PtdIns(3,4,5)P_3 Gets Its Message Across

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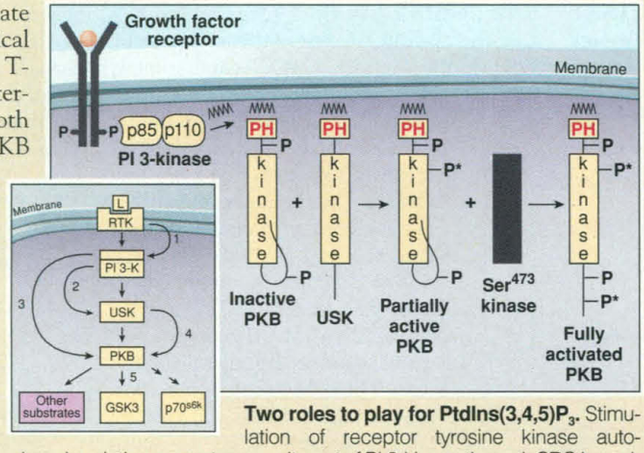
Phosphatidylinositol-3,4,5-trisphosphate [PtdIns(3,4,5)P_3] is clearly a key second messenger in cell regulation, but its mode of action has been somewhat elusive (*1*). Recent work indicates at least two likely roles—promotion of protein attachment to membranes and activation of protein kinase activity. The current paradigm for understanding PtdIns(3,4,5)P_3 action is regulation of the proto-oncogenic serine/threonine protein kinase B (PKB) (also called Akt) (*2*). On page 567 of this issue of *Science* and in a recent issue of *Current Biology*, Stokoe *et al.* (*3*) and Alessi *et al.* (*4*) provide new insights into PKB activation by phosphorylation (see figure).

Predicting that upstream kinases that activate PKB are regulated by PtdIns(3,4,5)P_3, both groups set out to isolate PtdIns(3,4,5)P_3–stimulated protein kinases able to phosphorylate PKB at either of the two critical regulatory sites, Thr^{308} in the T-loop and Ser^{473} in the carboxyl-terminal regulatory domain. Both groups identified an upstream PKB kinase (called USK, or PKBK as suggested earlier), which phosphorylates Thr^{308} and partially activates the kinase. The two activities, although similar, may actually represent isoforms of the same kinase family. Alessi *et al.* (*4*) termed the activity phospholipid-dependent protein kinase or PDK1. With these purified proteins in hand, the authors then dissected the role of PtdIns(3,4,5)P_3 in the activation of USK and subsequently of PKB.



Two roles to play for PtdIns(3,4,5)P_3. Stimulation of receptor tyrosine kinase autophosphorylation promotes recruitment of PI 3-kinase through SRC homology 2 (SH2) domain–phosphotyrosine interactions. Subsequently, PI 3-kinase phosphorylates PtdIns(4,5)P_2 to produce the second messenger PtdIns(3,4,5)P_3, which localizes PKB and possibly USK to the membrane and alters PKB's conformation. USK can then phosphorylate PKB on Thr^{308} and partially stimulate its activity. Complete PKB activation requires further phosphorylation of Ser^{473} by an as yet unidentified kinase. **(Inset) Information transfer.** Activation of PKB by receptor tyrosine kinase–stimulated PI 3-kinase and PtdIns(3,4,5)P_3-stimulated USK.

First, the activity of USK with PKB as substrate is stereospecifically stimulated by PtdIns(3,4,5)P_3 [suggesting that it may also contain a pleckstrin homology (PH) domain]. Second, previous work (*5*) established that the PH domain of PKB binds Ptd(3,4,5)P_3 and PtdIns(3,4)P_2 with high affinity and that the binding probably causes conformational changes in the kinase. These observations were verified through the use of PKB carrying mutations in its PH domain (Arg^{25} to Cys and Trp^{99} to Leu) for the in vitro analysis. Both residues appear critical for full activation of PKB in vivo. Phosphorylation of PKB by USK is abolished by mutations affecting PH domain function (presumably lipid binding) in PKB. As pointed out by Stokoe *et al.* (*3*), this suggests that the PH domain of PKB masks the Thr^{308} residue in the T-loop and that binding of PtdIns(3,4,5)P_3 relieves this constraint. Thus, the double role of PtdIns(3,4)P_2 in the activation of PKB appears to be resolved by the new findings. Upon addition of this lipid to a coupled kinase assay, PtdIns(3,4,)P_2 stimulated the phosphorylation of PKB in a PH domain–dependent manner but did not stimulate USK activity.

The conclusions drawn from these data largely coincide with the earlier predictions (*2*). PtdIns(3,4,5)P_3 appears to be pivotal for the activation process. If in fact USK has a PH domain, there is a strong case for PH domains as signal-dependent membrane adapters (*2*), probably by mediation of protein kinase complex assembly on the membrane. Within the tethered kinase complex, PtdIns(3,4,5)P_3 could also act as an allosteric activator of USK and promote phosphorylation of the downstream target, which itself is "opened up" by lipid binding to its PH domain. A full molecular description of the process will require identification of the predicted Ser^{473} kinase (at the present rate of discovery, this should be accomplished by the end of 1997).

The new model also indicates how information, that is, extracellular ligand binding to transmembrane receptors, is transferred into the cytoplasm. In this case, PKB activation uses almost the complete spectrum of regulatory mechanisms: tyrosine phosphorylation, protein recruitment, phosphorylation of lipids to act as second messengers, allosteric activation, and serine/threonine phosphorylation (see inset). Regulation of the regulators will obviously prove to be complex—but important in life and death decisions, as emphasized by the recent identification of an oncogenic form of the phosphoinositide 3-kinase (PI 3-kinase) (*6*).

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References

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