1. Phylogeny  
   Protein O-mannose kinase (POMK), also known as SGK196 or Sugen kinase 196, is classified as an atypical protein kinase that belongs to a divergent branch within the protein kinase‐like (PKL) clan. Early bioinformatics investigations using PSI‐BLAST and CLANS analyses of Pfam seed sequences identified SGK196 as a member of the FAM69 kinase family, a group that exhibits unique sequence features such as the replacement of the conventional catalytic [HY]RD motif by a distinctive MCD motif (dudkiewicz2013anovelpredicted pages 6-8). Phylogenetic analyses demonstrate that orthologs of POMK are present across Metazoa, including key early‐branching opisthokonts such as choanoflagellates and Capsaspora, and even trace back to remote homologs in plants, suggesting that the evolutionary origin of POMK antedates the divergence of major eukaryotic lineages (dudkiewicz2013anovelpredicted pages 8-8). Despite its atypical features, the overall kinase fold of POMK is conserved, and comparisons with canonical kinases such as protein kinase A (PKA) reveal that it retains the bilobal structure that is emblematic of the eukaryotic kinase superfamily (zhu2016structureofprotein pages 1-2, nagae20173dstructuralanalysis pages 1-2). The unusual positioning of key catalytic residues—exemplified by a lysine (e.g., Lys91 in Danio rerio POMK) and an aspartate (Asp227) occupying non‐canonical positions—marks a clear case of “active site migration” that underpins its classification as an atypical but catalytically active kinase (zhu2016structureofprotein pages 2-3, dudkiewicz2013anovelpredicted pages 6-8). Recent structurally validated sequence alignments of the human kinome further consolidate the placement of POMK within an evolutionary core of kinases that, although divergent from classical eukaryotic protein kinases, share ancestry from the Last Eukaryotic Common Ancestor (LECA) (modi2019astructurallyvalidatedmultiple pages 14-15, modi2019astructurallyvalidated pages 32-34). Thus, POMK occupies a phylogenetic position that attests both to its ancient origin and to its adaptation away from the conventional kinase machinery, setting it apart from the canonical AGC, CAMK, CK1, CMGC, STE, and TK groups described in early kinome studies (dudkiewicz2013anovelpredicted pages 6-8, zhu2016structureofprotein pages 1-2).
2. Reaction Catalyzed  
   POMK catalyzes the phosphorylation of a specific carbohydrate moiety attached to proteins. The enzyme mediates the transfer of a phosphate group from adenosine triphosphate (ATP) to the 6‐hydroxyl group of the mannose residue that is part of an O-mannosyl glycan. More specifically, POMK targets the trisaccharide motif composed of N‑acetylgalactosamine (GalNAc) linked via a β1,3 bond to N‑acetylglucosamine (GlcNAc), which is, in turn, β1,4‑linked to a mannose residue. The reaction is formalized as follows:

  ATP + [protein]–(GalNAc‑β1,3‑GlcNAc‑β1,4‑Man) → ADP + [protein]–(GalNAc‑β1,3‑GlcNAc‑β1,4‑(phosphate‑6-)Man) + H⁺

This phosphorylation event on the O-mannosyl trisaccharide is critical because it primes the glycan for subsequent elongation by additional glycosyltransferases, thereby enabling the formation of the matriglycan structure on alpha-dystroglycan, which is indispensable for high-affinity binding to extracellular matrix proteins such as laminin (zhu2016structureofprotein pages 1-2, dudkiewicz2013anovelpredicted pages 8-8).

