Meeting report

Complexities of protein kinase C regulation

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A report from the Keystone Protein kinase C: structure, regulation and cellular function meeting, New Mexico, February 5-10, 2000.

With the era of the human genomic sequence nearing completion, experiments describing the expression patterns of hundreds and thousands of genes and proteins, and a new '-omics' field being described weekly, it is noteworthy that there are still meetings devoted to the regulation of a single protein. Protein kinase C (PKC) was first discovered as an activity over 20 years ago, and initial clones were isolated in 1986. Since then, 12 separate isoforms have been described, each denoted by a Greek letter and a numeral and the keystone meeting covered aspects relating to all 12, as well as concepts that extend to the regulation and role of related protein kinases in mammals and other organisms. Here, I focus on talks that addressed one controversial aspect of PKC regulation (which also applies to other protein kinases), and highlight new results that give some fresh insights into the ability of growth factors to initiate cell cycle progression.

Protein kinase C is a member of a larger sub-class of protein kinases termed the AGC kinases (denoting protein kinases A, G and C, but also including protein kinase B (PKB)/akt, p70 and p90 ribosomal S6 kinases, and phosphoinositide-dependent kinase-1 (PDK-1)). Phosphorylation is important in the activation of all these kinases, although additional distinct regulatory mechanisms are involved for each one (Figure 1). PDK-1 was initially isolated as a protein kinase that could phosphorylate and activate PKB *in vitro*. It was subsequently shown to phosphorylate the equivalent phosphorylation site (termed the activation loop site) in the other AGC kinases *in vitro*. Phosphorylation at this site is critical for the activation of this class of kinases, placing PDK-1 as an important mediator of cell signaling responses; but, all members of this sub-class also undergo phosphorylation on at least one additional site at the carboxy-terminus of the protein, termed the hydrophobic motif site. The identity of the kinase(s) responsible for phosphorylation of this motif has remained elusive and controversial.

Peter Parker (Imperial Cancer Research Fund) showed that phosphorylation of the hydrophobic motif site of PKCδ, Ser662, did not occur in a Thr505A (activation loop site) mutant of PKCδ, which has reduced catalytic activity. Phosphorylation on Ser662 could be restored, however, by incubating cells with okadaic acid, a potent inhibitor of serine/threonine phosphatases. Moreover, when quiescent cells were treated with serum, wild-type PKCδ became phosphorylated on Thr505 and Ser662. Phosphorylation on Ser662 was not prevented by preincubating the cells with PKC inhibitors, but was prevented by both LY294002 and rapamycin, relatively specific inhibitors of phosphoinositide 3-kinase (PI3K) and the target of rapamycin kinase (mTOR), respectively. This suggests that the kinase responsible for phosphorylating Ser662 in PKCδ is distinct from PKCδ itself, and may act downstream of both PI3K and mTOR. PKC and mTOR have previously been shown to act downstream of P13K, but this is the first suggestion that PKC may act downstream of mTOR. In contrast to Parker's findings for PKCδ, Alexandra Newton (University of California, San Diego) presented data showing that purified PKCβII could phosphorylate itself on the hydrophobic motif site in a concentration-independent manner, suggesting autophosphorylation *in cis*. Similarly, Alex Toker (Harvard Medical School) showed that kinase-dead PKCε cannot be phosphorylated at its hydrophobic motif, again suggesting that autophosphorylation occurs at this site. These results could suggest differential regulation of this site among different PKC family members.

Toker also presented data showing that PKB could induce phosphorylation of its hydrophobic motif site, Ser473, in a manner consistent with autophosphorylation. Specifically, incubation of PKB with PDK1 *in vitro* caused phosphorylation of both Thr308 (activation loop site) and Ser473.

