Barry J. Thompson[2] & Neil Q. McDonald[1,3]

[1]Signalling and Structural Biology, [2]Epithelial Biology, The Francis Crick Institute, 1 Midland Road, London NW1 1AT

[3]Institute of Structural and Molecular Biology
School of Biological Science,
Birkbeck College, Malet Street
London WC1E 7HX, UK

Correspondence:
neil.mcdonald@crick.ac.uk
barry.thompson@crick.ac.uk

Title: Competitive inhibition of aPKC by Par-3/Bazooka and other substrates

Our original study reported the structure of an aPKC kinase domain bound to a Mg-ADP analogue and a Par-3 CR3 peptide (Soriano et al., 2016), with additional contacts to those described by Wang et al (Wang et al., 2012). The Par-3 CR3 peptide bound with high affinity to the aPKC kinase domain and we were barely able to detect its phosphorylation using an ADP-coupled assay. We also showed this Par-3 CR3 peptide (and an S>A variant) could competitively inhibit aPKC phosphorylation of a model substrate more potently than a Par-1 equivalent. Holly and Prehoda use a more sensitive radiometric assay to show the CR3 peptide substrate can be phosphorylated by aPKC and behaves as a competitive inhibitor. We agree with the general point these authors make that Par-3, Kibra and other aPKC substrates can all act as competitive inhibitors, albeit with widely varying potencies (Lin et al., 2000; Yoshihama et al., 2011). This dual function was proposed to be important for influencing the apical-junctional localisation of the Par-3 homolog Bazooka by Morais de-Sa et al (Morais-de-Sa et al., 2010), which our own data support. Our structural and *in vivo* evidence are consistent with a functional role for CR3 flanking regions in modulating apical-junctional localisation. Our major findings are not disputed by Prehoda and colleagues, and we are grateful for their new data and perspectives. We recognise that the use of peptide substrates as surrogates for full-length Par proteins will ultimately be superseded by the characterisation of intact Par-aPKC complex assemblies.

References

Lin, D., Edwards, A.S., Fawcett, J.P., Mbamalu, G., Scott, J.D., and Pawson, T. (2000). A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. Nat Cell Biol *2*, 540-547.

Morais-de-Sa, E., Mirouse, V., and St Johnston, D. (2010). aPKC phosphorylation of Bazooka defines the apical/lateral border in Drosophila epithelial cells. Cell *141*, 509-523.
Soriano, E.V., Ivanova, M.E., Fletcher, G., Riou, P., Knowles, P.P., Barnouin, K., Purkiss, A., Kostelecky, B., Saiu, P., Linch, M.*, et al.* (2016). aPKC Inhibition by Par3 CR3 Flanking Regions Controls Substrate Access and Underpins Apical-Junctional Polarization. Dev Cell *38*, 384-398.
Wang, C., Shang, Y., Yu, J., and Zhang, M. (2012). Substrate recognition mechanism of atypical protein kinase Cs revealed by the structure of PKCiota in complex with a substrate peptide from Par-3. Structure *20*, 791-801.
Yoshihama, Y., Sasaki, K., Horikoshi, Y., Suzuki, A., Ohtsuka, T., Hakuno, F., Takahashi, S., Ohno, S., and Chida, K. (2011). KIBRA suppresses apical exocytosis through inhibition of aPKC kinase activity in epithelial cells. Curr Biol *21*, 705-711.