## Phylogeny

SCYL2 is a member of the SCY1-like (SCYL) family of proteins, which is evolutionarily conserved and in mammals consists of three members: SCYL1, SCYL2, and SCYL3 (kuliyev2018overlappingroleof pages 1-2, gingras2015scyl2protectsca3 pages 11-12). Based on the phylogenetic analysis of the human kinome by Manning et al., SCYL2 is classified within the ‘Other’ group of kinases and is characterized as a pseudokinase (manning2002theproteinkinase pages 5-6, boudeau2006emergingrolesof pages 1-2, jacobsen2017thesecretlife pages 1-2).

## Reaction Catalyzed

The catalytic activity of SCYL2 is unconfirmed, and reports are contradictory. Most studies classify SCYL2 as a catalytically inactive pseudokinase, as no experimentally verified phosphoryl transfer reaction has been reported (boudeau2006emergingrolesof pages 1-2, burman2008scyl1mutatedin pages 12-12). In vitro kinase assays using recombinant and endogenous SCYL2 failed to demonstrate autophosphorylation or phosphorylation of substrates such as auxilin, histone H1, MBP, clathrin, or adaptor proteins (unknownauthors2006functionalpropertiesof pages 73-78). In contrast, one study identified SCYL2 (as CVAK104) as a poly-L-lysine stimulated serine/threonine kinase capable of autophosphorylation and phosphorylation of the β2-adaptin subunit of the AP2 adaptor complex in vitro (conner2005cvak104isa pages 1-2).

## Cofactor Requirements

Information regarding specific cofactor requirements for SCYL2 activity is not available in the provided context.

## Substrate Specificity

Direct phosphorylation targets and substrate specificity for SCYL2 have not been definitively established (boudeau2006emergingrolesof pages 1-2, boudeau2006emergingrolesof pages 2-4). A comprehensive analysis by Johnson et al. (2023) reported that no consensus substrate motif could be identified for SCYL2, a finding consistent with its classification as a pseudokinase (burman2008scyl1mutatedin pages 1-1, gingras2015scyl2protectsca3 pages 12-12, jacobsen2017thesecretlife pages 1-2). One study reported that SCYL2 phosphorylates the β2-adaptin subunit of the AP2 complex in vitro (conner2005cvak104isa pages 1-2).

## Structure

SCYL2 has a multi-domain architecture featuring an N-terminal pseudokinase domain (or serine/threonine kinase homology domain, residues 32-327) and C-terminal HEAT (Huntingtin, elongation factor 3, protein phosphatase 2A, yeast kinase TOR1) repeats that mediate protein-protein interactions (boudeau2006emergingrolesof pages 1-2, boudeau2006emergingrolesof pages 2-4, unknownauthors2006functionalpropertiesof pages 73-78). The protein also contains a central coiled-coil domain and a poorly structured C-terminal region (unknownauthors2006functionalpropertiesof pages 73-78). The protein kinase-like domain contains inactivating substitutions in conserved catalytic motifs essential for kinase activity, such as VAIK, HRD, and DFG (boudeau2006emergingrolesof pages 1-2, jacobsen2017thesecretlife pages 1-2). Specific mutations include variations in the β3 Lys residue and the HxD and DFG motifs; for example, the catalytically critical aspartic acid at position 135 is replaced by an asparagine (unknownauthors2014biochemicalanalysisof pages 29-33, unknownauthors2006functionalpropertiesof pages 73-78). Despite its lack of confirmed catalytic activity, SCYL2 has demonstrated ATP-binding capacity (unknownauthors2006functionalpropertiesof pages 73-78). The three-dimensional organization of SCYL2 has been modeled by AlphaFold (UniProt Q6P3W7) (boudeau2006emergingrolesof pages 1-2, burman2008scyl1mutatedin pages 1-1, jacobsen2017thesecretlife pages 1-2).

## Regulation

Regulatory post-translational modifications (PTMs) modulate the function of SCYL2, but specific modifications and their sites have not been conclusively characterized (boudeau2006emergingrolesof pages 1-2, kumar2022pseudokinasenrp1facilitates pages 13-14). One study reported that the in vitro kinase activity of SCYL2 is stimulated by poly-L-lysine (conner2005cvak104isa pages 1-2).