1. Cofactor Requirements  
   As with many kinases, the enzymatic activity of POMK is contingent upon the presence of divalent metal ion cofactors. Studies using crystallographic and biochemical assays have consistently demonstrated that POMK requires magnesium ions (Mg²⁺) to facilitate the proper binding and orientation of ATP within its active site (nagae20173dstructuralanalysis pages 2-5, zhu2016structureofprotein pages 2-3). In certain experimental conditions, manganese ions (Mn²⁺) have also been observed to support catalytic activity, but Mg²⁺ is generally recognized as the primary physiological cofactor (nagae20173dstructuralanalysis pages 2-5).
2. Substrate Specificity  
   Distinct from most serine/threonine protein kinases that target polypeptide substrates, POMK exhibits a highly selective substrate specificity. The enzyme specifically recognizes and phosphorylates the mannose residue only when it is presented as part of a well-defined O-mannosyl trisaccharide structure. The substrate must contain a disaccharide moiety comprising GalNAc linked β1,3 to GlcNAc attached to the 4‑position of mannose; this disaccharide segment is essential for substrate recognition and binding by POMK (zhu2016structureofprotein pages 1-2, dudkiewicz2013anovelpredicted pages 8-8). Structural studies have detailed how the enzyme’s active site is configured to form a precise binding groove that interacts specifically with the GalNAc‑β1,3‑GlcNAc moiety, thereby ensuring that the mannose is phosphorylated only when presented in the correct glycan context (nagae20173dstructuralanalysis pages 5-7, zhu2016structureofprotein pages 3-6). This strict requirement for a terminal disaccharide prioritizes substrate selectivity and distinguishes POMK from non-specific kinases (zhu2016structureofprotein pages 1-2, dudkiewicz2013anovelpredicted pages 8-8, walimbe2021proteinomannosekinasemediated pages 30-34).
3. Structure  
   POMK is an atypical type II transmembrane protein characterized by a modular architecture. Its overall structure can be divided into three primary regions: a short cytoplasmic N‑terminal segment, a single transmembrane helix, and a large luminal catalytic domain that resides in the endoplasmic reticulum (ER) lumen (nagae20173dstructuralanalysis pages 1-2). The catalytic domain, which comprises approximately 350 amino acids, adopts a bilobed kinase fold similar to that observed in canonical eukaryotic protein kinases. The N-terminal lobe is constructed primarily from five antiparallel β‑strands and two α-helices, serving critical roles in ATP binding, while the C‑terminal lobe is dominated by several α-helices and a β‑hairpin structure that contribute to substrate binding and the phosphotransfer reaction (nagae20173dstructuralanalysis pages 1-2, zhu2016structureofprotein pages 1-2).

Crystallographic studies of Danio rerio POMK have elucidated unique features within its catalytic domain. Despite its low overall sequence similarity to canonical kinases, the three-dimensional (3D) structure reveals that POMK maintains the core kinase fold. However, it diverges in its active site organization: the classical [HY]RD motif is replaced by a unique MCD motif, and key catalytic residues such as the lysine (e.g., Lys91) and aspartate (notably Asp227) are repositioned in a manner that reflects an “active site migration” relative to conventional kinases (dudkiewicz2013anovelpredicted pages 6-8, zhu2016structureofprotein pages 2-3). In addition, POMK is stabilized by several conserved disulfide bonds, which are formed among cysteine residues within its luminal domain and are essential for maintaining the proper 3D conformation in the oxidative environment of the ER (nagae20173dstructuralanalysis pages 2-5, walimbe2021proteinomannosekinasemediated pages 30-34).

Moreover, structural models, corroborated by X-ray crystallography, indicate that POMK possesses a distinctive substrate-binding groove that is shaped to accommodate the GalNAc‑β1,3‑GlcNAc disaccharide moiety tethered to the mannose substrate. This groove ensures precise positioning of the O-mannosyl glycan for phosphorylation at the C6 hydroxyl group (zhu2016structureofprotein pages 6-9, nagae20173dstructuralanalysis pages 5-7). Notably, the enzyme’s overall structure is further modulated by potential N‑linked glycosylation sites; these sites are postulated to contribute to both the stability and trafficking of POMK within the secretory pathway (nagae20173dstructuralanalysis pages 1-2). Collectively, these structural insights demarcate POMK as an atypical kinase with a conserved bilobed fold yet possessing specialized features imperative for glycan recognition and catalysis.

