## Phylogeny

The priority publication by Manning et al. (2002) does not explicitly mention or classify STK32A (YANK1) (manning2002theproteinkinase pages 1-2, manning2002theproteinkinase pages 3-3, manning2002theproteinkinase pages 7-8). According to other classifications, STK32A (YANK1) belongs to the AGC group of protein kinases and the STK32 family (sorrell2020stk32aisa pages 7-10, sorrell2020stk32aisa pages 18-22, arencibia2013agcproteinkinases pages 2-3). On the kinome phylogenetic tree, the STK32 family is placed adjacent to the Aurora kinases, but they possess the full C-terminal extension characteristic of the AGC group (sorrell2020stk32aisa pages 1-5, sorrell2020stk32aisa pages 5-7). A conflicting classification places the YANK family in the Miscellaneous kinase group (thiriet2013preambletocytoplasmic pages 1-4). STK32A shares approximately 36% sequence identity with its nearest other kinases, such as PRKACG or RSK2 (sorrell2020stk32aisa pages 1-5). It shares 69% and 65% sequence identity with its paralogs STK32B and STK32C, respectively (sorrell2020stk32aisa pages 5-7). Orthologs of STK32A are conserved across opisthonts and have been identified in fungi (Magnaporthe oryzae, Schizosaccharomyces pombe), insects, sea anemone, and various vertebrates (sorrell2020stk32aisa pages 1-5, sorrell2020stk32aisa pages 5-7, sorrell2020stk32aisa pages 24-29).

## Reaction Catalyzed

STK32A is a dual-specificity kinase that catalyzes the transfer of a γ-phosphate group from ATP to a serine, threonine, or tyrosine residue on a substrate protein (sorrell2020stk32aisa pages 1-5, sorrell2020stk32aisa pages 5-7, sorrell2020stk32aisa pages 7-10). STK32A + ATP → Phospho-STK32A + ADP (Autophosphorylation) Substrate protein + ATP → Phospho-substrate protein + ADP (Substrate phosphorylation)

## Cofactor Requirements

The catalytic activity of STK32A requires divalent cations (sorrell2020stk32aisa pages 15-18, sorrell2020stk32aisa pages 24-29). Both Mg²⁺ and Mn²⁺ support kinase activity, but autophosphorylation and substrate phosphorylation are enhanced in the presence of Mn²⁺ ions compared to Mg²⁺ (sorrell2020stk32aisa pages 5-7, sorrell2020stk32aisa pages 7-10).

## Substrate Specificity

Data for STK32A was not explicitly available in the provided context from the priority source, Johnson et al. (2023) (johnson2023anatlasof pages 1-2, sorrell2020stk32aisa pages 1-5, sorrell2020stk32aisa pages 24-29, sorrell2020stk32aisa pages 7-10, sorrell2020stk32aisa pages 15-18, sorrell2020stk32aisa pages 39-43). According to other sources, STK32A is a dual-specificity kinase with a preference for acidic substrates (sorrell2020stk32aisa pages 1-5). Mass spectrometry analysis revealed a consensus substrate recognition motif characterized by a preference for acidic residues (aspartate or glutamate) at positions P-4, P+1, P+2, and P+3 relative to the phosphorylated serine or threonine residue (sorrell2020stk32aisa pages 5-7, sorrell2020stk32aisa pages 7-10). The substrate binding groove contains the basic residues Arg109, Arg221, and Arg304, which likely confer specificity towards acidic peptides (sorrell2020stk32aisa pages 10-12, sorrell2020stk32aisa pages 12-15). STK32A can also phosphorylate primed substrates with nearby phosphorylated residues (sorrell2020stk32aisa pages 12-15).

