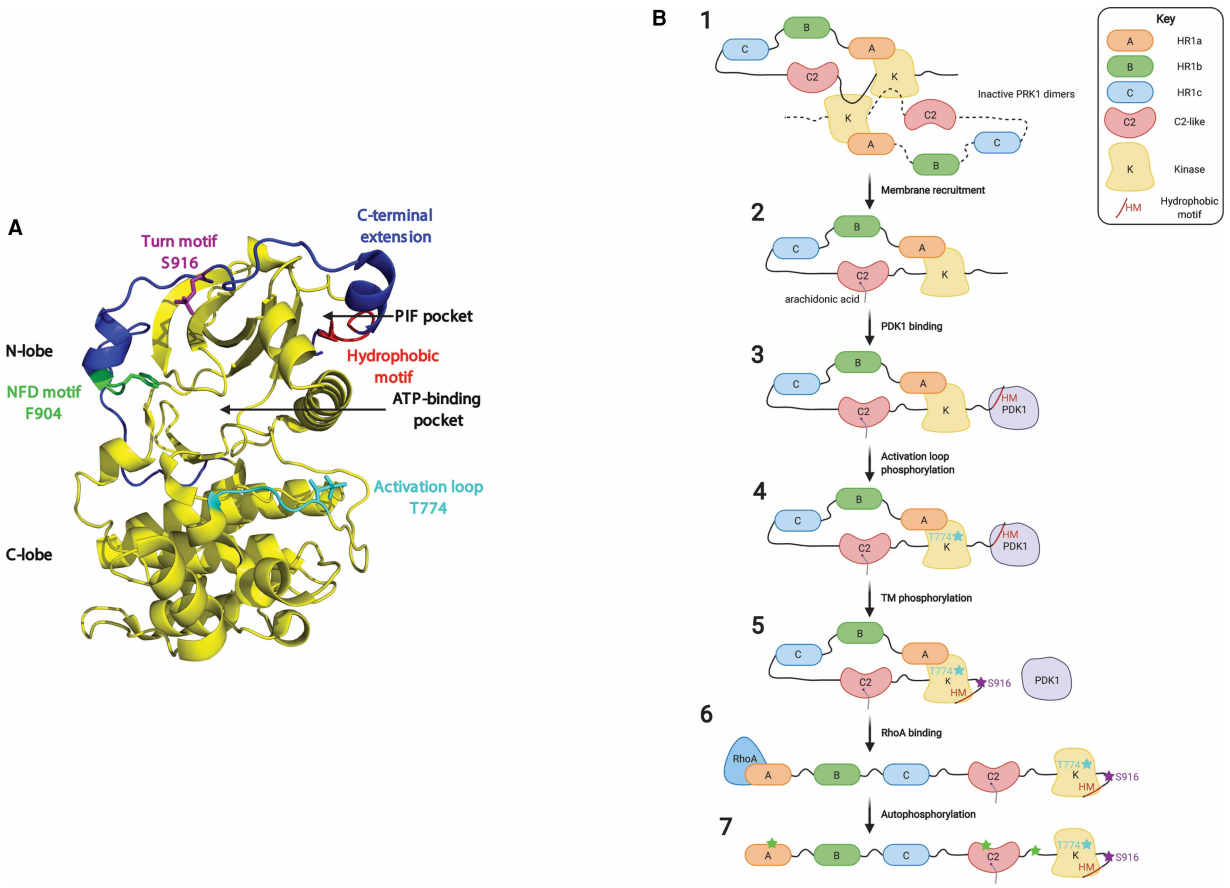

Figure 3. The PRK kinase domain and its activation.

(**A**) Crystal structure of the PRK1 kinase domain (apoprotein) [37] (PDB:4OTD). Key residues within the activation loop (cyan), hydrophobic motif (red), turn motif (purple) and NFD motif (green) are shown as sticks on the structure. The active site, ATP-binding pocket and the allosteric regulation site, the PIF pocket, are labelled. (**B**) PRK1 activation events. The events have been placed in a speculative temporal order partly based on work on PRK2 dimerization studies [51] (1–7). (1) Dimerization keeps PRK1 inactive by allosterically preventing the intramolecular HM/PIF pocket interaction. (2) Recruitment to the cell membrane via the C2-like domain may allow lipids such as arachidonic acid to disrupt this dimer. (3) HM phosphomimic can bind to the PDK1 PIF pocket. (4) PDK1 phosphorylates the activation loop. (5) TM phosphorylation by mTOR2/CDK1 releases PDK1 by promoting the intramolecular HM/PIF pocket interaction. (6) RhoA binds to the HR1a domain and relieves pseudosubstrate autoinhibition. (7) PRK1 autophosphorylates leading to additional activation. Phosphorylated residues [57] are denoted with a star.

(3-phosphoinositide dependent kinase) [38,39] to activate the kinase. The kinase domain has a C-terminal extension that encircles the catalytic domain and contains three conserved features found in AGC kinases, which govern the allosteric regulation of their catalytic domain (Figure 3A, Table 1):

(1) An NFD motif, whose phenylalanine contacts the adenine ring of the substrate ATP.

Table 1. Conserved motifs in the kinase domain and C-terminal extension of the PRKs

Protein	Activation loop	NFD	TM	HM
PRK1	T774	903-NFD-905	S916	932-FLDFDF-937
PRK2	T816	945-NFD-947	T958	974-FRDFDY-979
PRK3	T718	847-YFE-849	T860	876-FRDFDF-881

(2) A turn motif (TM) C-terminal to the NFD [40,41], which is phosphorylated to enhance intramolecular interactions involving the hydrophobic motif (HM) [37,42].
(3) HM at the extreme C-terminus. Phosphorylated HM interacts with a region of the N-lobe known as the PDK1-interacting fragment (PIF) pocket, which transmits conformational changes to the active site, allosterically activating the kinase [39]. The PRK HM includes a phosphomimic Asp residue, allowing constitutive interaction with the PIF pocket.