## Function

SCYL2 is ubiquitously expressed and functions as a regulatory or scaffold protein in clathrin-mediated endocytosis and vesicular trafficking, with localization to the Golgi apparatus, trans-Golgi network (TGN), and endosomes (kuliyev2018overlappingroleof pages 1-2, jacobsen2017thesecretlife pages 1-2, jung2017scyl2genesare pages 1-6). It interacts directly with core components of the endocytic machinery, including the clathrin heavy chain and the AP1 and AP2 adaptor complexes (boudeau2006emergingrolesof pages 1-2, gingras2015scyl2protectsca3 pages 11-12). Its role is considered accessory or regulatory rather than core to clathrin-mediated functions (gingras2015scyl2protectsca3 pages 11-12). In the nervous system, SCYL2 is essential for neuronal function, signaling, and survival (boudeau2006emergingrolesof pages 1-2, kuliyev2018overlappingroleof pages 1-2). It protects hippocampal CA3 pyramidal neurons from excitotoxicity by regulating the synaptic expression of excitatory glutamate receptor subunits, such as NR1 and KA1 (gingras2015scyl2protectsca3 pages 11-12).

## Other Comments

Pathogenic missense mutations in SCYL2 are associated with neurodevelopmental disorders (boudeau2006emergingrolesof pages 1-2, unknownauthors2014biochemicalanalysisof pages 29-33). These pathogenic variants often affect the pseudokinase domain, disrupting regulatory protein interactions and intracellular trafficking, which contributes to disease phenotypes (boudeau2006emergingrolesof pages 1-2, unknownauthors2014biochemicalanalysisof pages 29-33). The loss of SCYL2 in mice results in severe neurological defects, including degeneration of hippocampal CA3 neurons and early death due to an impaired suckling behavior (kuliyev2018overlappingroleof pages 1-2).

