## Phylogeny

MAP4K3 (also GLK/KHS2) is a member of the STE20 family, GCK-I subfamily. Its closest human paralogues are MAP4K1, MAP4K2, MAP4K4 and MAP4K5 (Silvian, 2017). Orthologues in Mus musculus (Map4k3), Rattus norvegicus (Map4k3), Drosophila melanogaster (happyhour), Caenorhabditis elegans (MIG-15) and Saccharomyces cerevisiae (Ste20p) indicate deep evolutionary conservation (Chuang & Tan, 2019). A longer activation loop than in MAP4K4 confers a distinctive, loose activation-loop-swapped dimer unique to MAP4K3 (Marcotte et al., 2017).

## Reaction Catalyzed

ATP + protein-Ser/Thr → ADP + protein-Ser/Thr-phosphate (Marcotte et al., 2017).

## Cofactor Requirements

Catalysis requires Mg²⁺ (~10 mM MgCl₂). Mn²⁺ is not needed for phosphotransfer and is used only during phosphatase control steps (Marcotte et al., 2017).

## Substrate Specificity

Validated substrates include PKCθ Thr538, TFEB Ser3, IQGAP1 Ser480, the activation loops of LATS1/2 and myelin basic protein (Chuang et al., 2016). A kinome-wide survey has not yet defined a consensus phosphorylation motif for MAP4K3 (Han et al., 2024).

## Structure

• Domain organisation: N-terminal kinase domain (residues 1–314), central proline-rich/PEST segment, C-terminal citron-homology domain (Thiriet, 2013).  
• 2.85 Å crystal structure (PDB 5J5T) shows an activation-loop-swapped dimer formed via APE and TYPW motifs (Marcotte et al., 2017).  
• Key catalytic features: Lys45–Glu61 salt bridge, intact HRD triad, aligned regulatory spine; phospho-Ser170 stabilises the activation loop, and the S170A mutant retains ATP binding but ≤ 3 % activity (Marcotte et al., 2017).  
• A straight P-loop and αK-helix Asn290 create an inhibitor-binding pocket, and an acidic C-terminal extension docks into a neighbouring basic groove, reminiscent of AGC-kinase PIF binding (Marcotte et al., 2017).

## Regulation

• Essential autophosphorylation on Ser170; dephosphorylated and inactivated by PP2A-PR61ε (Chuang et al., 2016; Silvian, 2017).  
• Growth factors induce phosphorylation at Tyr366, Tyr379, Tyr574 and Tyr735 (Chuang & Tan, 2019).  
• TRAF2-dependent K63-linked ubiquitination enhances kinase activation and JNK signalling (Chuang et al., 2016).  
• STRN4-containing STRIPAK complex binds and suppresses MAP4K3, restraining Hippo pathway output (Seo et al., 2020).  
• The activation-loop-swapped dimer collapses when Ser170 is dephosphorylated (Marcotte et al., 2017).

## Function

MAP4K3 is ubiquitously expressed and becomes up-regulated in T and B lymphocytes following TCR, TNF-α or Wnt3a stimulation (Diener et al., 1997).  
• Immune signalling: GLK binds SLP-76 and phosphorylates PKCθ Thr538, activating IKK/NF-κB and promoting Th1/Th2/Th17 cytokine production (Chuang et al., 2016).  
• Stress pathways: Over-expression stimulates the MEKK1→MKK4→JNK cascade but not ERK or p38 (Chuang et al., 2016).  
• Nutrient sensing & autophagy: Ser170 phosphorylation links amino-acid sufficiency to mTORC1 and phosphorylates TFEB Ser3, suppressing autophagy (Chuang & Tan, 2019).  
• Hippo pathway: MAP4K3 phosphorylates LATS1/2 to activate Hippo signalling, an effect antagonised by STRIPAK (Seo et al., 2020).  
• Cell migration: Phosphorylation of IQGAP1 Ser480 promotes Cdc42-mediated migration (Chuang & Tan, 2019).  
• Apoptosis: Via JNK, GLK stabilises BH3-only proteins and phosphorylates BIM, driving apoptosis (Lam et al., 2009).