1. Regulation  
   The regulation of POMK is fundamentally tied to its structural conformation and its precise folding in the ER lumen. Although detailed mechanistic studies on its regulation are not as extensive as those for canonical kinases, available evidence indicates that the activity of POMK is modulated through conformational adjustments induced upon substrate binding. Molecular dynamics simulations have revealed that upon ligand occupancy, subtle movements within regions such as the α2 helix occur; residues in this region (for example, D117 and H120) are seen to shift towards the substrate‐binding groove, thereby enhancing interactions with the O-mannosyl glycan (nagae20173dstructuralanalysis pages 5-7, zhu2016structureofprotein pages 3-6).

Additionally, post‑translational modifications—most notably N‑linked glycosylation—play a crucial role in ensuring that the catalytic domain attains and maintains its correct three-dimensional structure (nagae20173dstructuralanalysis pages 1-2). There is no detailed evidence from the provided literature to suggest that POMK undergoes extensive autophosphorylation or is directly modified by other kinases; rather, its regulation appears to depend primarily on the integrity of its disulfide bond network and proper domain folding. The critical importance of its structural configuration is further underscored by the observation that mutations affecting key active site residues (such as V302D, L137R, Q258R, and premature truncations at residues F96 or Q109) invariably result in loss of catalytic function, which in turn leads to defective glycosylation of alpha-dystroglycan (zhu2016structureofprotein pages 9-11, walimbe2021proteinomannosekinasemediated pages 30-34, dudkiewicz2013anovelpredicted pages 8-9). In summary, while direct allosteric regulators and inhibitory proteins have not been comprehensively characterized for POMK, its catalytic activity is exquisitely sensitive to the structural integrity of its modified active site and its correct localization within the ER.

1. Function  
   POMK occupies a critical role in the post‑translational modification of alpha‑dystroglycan, a glycoprotein that mediates the link between the extracellular matrix and the cytoskeleton. The phosphorylation reaction catalyzed by POMK is a pivotal step in the biosynthetic pathway of dystroglycan glycosylation. By specifically phosphorylating the mannose residue in the O-mannosyl trisaccharide (GalNAc‑β1,3‑GlcNAc‑β1,4‑Man), POMK generates a phosphorylated structure that is essential for the subsequent addition of tandem ribitol-phosphate moieties and the formation of the mature repeating disaccharide chain (matriglycan). This structural modification is required for high-affinity binding of laminin and other extracellular matrix ligands to alpha‑dystroglycan (zhu2016structureofprotein pages 1-2, nagae20173dstructuralanalysis pages 1-2).

Expression studies have revealed that POMK is expressed in multiple tissues—including the brain, skeletal muscle, heart, and kidneys—with particularly high expression levels during fetal development (nagae20173dstructuralanalysis pages 1-2, zhu2016structureofprotein pages 2-3). Functional assays and patient-derived data have implicated loss-of-function mutations in POMK as causative for a spectrum of congenital muscular dystrophies, collectively termed dystroglycanopathies. In these disorders, impaired phosphorylation by POMK leads to hypoglycosylation of alpha‑dystroglycan, thereby compromising its ability to bind extracellular matrix components, which ultimately results in muscle weakness and brain malformations (nagae20173dstructuralanalysis pages 9-11, zhu2016structureofprotein pages 17-18).

Furthermore, the activity of POMK is integral to a broader glycosylation cascade that involves subsequent enzymatic modifications by glycosyltransferases such as fukutin and FKRP. These downstream modifications are necessary to generate the full-length matriglycan required for proper extracellular matrix assembly and cellular adhesion (dudkiewicz2013anovelpredicted pages 8-9, walimbe2021proteinomannosekinasemediated pages 30-34). Thus, POMK functions not merely as an isolated kinase but as a key initiator of a complex glycosylation pathway that is indispensable for maintaining tissue integrity, particularly in muscle and neural tissues.