Activation and autoregulation

Phosphorylation of the activation loop threonine by PDK1 is aided by interactions between the PRK HM motif and the PDK1 PIF pocket, which activates PDK1 and brings the two proteins into close proximity. However, this intermolecular interaction prevents the immediate allosteric activation of the PRK kinase. This requires phosphorylation of the PRK TM, leading to the release of PDK1, PRK intramolecular HM/PIF pocket interaction and kinase activation [17,43–45]. The mammalian target of rapamycin (mTOR) 2, a protein complex with diverse signalling roles, phosphorylates the PRK1 TM [43], as does cyclin-dependent kinase (CDK) 1 [45], suggesting multiple pathways feed into PRK activation.

Kitagawa et al. [46] first suggested autoregulation by the N-terminus when they showed that a peptide corresponding to amino acids 39–53 of PRK1 HR1a, with Ile46 mutated to serine, was phosphorylated by PRK1. They suggested that HR1a contains a pseudosubstrate motif that autoinhibits the kinase, which is released when RhoA binds to HR1a. The PRK1 consensus is not well-defined, however, and the pseudosubstrate hypothesis has not been confirmed [47,48]. RhoA may enhance PRK activation in other ways, e.g. when PRK1 binds to RhoA, activation loop phosphorylation by PDK1 increases [49]. The *Drosophila* orthologue Pkn is activated by RhoA and Rac1 [50], while RhoC has also been linked to increased PRK3 activity [17], but exactly how these Rho GTPases achieve this is not understood.

The PRK2 N-terminus inhibits the catalytic domain by preventing its interaction with PDK1 [51]; this may be relieved by RhoA binding. In addition, PRK2 activity appears to be inhibited *in trans* by intermolecular dimer formation, mediated by residues 464–500 (Figure 1). Peptides based on this region inhibit the catalytic activity of all three PRKs [48,51,52]. It is thought that when the intramolecular HM/PIF pocket interaction is stabilized, the ensuing conformational changes displace the dimerization motif, leading to PRK monomerization and activation. The intramolecular HM/PIF pocket and dimerization interactions are, therefore, allosterically mutually exclusive, with opposing effects on catalytic activity. Intermolecular interactions involving other regions in the N-terminus have been suggested [51], implying that PRK dimerization may be mediated by multiple, cooperative events.

PRK1 and PRK2 can also be activated by proteolysis, e.g. by caspase-3 during apoptosis [53–55]. The constitutively active PRK1 fragment does not seem to play a direct role in apoptotic events but may be involved in morphological changes observed in microglia [56].

PRK1 is activated by phosphatidylinositol 4,5-bisphosphate (PIP_2), phosphatidylinositol 3,4,5-triphosphate (PIP_3) [32] and arachidonic acid [53]. Arachidonic acid is thought to remove the autoinhibition exhibited by a region of PRK1 and PRK2 that overlaps with or is adjacent to the C2-like domain [57], highlighting the need for the PRKs to be recruited to a membrane to be successfully activated. Arachidonic acid also activates PRK3, although its activation of PRK2/PRK3 is less potent than that of PRK1 [4,52,58]. PRKs are activated by other lipids, including linoleic acid, oleic acid and cardiolipin [5,58–61], and the isoforms show distinct lipid activation profiles and possibly activation mechanisms [52]. Phospholipids also bind directly to PRK1 HR1a, suggesting that HR1 domains may be involved in membrane binding [62].

Taken together, it is clear that PRK regulation is complex, involving a combination of Rho protein binding to at least two HR1 domains, competition between inter- and intramolecular interactions, oligomerization, autophosphorylation and lipid binding. This complexity allows PRKs to be activated in response to many different signalling cues, or in multiple locations, and opens the possibility of PRKs having multiple levels of activation, rather than being a binary 'on-off' switch. Figure 3B postulates a temporal order of activation.

Physiological roles

Many physiological roles of PRKs have been elucidated by identifying direct substrates for their enzyme activity [47,63] and Table 2 lists some of these. PRK1 is the best-characterized isoform and its roles are summarized in Figure 4.

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Table 2. Key PRK substrates. The PRK isoform, substrate and corresponding cellular process/function are listed

PRK	Substrate	Cell process/function	Reference
PRK1	α-actinin	Cytoskeletal regulation	[64]
PRK1	vimentin	Cytoskeletal regulation	[71]
PRK2	Cortactin	Cytoskeletal regulation	[66]
PRK3	p130^{cas}	Cytoskeletal regulation	[123]
PRK1, PRK2	HDAC5, HDAC7, HDAC9	Chromatin remodelling	[88]
PRK1	H3 Thr11	Androgen receptor signalling	[85]
PRK1, PRK2	Cdc25	Cell cycle	[15,156]
PRK1, PRK2	Pyrin	Immune response	[98]
PRK1	RHP3A	Vesicular trafficking	[157]
PRK1	GSK-3β	Glucose metabolism	[104]
PRK1, PRK2	PI3KC2-β	Nutrient signalling	[106]
PRK2	DUSP6	Inhibition of transcription	[120]
PRK1	Tau	Microtubule disruption	[73,74,104]
PRK1	α Crystallin B	Molecular chaperone	[125]
PRK1	HPV E6 oncoprotein	Cell immortalization	[158]
PRK2	HCV RNA Pol	HCV replication	[136,138]

Cytoskeletal regulation and cell adhesion

PRK1 interacts with the actin cross-linking protein α-actinin [64] and its overexpression in fibroblasts resulted in actin reorganization [65]. Cortactin, which cross-links F-actin, is inhibited by Rac1-activated PRK2, leading to key cytoskeletal rearrangements in astrocyte migration [66]. The PRKs also regulate the formation of stress fibres, actin-rich bundles essential for cell migration. Exogenous expression of PDK1 and PRK1 in mammalian cells led to insulin-induced actin cytoskeletal reorganization and a reduction in the number of stress fibres as well as membrane ruffling [67]. Expression of active PRK2 caused the formation of fewer, larger stress fibres but its kinase-dead mutant led to their almost complete loss [7]. Finally, RNAi-mediated knockdown of PRK3 in human umbilical endothelial cells (HUVECs) compromised their migration [68] and their ability to form stress fibres downstream of TNF-α [69]. More recently, a link between membrane tension, RhoA and ROCK1/PRK2 provided a mechanism for rear retraction and therefore directional migration [70]. Together, these studies suggest that PRKs are involved in the modulation of actin stress fibres and the cellular reorganization of F-actin, although their precise roles may differ.

