## Phylogeny

Death-associated protein kinase 3 (DAPK3) is a serine/threonine kinase belonging to the death-associated protein kinase (DAPK) family, which also includes DAPK1, DAPK2 (DRP-1), DRAK1, and DRAK2 (usui2014zipperinteractingprotein pages 1-2, gozuacik2006dapkproteinfamily pages 1-2, bialik2006thedeathassociatedprotein pages 1-2). Based on comprehensive kinome analysis, the DAPK family is phylogenetically classified within the Ca2+/calmodulin-dependent protein kinase (CAMK) group (gozuacik2006dapkproteinfamily pages 1-2, bialik2006thedeathassociatedprotein pages 1-2, farag2019death‐associatedproteinkinase pages 2-4). DAPK3 is part of a distinct CAMK branch that also includes myosin light-chain-related kinases (MLCKs) and triple functional domain protein-related (TRIO) kinases (temmerman2013structuralandfunctional pages 1-2). DAPK3 shares approximately 80-83% amino acid identity in its catalytic domain with DAPK1 and DAPK2 and is more distantly related to the DRAK kinases (bialik2006thedeathassociatedprotein pages 2-4, farag2019death‐associatedproteinkinase pages 2-4, shani2004deathassociatedproteinkinase pages 1-2). Homologs are primarily found in mammals (bialik2006thedeathassociatedprotein pages 2-4). Unlike other DAPK family members, DAPK3 is a calcium/calmodulin-independent kinase (shoval2011newmodularityof pages 11-11, farag2019death‐associatedproteinkinase pages 36-37).

## Reaction Catalyzed

DAPK3 is a serine/threonine kinase that catalyzes the transfer of a phosphate group from ATP to serine or threonine residues on substrate proteins (alghabkari2024cooperativeinvolvementof pages 13-15, bialik2006thedeathassociatedprotein pages 1-2). The reactions are: 1. ATP + a [protein]-L-serine = ADP + a [protein]-L-serine phosphate (shoval2011newmodularityof pages 11-11, bialik2006thedeathassociatedprotein pages 2-4). 2. ATP + a [protein]-L-threonine = ADP + a [protein]-L-threonine phosphate (shoval2011newmodularityof pages 11-11, bialik2006thedeathassociatedprotein pages 2-4).

## Cofactor Requirements

The catalytic activity of DAPK3 requires ATP as a cofactor (alghabkari2024cooperativeinvolvementof pages 13-15). The kinase reaction also utilizes divalent cation cofactors such as Mg2+ (bialik2006thedeathassociatedprotein pages 2-4). Its activity is independent of Ca2+/calmodulin (CaM), which distinguishes it from other DAPK family members such as DAPK1 and DRP-1 (bialik2006thedeathassociatedprotein pages 4-6, usui2014zipperinteractingprotein pages 1-2, shani2004deathassociatedproteinkinase pages 1-2).

## Substrate Specificity

A comprehensive atlas of substrate specificities for the human serine/threonine kinome has defined a precise consensus motif for DAPK3 (farag2019death‐associatedproteinkinase pages 6-9). The substrate motif is characterized by a preference for basic residues at positions -3 and -2, acidic residues at the +1 position, and hydrophobic residues at the +3 position relative to the phosphorylated serine or threonine (farag2019death‐associatedproteinkinase pages 6-9).

Key physiological substrates include: - **MYPT1** (myosin phosphatase target subunit 1) (alghabkari2024cooperativeinvolvementof pages 13-15, bialik2006thedeathassociatedprotein pages 1-2, bialik2006thedeathassociatedprotein pages 4-6). - **MYL9 / MLC** (myosin regulatory light chain 9) (alghabkari2024cooperativeinvolvementof pages 13-15, bialik2006thedeathassociatedprotein pages 1-2, bialik2006thedeathassociatedprotein pages 4-6). - **LC20 / MYL12B** (myosin light chain 20) (usui2014zipperinteractingprotein pages 1-2, usui2014zipperinteractingprotein pages 2-4). - **STAT3** (at Ser727) (farag2019death‐associatedproteinkinase pages 10-12). - **Beclin-1** (at Ser90) (farag2019death‐associatedproteinkinase pages 10-12). - **Histone H3** (bialik2006thedeathassociatedprotein pages 8-10). - **p21WAF1/p21Cip1** (bialik2006thedeathassociatedprotein pages 8-10, bialik2006thedeathassociatedprotein pages 4-6). - **GAIT complex component L13A** (bialik2014thedapkinaseinteractome pages 5-6).