1. Other Comments  
   Although the development of specific inhibitors targeting POMK has not been extensively reported to date, the enzyme’s unique structural attributes and its indispensable role in glycan phosphorylation make it a promising target for therapeutic intervention in dystroglycanopathies. Notably, several disease-associated mutations in POMK—such as missense mutations (V302D, L137R, Q258R) and truncating mutations (F96 and Q109)—have been directly linked to severe congenital muscular dystrophies, underscoring the clinical importance of ensuring proper POMK function (zhu2016structureofprotein pages 9-11, nagae20173dstructuralanalysis pages 7-9).  
   Historically, POMK was annotated as a pseudokinase owing to its divergence from canonical protein kinase motifs; however, biochemical and structural studies have since confirmed its bona fide catalytic activity, thereby necessitating a re-evaluation of its classification within the kinome (dudkiewicz2013anovelpredicted pages 6-8, zhu2016structureofprotein pages 2-3). Given its evolutionary conservation and the severity of the associated dystroglycanopathies, continued research into the regulation, substrate interactions, and potential pharmacological targeting of POMK is warranted. Future studies may focus on exploiting its unique active site configuration for the design of selective modulators capable of restoring proper glycosylation in patients with dystroglycan-related muscular dystrophies (walimbe2021proteinomannosekinasemediated pages 30-34, modi2019astructurallyvalidatedmultiple pages 14-15).
2. References
3. dudkiewicz2013anovelpredicted pages 6-8
4. dudkiewicz2013anovelpredicted pages 8-8
5. nagae20173dstructuralanalysis pages 1-2
6. nagae20173dstructuralanalysis pages 2-5
7. nagae20173dstructuralanalysis pages 5-7
8. nagae20173dstructuralanalysis pages 7-9
9. nagae20173dstructuralanalysis pages 9-11
10. zhu2016structureofprotein pages 1-2
11. zhu2016structureofprotein pages 2-3
12. zhu2016structureofprotein pages 3-6
13. zhu2016structureofprotein pages 6-9
14. zhu2016structureofprotein pages 9-11
15. dudkiewicz2013anovelpredicted pages 8-9
16. walimbe2021proteinomannosekinasemediated pages 30-34
17. zhu2016structureofprotein pages 17-18
18. modi2019astructurallyvalidatedmultiple pages 14-15
19. modi2019astructurallyvalidated pages 32-34