As well as regulating the actin cytoskeleton, PRK1 phosphorylates the intermediate filament protein vimentin, leading to vimentin filament disruption *in vitro*[71], while PRK3 knockdown also led to vimentin disruption [17]. PRK1 also disrupts neurofilament assembly and axonal transport [72]. In neurons affected with Alzheimer's disease, PRK1 has been linked to neurofibrillary tangles and their key component, the protein tau. PRK1 phosphorylates the tau protein leading to disruption of the microtubule assembly [73,74].

In osteoclasts, PRK3 is activated downstream of the Wnt5–RhoA signalling axis and binds the non-receptor tyrosine kinases c-Src and Pyk2 (Figure 5A). This activates c-Src, which is essential for actin ring formation, a requirement for osteoclast bone resorption [75,76]. Therefore, PRK3 has a role in bone resorption *in vivo* and could be a therapeutic target for bone-loss diseases.

Given their involvement in cytoskeletal regulation, it is logical that the PRKs have also been linked to cell adhesion. PRK3-depleted HUVECs showed the irregular distribution of VE-cadherin/β-catenin, which link adherens junctions to the actin cytoskeleton [69], and a reduction in ICAM-1 and in Pyk2 phosphorylation, both of which are involved in cell adhesion. A study using PRK3 knockout mice implicated PRK3 in the regulation of glycosylation of ICAM-1 and integrins, suggesting that PRK3 may directly affect cell adhesion [8]. In addition, the PRK3 knockout mice showed decreased micro-vessel sprouting, while MEFs displayed reduced migration which was not rescued by growth factors. Taken together, these results suggest that PRK3 has a

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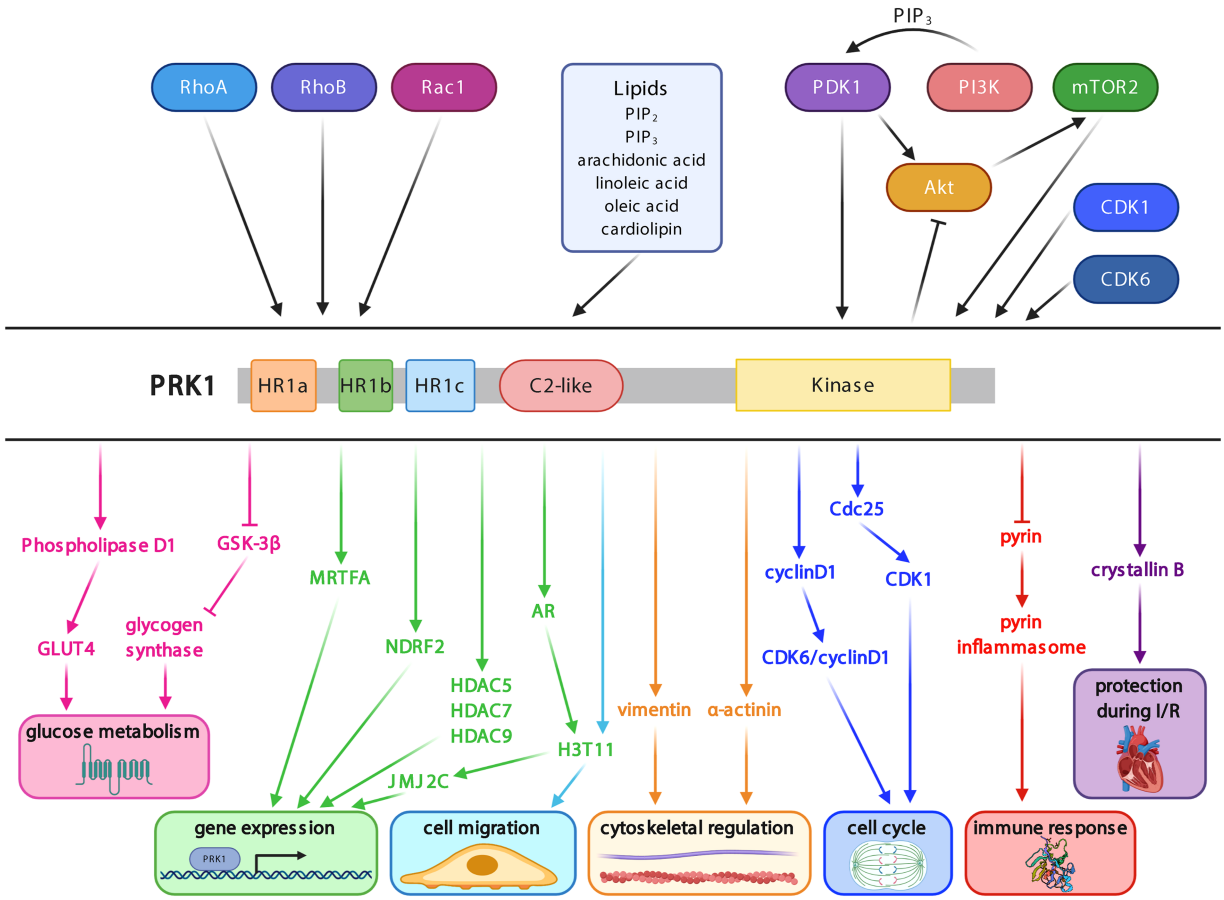

Figure 4. Physiological roles of PRK1.

