## Phylogeny

SCYL1 (also called NTKL) belongs to the SCY1-like pseudokinase family, which forms part of the ‘Atypical’ branch of the human kinome and is conserved across eukaryotes (Manning et al., 2002; Burman et al., 2008; Schmidt et al., 2007). Orthologues are present in mouse (*Scyl1*), yeast (*Scy1*), chimpanzee, dog, cow, chicken, fruit fly, mosquito and plants (e.g., *Arabidopsis* SCYL2A/B) (Unknown Authors, 2012; Jung et al., 2017). Human SCYL1 is a distant homologue of SCYL2/CVAK104 (Burman et al., 2008).

## Reaction Catalyzed

SCYL1 lacks the catalytic residues required for phospho-transfer and shows no detectable kinase activity in vitro; therefore, no ATP-dependent phosphorylation reaction has been observed (Johnson et al., 2023; Burman et al., 2008; Unknown Authors, 2013).

## Cofactor Requirements

Because SCYL1 is catalytically inactive, no Mg²⁺/ATP or other cofactors are required (Johnson et al., 2023; Unknown Authors, 2014).

## Substrate Specificity

High-throughput peptide profiling failed to identify a consensus phosphorylation motif, and no peptide or protein substrates could be detected, consistent with pseudokinase status (Johnson et al., 2023; Unknown Authors, 2013).

## Structure

The protein contains an N-terminal pseudokinase domain followed by coiled-coil/HEAT repeats (Manning et al., 2002; Thiriet, 2013). Critical catalytic motifs are replaced: β3 Lys (VAIK) → Phe, HRD → HNN, and DFG → GLD (Thiriet, 2013; Unknown Authors, 2014). AlphaFold predicts a protein-kinase-like fold despite these substitutions (Manning et al., 2002). A C-terminal RKXX-COO⁻ sequence (RKLD) and internal KK motifs mediate binding to COPI coat proteins (Burman et al., 2008).

## Regulation

Protein arginine methyltransferase 1 (PRMT1) methylates the C-terminal arginine of SCYL1; this modification enhances binding to the γ2-COP subunit and is essential for Golgi morphogenesis and neurite outgrowth (Amano et al., 2020). No phospho-regulatory data are reported.

## Function

SCYL1 is ubiquitously expressed with high levels in neuronal cell bodies, notably cerebellar Purkinje cells (Burman et al., 2008; Schmidt et al., 2007). The protein localises to the ER-Golgi intermediate compartment and cis-Golgi, where it acts as a scaffold that cooperates with Arf1 to recruit COPI components and regulate retrograde Golgi-to-ER trafficking, thereby maintaining Golgi structure (Burman et al., 2008; Frappaolo et al., 2020). Interaction partners include COPI coat subunits (γ-COP/γ2-COP) and class II Arfs, as well as the α- and β2-ear domains of AP-2, linking SCYL1 to clathrin-mediated endocytosis (Enkler et al., 2018; Burman et al., 2008).

## Other Comments

Recessive mutations in *SCYL1* cause spinocerebellar neurodegeneration and a syndrome featuring recurrent liver failure, peripheral neuropathy and ataxia (Burman et al., 2008; Schmidt et al., 2007; Enkler et al., 2018). The *mdf* mouse, carrying a frameshift null allele, exhibits motor-neuron degeneration and cerebellar atrophy (Schmidt et al., 2007). Mutation of the RK residues in the C-terminal COPI-binding motif disrupts COPI interaction, and only three of six splice variants retain this motif (Burman et al., 2008).

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