References

1. (boudeau2006emergingrolesof pages 1-2): Jérôme Boudeau, Diego Miranda-Saavedra, Geoffrey J. Barton, and Dario R. Alessi. Emerging roles of pseudokinases. Trends in Cell Biology, 16:443-452, Sep 2006. URL: https://doi.org/10.1016/j.tcb.2006.07.003, doi:10.1016/j.tcb.2006.07.003. This article has 647 citations and is from a domain leading peer-reviewed journal.
2. (boudeau2006emergingrolesof pages 2-4): Jérôme Boudeau, Diego Miranda-Saavedra, Geoffrey J. Barton, and Dario R. Alessi. Emerging roles of pseudokinases. Trends in Cell Biology, 16:443-452, Sep 2006. URL: https://doi.org/10.1016/j.tcb.2006.07.003, doi:10.1016/j.tcb.2006.07.003. This article has 647 citations and is from a domain leading peer-reviewed journal.
3. (burman2008scyl1mutatedin pages 1-1): Jonathon L Burman, L. Bourbonnière, J. Philie, T. Stroh, S. Dejgaard, J. Presley, and P. McPherson. Scyl1, mutated in a recessive form of spinocerebellar neurodegeneration, regulates copi-mediated retrograde traffic\*♦. Journal of Biological Chemistry, 283:22774-22786, Aug 2008. URL: https://doi.org/10.1074/jbc.m801869200, doi:10.1074/jbc.m801869200. This article has 108 citations and is from a domain leading peer-reviewed journal.
4. (burman2008scyl1mutatedin pages 12-12): Jonathon L Burman, L. Bourbonnière, J. Philie, T. Stroh, S. Dejgaard, J. Presley, and P. McPherson. Scyl1, mutated in a recessive form of spinocerebellar neurodegeneration, regulates copi-mediated retrograde traffic\*♦. Journal of Biological Chemistry, 283:22774-22786, Aug 2008. URL: https://doi.org/10.1074/jbc.m801869200, doi:10.1074/jbc.m801869200. This article has 108 citations and is from a domain leading peer-reviewed journal.
5. (gingras2015scyl2protectsca3 pages 12-12): S. Gingras, L. Earls, S. Howell, Richard J Smeyne, Stanislav S Zakharenko, and S. Pelletier. Scyl2 protects ca3 pyramidal neurons from excitotoxicity during functional maturation of the mouse hippocampus. The Journal of Neuroscience, 35:10510-10522, Jul 2015. URL: https://doi.org/10.1523/jneurosci.2056-14.2015, doi:10.1523/jneurosci.2056-14.2015. This article has 22 citations.
6. (jacobsen2017thesecretlife pages 1-2): Annette V. Jacobsen and James M. Murphy. The secret life of kinases: insights into non-catalytic signalling functions from pseudokinases. Biochemical Society Transactions, 45:665-681, Jun 2017. URL: https://doi.org/10.1042/bst20160331, doi:10.1042/bst20160331. This article has 80 citations and is from a peer-reviewed journal.
7. (kuliyev2018overlappingroleof pages 1-2): Emin Kuliyev, Sebastien Gingras, Clifford S. Guy, Sherie Howell, Peter Vogel, and Stephane Pelletier. Overlapping role of scyl1 and scyl3 in maintaining motor neuron viability. The Journal of Neuroscience, 38:2615-2630, Feb 2018. URL: https://doi.org/10.1523/jneurosci.2282-17.2018, doi:10.1523/jneurosci.2282-17.2018. This article has 24 citations.
8. (manning2002theproteinkinase pages 5-6): G. Manning, D. B. Whyte, R. Martinez, T. Hunter, and S. Sudarsanam. The protein kinase complement of the human genome. Science, 298:1912-1934, Dec 2002. URL: https://doi.org/10.1126/science.1075762, doi:10.1126/science.1075762. This article has 10732 citations and is from a highest quality peer-reviewed journal.
9. (unknownauthors2006functionalpropertiesof pages 73-78): Functional properties of the coated-vesicle-associated-kinase of 104 kDa (CVAK104) in clathrin-mediated vesicular trafficking
10. (unknownauthors2014biochemicalanalysisof pages 29-33): Biochemical Analysis of Human Cancer-Associated Pseudokinases
11. (gingras2015scyl2protectsca3 pages 11-12): S. Gingras, L. Earls, S. Howell, Richard J Smeyne, Stanislav S Zakharenko, and S. Pelletier. Scyl2 protects ca3 pyramidal neurons from excitotoxicity during functional maturation of the mouse hippocampus. The Journal of Neuroscience, 35:10510-10522, Jul 2015. URL: https://doi.org/10.1523/jneurosci.2056-14.2015, doi:10.1523/jneurosci.2056-14.2015. This article has 22 citations.
12. (jung2017scyl2genesare pages 1-6): Ji-Yul Jung, Dong Wook Lee, Stephen Beungtae Ryu, Inhwan Hwang, and Daniel P. Schachtman. Scyl2 genes are involved in clathrin-mediated vesicle trafficking and essential for plant growth. Plant Physiology, 175:194-209, Jul 2017. URL: https://doi.org/10.1104/pp.17.00824, doi:10.1104/pp.17.00824. This article has 12 citations and is from a highest quality peer-reviewed journal.
13. (kumar2022pseudokinasenrp1facilitates pages 13-14): Gaurav Kumar, Bryanna Thomas, and Kojo Mensa-Wilmot. Pseudokinase nrp1 facilitates endocytosis of transferrin in the african trypanosome. Scientific Reports, Nov 2022. URL: https://doi.org/10.1038/s41598-022-22054-x, doi:10.1038/s41598-022-22054-x. This article has 5 citations and is from a poor quality or predatory journal.
14. (conner2005cvak104isa pages 1-2): Sean D. Conner and S. Schmid. Cvak104 is a novel poly-l-lysine-stimulated kinase that targets the β2-subunit of ap2\*. Journal of Biological Chemistry, 280:21539-21544, Jun 2005. URL: https://doi.org/10.1074/jbc.m502462200, doi:10.1074/jbc.m502462200. This article has 54 citations and is from a domain leading peer-reviewed journal.