The key activatory pathways are highlighted. The various processes in which PRK1 is implicated are highlighted in different colours.

unique physiological role in angiogenesis that is linked to cell adhesion. In this setting, PRK3 bound preferentially to RhoC, rather than RhoA/B suggesting that RhoC plays a role in angiogenesis. The PRK3 knockout mice showed reduced metastasis but no change in tumour angiogenesis, so its role in metastasis is not through increased angiogenesis.

PRK1 and PRK2 also play a part in cell adhesion. ROCK/PRK1 disrupt adhesion of endothelial cells downstream of thrombin [77]. PRK1 regulates both tight junctions [78] and adherens junctions; in the latter, PRK1 kinase activity was necessary for cell-surface expression of N-cadherin and integrins in fibroblasts [79]. In contrast, PRK2 regulates apical junction formation in bronchial endothelial cells [20], while RhoA-activated PRK2 induces Fyn/Src-dependent phosphorylation of β/γ-catenin and $p120^{ctn}$, promoting keratinocyte cell–cell adhesion [80]. The role of PRK2 in cell adhesion has been further demonstrated in myoblasts [81], where it interacts directly with Cdo, a cell-surface protein, Akt and the adaptor protein APPL1, leading to Akt activation and differentiation. This is in contrast with PRK1, which is a negative regulator of Akt [82–84].

Androgen receptor signalling, chromatin remodelling and transcriptional regulation

Metzger et al.[16] implicated PRK1 and PRK2 in androgen receptor (AR) signalling (Figure 5B), showing that PRK1 binds to the transactivation domain of the AR *in vitro* and that the PRK1/AR complex binds androgen response elements of AR-regulated genes. A subsequent study [85] showed that PRK1 phosphorylates Thr11 of histone H3. H3T11 phosphorylation recruits JMJD2C, which demethylates H3 Lys9, activating AR-dependent gene expression. All three PRKs are activated downstream of the thromboxane receptors TPα and TPβ [86]. PRK1/PRK2 activation in thromboxane signalling is crucial for chromatin remodelling through

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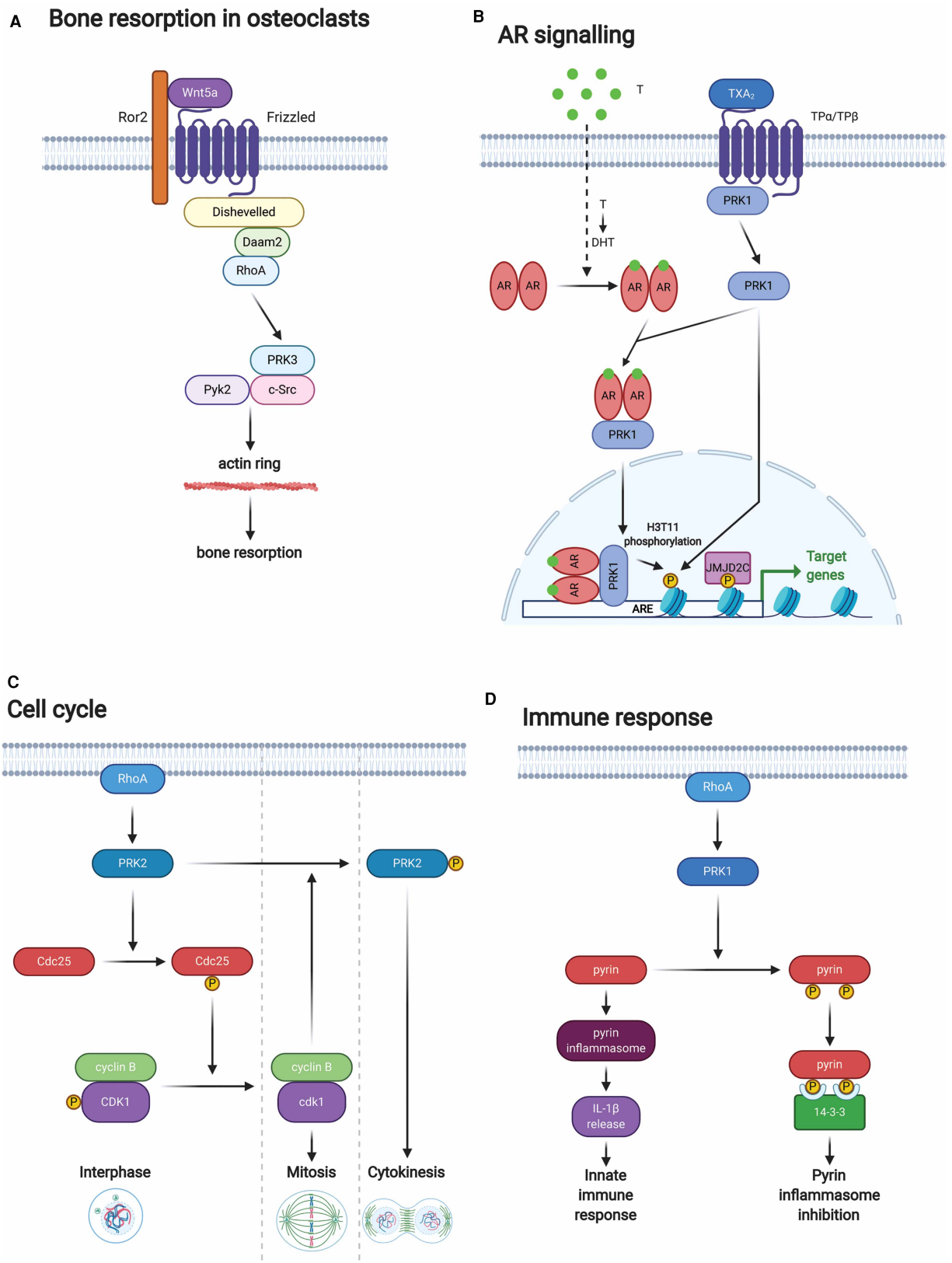

