## Phylogeny

ULK2 belongs to the unc-51 like autophagy activating kinase (ULK) family, which is part of the serine/threonine kinase group within the human kinome as classified by Manning et al. (chaikuad2019conservationofstructure pages 1-4, karmacharya2023smallmoleculeinhibitors pages 1-2). The ULK family is assigned to the CAMK (Ca2+/calmodulin-dependent kinase) group (chaikuad2019conservationofstructure pages 12-15, kumar2020apancancerassessment pages 2-3). ULK2 is a human paralog of ULK1 and a human ortholog of yeast Atg1 and *C. elegans* UNC-51 kinase (demeter2020ulk1andulk2 pages 3-5, demeter2020ulk1andulk2 pages 1-3, yan1999mouseulk2a pages 1-3). The ULK1 and ULK2 genes arose from a duplication event of a single Atg1 ortholog at the base of the Chordates approximately 500 million years ago (demeter2020ulk1andulk2 pages 3-5). Orthologs of ULK2 are conserved across species involved in autophagy regulation (chaikuad2019conservationofstructure pages 17-20).

## Reaction Catalyzed

ULK2 catalyzes the phosphotransfer reaction of transferring the gamma-phosphate from an ATP molecule to the hydroxyl group of serine or threonine residues on substrate proteins (chaikuad2019conservationofstructure pages 1-4, chaikuad2019conservationofstructure pages 17-20, karmacharya2023smallmoleculeinhibitors pages 1-2).

## Cofactor Requirements

The catalytic activity of ULK2 requires divalent metal ions, specifically Mg2+, to stabilize ATP binding and facilitate catalysis (chaikuad2019conservationofstructure pages 1-4, chaikuad2019conservationofstructure pages 17-20, demeter2020ulk1andulk2 pages 6-10, chaikuad2019conservationofstructure pages 4-7).

## Substrate Specificity

Experimental substrate specificity profiling using positional scanning peptide libraries (PSPA) places ULK2 in Cluster 1 of basophilic kinases (johnson2023anatlasof pages 12-18). As a basophilic kinase, it favors basic residues such as arginine or lysine upstream of the phosphorylation site, with a consensus motif format described as R-x-x-S/T or similar (johnson2023anatlasof pages 12-18). The specificity is also influenced by negative selection against certain residues, which helps insulate phosphosites from phosphorylation by unrelated kinases (johnson2023anatlasof pages 3-4). The kinase has a preference for phosphorylating serine residues when a large hydrophobic residue is present at the DFG+1 position of the kinase, a feature consistent with its homolog ULK1 (kumar2020apancancerassessment pages 3-4).

## Structure

ULK2 is a 1033 amino acid protein composed of three primary domains: an N-terminal kinase domain (KD) responsible for catalysis, a central proline/serine-rich (PS) domain, and a C-terminal domain (CTD) that mediates protein-protein interactions and complex formation (chaikuad2019conservationofstructure pages 1-4, karmacharya2023smallmoleculeinhibitors pages 1-2, demeter2020ulk1andulk2 pages 1-3, yan1999mouseulk2a pages 1-3). The KD of ULK2 shares 78.71% sequence identity with ULK1 (demeter2020ulk1andulk2 pages 6-10).

Crystal structures of ULK2 (e.g., PDB IDs 6QAS, 6QAT) show that it forms a dimer via an activation segment domain exchange, a mechanism not observed for the typically monomeric ULK1 (chaikuad2019conservationofstructure pages 1-4, chaikuad2019conservationofstructure pages 7-10, karmacharya2023smallmoleculeinhibitors pages 2-5). This dimerization is thought to facilitate trans-autophosphorylation and activation (chaikuad2019conservationofstructure pages 7-10). The kinase domain features conserved catalytic motifs like DFG and APE, and a salt bridge between K46 and E63 that is essential for activity, analogous to ULK1 (kumar2020apancancerassessment pages 3-4). Structurally unique features include a flexible methionine gatekeeper residue and an outward-facing phenylalanine in the P-loop, creating an unusually large inhibitor-binding pocket (chaikuad2019conservationofstructure pages 10-12).