## Structure

DAPK3 consists of 454 amino acids with an approximate molecular weight of 52.5 kDa (farag2019death‐associatedproteinkinase pages 2-4, usui2014zipperinteractingprotein pages 1-2). While full-length crystal structures have not been reported, six structures of the kinase domain are available (farag2019death‐associatedproteinkinase pages 2-4). - **Domain Organization**: DAPK3 contains an N-terminal kinase domain and a C-terminal extension with multiple functional domains (bialik2006thedeathassociatedprotein pages 4-6, usui2014zipperinteractingprotein pages 1-2). It lacks the ankyrin repeats and the C-terminal death domain characteristic of DAPK1 (bialik2006thedeathassociatedprotein pages 1-2, shani2004deathassociatedproteinkinase pages 1-2, farag2019death‐associatedproteinkinase pages 2-4). One source provides contradictory information, stating that DAPK3 contains ankyrin repeats and a death domain (usui2014zipperinteractingprotein pages 1-2). The C-terminal region contains a leucine zipper domain (residues 427–441) essential for homodimerization and kinase activation, and there are four putative nuclear localization signals (NLS) (farag2019death‐associatedproteinkinase pages 4-6, shani2004deathassociatedproteinkinase pages 1-2). The kinase also has an autoinhibitory domain (alghabkari2024cooperativeinvolvementof pages 13-15). - **Key Features**: The catalytic domain contains 11 typical kinase subdomains, a hinge region allowing lobe flexibility, a conserved lysine (K42) essential for phosphotransfer, and a unique basic loop that facilitates dimerization (farag2019death‐associatedproteinkinase pages 4-6, bialik2014thedapkinaseinteractome pages 5-6). It also features a distinct HF/LD motif that replaces the canonical HRD motif found in other CAMK-group kinases (temmerman2013structuralandfunctional pages 1-2).

## Regulation

DAPK3 activity is controlled by autophosphorylation, phosphorylation by upstream kinases, protein-protein interactions, and ubiquitination (bialik2006thedeathassociatedprotein pages 4-6, bialik2006thedeathassociatedprotein pages 8-10). - **Autophosphorylation**: DAPK3 is regulated by positive autophosphorylation, which enhances its catalytic activity (bialik2006thedeathassociatedprotein pages 4-6). Key autophosphorylation sites that are critical for activity include Thr180, Thr225, and Thr265 (usui2014zipperinteractingprotein pages 1-2, bialik2006thedeathassociatedprotein pages 4-6). Phosphorylation at Thr265 modulates kinase activity and subcellular localization, while phosphorylation at Thr299 (analogous to Thr265) affects cytoplasmic localization and cell death-promoting activity (alghabkari2024cooperativeinvolvementof pages 13-15, bialik2006thedeathassociatedprotein pages 8-10). - **Upstream Kinases**: DAPK3 is a downstream target in a kinase hierarchy where DAPK1 phosphorylates it at multiple sites, including six residues in the C-terminal domain, which modulates its localization and amplifies cell death signals (bialik2006thedeathassociatedprotein pages 8-10, shani2004deathassociatedproteinkinase pages 1-2). ROCK1 also phosphorylates and activates DAPK3 (shoval2011newmodularityof pages 11-11). The MAPK/ERK cascade kinases ERK and RSK can phosphorylate DAPK3 with opposing effects (bialik2006thedeathassociatedprotein pages 8-10). - **Protein Interactions and Other Modifications**: Homodimerization via its leucine zipper domain is essential for kinase activation (farag2019death‐associatedproteinkinase pages 6-9, shani2004deathassociatedproteinkinase pages 1-2). DAPK3 interacts with the molecular chaperone Hsp90, which stabilizes the kinase (farag2019death‐associatedproteinkinase pages 4-6). The E3 ubiquitin ligase DIP-1 mediates its ubiquitination and turnover (bialik2006thedeathassociatedprotein pages 4-6). The phosphatase PP2A can dephosphorylate DAPK family members (farag2019death‐associatedproteinkinase pages 30-31). DAPK3 expression is inducible by stress signals such as TGFβ and p53 activation (bialik2006thedeathassociatedprotein pages 8-10).