Figure 5. Detailed insight into selected PRK pathways. Part 1 of 2

(**A**) The role of PRK3 in bone resorption by osteoclasts. PRK3 is activated downstream of the Wnt5/RhoA signalling axis and it forms a complex via

Figure 5. Detailed insight into selected PRK pathways. Part 2 of 2

its proline-rich region with the non-receptor tyrosine kinases c-Src and Pyk2. This leads to c-Src activation, which is essential for actin ring formation, a requirement for bone resorption in osteoclasts. (**B**) The role of PRK1 in AR signalling and chromatin remodelling. The steroid hormone testosterone (T) is converted into dihydrotestosterone (DHT) which binds to the androgen receptor (AR). The AR interacts with PRK1 and the complex translocates to the nucleus where it binds to androgen-responsive elements (AREs). PRK1 phosphorylates Thr11 of Histone3, recruiting the demethylase JMJD2C and leading to increased transcription of target genes. The thromboxane receptors TPα and TPβ, activated by thromboxane A_2 (TXA_2), induce Histone3 Thr11 phosphorylation by PRK1 and enhance recruitment of the AR, which also leads to Histone3 Thr11 phosphorylation. (**C**) PRK2 phosphorylates and activates the Cdc25 phosphatase, which in turn activates CDK1/cyclin B to enter mitosis. CDK1/cyclin B then phosphorylates PRK2 which is also important during cytokinesis. D) PRK1-dependent down-regulation of the pyrin inflammasome. Pyrin is part of the pyrin inflammasome which promotes IL-1β release, leading to inflammation during the innate immune response. PRK1 phosphorylates pyrin causing it to be sequestered by 14-3-3 proteins.

phosphorylation of H3T11 (Figure 5B) and enhanced recruitment of the AR [87]. The histone deacetylases HDAC5/7/9 are phosphorylated by PRK1/PRK2 within their nuclear localization signal [88]. This is thought to reduce nuclear import by allowing the binding of 14-3-3 scaffolding proteins to HDACs, thereby preventing the HDACs accessing the nucleus to repress transcription. Overall, the PRKs directly affect chromatin structure leading to transcriptional up-regulation.

PRK1 has been implicated in regulating transcription factors and is localized to the nucleus under stress conditions [89], where it interacts with a neurone-specific helix-loop-helix transcription factor, NDRF2, to amplify transcription [90]. PRK1 also stimulates expression of atrial natriuretic factor (ANF) in cardiomyocytes [91], binds to cyclin T2a to enhance myoD-dependent transcription [92] and up-regulates transcription of focal adhesion proteins such as paxillin [93].

Cell cycle regulation

PRK1 and PRK2 move to the cleavage furrow of HeLa cells during telophase and the midbody during cytokinesis [15]. Furthermore, siRNA-depletion of PRK2 led to failed abscission, suggesting a direct role in cytokinesis. PRK2 depletion delays the G2/M transition, suggesting a role in mitotic entry (Figure 5C) and the pro-mitotic Cdc25 phosphatase is activated by PRK2 phosphorylation [15]. PRK1 and PRK2 are, therefore, involved in both mitotic entry and exit in human cells.

The G1 phase cyclinD1–CDK6 complex phosphorylates PRK1 and modulates vascular smooth muscle cell division and migration [94,95]. RhoA was shown to transcriptionally regulate cyclinD1 expression via PRK1 in human embryonic stem cells leading to cell proliferation [96]. This suggests that a feedback loop exists between PRK1 and cyclinD1.

The immune response

Pyrin is involved in the formation of a protein complex known as the pyrin inflammasome, part of the innate immune system that forms in response to bacterial infection and induces interleukin (IL) release from macrophages to combat infection. Some toxins, such as *Clostridium botulinum* C3 and *Clostridium difficile* TcdB inactivate RhoA, activating the inflammasome and therefore the innate immune response [97]. Depletion of PRK1 and PRK2 in macrophages leads to inflammasome-dependent IL-1β release, suggesting that these PRKs inhibit pyrin inflammasome activation [98]. The PRKs phosphorylate pyrin, leading to recruitment of 14-3-3 proteins and inhibition of inflammasome activation (Figure 5D). Mutation in the pyrin gene in patients with the autoinflammatory disease Familial Mediterranean Fever result in a pyrin variant with reduced binding to PRKs and increased inflammasome activation. Another autoinflammatory disease, hyperimmunoglobulinemia D syndrome (HIDS), is caused by mutations in the gene for mevalonate kinase, which is necessary for the correct lipid modification and therefore localization of RhoA, underlining the importance of the RhoA-PRK signalling axis in inflammasome regulation.

PRK1 has been implicated in lymphocyte trafficking by studies with a PRK1 kinase-inactive knock-in mouse [99], whose lymphocytes displayed reduced migration from secondary lymphoid organs to the blood and lymph. The same mouse model indicated that PRK1 also regulates trafficking of all leukocytes [100].

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Glucose metabolism and nutrient signalling

Actin cytoskeletal reorganization and membrane ruffling was observed in adipocytes and fibroblasts expressing the insulin receptor upon insulin treatment or when PRK1 or PDK1 were expressed [67]. Non-phosphorylatable PRK1 or kinase-dead PDK1 had no effect, suggesting that activation of PRK1 via PDK1 is vital for signal transduction from the insulin receptor to the actin cytoskeleton.

