## Phylogeny

According to the classification by Manning et al. (2002), PINK1 is placed in the TKL (tyrosine kinase-like) group and assigned to the PINK kinase family (manning2002theproteinkinase pages 3-3). However, another analysis based on the same classification framework suggests that due to a lack of significant relationship with the nine main kinase groups, human PINK1 falls into the ‘OTHER’ category (modi2019astructurallyvalidatedmultiple pages 8-10). PINK1 shows high homology to the Ca2+/calmodulin-dependent kinase (CAMK) family and is also classified within the NSKs (nervous system function and development kinase) family (unknownauthors2012pink1acritical pages 47-49, unknownauthors2012pink1acritical pages 46-47). PINK1 is a highly conserved kinase in metazoans, with known orthologs in *Homo sapiens* (human), *Macaca mulatta* (rhesus macaque), *Mus musculus* (mouse), *Gallus gallus* (chicken), *Danio rerio* (zebrafish), *Triboleum castaneum* (beetle), *Drosophila melanogaster* (fruit fly), and *Caenorhabditis elegans* (nematode); it is absent from yeast (unknownauthors2012pink1acritical pages 46-47, goncalves2024pink1g411smutantincreases pages 29-31, unknownauthors2016theparkinsonsdiseaserelated pages 15-18). Human PINK1 shares 97% sequence identity with its rhesus macaque ortholog, 81% with mouse, 64% with chicken, 54% with zebrafish, approximately 40-45% with insects, and 32% with *C. elegans* (unknownauthors2012pink1acritical pages 46-47).

## Reaction Catalyzed

PINK1 catalyzes the transfer of the γ-phosphate from ATP to the hydroxyl group of serine or threonine residues on substrate proteins (unknownauthors2012pink1acritical pages 47-49, unknownauthors2012pink1acritical pages 49-53).

## Cofactor Requirements

Catalytic activity requires the presence of divalent metal ion cofactors, specifically Mg²⁺ or Mn²⁺ (goncalves2024pink1g411smutantincreases pages 29-31, unknownauthors2012pink1acritical pages 46-47). The DFG motif within the activation loop is responsible for chelating Mg²⁺ to assist in ATP coordination and phosphoryl transfer (unknownauthors2012pink1acritical pages 46-47).

## Substrate Specificity

A comprehensive analysis of the human kinome found that PINK1 clusters with kinases LKB1, CAMKK, and PBK (cluster 14), which possess a distinct substrate recognition motif (johnson2023anatlasof pages 2-3). The PINK1 consensus phosphorylation motif favors a serine or threonine residue preceded by acidic or hydrophobic residues, with a preference for a proline at the +1 position and an acidic residue at the -3 position (unknownauthors2024characterizationofa pages 38-42). There is also a preference for hydrophobic residues at the P+1 position, with no strict polarity requirement at the P+2 position (quinn2020pink1parkinsignallingin pages 4-6).

## Structure

PINK1 is a 581-amino acid kinase with several functional domains: an N-terminal mitochondrial targeting sequence (MTS, residues 1-34), an N-terminal α-helical extension (NTE), a transmembrane domain (TMD, residues 89-111), a catalytic kinase domain (residues 156-510), and a C-terminal extension (CTE, residues 511-581) (kakade2022mappingofa pages 1-2, unknownauthors2019characterizationofparlmediated pages 39-45). A critical intramolecular interface forms between the NTE and the CTE, which is required for PINK1 stabilization, autophosphorylation, and activation (kakade2022mappingofa pages 1-2). The kinase domain contains conserved features typical of Ser/Thr kinases, including the AIK motif in subdomain II for ATP binding, a catalytic loop with the HRD motif (subdomain VI), an activation loop containing the DFG motif for Mg²⁺ chelation and ATP orientation (subdomain VII), and an APE motif in subdomain VIII that stabilizes the domain (unknownauthors2012pink1acritical pages 46-47, unknownauthors2012pink1acritical pages 47-49). The kinase domain also possesses unique structural features, such as the Ins3 domain insertion (biswas2023aninsilicoapproach pages 1-5).