## Function

DAPK3 is a multifunctional kinase involved in the regulation of smooth muscle contraction, apoptosis, autophagy, and cytoskeletal dynamics (alghabkari2024cooperativeinvolvementof pages 13-15, bialik2006thedeathassociatedprotein pages 1-2). It is localized to both the nucleus and cytoplasm (shani2004deathassociatedproteinkinase pages 1-2). - **Signaling Pathways**: DAPK3 functions downstream of DAPK1 in a kinase cascade and operates downstream of G-protein–coupled receptor pathways involving Gα12/13 and Gαq/11 (bialik2006thedeathassociatedprotein pages 8-10, farag2019death‐associatedproteinkinase pages 19-22). - **Smooth Muscle Function**: It regulates vascular smooth muscle cell migration and contraction, thereby maintaining vascular tone (alghabkari2024cooperativeinvolvementof pages 13-15). It achieves this by phosphorylating MYL9 and MYPT1, which leads to inhibition of myosin phosphatase and enhances Ca2+ sensitization (usui2014zipperinteractingprotein pages 2-4, alghabkari2024cooperativeinvolvementof pages 13-15). - **Cell Death and Autophagy**: DAPK3 promotes apoptosis and membrane blebbing via phosphorylation of myosin light chain (MLC) (shani2004deathassociatedproteinkinase pages 1-2, bialik2006thedeathassociatedprotein pages 4-6). It is also involved in starvation-induced autophagy through the phosphorylation of Beclin-1 at Ser90 (farag2019death‐associatedproteinkinase pages 10-12). - **Cytoskeletal Regulation**: The kinase plays a role in actin filament assembly, focal adhesion dynamics, and cell motility (alghabkari2024cooperativeinvolvementof pages 13-15). It interacts with proteins such as PAR4 to influence actin filament dynamics (usui2014zipperinteractingprotein pages 1-2). - **Other Cellular Roles**: DAPK3 participates in transcriptional regulation by binding to ATF4 and phosphorylating histone H3 (farag2019death‐associatedproteinkinase pages 2-4, bialik2006thedeathassociatedprotein pages 8-10). It is also involved in inflammatory responses by phosphorylating the GAIT complex component L13A during IFN-γ signaling (bialik2014thedapkinaseinteractome pages 5-6). It cooperatively functions with the phosphatase CDC14A in vascular smooth muscle cell migration (alghabkari2024cooperativeinvolvementof pages 13-15).

## Inhibitors

Several classes of selective, ATP-competitive inhibitors for DAPK3 have been identified (carlson2013fluorescencelinkedenzyme pages 3-4, farag2019death‐associatedproteinkinase pages 19-22). - **Pyrazolo[3,4-d]pyrimidinone derivatives**: These compounds potently inhibit DAPK3 activity and suppress smooth muscle calcium sensitization (alghabkari2024cooperativeinvolvementof pages 13-15). Compound 9 from this class inhibits DAPK3 with nanomolar affinity and reduces phosphorylation of MYPT1 and MYL9 (farag2019death‐associatedproteinkinase pages 19-22). - **HS38**: This thiol-substituted pyrazolo[3,4-d]pyrimidinone derivative is a potent and selective inhibitor of DAPK3 (ZIPK) and DAPK1, with a Kd of ~280 nM for ZIPK, and shows minimal off-target effects on ROCK2 or smMLCK (carlson2013fluorescencelinkedenzyme pages 3-4). - **Other Scaffolds**: Synthetic small molecules based on aminopyridazine and imidazo[1,2-b]pyridazine scaffolds have been characterized as inhibitors of the DAPK family (farag2019death‐associatedproteinkinase pages 17-19).

## Other Comments

Dysregulation of DAPK3 is associated with several human diseases (usui2014zipperinteractingprotein pages 1-2, alghabkari2024cooperativeinvolvementof pages 13-15). - **Disease Associations**: DAPK3 contributes to hypertension by increasing vascular smooth muscle contractility (alghabkari2024cooperativeinvolvementof pages 13-15, usui2014zipperinteractingprotein pages 2-4). It also acts as a tumor suppressor, with its expression often decreased in cancers such as gastric cancer and squamous cell carcinoma (usui2014zipperinteractingprotein pages 1-2). The kinase is also implicated in cancer progression, stroke, neurodegeneration, and ischemia-induced neuronal damage (alghabkari2024cooperativeinvolvementof pages 13-15, bialik2014thedapkinaseinteractome pages 11-12, bialik2006thedeathassociatedprotein pages 1-2). - **Mutations**: Mutations in the leucine zipper domain, including V422A, V429A, and L436A, significantly reduce DAPK3 homodimerization and autophosphorylation, thereby affecting its kinase activity (farag2019death‐associatedproteinkinase pages 6-9).

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