The effect of PRK1 on insulin-stimulated glucose transport was dependent on both active RhoA and phosphoinositide-3-kinase (PI3K) [101], and expression of PRK1 and active RhoA increased the levels of GLUT4 glucose transporters translocated to the membrane (Figure 6A). Both PRK1 and PRK3 interact with phospholipase D1 [102,103], which could provide the link between PRK1 and GLUT4 vesicle translocation. PRK1 also phosphorylates and inhibits glycogen synthase kinase, GSK-3β, leading to activation of glycogen synthase [104].

In skeletal muscle cells, PRK2 responds to insulin stimulation by promoting glucose uptake, glycogen synthesis and glucose oxidation [105]. Here, PRK2 knockdown led to reduced mTOR phosphorylation and protein synthesis and increased AMP-activated protein kinase (AMPK) signalling, suggesting that PRK2 inhibits AMPK and promotes energy storage and cell growth.

PRK1 and PRK2 both phosphorylate PI3KC2-β, a class II PI3K which catalyzes PIP_2 formation, leading to mTOR1 inactivation [106]. Phosphorylated PI3KC2-β is sequestered by 14-3-3, allowing mTOR1 activation when nutrients and growth factors are abundant. Therefore, mTOR2, which phosphorylates the TM of PRK1 and PRK2 activating them, promotes mTOR1-dependent nutrient signalling in response to growth factors and nutrients (Figure 6B). Since PRK2 also activates Akt [81], an activator of mTOR1, it appears PRK2 has a synergistic effect on cell growth. All the above may be under the control of class I PI3Ks, which can activate PRK1 and PRK2 via mTOR2-dependent TM phosphorylation and PI3K/PDK1-dependent activation loop phosphorylation [43].

Development

PRK2, but not PRK1/PRK3, is essential during mouse embryogenesis. PRK2 knockout embryos showed severe cardiovascular abnormalities, a collapsed mesenchyme, insufficient axial turning, a neural crest migration defect [107] and neural tube closure defects [108], effects which were unobserved with PRK1 and PRK3 knockouts.

Recently, PRK1 has been identified as a key player in mouse cerebellar development [83], indicated by PRK1 knockout mice having problems with axonal outgrowth and with synapse formation in Purkinje cells. Additionally, the *Drosophila melanogaster* orthologue dPkn affects the spatiotemporal regulation of myosin, leading to its relocalization, an event thought to promote the asymmetric division of neural stem cells [109]. A mutation in the dPkn gene has also been linked to wing morphogenesis in the context of the Jun-terminal kinase pathway [110], while dPkn activity has also been associated with negative regulation of actin-myosin contraction in other developmental processes [111] and with dorsal closure [50].

Role in disease

Cancer

PRK1 is overexpressed in human prostate cancer [16] and promotes transcription of AR-regulated genes by phosphorylating histone H3T11, an epigenetic marker of prostate cancer [85]. The importance of PRK1 in prostate cancer is further emphasized as its inhibition abrogates the proliferation of AR-induced tumour cells. In addition, PRK1 and PRK2, activated by thromboxane receptors, mimic and enhance androgen-dependent chromatin remodelling and promote the migration and proliferation of prostate cancer cells [87]. PRK1 is a key driver of metastasis of androgen-independent prostate cancer cells *in vivo,* based on both knockdown of PRK1 or use of a PRK1 inhibitor [93]. Additionally, PRK3, downstream of PI3K, is necessary for the migration of PC-3 cells, while PRK3 inhibition abrogated lymph node metastasis in an orthotopic mouse prostate tumour model [112]. Contrastingly PRK1 and PRK2, but not PRK3, were important for migration in PC-3 cells when the PRKs are activated downstream of the TPα and TPβ receptors [86,87].

PRK1 mRNA is abundant in several other malignant tissues, particularly ovarian cancer, suggesting that PRK1 is implicated in several cancers [113]. A PRK1 mutation (E216K) in the HR1c domain has been described in rhabdomyosarcoma and linked to inhibition of myogenic differentiation [114], while a nuclear factor 1 X-type (NFIX)-PRK1 fusion has been described in a patient with secretory skin carcinoma [115].