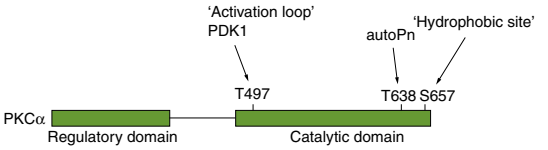

Figure 1
Regulatory phosphorylation sites on AGC kinases. PKCα is taken as an example; all AGC kinases have this generalized structure. It has been proposed that the hydrophobic site of PKB is phosphorylated by one of the following: a separate enzyme - PDK2; PDK1 in the presence of PIF; or autophosphorylation (autoPn), either in the presence or absence of PIF.

Phosphorylation of Ser473, but not of Thr308, was abolished by inactivating PKB prior to the incubation. Similarly, insulin-like growth factor-1 (IGF-1) caused activation of PKB in cells accompanied by phosphorylation of both sites. Kinase-dead PKB was only phosphorylated on Thr308 in response to IGF-1, however. This contrasts with earlier reports from Dario Alessi and Philip Cohen (Dundee University), suggesting that Ser473 phosphorylation is due either to a distinct protein kinase (tentatively termed PDK-2), or to PDK-1 itself, when complexed to a distinct moiety dubbed PIF (PDK-1 interacting factor). The reported discrepancies could be due to the differences in the methodology used between the different groups: phospho-peptide mapping for the Dundee group versus phospho-specific antibodies utilized by Toker. Phospho-specific antibodies may be a more sensitive method to detect lower levels of phosphorylation. The stochiometry of phosphorylation was not addressed by either group, and is difficult to address using the antibody approach. Intriguingly, it could also suggest that PIF could actually influence the ability of PKB to autophosphorylate itself, rather than influence the substrate specificity of PDK-1.

Experiments performed over 20 years ago showed that in order for quiescent cells to undergo DNA synthesis, growth factors must be present for several hours, after which point they are dispensable. This point, termed the restriction point, occurs in the mid-G1 phase of the cell cycle, several hours before the initiation of S phase and DNA replication. Although much work has been done since then to increase our understanding of the signals that are generated following addition of growth factors to quiescent cells, the minimal requirements to push cells into S phase are still surprisingly unclear. Platelet-derived growth factor (PDGF) has been shown to activate a plethora of signaling enzymes, though many of these are transient in nature and are downregulated after an hour or so. Andrius Kazlauskas (Harvard Medical School) recently reported that PI3K lipid products, previously thought to be among the transient products generated in response to PDGF, are actually activated biphasically, with the second wave occurring 4-5 hours later. This second wave was shown to be crucial for the ability of PDGF to induce DNA synthesis, in stark contrast to the first wave, which was entirely dispensable. Addition of synthetic forms of PI3K lipid products to cells at times corresponding to this second wave allowed DNA synthesis, but only in combination with additional PDGF-mediated signal(s). In his talk at this meeting, Kazlauskas extended this earlier work by showing that activation of PKCε (as well as classical PKCs) by PDGF also occurs in a biphasic manner. As in the experiment with PI3K activation, the first wave of PKC activation was dispensable for the induction of DNA synthesis, whereas activation of the second wave was required. To attempt to dissect the relevant contributions of the first and second waves of PDGF function, Kazlauskas showed that the continued presence of PDGF was not in fact required, and could be substituted by an initial exposure which could be washed out after 30 minutes, followed by a second addition eight hours later. Interestingly, the signals downstream of PDGF that are required for DNA synthesis are different at the two stages: only the second stage seems to require PKC and PI3K activation. The relevant signals supplied by the first stage remain unknown, although Kaslauskas showed that they could be supplied by mutants of the PDGF receptor that do not support association of the receptor with various signaling molecules: PI3K, phospholipase Cγ, the tyrosine phosphatase SHP2 or the Ras GTPase-activating protein (RasGAP).

In summary, progress is being made regarding the regulation and function of the protein kinase C family. With at least another 100,000 proteins to work on, analysis of this type seems likely to keep biochemists busy for many years.