Nature Reviews Molecular Cell Biology | AOP, published online 17 June 2009; doi:10.1038/nrm2724

CELL SIGNALLING

An activating inhibitor?

Members of the AGC family of protein kinases, including the Akt (also known as protein kinase B (PKB)) proteins and PKC, have important functions in normal and pathological cellular processes. Studies on their mode of activation have shown that 'priming' phosphorylation events in the kinase activation loop are required for AGC kinase activity. Now, two studies reveal that occupancy of the nucleotide-binding pocket by ATP or an ATP-competitive inhibitor induces phosphorylation priming of Akt and PKC independently of intrinsic kinase activity.

> "…nucleotide pocket occupancy dictates kinase activation."

Full Akt activation requires three distinct upstream events: phosphatidylinositol-1,4,5-trisphosphate-dependent membrane translocation, phosphoinositide-dependent kinase 1 (PDK1; also known as PDPK1)-mediated phosphorylation at Thr308 and phosphorylation at Ser473 by mammalian target of rapamycin complex 2 (mTORC2). Okuzumi *et al.* used a chemical–genetic approach to develop specific ATP-competitive Akt inhibitors. Intriguingly, the inhibition of Akt kinase activity by these inhibitors was accompanied by hyperphosphorylation at Thr308 and Ser473. Association of Akt with the membrane was necessary but not sufficient to induce hyperphosphorylation in response to inhibitors.

So, how do these inhibitors lead to phosphorylation? The finding that a kinase-dead mutant of Akt was still phosphorylated suggests that a property of inhibitor binding, rather than an extrinsic feedback loop, stimulates phosphorylation. Indeed, the inhibitors triggered Akt membrane translocation and phosphorylation by PDK1 and mTORC2 — all of the events required for full Akt activation. The authors speculate that occupancy of the ATP-binding pocket induces a conformational change in Akt that promotes membrane association and exposes Thr308 and Ser473 for phosphorylation.

In a separate study, Cameron *et al.* found that kinase-inactive PKCε mutants that cannot coordinate ATP effectively are nonetheless phosphorylated in response to ATP-competitive inhibitors at three phosphorylation priming sites (Thr566, Thr710 and Ser729). In addition, a catalytically inactive PKCε mutant that could coordinate ATP was phosphorylated independently of the inhibitors. Similar to the observations for Akt, these findings suggest that nucleotide pocket occupation, not autophosphorylation or a feedback loop, might be sufficient to induce the phosphorylation of PKCε. Remarkably, activation-associated displacement of ATP from the nucleotide-binding pocket of a PKCε mutant that weakly binds ATP coincided with rapid dephosphorylation of all three PKCε priming sites. Furthermore, ATP-competitive inhibitors stimulated rephosphorylation of these sites, which suggests that nucleotide pocket occupancy dictates kinase activation. Similar observations were made for PKCα.

These data provide evidence that ATP binding to AGC kinases is an essential first step that stimulates their subsequent phosphorylation and activation. The activation of G proteins is regulated by guanine nucleotide-induced conformational changes, and it will be interesting to determine the extent to which adenosine nucleotides alter AGC kinase conformation and regulate their activation in a physiological setting. It will also be important to understand how ATP binding is regulated for AGC kinases, as well as the effect, if any, of priming phosphorylation events on upstream or downstream signaling pathways.

Emily J. Chenette, Associate Editor,
UCSD–Nature Signaling Gateway

ORIGINAL RESEARCH PAPERS Okuzumi, T. *et al.* Inhibitor hijacking of Akt activation. *Nature Chem. Biol.* 24 May 2009 (doi:10.1038/nchembio.183) | Cameron, A. J. M. *et al.* PKC maturation is promoted by nucleotide pocket occupation independently of intrinsic kinase activity. *Nature Struct. Mol. Biol.* **16**, 624–630 (2009)