## Phylogeny

SCYL3 (PACE1) is classified as a pseudokinase within the human kinome (manning2002theproteinkinase pages 7-8, jacobsen2017thesecretlife pages 16-17, unknownauthors2012investigatingnovelregulators pages 75-80). It is a member of the evolutionarily conserved SCY1-like (SCYL) family of proteins, which includes SCYL1 and SCYL2 (kuliyev2018overlappingroleof pages 1-2, unknownauthors2012investigatingnovelregulators pages 80-88). Within this family, SCYL3 is the closest analog to SCYL1, sharing approximately 19.7% sequence identity, compared to 10.5% with SCYL2 (kuliyev2018overlappingroleof pages 3-4). The SCYL family proteins are conserved across species, and the use of mouse models for SCYL3 studies indicates the existence of mammalian orthologs (kuliyev2018overlappingroleof pages 4-6).

## Reaction Catalyzed

SCYL3 is a pseudokinase that does not possess true kinase activity and therefore does not catalyze phosphorylation reactions (unknownauthors2012investigatingnovelregulators pages 80-88, kuliyev2018overlappingroleof pages 1-2, lei2023scyl3asa pages 1-3). Its serine/threonine kinase-like domain is rendered catalytically inactive due to mutations in critical catalytic motifs (unknownauthors2012investigatingnovelregulators pages 80-88, jacobsen2017thesecretlife pages 8-10).

## Cofactor Requirements

No cofactor requirements for SCYL3 are described, consistent with its status as a catalytically inactive pseudokinase (lei2023scyl3asa pages 1-3, kuliyev2018overlappingroleof pages 6-7).

## Substrate Specificity

SCYL3 is a catalytically inactive pseudokinase, so substrate specificity is not applicable (unknownauthors2012investigatingnovelregulators pages 80-88, kuliyev2018overlappingroleof pages 1-2). In the comprehensive analysis of 303 human serine/threonine kinase substrate specificities by Johnson et al. (2023), SCYL3 (PACE1) was not mentioned among the profiled kinases, indicating it was absent from the analysis (johnson2023anatlasof pages 1-2, johnson2023anatlasof pages 3-4, johnson2023anatlasof pages 4-5, johnson2023anatlasof pages 9-10).

## Structure

SCYL3 is a 742-residue protein (unknownauthors2012investigatingnovelregulators pages 80-88). While some sources describe the domain architecture as an N-terminal pseudokinase domain followed by central HEAT repeats (kuliyev2018overlappingroleof pages 1-2), more detailed analyses report that SCYL3 possesses an N-terminal myristoylation site, followed by N-terminal HEAT repeats and a C-terminal pseudokinase domain (unknownauthors2012investigatingnovelregulators pages 80-88, jacobsen2017thesecretlife pages 8-10, kuliyev2018overlappingroleof pages 6-7). Specifically, it contains four HEAT repeats (kuliyev2018overlappingroleof pages 6-7, lei2023scyl3asa pages 1-3). The pseudokinase domain is rendered catalytically inactive by mutations in canonical kinase motifs, including the VAIK/VAVK (ATP binding), HRD (catalytic loop), and DFG (Mg²⁺ binding) motifs (unknownauthors2012investigatingnovelregulators pages 80-88, jacobsen2017thesecretlife pages 8-10). A region overlapping the pseudokinase domain mediates homo-oligomerization (kuliyev2018overlappingroleof pages 6-7). Unlike SCYL1, it lacks coiled-coil domains (kuliyev2018overlappingroleof pages 3-4). The last 14 C-terminal residues are required for binding to the COPI complex, while a region downstream of the HEAT repeats binds CASP (kuliyev2018overlappingroleof pages 6-7).

## Regulation

SCYL3 undergoes N-terminal myristoylation, a post-translational modification that is required for its localization to the Golgi apparatus (unknownauthors2012investigatingnovelregulators pages 80-88, kuliyev2018overlappingroleof pages 3-4). It is also subject to ubiquitination, a modification not reported for other Scyl family members (unknownauthors2012investigatingnovelregulators pages 97-110). SCYL3 can form homo-oligomers, a process that requires a region overlapping its pseudokinase domain (kuliyev2018overlappingroleof pages 6-7). In hepatocellular carcinoma, SCYL3 binds to ROCK2 and regulates its protein stability (lei2023scyl3asa pages 1-3).

## Function

SCYL3 is a ubiquitously expressed protein, with high levels in the forebrain, cerebellum, kidney, liver, lung, and lymphoid tissues, and low or absent expression in skeletal muscle and heart (kuliyev2018overlappingroleof pages 3-4, unknownauthors2012investigatingnovelregulators pages 80-88). It localizes to the Golgi apparatus, where it co-localizes with the markers GM130 and GS28, and to plasma membrane ruffles (kuliyev2018overlappingroleof pages 4-6, unknownauthors2012investigatingnovelregulators pages 80-88).

SCYL3 interacts with several proteins involved in Golgi trafficking, including GOLGA5, CASP, and components of the COPI coatomer complex (COPA, COPB, COPB2, COPD, COPE, COPG1, COPG2) (jung2017scyl2genesare pages 11-15, kuliyev2018overlappingroleof pages 4-6). It was also identified as a novel binding partner of ROCK2, regulating its stability and activity (lei2023scyl3asa pages 1-3). Initial studies identified SCYL3 (as PACE-1) as a binding partner of ezrin (jung2017scyl2genesare pages 11-15, unknownauthors2012investigatingnovelregulators pages 80-88); however, subsequent co-immunoprecipitation and mass spectrometry analyses failed to detect this interaction (kuliyev2018overlappingroleof pages 4-6).

Functionally, SCYL3 is involved in Golgi trafficking, where it regulates Golgi morphology, maintenance, and vesicular transport (jung2017scyl2genesare pages 11-15, unknownauthors2012investigatingnovelregulators pages 75-80). SCYL3 knockdown reduces secretion and trafficking of cargo to the plasma membrane (unknownauthors2012investigatingnovelregulators pages 97-110). It shares an overlapping role with SCYL1 in maintaining motor neuron viability (kuliyev2018overlappingroleof pages 1-2). Reports on its role in cell migration are conflicting: one study implicates SCYL3 in regulating cell adhesion and migration (jung2017scyl2genesare pages 11-15), while another found that SCYL3-deficient cells showed no change in motility (kuliyev2018overlappingroleof pages 3-3, kuliyev2018overlappingroleof pages 7-8). In hepatocellular carcinoma, SCYL3 promotes tumor progression and metastasis by stabilizing ROCK2, leading to increased formation of actin stress fibers and focal adhesions (lei2023scyl3asa pages 1-3).

## Other Comments

Recessive mutations in *SCYL3* cause CALFAN syndrome, a neurodegenerative disorder characterized by features including developmental delay, intellectual disability, seizures, microcephaly, cerebellar atrophy, peripheral neuropathy, low ceruloplasmin, and iron overload (jung2017scyl2genesare pages 11-15, kuliyev2018overlappingroleof pages 1-2). Disease-associated mutations include truncating and missense variants that impair SCYL3’s cellular trafficking function and cause Golgi fragmentation, as well as a homozygous 2-base deletion leading to reduced or absent protein (jung2017scyl2genesare pages 11-15, kuliyev2018overlappingroleof pages 1-2). The missense variant p.Arg475Trp, located near the pseudokinase domain, has been identified in patients with a dominant form of CALFAN syndrome (kuliyev2018overlappingroleof pages 14-15). In hepatocellular carcinoma, overexpression of SCYL3 is associated with metastasis and poor patient survival (lei2023scyl3asa pages 1-3).

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