## Phylogeny

3-Phosphoinositide-dependent protein kinase-1 (PDK1; gene PDPK1) is the sole representative of the PDK1 subfamily within the AGC group of the human kinome (mora2004pdk1themaster pages 1-2, biondi2004phosphoinositidedependentproteinkinase pages 2-3). Orthologs with conserved catalytic and PH domains are reported in Mus musculus (Pdk1) (calleja2014acuteregulationof pages 1-2), Rattus norvegicus (sequence identity highlighted to human PDK1) (falasca20133phosphoinositidedependentproteinkinase1 pages 2-3), Xenopus laevis (functional studies not detailed here) (mora2004pdk1themaster pages 1-2), and Drosophila melanogaster where DSTPK61 exhibits strong structural and functional homology (alessi19973phosphoinositidedependentproteinkinase1 pages 3-5). PDK1 shares the conserved bilobal kinase core with other AGC kinases but uniquely lacks the intramolecular C-terminal hydrophobic motif, leaving its PIF pocket free to dock substrates in trans (biondi2004phosphoinositidedependentproteinkinase pages 2-3).

## Reaction Catalyzed

ATP + protein-Ser/Thr → ADP + protein-O-phospho-Ser/Thr (biondi2004phosphoinositidedependentproteinkinase pages 1-2).

## Cofactor Requirements

Catalytic activity requires millimolar Mg²⁺; Ser241 autophosphorylation and substrate phosphorylation proceed in its presence. Mn²⁺ can substitute in vitro with reduced efficiency (gao2006roleofthe pages 11-13, hindie2009structureandallosteric pages 1-4).

## Substrate Specificity

PDK1 phosphorylates activation-loop Ser/Thr residues embedded in the basic motif R-x-R-x-x-S/T (reinhardt2023acriticalevaluation pages 2-3). High-affinity phosphorylation of many AGC kinases additionally depends on a C-terminal hydrophobic motif Φ-x-x-Φ whose phosphorylated form docks into the PIF pocket, aligning the activation loop for catalysis (mora2004pdk1themaster pages 3-5, biondi2004phosphoinositidedependentproteinkinase pages 3-5).

## Structure

Domain organisation  
• N-terminal kinase domain (residues ~70-359) displaying the canonical bilobal fold with a solvent-exposed PIF pocket between β4/β5 and helices αB/αC (mora2004pdk1themaster pages 3-5).  
• Linker (residues ~360-407) carrying autophosphorylation site Thr513 (calleja2014acuteregulationof pages 2-3).  
• C-terminal pleckstrin homology (PH) domain (residues ~408-556) containing an additional two-β-strand/α-helix “bud” and a spacious phosphoinositide pocket (gao2006roleofthe pages 1-3).

3D structural information  
Crystal structures 1H1W and 3HRF reveal an assembled hydrophobic spine, an “in” C-helix, a catalytically aligned DFG motif, and an empty PIF pocket (mora2004pdk1themaster pages 3-5, hindie2009structureandallosteric pages 4-7). In full-length models, PH-domain homodimerisation buries Ser241 and occludes the PIF pocket; PtdIns(3,4,5)P₃ binding or Thr513 phosphorylation disrupts this interface (calleja2014acuteregulationof pages 3-4). Membrane-anchored kinase domains can further dimerise via their αG helices to enable trans-autophosphorylation (reinhardt2023acriticalevaluation pages 7-8).

## Regulation

Post-translational modifications  
Residue / enzyme / effect:  
Ser241 – constitutive trans-autophosphorylation, required for activity (gao2006roleofthe pages 11-13).  
Thr513 – autophosphorylation, destabilises PH–PH dimer, increases activity (calleja2014acuteregulationof pages 2-3).  
Ser160 – PI3K-dependent phosphorylation, stabilises active conformation (calleja2014acuteregulationof pages 1-2).  
Tyr9, Tyr373, Tyr376 – Src family phosphorylation, enhances activity and alters localisation (calleja2014acuteregulationof pages 2-3).  
Ser504, Ser532 – PKCθ phosphorylation, inhibits kinase under palmitate stress (calleja2014acuteregulationof pages 1-2).  
Ser394, Ser398, Thr354 – ASK1/MPK38 phosphorylation, promotes 14-3-3 binding and inhibition (calleja2014acuteregulationof pages 2-3).  
Ser549 – S6K1 phosphorylation, strengthens 14-3-3 binding, enhances homodimerisation, reduces PIP₃ affinity, dampens AKT activation (jiang2022s6k1mediatedphosphorylationof pages 1-2).