## Inhibitors

• Verteporfin, IC₅₀ ≈ 1.15 nM, reduces IL-17A production (Chuang & Tan, 2019).  
• Crizotinib analogues inhibit MAP4K3 in vitro but have limited in-vivo exposure (Chuang & Tan, 2019).  
• Pyrrolo-pyridinylamine “Compound 1”, IC₅₀ ≈ 110 nM, co-crystallised with MAP4K3 and selective within GCK-I kinases (Marcotte et al., 2017).  
• Astragalus polysaccharide and 10-hydroxycamptothecin also suppress kinase activity and downstream mTORC1 signalling (Chuang & Tan, 2019).

## Other Comments

GLK-null mice show defective antibody responses and resistance to experimental autoimmune encephalomyelitis (Chuang et al., 2016). T-cell MAP4K3 levels correlate with disease activity in systemic lupus erythematosus, rheumatoid arthritis and adult-onset Still’s disease (Chuang & Tan, 2019). High MAP4K3–IQGAP1 Ser480 complex abundance predicts metastasis and poor survival in non-small-cell lung carcinoma (Chuang & Tan, 2019). A pancreatic cancer mutant (E351K) increases kinase activity (Chuang & Tan, 2019).

## 9. References

Chuang, H.-C., Wang, X., & Tan, T.-H. (2016). MAP4K family kinases in immunity and inflammation. Advances in Immunology, 129, 277–314. https://doi.org/10.1016/bs.ai.2015.09.006

Chuang, H.-C., & Tan, T.-H. (2019). MAP4K3/GLK in autoimmune disease, cancer and aging. Journal of Biomedical Science. https://doi.org/10.1186/s12929-019-0570-5

Diener, K., Wang, X., Chen, C., Meyer, C., Keesler, G. A., Zukowski, M., Tan, T., & Yao, Z. (1997). Activation of the c-Jun N-terminal kinase pathway by a novel protein kinase related to human germinal center kinase. Proceedings of the National Academy of Sciences of the United States of America, 94(18), 9687–9692. https://doi.org/10.1073/pnas.94.18.9687

Han, H., Huang, Z., Xu, C., Seo, G., An, J., Yang, B., Liu, Y., Lan, T., Yan, J., Ren, S., Xu, Y., Xiao, D., Yan, J. K., Ahn, C., Fishman, D. A., Meng, Z., Qi, R., Luo, R., & Wang, W. (2024). Functional annotation of the Hippo pathway somatic mutations in human cancers. Nature Communications. https://doi.org/10.1038/s41467-024-54480-y

Lam, D., Dickens, D., Reid, E. B., Loh, S. H. Y., Moisoi, N., & Martins, L. M. (2009). MAP4K3 modulates cell death via the post-transcriptional regulation of BH3-only proteins. Proceedings of the National Academy of Sciences, 106, 11978–11983. https://doi.org/10.1073/pnas.0900608106

Marcotte, D., Rushe, M., Arduini, R. M., Lukacs, C., Atkins, K., Sun, X., Little, K., Cullivan, M., Paramasivam, M., Patterson, T. A., Hesson, T., McKee, T. D., May-Dracka, T. L., Xin, Z., Bertolotti-Ciarlet, A., Bhisetti, G. R., Lyssikatos, J. P., & Silvian, L. F. (2017). Germinal-center kinase-like kinase co-crystal structure reveals a swapped activation loop and C-terminal extension. Protein Science. https://doi.org/10.1002/pro.3062

Seo, G., Han, H., Vargas, R., Yang, B., Li, X., & Wang, W. (2020). MAP4K interactome reveals STRN4 as a key STRIPAK complex component in Hippo pathway regulation. Cell Reports, 32(1), 107860. https://doi.org/10.1016/j.celrep.2020.107860

Silvian, L. (2017). How can the structure of germinal-center kinase-like kinase help us in drug discovery? Future Medicinal Chemistry, 9, 1583–1585. https://doi.org/10.4155/fmc-2017-0133

Thiriet, M. (2013). Cytoplasmic protein serine/threonine kinases. In M. Thiriet (Ed.), Biomathematical and Biomechanical Modeling of the Circulatory and Ventilatory Systems (pp. 175–310). Springer. https://doi.org/10.1007/978-1-4614-4370-4\_5