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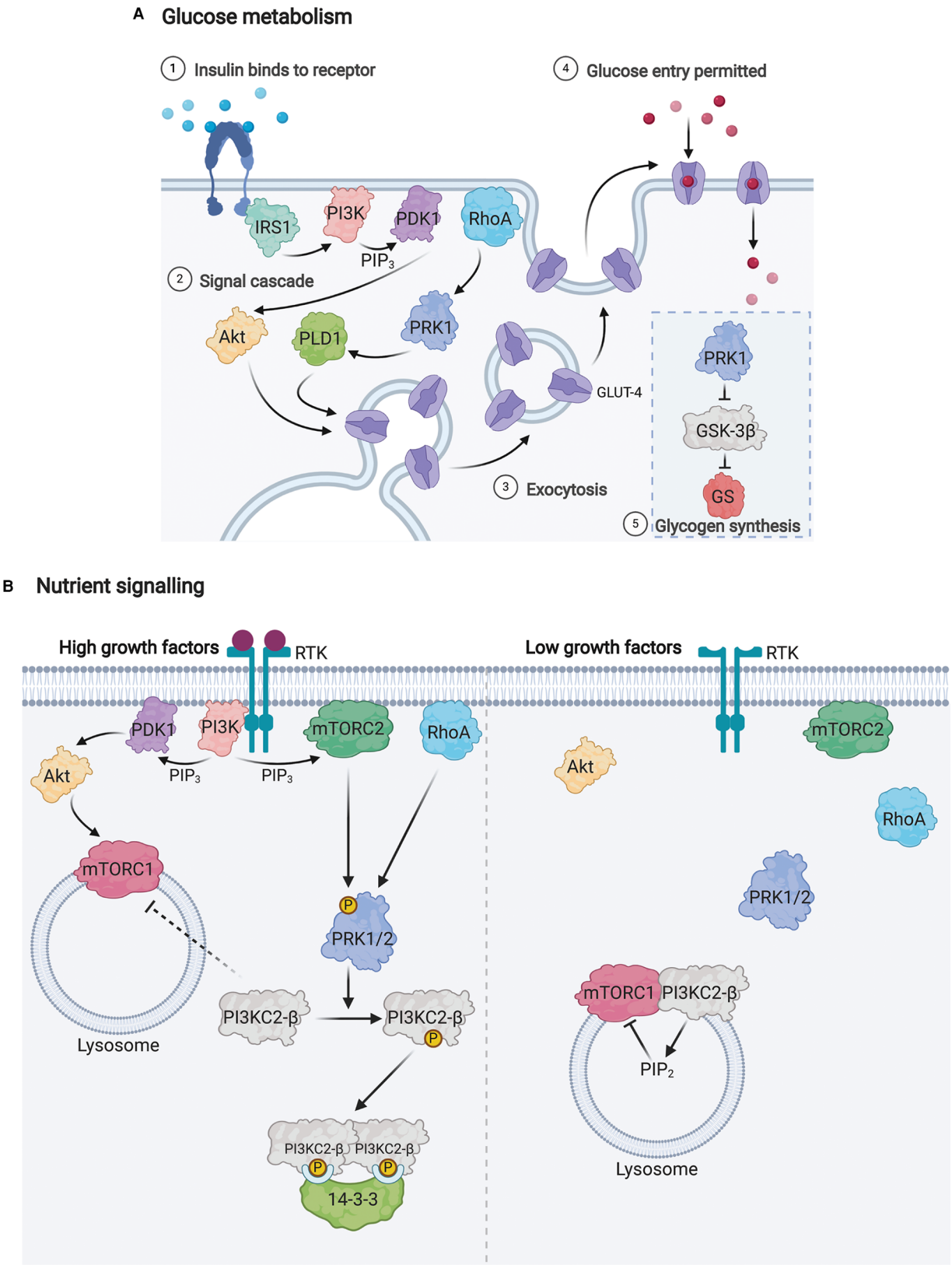

Figure 6. The role of PRK1 in glucose metabolism and nutrient signalling. Part 1 of 2

(**A**) The role of PRK1 in glucose metabolism. (1) Insulin binds to its receptor initiating a signal cascade (2) involving the insulin receptor substrate 1

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Figure 6. The role of PRK1 in glucose metabolism and nutrient signalling. Part 2 of 2

(IRS1)-dependent activation of phosphoinositide 3-kinase (PI3K). This activates PDK1 which in turn activates PRK1. PRK1 interacts with phospholipase D1 (PLD1) to enhance the translocation (3) of GLUT4 receptors to the membrane permitting glucose entry (4). PRK1 also inhibits GSK-3β, an inhibitor of glycogen synthase (GS), to promote glycogen synthesis (5). B) The role of PRK1 in nutrient signalling. PRK1 and PRK2, which are activated by mTOR2, phosphorylate PI3KC2-β, a class II PI3K. This enzyme catalyzes PIP_2 formation causing the sequestration of PI3KC2-β by 14-3-3 proteins and subsequent mTOR1 activation when nutrients are abundant. When nutrients are scarce, PI3KC2-β activity leads to mTOR1 inhibition.

Other PRK1 fusions have been described in melanocytoma and lung squamous cell and hepatocellular carcinomas [116,117], which lack the N-terminal domains and are, therefore, presumed to be constitutively activated.

PRK2, and to a lesser extent PRK1, is vital for cell migration and invasion of a bladder tumour cell line [118]. Consistent with this, silencing of PRK2 led to decreased cell proliferation, migration and colony formation in smoke-exposed keratinocytes, while in unsilenced cells PRK2 was overexpressed and hyperphosphorylated at the activation loop threonine [119]. In contrast, in colon cancer cells PRK2 activated the DUSP6 phosphatase, reducing phosphorylation of Erk1/2 and activation of the CREB/Elk1 transcription factors [120]. This inhibited the transcription of IL4/10, potentially explaining the reduced levels of tumour-associated macrophages in human colon cancer. This is, however, the only anti-tumourigenic role of PRK2 described so far.

PRK2 is highly expressed in triple-negative breast cancer (TNBC) and its depletion in mouse TNBC cells impaired cell proliferation. PRK2 and PRK1 localize to the transition zone of cilia, in agreement with their ability to interact with several cilia proteins and contribute to cilia size and signalling, while PRK2 depletion in polarized epithelial cells impaired planer polarity and anchorage-independent growth [121]. In contrast, CDK10/Cyclin M-dependent phosphorylation of two threonines in the loop between the PRK2 HR1a and HR1b domains may stabilize the interaction of PRK2 with RhoA, leading to actin polymerization and repression of ciliogenesis during cell cycle exit [122].

The role of PRK3 in cancer, particularly metastasis, is being increasingly characterized. Its importance in primary tumour growth and lymph node metastasis was confirmed in an orthotopic mouse prostate cancer model [17], where RhoC was identified its preferred binding partner. PRK3 and RhoC are overexpressed in breast cancer cell lines and PRK3 is critical for their migration in a RhoC-dependent manner. Additionally, PRK3 is vital for melanoma metastasis in mice and has been implicated in the glycosylation of ICAM-1, which is involved in signalling between tumour cells during metastasis [8]. PRK3 also co-localizes with the actin cytoskeleton remodelling adaptor protein $p130^{cas}$ in pro-invasive cell structures and both are overexpressed in breast and prostate cancer. PRK3 phosphorylates $p130^{cas}$ and this is required for PRK3-dependent malignant growth and invasiveness of MEFs expressing constitutively active c-Src [123]. PRK3, therefore, drives metastasis, a hallmark of cancer.