Allosteric inputs  
• Binding of PtdIns(3,4,5)P₃ or PtdIns(3,4)P₂ to the PH domain relieves autoinhibition and co-localises PDK1 with membrane-tethered substrates (alessi19973phosphoinositidedependentproteinkinase1 pages 9-10, falasca20133phosphoinositidedependentproteinkinase1 pages 2-3).  
• Occupancy of the PIF pocket by a phosphorylated hydrophobic motif allosterically accelerates catalysis 3- to 4-fold (biondi2004phosphoinositidedependentproteinkinase pages 3-5).  
• HSP90 stabilises PDK1 and facilitates its interaction with Src; HSP90 inhibition reduces PDK1 stability (calleja2014acuteregulationof pages 2-3).

## Function

Expression and localisation  
PDK1 mRNA and protein are ubiquitous; detectable in heart, skeletal muscle and other organs (alessi19973phosphoinositidedependentproteinkinase1 pages 1-2, garciaviloca2022molecularinsightsinto pages 12-13). PH-domain binding to 3-phosphorylated phosphoinositides targets the kinase to the plasma membrane (falasca20133phosphoinositidedependentproteinkinase1 pages 2-3).

Upstream regulators  
Class-I PI3-kinase products PtdIns(3,4,5)P₃/PtdIns(3,4)P₂, Src family kinases, and feedback phosphorylation by S6K1 modulate activity, whereas PKCθ and ASK1/MPK38 deliver inhibitory phosphorylations (calleja2014acuteregulationof pages 1-2, jiang2022s6k1mediatedphosphorylationof pages 1-2).

Downstream substrates and signalling pathways  
PDK1 phosphorylates the activation loop of at least 23 AGC kinases including PKB/AKT1-3, S6K1, RSK1-3, SGK1-3, conventional and atypical PKCs, PKN1/2 and PAK1, integrating PI3K, mTOR and MAPK pathways that govern metabolism, growth, survival and migration (biondi2004phosphoinositidedependentproteinkinase pages 1-2, blasio2017serinethreoninekinase3phosphoinositidedependent pages 1-3). Additional substrates such as MRCKα, ROCK1, PLCγ1 and β3-integrin link PDK1 to cytoskeletal reorganisation and cell motility (blasio2017serinethreoninekinase3phosphoinositidedependent pages 1-3).

## Inhibitors

GSK2334470 – ATP-competitive; IC₅₀ ≈ 10 nM on purified PDK1; >100-fold selectivity over 93 kinases; blocks SGK and S6K T-loop phosphorylation in cells (najafov2011characterizationofgsk2334470 pages 1-2, emmanouilidi2017targetingpdk1for pages 11-13).  
BX-912 – substituted pyrimidine; reduces AKT Thr308 phosphorylation in PTEN-deficient PC-3 cells (peifer2008small‐moleculeinhibitorsof pages 1-3).  
BX-795 and BX-320 – indolyl azaindoles; BX-795 IC₅₀ ≈ 0.3 µM on PDK1 and diminishes AKT and S6K phosphorylation in cells (peifer2008small‐moleculeinhibitorsof pages 10-11).  
OSU-03012 – celecoxib derivative; reported cellular PDK1 inhibition with anticancer activity (peifer2008small‐moleculeinhibitorsof pages 1-3).  
2-O-Bn-InsP₅ – PH-domain ligand that prevents membrane recruitment and downstream AKT activation (emmanouilidi2017targetingpdk1for pages 11-13).

## Other Comments

Over-expression or hyperactivation of PDK1 is associated with oncogenic transformation, epithelial-mesenchymal transition, metastasis and therapy resistance in melanoma, colorectal, ovarian, gallbladder and other cancers (blasio2017serinethreoninekinase3phosphoinositidedependent pages 16-17). A knock-in PIF-pocket mutant (L155E) causes embryonic lethality, whereas PH-domain lipid-binding mutants (K465E or “LLL”) produce growth retardation and insulin resistance in mice (bayascas2008dissectingtherole pages 3-4). Patient-derived variants that lower Ser549 phosphorylation or 14-3-3 binding hyperactivate AKT and enhance tumourigenicity (jiang2022s6k1mediatedphosphorylationof pages 1-2).

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