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An Unnatural PIP Simulates Growth Factor Signaling

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In this issue of *Chemistry & Biology*, Laketa et al. describe the synthesis of a membrane permeant phosphoinositide lipid that acts to stimulate PI(3,4,5)P_3-dependent signaling without the need of growth factor stimulation.

The translation of extracellular signals to a cellular response relies on temporal and spatial cues provided by a complex network of protein recruitment and modification on membrane subdomains. A critical cue of both the location of a given membrane in the cell and its state of activity is the phosphorylation state of a subgroup of acidic phospholipids, the phosphoinositide lipids.

Naturally occurring phosphoinositides (PIs) can be phosphorylated on any combination of the 3′, 4′, and 5′ positions of their inositol head group, giving rise to seven possible lipid species with unique distributions on cellular membranes (Figure 1). In these membranes, PI species often serve as part of a recruitment mechanism for many cytosolic proteins, thus targeting both a time and a place where signaling cascades can be assembled. Likewise, PIs are themselves precursors of the second messengers, inositol triphosphate (IP_3) and diacylglycerol.

The fact that different PI species are confined to separate membrane domains suggests a high degree of regulation by PI kinases and phosphatases, in fact, a highly coordinated series of lipid phosphorylation and dephosphorylation events are required for normal cellular signaling and protein trafficking. Misregulation of PI metabolism contributes to several human congenital diseases (Attree et al., 1992; Bielas et al., 2009; Bolino et al., 2000; Jacoby et al., 2009; Laporte et al., 1996). More acutely, during the lifetime of individuals, phosphoinositide dysregulation contributes to several forms of cancers. Particularly prominent among oncogenic mutations are mutations in the cycle of enzymes modulating the production of PI(3,4,5)P_3 in response to growth factor cues (Denley et al., 2009; Li et al., 1997), all mutations that lead to the increase of the highly charged PI, PI(3,4,5)P_3 (or PIP_3).

Recent studies show that more than two hundred individual proteins specifically associate with PIP_3-containing complexes alone (Catimel et al., 2009). Given the plethora of possible signaling interactions found and their pleiotropic effects on cell morphology and survival, unraveling the effects of individual phosphoinositide species in different cellular contexts is of critical interest to the field of cell biology. However, given that many PI binding domains show high affinity for their substrate coupled with low specificity, it can prove difficult in many cases to unravel the influence of individual PIs on protein targeting and function in vivo.

How then to sort out all these effects? Genetic approaches have been fruitful in assigning roles of individual PI metabolizing enzymes to cell biological processes, but the lipid product itself is often compensated for by other enzymes. Pharmacology has also been used to tackle these themes, such as the use of wortmannin and LY294002 to inhibit PI-3′ kinases, but for many enzymes there is no compound available for specific pharmacological manipulations.

Recent advances have been made in making acute, targeted changes in local PI concentration using a bipartite system of PI phosphatases (Fili et al., 2006; Varnai et al., 2006) or kinases (Suh et al., 2006) that heterodimerize to a targeting construct upon the addition of the small molecule rapamycin. These approaches have been extremely powerful for resolving the contribution of individual phosphoinositide lipids to many cellular processes, but they rely on easily transfectable cells, requiring as they do the incorporation of three plasmids: the two halves of the dimerizable construct and a reporter plasmid.

Laketa et al. (2009) add another string to the bows of biologists looking at the signaling effects mediated by PI metabolism by directly adding a novel PI analog, PI(3,4,5,6)P_4, to cells. To facilitate the transfer of these lipids to the inner leaflet of the plasma membrane, they

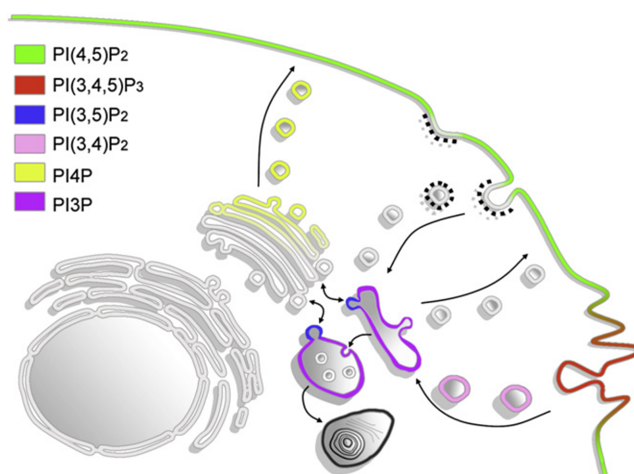

Figure 1. The Distribution of Different PIP Species in Cellular Membranes
The most abundant PIP species, PI(4,5)P_2 and PI4P, are predominantly found in the plasma membrane and Golgi, respectively. PI(3,4,5)P_3 is transiently produced at the plasma membrane by PI 3′-kinases acting on PI(4,5)P_2 in response to growth factor receptor activation. Image kindly provided by Dr. Andrea Raimondi (De Camilli Lab).

