UPDATE: SIGNAL TRANSDUCTION

PH Domains—A Universal Membrane Adapter

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As discussed recently (*1*) in *Science* and elsewhere, phosphoinositide 3-kinase (PI 3-kinase) stimulates the activity of the proto-oncogenic serine-threonine kinase, Akt (or PKB). Akt is activated when growth factors stimulate PI 3-kinase–mediated synthesis of phospholipid second messengers. The pleckstrin homology (PH) domain of Akt is the target of these phospholipid second messengers. A number of cellular processes are regulated in this way, ranging from insulin signaling and protein synthesis to differentiation and cell survival. Recent work from several different perspectives has implicated PI 3-kinase in the regulation of many other cellular functions—cytoskeletal reorganization, secretion, vesicular sorting, cell migration, and neutrophil and platelet activation (*2*). And on page 1927 in this issue of *Science*, Klarlund *et al.* (*3*) show that the link between these additional cellular functions and PI 3-kinase may also be mediated by PH domains. These data thus provide a framework whereby the diverse effects of PI 3-kinase can be understood mechanistically (see figure).

Since PH domains were first described in 1993, over 100 proteins (*4*) have been shown to contain this module of about 100 amino acids. Characterization of this domain has been rapid; after the first description of the three-dimensional structure of a PH domain, Harlan and colleagues reported that this module bound phospholipids with moderate affinity (*5*). Subsequent studies have mapped the binding site for the inositol phosphate head group of the physiologically relevant phospholipids for two different PH domains (*6*). The general picture that has emerged so far is that PH domains function as signal-dependent membrane adapters.

Klarlund *et al.* used a novel expression cloning approach to identify proteins that bind phosphatidylinositol trisphosphate [PtdIns(3,4,5)P_3] with high affinity. The protein identified, GRP1 (general receptor for phosphoinositides), consists of an NH_2-terminally located coiled-coil followed by a Sec7 domain and a COOH-terminal PH domain. Mapping experiments confirmed that the PH domain preferentially bound PtdIns(3,4,5)P_3 with high affinity, potentially making it a direct target for PI 3-kinase regulation. Significantly, the GRP1 PH domain also bound inositol(1,3,4,5)P_4 with high affinity, which could have significance in down-regulation of signaling by this adapter protein.

Two other closely related proteins have similar structures (*7*) with both Sec7 and PH domains. The first, cytohesin-1, was identified on the basis of its ability to bind to the cytoplasmic tail of the integrin β2 receptor. The other, ARNO, is a nucleotide exchange factor for the small GTP-binding protein, ARF. The isolation of all of these PH-containing proteins by three distinct strategies offers a possible explanation of how PI 3-kinase can regulate diverse cellular processes.

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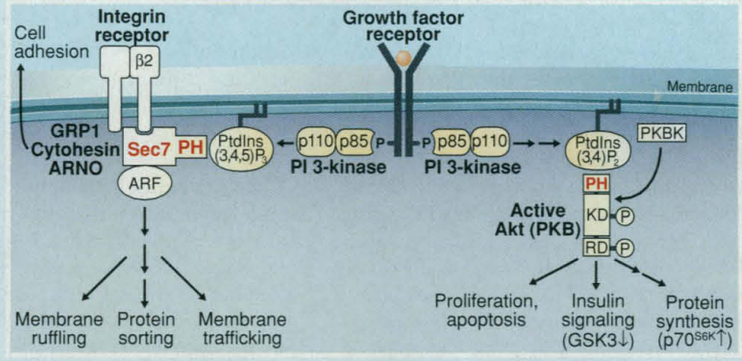
Cytohesin-1 is thought to participate in inside-outside signaling linking growth factor receptor stimulation of PI 3-kinase to cell adhesion. ARNO specifically stimulates nucleotide exchange of ARF through its Sec7 domain. Interestingly, the activity of the Sec7 domain is dramatically stimulated by PtdIns(4,5)P_2 binding to its PH domain. These observations potentially link PI 3-kinase by way of ARNO to the regulation of protein sorting and membrane trafficking. Adding further significance to the PI 3-kinase–PH domain connection is the fact that both GEFs and GAPs [proteins that either stimulate guanosine diphosphate–guanosine triphosphate (GDP-GTP) exchange or stimulate the guanosine triphosphatase activity] of small GTP-binding proteins of the Ras and Rho families have PH domains. [Cytohesin 1 was also recently demonstrated to have GEF activity with ARF (*8*)].The available data indicate that the PH domains are critical for their regulatory roles



PI 3-kinase–PH domain signaling modules. Stimulation of PI 3-kinase by growth factor receptors activates two distinct signaling modules through the membrane recruitment of PH domain–containing proteins. After membrane attachment, the activity of the signaling proteins is increased, ultimately leading to a physiological response. KD, kinase domain; RD, regulatory domain.

The question that emerges from the current studies is whether the cellular array of PH domains contained in numerous target proteins are all receptors for signals emanating from the PI 3-kinase family of lipid kinases. Or, to borrow a football analogy, do PH domains always play the wide receiver to the PI 3-kinase quarterback?

References

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