## Phylogeny

Phosphoinositide-dependent protein kinase-1 (PDK1; gene PDPK1) is the single member of the PDK1 sub-family within the AGC group of the human kinome (Mora et al., 2004; Biondi, 2004). Orthologues with highly conserved catalytic and pleckstrin-homology (PH) domains are present in Mus musculus, Rattus norvegicus, Xenopus laevis and Drosophila melanogaster (Alessi et al., 1997; Calleja et al., 2014; Falasca & Fyffe, 2013). Like other AGC kinases, PDK1 possesses the conserved bilobal kinase core but uniquely lacks a C-terminal hydrophobic motif, leaving its PIF pocket available for docking partner kinases in trans (Biondi, 2004).

## Reaction Catalyzed

ATP + protein-Ser/Thr ⇌ ADP + protein-O-phospho-Ser/Thr (Biondi, 2004).

## Cofactor Requirements

Catalysis requires millimolar Mg²⁺; Mn²⁺ can substitute in vitro with lower efficiency (Gao & Harris, 2006; Hindie et al., 2009).

## Substrate Specificity

PDK1 phosphorylates activation-loop Ser/Thr residues within the basic consensus R-x-R-x-x-S/T (Reinhardt & Leonard, 2023). High-affinity phosphorylation of many AGC kinases additionally depends on a C-terminal Φ-x-x-Φ hydrophobic motif whose phosphorylated form docks into the PIF pocket to align the activation loop (Mora et al., 2004; Biondi, 2004).

## Structure

• N-terminal kinase domain (~residues 70-359) containing an exposed PIF pocket between β4/β5 and αB/αC (Mora et al., 2004).  
• Linker (~360-407) harbouring the regulatory autophosphorylation site Thr513 (Calleja et al., 2014).  
• C-terminal PH domain (~408-556) with an additional two-β-strand/α-helix bud and a spacious phosphoinositide-binding pocket (Gao & Harris, 2006).

Crystal structures (e.g., PDB 1H1W, 3HRF) show an active kinase core with an assembled hydrophobic spine, an “in” C-helix, and an empty PIF pocket (Mora et al., 2004; Hindie et al., 2009). Full-length models reveal PH-domain homodimerisation that buries Ser241 and occludes the PIF pocket; binding of PtdIns(3,4,5)P₃ or phosphorylation of Thr513 disrupts this interface (Calleja et al., 2014). Membrane-anchored kinase domains can further dimerise via their αG helices to enable trans-autophosphorylation (Reinhardt & Leonard, 2023).

## Regulation

Post-translational modifications  
• Ser241 – constitutive autophosphorylation, essential for activity (Gao & Harris, 2006).  
• Thr513 – autophosphorylation; disrupts PH–PH dimer and increases activity (Calleja et al., 2014).  
• Ser160 – PI3K-dependent phosphorylation that stabilises the active conformation (Calleja et al., 2014).  
• Tyr9, Tyr373, Tyr376 – Src-family phosphorylation, enhancing activity and modulating localisation (Calleja et al., 2014).  
• Ser504, Ser532 – PKCθ-mediated phosphorylation, inhibiting PDK1 under palmitate stress (Calleja et al., 2014).  
• Ser394, Ser398, Thr354 – ASK1/MPK38 phosphorylation, promoting 14-3-3 binding and inhibition (Calleja et al., 2014).  
• Ser549 – S6K1 phosphorylation, strengthening 14-3-3 binding, diminishing PIP₃ affinity and dampening AKT activation (Jiang et al., 2022).

Allosteric inputs  
PtdIns(3,4,5)P₃ or PtdIns(3,4)P₂ binding to the PH domain relieves autoinhibition and recruits PDK1 to membranes; occupation of the PIF pocket by a phosphorylated hydrophobic motif accelerates catalysis; HSP90 binding stabilises the kinase (Alessi et al., 1997; Falasca & Fyffe, 2013; Biondi, 2004; Calleja et al., 2014).

## Function

Expression is ubiquitous, with notable levels in heart and skeletal muscle (Alessi et al., 1997; Garcia-Viloca et al., 2022). PH-domain engagement with 3-phosphorylated phosphoinositides targets PDK1 to the plasma membrane (Falasca & Fyffe, 2013).

Upstream regulators include class I PI3-kinase lipid products, Src kinases, feedback phosphorylation by S6K1, and inhibitory inputs from PKCθ and ASK1/MPK38 (Calleja et al., 2014; Jiang et al., 2022).

PDK1 phosphorylates at least 23 AGC kinases—such as AKT1-3, S6K1, RSK1-3, SGK1-3, conventional/atypical PKCs, PKN1/2 and PAK1—thereby integrating PI3K, mTOR and MAPK signalling pathways that control metabolism, growth, survival and migration; additional substrates (MRCKα, ROCK1, PLCγ1, β3-integrin) link PDK1 to cytoskeletal regulation and motility (Biondi, 2004; di Blasio et al., 2017).

## Inhibitors

• GSK2334470 – ATP-competitive; IC₅₀ ≈ 10 nM; >100-fold selective; blocks SGK and S6K phosphorylation in cells (Najafov et al., 2011; Emmanouilidi & Falasca, 2017).  
• BX-912 – reduces AKT Thr308 phosphorylation (Peifer & Alessi, 2008).  
• BX-795 and BX-320 – indolyl azaindoles; BX-795 IC₅₀ ≈ 0.3 µM (Peifer & Alessi, 2008).  
• OSU-03012 – celecoxib derivative with reported cellular activity (Peifer & Alessi, 2008).  
• 2-O-Bn-InsP₅ – PH-domain ligand that prevents membrane recruitment (Emmanouilidi & Falasca, 2017).

## Other Comments

PDK1 over-expression or hyperactivation is linked to oncogenic transformation, EMT, metastasis and therapy resistance in multiple cancers (di Blasio et al., 2017). A PIF-pocket knock-in mutant (L155E) is embryonically lethal, whereas PH-domain lipid-binding mutants (K465E or “LLL”) cause growth retardation and insulin resistance in mice (Bayascas, 2008). Patient variants that diminish Ser549 phosphorylation or 14-3-3 binding hyperactivate AKT and enhance tumourigenicity (Jiang et al., 2022).

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