Heart disease

PRK1 activation has been linked to cardiac myocyte survival, increased production and phosphorylation of α crystallin B (a molecular chaperone) and increased cardiac proteasome activity, all of which would protect the heart during ischaemia [124,125]. Hypotonic swelling of cardiac myocytes activates PRK1 and may involve signalling between Src, RhoA, PRK1 and ERK leading to survival [126]. During ischaemia/reperfusion (I/R), PRK1 localizes to the sarcoplasmic reticulum and associates with Ca^{2+}-calmodulin-dependent kinase 2 delta (CamKIIδ) [127], preventing its phosphorylation of phospholamban, which may explain PRK1-dependent cardioprotection *in vivo*. PRK1 knockouts exhibit slight systolic and diastolic dysfunction and show greater I/R injury [127], consistent with a role in cardioprotection. The involvement of PRK1 in cardioprotection is also suggested by its involvement in the transcriptional up-regulation of ANF, which is secreted by the heart to offset high pressure [91,128]. In humans with chromosome 19p13.12 microdeletion syndrome, PRK1 is lost, leading to several congenital cardiac defects and further implicating a role for PRK1 in normal heart physiology [129].

PRK1 is part of a signalling complex assembled by AKAP-Lbc, which includes PRK1, p38α MAPK, MLTK and MKK3: RhoA/PRK1 signalling is needed to activate p38α in cardiomyocytes leading to compensatory cardiac hypertrophy and preservation of cardiac function following pressure overload [130–132]. PRK1 and

PRK2 phosphorylate Myocardin-related transcription factor A (MRTFA), which can then up-regulate cardiac hypertrophic gene expression [133]. However, knockout of PRK1/PRK2 in cardiomyocytes led to greater resistance to cardiac dysfunction, also implicating the PRKs in pathological cardiac hypertrophy and fibrosis regulation [133].

Pathogen infection

Salmonella inject cells with an E3 ubiquitin ligase, SspH1, which selectively removes host proteins. The leucine-rich repeat region of SspH1 binds to PRK1 HR1b (Figure 2D), relieving its own autoinhibition [134]. This leads to SspH1-mediated proteasomal degradation of PRK1, which, given the role of PRK1 in cellular immunity, may be beneficial to the infecting pathogen.

The CagA toxin was responsible for the recruitment of PRK2 from the cytosol to the plasma membrane in *Helicobacter pylori*-infected gastric adenocarcinoma cells [135]. Surprisingly, while CagA inhibited PRK2 activity, cells infected with CagA showed increased cell motility and invasion, and it was suggested that this may be due to disrupted PRK2-dependent cell adhesion, leading to loss of cellular polarity.

Some pathogens, rather than inhibiting PRK2, depend on it for virulence, e.g. the hepatitis C virus (HCV), where PRK2 phosphorylates its RNA-dependent RNA polymerase allowing viral replication [136–138]. Another example is the interaction of PRK2 with the leucine-rich repeat domains of the YopM proteins of *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*; this interaction is necessary for full virulence [139,140] and leads to PRK-dependent pyrin inflammasome inactivation [141,142].

Conclusions

Here, we have summarized the current knowledge about the regulation of the PRKs and the multiple signalling pathways in which they operate.

Several attempts to target the PRK kinase domain have been made with inhibitors that target the active site [37,52,143–148] but, as expected, the well-documented issues with specificity around small molecule inhibitors of kinases apply equally to PRK inhibitors [149]. The complex regulation that we have described above suggests that engineering more specific inhibitors, e.g. to block the PIF pocket, is a potential alternative approach [150]. Extensive understanding of the kinase domain structure, dynamics and allosteric regulation will be necessary for the rational design of such inhibitors.

Attempts have been made to target PRK2 in HCV infection in mice, with siRNA-PRK2 treatment showing potent anti-HCV efficacy, although this needs to be tested in more animal models [151,152]. The low expression of PRK3 in normal tissues and its high expression in cancer suggest that it will have a good therapeutic window. The siRNA/lipoplex complex known as Atu027, designed to inactivate PRK3, showed efficacy in mouse prostate and pancreatic cancer models [68] and very low toxicity in patients [153]. This is currently in Phase II clinical trials for pancreatic cancer [154] and a preliminary Phase Ib/IIa study has demonstrated promising results [155].

As more is discovered about the roles of PRKs in several pathologies, specific inhibitors that could represent useful therapeutics will be eagerly sought.

Perspective

- The PRKs are understudied kinases and there is immediate need for further deciphering of their structure and function.
- More studies are needed to identify novel activation mechanisms, substrates and interacting partners.
- Inhibitors that are specific for each PRK isoform with few off target effects are required to dissect the role of the PRKs in signalling pathways and as lead therapeutics in several pathologies.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contributions

All authors were involved in writing the manuscript. GS produced all the figures.

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Abbreviations

AMPK, AMP-activated protein kinase; ANF, atrial natriuretic factor; AR, androgen receptor; CDK, cyclin-dependent kinase; GPCR, G protein-coupled receptor; GSK-3β, glycogen synthase kinase 3β; HCV, hepatitis C virus; HDAC, histone deacetylase; HIDS, hyperimmunoglobulinemia D syndrome; HM, hydrophobic motif; HPV, human papilloma virus; HR1, homology region 1; HUVECs, human umbilical endothelial cells; I/R, ischaemia/reperfusion; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; MTRFA, Myocardin-related transcription factor A; PDK1, 3-phosphoinositide dependent kinase; PI3K, phosphoinositide-3-kinase; PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP_3, phosphatidylinositol 3,4,5-triphosphate; PKB, protein kinase B; PKC, protein kinase C; PP2A, protein phosphatase 2A; PRK, protein kinase C-related kinase; PTEN, phosphatase and tensin homologue; ROCK, Rho-associated coiled coil kinase; TM, turn motif; TP, thromboxane receptor; TXA_2, thromboxane A_2.

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