## Regulation

PINK1 activity is regulated by mitochondrial health, primarily through post-translational modifications and proteolytic cleavage (quinn2020pink1parkinsignallingin pages 4-6, choubey2021molecularmechanismsand pages 2-4). Under normal mitochondrial membrane potential (ΔΨm), PINK1 is imported into the inner mitochondrial membrane (IMM) via TOM/TIM23 complexes and sequentially cleaved, first by mitochondrial processing peptidase (MPP) and then by the rhomboid protease PARL at residue Ala103 (quinn2020pink1parkinsignallingin pages 4-6, unknownauthors2019characterizationofparlmediated pages 39-45). The resulting 52 kDa fragment is retro-translocated to the cytosol and targeted for degradation by the N-end rule pathway, involving UBR1, UBR2, and UBR4 E3 ligases (quinn2020pink1parkinsignallingin pages 4-6). Upon mitochondrial depolarization, PINK1 import stalls, leading to its accumulation on the outer mitochondrial membrane (OMM) (goncalves2024pink1g411smutantincreases pages 1-5). On the OMM, PINK1 dimerizes and undergoes autophosphorylation at residues Ser228 and Ser402, leading to its activation (goncalves2024pink1g411smutantincreases pages 1-5, kakade2022mappingofa pages 1-2). PINK1 is also ubiquitinated at Lys137, marking it for proteasomal degradation (unknownauthors2024characterizationofa pages 38-42).

## Function

PINK1 mRNA is expressed in many tissues, including the brain, where it is found in cortical neurons, Purkinje cells, the striatum, and the brainstem (unknownauthors2012pink1acritical pages 44-46). PINK1 acts as a primary sensor of mitochondrial damage, initiating quality control mechanisms like mitophagy (brunelli2022pink1protectsagainst pages 15-15, choubey2021molecularmechanismsand pages 2-4). Upon accumulation on the OMM of damaged mitochondria, activated PINK1 phosphorylates two key substrates to trigger mitophagy: ubiquitin at Ser65 and the E3 ubiquitin ligase Parkin at Ser65 within its ubiquitin-like (Ubl) domain (biswas2023aninsilicoapproach pages 1-5, harper2018buildinganddecoding pages 4-5). Phosphorylation of Parkin releases its autoinhibition, and its subsequent binding to PINK1-phosphorylated ubiquitin (pSer65-Ub) leads to full Parkin activation, creating a positive feedback loop (harper2018buildinganddecoding pages 4-5, choubey2021molecularmechanismsand pages 4-6). Activated Parkin ubiquitinates numerous OMM proteins, flagging the mitochondrion for autophagic degradation (harper2018buildinganddecoding pages 4-5). Beyond mitophagy, PINK1 phosphorylates other substrates, such as MFN2, Miro, HtrA2, and TRAP1, to regulate mitochondrial dynamics, calcium homeostasis, and oxidative stress responses (unknownauthors2024characterizationofa pages 38-42). It also interacts with Beclin1 to inhibit apoptosis and can mitigate STING-induced inflammation (brunelli2022pink1protectsagainst pages 15-15).

## Other Comments

Loss-of-function mutations in the *PINK1* gene are a cause of autosomal recessive early-onset Parkinson’s disease (ARJP) (biswas2023aninsilicoapproach pages 1-5). More than 130 pathogenic mutations have been identified, most of which are located in the kinase domain and result in impaired or abolished mitophagy, leading to the accumulation of damaged mitochondria and neuronal degeneration (goncalves2024pink1g411smutantincreases pages 1-5, biswas2023aninsilicoapproach pages 1-5). Specific mutations such as G309D and L347P significantly impair kinase activity (unknownauthors2024characterizationofa pages 38-42). Other mutations like P296L (in the Ins3 region) and Q126P (in the NTE:CTE interface) disrupt PINK1’s interaction with Parkin or impair its stabilization and activation, respectively (biswas2023aninsilicoapproach pages 1-5, kakade2022mappingofa pages 1-2). In contrast, the G411S variant is a gain-of-function mutation that enhances kinase activity by increasing the stability of the ATP-binding pocket, though its role in PD risk is controversial (goncalves2024pink1g411smutantincreases pages 1-5).

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