References

1. (dudkiewicz2013anovelpredicted pages 6-8): Małgorzata Dudkiewicz, Anna Lenart, and Krzysztof Pawłowski. A novel predicted calcium-regulated kinase family implicated in neurological disorders. PLoS ONE, 8:e66427, Jun 2013. URL: https://doi.org/10.1371/journal.pone.0066427, doi:10.1371/journal.pone.0066427. This article has 56 citations and is from a peer-reviewed journal.
2. (dudkiewicz2013anovelpredicted pages 8-8): Małgorzata Dudkiewicz, Anna Lenart, and Krzysztof Pawłowski. A novel predicted calcium-regulated kinase family implicated in neurological disorders. PLoS ONE, 8:e66427, Jun 2013. URL: https://doi.org/10.1371/journal.pone.0066427, doi:10.1371/journal.pone.0066427. This article has 56 citations and is from a peer-reviewed journal.
3. (nagae20173dstructuralanalysis pages 1-2): Masamichi Nagae, Sushil K. Mishra, Makiko Neyazaki, Rika Oi, Akemi Ikeda, Naohiro Matsugaki, Satoko Akashi, Hiroshi Manya, Mamoru Mizuno, Hirokazu Yagi, Koichi Kato, Toshiya Senda, Tamao Endo, Terukazu Nogi, and Yoshiki Yamaguchi. 3d structural analysis of protein o‐mannosyl kinase, pomk, a causative gene product of dystroglycanopathy. Genes to Cells, Apr 2017. URL: https://doi.org/10.1111/gtc.12480, doi:10.1111/gtc.12480. This article has 30 citations and is from a peer-reviewed journal.
4. (nagae20173dstructuralanalysis pages 2-5): Masamichi Nagae, Sushil K. Mishra, Makiko Neyazaki, Rika Oi, Akemi Ikeda, Naohiro Matsugaki, Satoko Akashi, Hiroshi Manya, Mamoru Mizuno, Hirokazu Yagi, Koichi Kato, Toshiya Senda, Tamao Endo, Terukazu Nogi, and Yoshiki Yamaguchi. 3d structural analysis of protein o‐mannosyl kinase, pomk, a causative gene product of dystroglycanopathy. Genes to Cells, Apr 2017. URL: https://doi.org/10.1111/gtc.12480, doi:10.1111/gtc.12480. This article has 30 citations and is from a peer-reviewed journal.
5. (nagae20173dstructuralanalysis pages 5-7): Masamichi Nagae, Sushil K. Mishra, Makiko Neyazaki, Rika Oi, Akemi Ikeda, Naohiro Matsugaki, Satoko Akashi, Hiroshi Manya, Mamoru Mizuno, Hirokazu Yagi, Koichi Kato, Toshiya Senda, Tamao Endo, Terukazu Nogi, and Yoshiki Yamaguchi. 3d structural analysis of protein o‐mannosyl kinase, pomk, a causative gene product of dystroglycanopathy. Genes to Cells, Apr 2017. URL: https://doi.org/10.1111/gtc.12480, doi:10.1111/gtc.12480. This article has 30 citations and is from a peer-reviewed journal.
6. (nagae20173dstructuralanalysis pages 7-9): Masamichi Nagae, Sushil K. Mishra, Makiko Neyazaki, Rika Oi, Akemi Ikeda, Naohiro Matsugaki, Satoko Akashi, Hiroshi Manya, Mamoru Mizuno, Hirokazu Yagi, Koichi Kato, Toshiya Senda, Tamao Endo, Terukazu Nogi, and Yoshiki Yamaguchi. 3d structural analysis of protein o‐mannosyl kinase, pomk, a causative gene product of dystroglycanopathy. Genes to Cells, Apr 2017. URL: https://doi.org/10.1111/gtc.12480, doi:10.1111/gtc.12480. This article has 30 citations and is from a peer-reviewed journal.
7. (nagae20173dstructuralanalysis pages 9-11): Masamichi Nagae, Sushil K. Mishra, Makiko Neyazaki, Rika Oi, Akemi Ikeda, Naohiro Matsugaki, Satoko Akashi, Hiroshi Manya, Mamoru Mizuno, Hirokazu Yagi, Koichi Kato, Toshiya Senda, Tamao Endo, Terukazu Nogi, and Yoshiki Yamaguchi. 3d structural analysis of protein o‐mannosyl kinase, pomk, a causative gene product of dystroglycanopathy. Genes to Cells, Apr 2017. URL: https://doi.org/10.1111/gtc.12480, doi:10.1111/gtc.12480. This article has 30 citations and is from a peer-reviewed journal.
8. (zhu2016structureofprotein pages 1-2): Qinyu Zhu, David Venzke, Ameya S Walimbe, Mary E Anderson, Qiuyu Fu, Lisa N Kinch, Wei Wang, Xing Chen, Nick V Grishin, Niu Huang, Liping Yu, Jack E Dixon, Kevin P Campbell, and Junyu Xiao. Structure of protein o-mannose kinase reveals a unique active site architecture. eLife, Nov 2016. URL: https://doi.org/10.7554/elife.22238, doi:10.7554/elife.22238. This article has 40 citations and is from a domain leading peer-reviewed journal.
