

# The long-awaited structure of HIPK2

DOI 10.1074/jbc.H119.010675

James M. Murphy<sup>1</sup>

From the Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia and the Department of Medical Biology, University of Melbourne, Parkville, Victoria 3052, Australia

Edited by Alex Toker

Homeodomain-interacting protein kinases (HIPKs) are kinases that phosphorylate transcription factors involved in cell proliferation, differentiation, and apoptosis. Their structures have been long sought because of their potential as drug targets in cancers and fibrosis. Agnew and colleagues present the first crystal structure of the HIPK2 kinase domain, complexed with the small-molecule inhibitor CX-4945, revealing important structural differences from related protein kinases of the DYRK family. This structure provides a starting point to exploit HIPK2's distinct structural features to develop selective small-molecule inhibitors of this kinase.

Protein kinases are essential signal-transducing molecules in all organisms. These enzymes adopt a highly conserved bilobal structure; the N-lobe comprises mostly  $\beta$ -strands and a key regulatory helix ( $\alpha$ C), and the larger C-lobe contains mostly helices, with the cleft between lobes where phosphate is transferred from ATP to the protein substrate (1). Differences among these kinases in the dispositions and lengths of loops and secondary structure elements are responsible for the differences in how these kinases are regulated and in their abilities to distinguish substrates and partner proteins.

The HIPK family<sup>2</sup> proteins have emerged as promising drug targets in cancers and chronic fibrosis, but their structures have long remained elusive. Agnew *et al.* (2) now fill this gap by determining the X-ray crystal structure of the kinase domain of HIPK2. This structure provides the first high-resolution glimpse into a HIPK kinase, enabling detailed structural comparisons with the related, and better-understood, dual-specificity tyrosine-regulated protein kinases of the DYRK family.

It is typical to stabilize active protein kinase conformations to facilitate crystallization by complexing with ATP or analogs. However, here the authors did not obtain crystals when HIPK2 was complexed with ATP or analogs, but only when bound to the casein kinase 2 subunit  $\alpha$  (CK2 $\alpha$ ) inhibitor CX-4945. The structure revealed unique features of the "foot" on the HIPK2 kinase domain, the so-called "CMGC insert," and a helix unique

to the N-lobe of HIPK2 between  $\alpha$ C and  $\beta$ -strand 4 (Fig. 1). The CMGC insert is characteristic of CMGC kinases and highly variable among them, serving as a binding region for signaling partner proteins or for oligomerization (3). This insert arises from an extended sequence connecting  $\alpha$ G and  $\alpha$ H. Its N terminus contains two short helices ( $\alpha$ L and  $\alpha$ L') also present in the closest HIPK2 relatives, the DYRK kinases. In contrast, the adjacent sequence exhibits features unique to HIPK2 (Fig. 1): a  $\beta$ -hairpin longer than that previously observed in DYRK1A and distinct from the loop present in the DYRK2 and DYRK3 structures, a novel helix (termed  $\alpha$ M), and a feature more typical of CMGC kinases, termed the  $\alpha$ N helix by the authors. Distinct from other CMGC kinases in which this helix is connected to  $\alpha$ H via a short loop, the C-terminal end of the CMGC insert contributes to the  $\alpha$ H helix, leading to a notably longer helix than that observed in structures of other CMGC family members. As a result, the CMGC insert interacts with the HIPK2 kinase domain C-lobe via a conformation distinctly different from those of other CMGC kinases, including the DYRK family.

Agnew and colleagues noted two phosphorylation sites in HIPK2: pTyr<sup>361</sup> in the activation loop and pSer<sup>441</sup> in the CMGC insert (Fig. 1). As in the related DYRK kinases, phosphorylated Tyr<sup>361</sup> did not interact with the catalytic loop HRD motif (HAD in HIPK2) and instead engaged a conserved Gln at the base of the substrate-binding pocket. Such an interaction leads to a strained activation loop and is thought to govern substrate switching between Tyr and Ser/Thr. Ser<sup>441</sup> is solvent-exposed, positioned in the loop connecting the two strands of the  $\beta$ -hairpin in the CMGC insert. Using molecular dynamics simulations, the authors found that Ser<sup>441</sup> phosphorylation does not stabilize the CMGC insert structure. Both pSer<sup>441</sup> and the adjacent  $\alpha$ M helix, which occludes a protein interaction site (the "P + 3 pocket") on the C-lobe, likely regulate protein-protein interactions. The authors posit that Ser<sup>441</sup> phosphorylation might dictate HIPK2's propensity to dimerize.

The authors identified additional defining features in the N-lobe of HIPK2. They noted the absence of helical and strand motifs that variously crown the N-lobes of DYRK kinases and also observed a short helix within the loop between  $\alpha$ C and  $\beta$ -strand 4 in HIPK2's N-lobe. A sequence analysis revealed that this helix is conserved among the HIPK kinases. The key residue within the  $\alpha$ C- $\beta$ 4 insert helix, Tyr<sup>258</sup>, engaged in interactions with Lys<sup>314</sup> in  $\alpha$ E and with Glu<sup>253</sup> immediately preceding  $\alpha$ C. The precise role of the  $\alpha$ C- $\beta$ 4 insert helix is currently unclear, but the authors propose that it may regulate the position of the  $\alpha$ C helix for catalysis or that it may function

This work was supported by Fellowship 1105754, Grants 1124735 and 1124737, and IRISS 9000433 (to J. M. M.) from the National Health and Medical Research Council of Australia and the Victorian Government Operational Infrastructure Support Scheme. The author declares that he has no conflicts of interest with the contents of this article.