have shielded the charged groups of the inositol head group with butyrate and AM esters, which are subsequently hydrolyzed in the cell cytosol. Intriguingly, it would appear that, despite the inclusion of a novel phosphate on the 6' position, many PIP_3 binding and metabolizing proteins are capable of being recruited by the PIP_4 molecule. Laketa et al. (2009) show that PIP_4/AM is a more potent stimulator of several PIP_3-dependent processes in the cell than is the related derivative PIP_3/AM, suggesting that the novel PIP_4 is not as accessible to degradative enzymes (such as endogenous 3′ or 5′ phosphatases) as is PIP_3 once cytosolic hydrolases have removed the AM and butyrate groups from the inositol ring. Using this technique, they are able to bypass the requirement for growth factor stimulation and subsequent PI 3′-kinase activation, to look directly at a subset of PI dependent signaling in the cell, including their feedback onto growth factor receptors themselves. An exciting possibility is that the inclusion of the 6' phosphate group in PIP_4 will render this analog inaccessible to some PIP_3 binding proteins, thus becoming a growth factor-independent agonist of pathways such as the PDK1, AKT/PKB pathway, which is characterized in great detail in this article.

Use of this new molecule, in combination with other methods of manipulating PI metabolism, has the potential to untangle some of the complex web of protein modification and signaling feedback that growth factor signaling evokes in models of health and disease.

REFERENCES

Attree, O., Olivos, I.M., Okabe, I., Bailey, L.C., Nelson, D.L., Lewis, R.A., McInnes, R.R., and Nussbaum, R.L. (1992). Nature *358*, 239–242.

Bielas, S.L., Silhavy, J.L., Brancati, F., Kisseleva, M.V., Al-Gazali, L., Sztriha, L., Bayoumi, R.A., Zaki, M.S., Abdel-Aleem, A., Rosti, R.O., et al. (2009). Nat. Genet. *41*, 1032–1036.

Bolino, A., Muglia, M., Conforti, F.L., LeGuern, E., Salih, M.A., Georgiou, D.M., Christodoulou, K., Hausmanowa-Petrusewicz, I., Mandich, P., Schenone, A., et al. (2000). Nat. Genet. *25*, 17–19.

Catimel, B., Yin, M.X., Schieber, C., Condron, M., Patsiouras, H., Catimel, J., Robinson, D.E., Wong, L.S., Nice, E.C., Holmes, A.B., and Burgess, A.W. (2009). J. Proteome Res. *8*, 3712–3726.

Denley, A., Gymnopoulos, M., Kang, S., Mitchell, C., and Vogt, P.K. (2009). Mol. Cancer Res. *7*, 1132–1138.

Fili, N., Calleja, V., Woscholski, R., Parker, P.J., and Larijani, B. (2006). Proc. Natl. Acad. Sci. USA *103*, 15473–15478.

Jacoby, M., Cox, J.J., Gayral, S., Hampshire, D.J., Ayub, M., Blockmans, M., Pernot, E., Kisseleva, M.V., Compere, P., Schiffmann, S.N., et al. (2009). Nat. Genet. *41*, 1027–1031.

Laketa, V., Zarbakhsh, S., Morbier, E., Subramanian, D., Dinkel, C., Burmbaugh, J., Zimmerman, P., Pepperkok, R., and Schultz, C. (2009). Chem. Biol. *16*, this issue, 1190–1196.

Laporte, J., Hu, L.J., Kretz, C., Mandel, J.L., Kioschis, P., Coy, J.F., Klauck, S.M., Poustka, A., and Dahl, N. (1996). Nat. Genet. *13*, 175–182.

Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S.I., Puc, J., Miliaresis, C., Rodgers, L., McCombie, R., et al. (1997). Science *275*, 1943–1947.

Suh, B.C., Inoue, T., Meyer, T., and Hille, B. (2006). Science *314*, 1454–1457.

Varnai, P., Thyagarajan, B., Rohacs, T., and Balla, T. (2006). J. Cell Biol. *175*, 377–382.

A Versatile Actor Finds a New Role

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RNA stars in many roles within the cell. In a recent paper published in *Cell*, Loh et al. demonstrate a new mechanism of action for natural riboswitches and provide important insights into the regulation of virulence in a pathogenic bacterium.

Whether it's on the big screen or on the small one, in the lead role or in support, some actors seem to be able to play any type of role. Two of the most versatile actors of today, Christopher Walken and Meryl Streep, appeared together in the 1978 film ‘‘The Deer Hunter,’’ for which Walken was awarded an Oscar for Best Supporting Actor, while Streep was nominated for Best Supporting Actress. Since then, both actors have gone on to appear in countless memorable roles, and I'm never surprised to see them in something unusual, such as Walken in ‘‘Hairspray,’’ or Streep as Julia Child in ‘‘Julie and Julia.’’

Such versatility appears at the molecular level as well, where RNA is frequently discovered in new roles. Coincidentally, it was also in 1978 when Sidney Altman and coworkers showed that RNA plays an essential role in the activity of the enzyme RNase P (Stark et al., 1978). In the three decades since, we have begun to appreciate the versatility of RNA as it stars in an increasing variety of roles in biological systems. Far from being a bit player mediating translation, RNA appears in diverse starring roles in both eubacteria (Waters and Storz, 2009) and eukaryotes (Rana, 2007).

The bacterial kingdom also has its share of versatile players, including the pathogen *Listeria monocytogenes*, which excels at playing the bad guy. In addition to being a leading cause of food-borne illness in humans, *L. monocytogenes* can

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