## Regulation

ULK2 activity is regulated by phosphorylation. The kinase undergoes autophosphorylation on its kinase and PS domains (yan1999mouseulk2a pages 1-3). The dimeric structure facilitates trans-autophosphorylation on threonine 173 (T173) in the activation loop, a modification crucial for kinase activation (chaikuad2019conservationofstructure pages 7-10).

Upstream kinases modulate ULK2 activity in response to cellular nutrient status. Under nutrient-replete conditions, mTORC1 phosphorylates ULK2, leading to its inactivation (demeter2020ulk1andulk2 pages 1-3, karmacharya2023smallmoleculeinhibitors pages 2-5). In response to nutrient starvation or energy stress, mTORC1 dissociates, and AMPK activates ULK2 through phosphorylation at specific sites (chaikuad2019conservationofstructure pages 1-4, chaikuad2019conservationofstructure pages 17-20, karmacharya2023smallmoleculeinhibitors pages 2-5). ULK2 also contains unique protein motifs absent in ULK1, such as a TRAF6 binding site, MAPK docking motifs, and calcium-dependent binding motifs, which suggest distinct regulatory inputs from cytokine, MAPK, and calcium signaling pathways (demeter2020ulk1andulk2 pages 13-14, demeter2020ulk1andulk2 pages 5-6).

## Function

ULK2 mRNA is broadly expressed in adult mouse tissues, with notable expression during nervous system development and higher levels in the spinal cord, corpus callosum, and testis (yan1999mouseulk2a pages 1-3, demeter2020ulk1andulk2 pages 6-10).

ULK2 is a key initiator of autophagy, acting upstream of the phosphatidylinositol 3-kinase PIK3C3 (sidat2022ulk12inhibitoressential pages 8-9). It forms a complex with ATG13, RB1CC1/FIP200, and ATG101, which is essential for the formation of autophagophores (chaikuad2019conservationofstructure pages 1-4). Activated ULK2 phosphorylates downstream autophagy components like VPS34 and Beclin-1 (karmacharya2023smallmoleculeinhibitors pages 2-5, sidat2022ulk12inhibitoressential pages 8-9). While functionally overlapping with ULK1, ULK2 has distinct roles; it uniquely interacts with WIPI2 in xenophagy and its interactors are enriched in nitrogen compound metabolic processes (demeter2020ulk1andulk2 pages 3-5, demeter2020ulk1andulk2 pages 10-12). Beyond autophagy, ULK2 is involved in neuronal development, particularly axon formation, and lipid metabolism (chaikuad2019conservationofstructure pages 1-4, demeter2020ulk1andulk2 pages 1-3).

## Inhibitors

Several ATP-competitive small molecule inhibitors target the highly conserved kinase domains of both ULK1 and ULK2 (chaikuad2019conservationofstructure pages 1-4). These include MRT68921 (IC50 = 1.1 nM; KD ~4.9 nM), MRT67307 (IC50 = 38 nM), and SBI-0206965 (chaikuad2019conservationofstructure pages 1-4, chaikuad2019conservationofstructure pages 10-12, sidat2022ulk12inhibitoressential pages 8-9). Other compounds developed as ULK1/2 inhibitors are ULK-101 and PF-03814735 (chaikuad2019conservationofstructure pages 1-4, chaikuad2019conservationofstructure pages 17-20). The compound DCC-3116 is currently in clinical trials for cancer therapy (karmacharya2023smallmoleculeinhibitors pages 1-2). A common issue with many of these inhibitors is off-target activity against Aurora A kinase (chaikuad2019conservationofstructure pages 1-4, chaikuad2019conservationofstructure pages 12-15).

## Other Comments

Dysregulation of ULK2 is linked to multiple human diseases, including cancer (lung, neuroblastoma, leukemia), Crohn’s disease, neurodegenerative disorders, and autoimmune disorders (chaikuad2019conservationofstructure pages 17-20, karmacharya2023smallmoleculeinhibitors pages 1-2). ULK2 has been reported to be downregulated in colon biopsies from patients with inactive ulcerative colitis (demeter2020ulk1andulk2 pages 13-14, demeter2020ulk1andulk2 pages 12-13). The combined loss of both ULK1 and ULK2 in mice results in neonatal lethality, underscoring their critical and shared importance (demeter2020ulk1andulk2 pages 3-5).

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