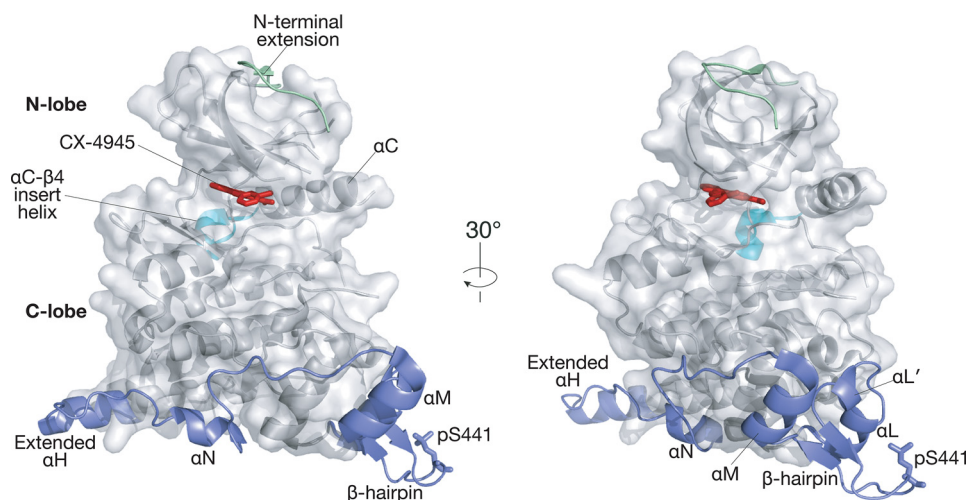
<sup>1</sup> To whom correspondence may be addressed: Dr. James M. Murphy, Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia. Tel.: 61-3-93452407; E-mail: jamesm@wehi.edu.au.

<sup>2</sup> The abbreviations used are: HIPK, homeodomain-interacting protein kinase; CK2 $\alpha$ , casein kinase 2 subunit  $\alpha$ ; DYRK, dual-specificity tyrosine-regulated kinase.

This is an open access article under the [CC BY](#) license.

13560 J. Biol. Chem. (2019) 294(37) 13560–13561





**Figure 1. The HIPK2 kinase domain possesses many distinguishing features.** The CMGC insert is shown in *slate blue*, the  $\alpha$ C- $\beta$ 4 insert helix in *cyan*, the N-terminal extension in *green*, and the small molecule CX-4945 in *red*. A comprehensive comparison with DYRK kinases is shown in Figs. 4 and 5 of Agnew *et al.* (2).

as a site for intra- or intermolecular regulatory protein–protein interactions.

Agnew *et al.* identified several interesting features within the HIPK2 structure that could not have been predicted by homology with the related DYRK kinases and with CMGC family kinases more broadly. The authors have identified intriguing structural features whose functions remain to be determined, opening up a new area for exploration. Of primary interest, whether these structural elements mediate protein–protein interactions, and thereby contribute to noncatalytic functions of the kinase (4, 5), remains to be established.

The structure reported by Agnew and colleagues also provides important insights into how HIPK2 might be targeted with small-molecule inhibitors. As noted, HIPK2 was crystallized in complex with the CK2 $\alpha$  inhibitor CX-4945, revealing a strong conservation of key compound-binding residues between CK2 $\alpha$  and HIPK2. There are some notable differences, however; the Gly-rich loop in HIPK2 is not involved in inhibitor binding, and the CX-4945-binding residue His<sup>160</sup> in the active site of CK2 $\alpha$  is absent in HIPK2. These differences indicate possible avenues that could be explored by medicinal chemistry

to generate selective HIPK2 inhibitors. Such inhibitors would have value in cancer and fibrosis therapies and could provide crucial tools to further explore the physiological functions of HIPK2 in health and disease.

#### References

1. Knighton, D. R., Zheng, J. H., Ten Eyck, L. F., Ashford, V. A., Xuong, N. H., Taylor, S. S., and Sowadski, J. M. (1991) Crystal structure of the catalytic subunit of cyclic adenosine monophosphate-dependent protein kinase. *Science* **253**, 407–414 [CrossRef Medline](#)
2. Agnew, C., Liu, L., Liu, S., Xu, W., You, L., Yeung, W., Kannan, N., Jablons, D., and Jura, N. (2019) The crystal structure of the protein kinase HIPK2 reveals a unique architecture of its CMGC-insert region. *J. Biol. Chem.* **294**, 13545–13559 [CrossRef Medline](#)
3. Kannan, N., and Neuwald, A. F. (2004) Evolutionary constraints associated with functional specificity of the CMGC protein kinases MAPK, CDK, GSK, SRPK, DYRK, and CK2 $\alpha$ . *Protein Sci.* **13**, 2059–2077 [CrossRef Medline](#)
4. Jacobsen, A. V., and Murphy, J. M. (2017) The secret life of kinases: insights into non-catalytic signalling functions from pseudokinases. *Biochem. Soc. Trans.* **45**, 665–681 [CrossRef Medline](#)
5. Kung, J. E., and Jura, N. (2016) Structural basis for the non-catalytic functions of protein kinases. *Structure* **24**, 7–24 [CrossRef Medline](#)