9. (zhu2016structureofprotein pages 2-3): Qinyu Zhu, David Venzke, Ameya S Walimbe, Mary E Anderson, Qiuyu Fu, Lisa N Kinch, Wei Wang, Xing Chen, Nick V Grishin, Niu Huang, Liping Yu, Jack E Dixon, Kevin P Campbell, and Junyu Xiao. Structure of protein o-mannose kinase reveals a unique active site architecture. eLife, Nov 2016. URL: https://doi.org/10.7554/elife.22238, doi:10.7554/elife.22238. This article has 40 citations and is from a domain leading peer-reviewed journal.
10. (zhu2016structureofprotein pages 3-6): Qinyu Zhu, David Venzke, Ameya S Walimbe, Mary E Anderson, Qiuyu Fu, Lisa N Kinch, Wei Wang, Xing Chen, Nick V Grishin, Niu Huang, Liping Yu, Jack E Dixon, Kevin P Campbell, and Junyu Xiao. Structure of protein o-mannose kinase reveals a unique active site architecture. eLife, Nov 2016. URL: https://doi.org/10.7554/elife.22238, doi:10.7554/elife.22238. This article has 40 citations and is from a domain leading peer-reviewed journal.
11. (zhu2016structureofprotein pages 6-9): Qinyu Zhu, David Venzke, Ameya S Walimbe, Mary E Anderson, Qiuyu Fu, Lisa N Kinch, Wei Wang, Xing Chen, Nick V Grishin, Niu Huang, Liping Yu, Jack E Dixon, Kevin P Campbell, and Junyu Xiao. Structure of protein o-mannose kinase reveals a unique active site architecture. eLife, Nov 2016. URL: https://doi.org/10.7554/elife.22238, doi:10.7554/elife.22238. This article has 40 citations and is from a domain leading peer-reviewed journal.
12. (zhu2016structureofprotein pages 9-11): Qinyu Zhu, David Venzke, Ameya S Walimbe, Mary E Anderson, Qiuyu Fu, Lisa N Kinch, Wei Wang, Xing Chen, Nick V Grishin, Niu Huang, Liping Yu, Jack E Dixon, Kevin P Campbell, and Junyu Xiao. Structure of protein o-mannose kinase reveals a unique active site architecture. eLife, Nov 2016. URL: https://doi.org/10.7554/elife.22238, doi:10.7554/elife.22238. This article has 40 citations and is from a domain leading peer-reviewed journal.
13. (dudkiewicz2013anovelpredicted pages 8-9): Małgorzata Dudkiewicz, Anna Lenart, and Krzysztof Pawłowski. A novel predicted calcium-regulated kinase family implicated in neurological disorders. PLoS ONE, 8:e66427, Jun 2013. URL: https://doi.org/10.1371/journal.pone.0066427, doi:10.1371/journal.pone.0066427. This article has 56 citations and is from a peer-reviewed journal.
14. (modi2019astructurallyvalidated pages 32-34): Vivek Modi and Roland L. Dunbrack. A structurally validated sequence alignment of all 497 typical human protein kinase domains. bioRxiv, Sep 2019. URL: https://doi.org/10.1101/776740, doi:10.1101/776740. This article has 8 citations.
15. (modi2019astructurallyvalidatedmultiple pages 14-15): Vivek Modi and Roland L. Dunbrack. A structurally-validated multiple sequence alignment of 497 human protein kinase domains. Scientific Reports, Dec 2019. URL: https://doi.org/10.1038/s41598-019-56499-4, doi:10.1038/s41598-019-56499-4. This article has 118 citations and is from a poor quality or predatory journal.
16. (walimbe2021proteinomannosekinasemediated pages 30-34): Ameya Shirish Walimbe. Protein O-Mannose Kinase-mediated regulation of α-dystroglycan function. PhD thesis, The University of Iowa, 2021. URL: https://doi.org/10.17077/etd.006103, doi:10.17077/etd.006103.
17. (zhu2016structureofprotein pages 17-18): Qinyu Zhu, David Venzke, Ameya S Walimbe, Mary E Anderson, Qiuyu Fu, Lisa N Kinch, Wei Wang, Xing Chen, Nick V Grishin, Niu Huang, Liping Yu, Jack E Dixon, Kevin P Campbell, and Junyu Xiao. Structure of protein o-mannose kinase reveals a unique active site architecture. eLife, Nov 2016. URL: https://doi.org/10.7554/elife.22238, doi:10.7554/elife.22238. This article has 40 citations and is from a domain leading peer-reviewed journal.