## Structure

The crystal structure of STK32A (PDB: 4FR4) reveals a canonical AGC kinase fold comprising N- and C-terminal lobes, with the ATP-binding site located between them (sorrell2020stk32aisa pages 1-5, sorrell2020stk32aisa pages 7-10, sorrell2020stk32aisa pages 24-29). The kinase domain contains conserved AGC family motifs, including the glycine-rich phosphate-binding loop, the catalytic HRD motif, and the APE motif (sorrell2020stk32aisa pages 5-7). Unique structural features include a novel alpha-helix, termed the ‘HF motif helix’, between the turn and hydrophobic motifs, and an altered binding mode of the hydrophobic motif (HF motif) to the N-lobe (sorrell2020stk32aisa pages 1-5, sorrell2020stk32aisa pages 5-7). The HF motif (sequence F-X-X-F-N-R) lacks a canonical phosphorylatable serine/threonine, containing an asparagine instead (sorrell2020stk32aisa pages 7-10, sorrell2020stk32aisa pages 5-7). The ATP-binding site features a small gatekeeper residue (Val100), which creates a larger binding pocket (sorrell2020stk32aisa pages 10-12, sorrell2020stk32aisa pages 12-15). SAXS analysis shows that STK32A is a monomer in solution (sorrell2020stk32aisa pages 10-12, sorrell2020stk32aisa pages 12-15).

## Regulation

STK32A activity is regulated by autophosphorylation on multiple serine, threonine, and tyrosine residues (sorrell2020stk32aisa pages 5-7). Identified autophosphorylation sites include S227, T229, S230, and S231 in the αF-αG loop; S320 in the turn motif; and S354 near the hydrophobic motif (sorrell2020stk32aisa pages 5-7). The C-terminal HF motif binds the N-lobe to stabilize the αC-helix in an active conformation without requiring phosphorylation in the C-terminal tail (sorrell2020stk32aisa pages 7-10). HPLC-SAXS analysis shows conformational differences between the unphosphorylated and autophosphorylated states of the enzyme (sorrell2020stk32aisa pages 29-39, sorrell2020stk32aisa pages 43-47). The paralog YANK2 is phosphorylated by the Fyn kinase at the conserved tyrosine 110, which increases YANK2 stability and activity (shi2024yank2activatedby pages 5-7, shi2024yank2activatedby pages 9-13). It is not known if STK32A is regulated by PDK1 (sorrell2020stk32aisa pages 7-10).

## Function

STK32A RNA levels are high in brain and endocrine tissues (sorrell2020stk32aisa pages 1-5). The protein localizes to the centrosome, while overexpressed protein is primarily cytosolic (sorrell2020stk32aisa pages 1-5, sorrell2020stk32aisa pages 15-18). In the mouse inner ear, STK32A is expressed in EMX2-negative vestibular hair cells, where it aligns the polarity of stereociliary bundles and regulates the subcellular distribution of the GPCR GPR156 (jia2023thedarkkinase pages 2-3). In vitro, STK32A phosphorylates full-length beta-casein and peptides from the p38α MAP kinase activation loop (sorrell2020stk32aisa pages 7-10, sorrell2020stk32aisa pages 15-18). The paralog YANK2 phosphorylates p70S6K at T389 in an mTOR-independent pathway (shi2024yank2activatedby pages 9-13). No interacting partners for STK32A have been reported (sorrell2020stk32aisa pages 1-5).

## Inhibitors

STK32A binds to several clinically used kinase inhibitors in vitro, including broad-spectrum type-I inhibitors targeting ALK (Ceritinib), BRAF (Dabrafenib), PAK4 (PF-03758309), SYK (PRT062607), and Aurora kinases (Danusertib), as well as the broad-spectrum inhibitor staurosporine (sorrell2020stk32aisa pages 12-15). Due to its small gatekeeper residue (Val100), STK32A also binds bulky inhibitors designed for analogue-sensitive kinases, such as 1NM-PP1 and PP-121 (sorrell2020stk32aisa pages 12-15, sorrell2020stk32aisa pages 29-39).

## Other Comments

Genetic studies link STK32A to several conditions. Single nucleotide polymorphisms (SNPs) in the STK32A gene, located at chromosomal locus 5q31–q33, are associated with coeliac disease (sorrell2020stk32aisa pages 1-5, arencibia2013agcproteinkinases pages 4-4). The gene is also associated with lung cancer susceptibility loci and smoking-related methylation changes (sorrell2020stk32aisa pages 1-5). A cancer-associated S89F missense mutation has been found in melanoma (sorrell2020stk32aisa pages 1-5). The YANK kinase family has been implicated in neurological diseases, and the paralog YANK2 promotes glioma tumorigenesis (shi2024yank2activatedby pages 8-9, shi2024yank2activatedby pages 5-7).

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