MINIREVIEW

Inositols do it all

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Inositol polyphosphate 5-phosphatases (IP5Pases) are Mg^{2+} -dependent enzymes of the phosphatidylinositol signaling pathway. They were initially identified by their ability to hydrolyze inositol 1,4,5-trisphosphate [$Ins(1,4,5)P_3$] to inositol 1,4-bisphosphate (Majerus 1992). Because the substrate mobilizes intracellular calcium ions through the $Ins(1,4,5)P_3$ receptor and the product is inactive in this respect, it was presumed that this enzyme was a signal-terminating enzyme analogous to cyclic nucleotide phosphodiesterase in the cAMP system. However, multiple genes encoding IP5Pases have been discovered in the last few years indicating a much broader role in cell signaling for this family of enzymes (Jefferson et al. 1995).

The most recently discovered IP5Pases are a unique group of enzymes that form complexes with other signaling molecules in response to hematopoietic cytokines and growth factors. These newly discovered enzymes provide the first linkage of IP5Pases to tyrosine kinase cell signaling. In this case, binding of macrophage colony-stimulating factor (M-CSF) to its receptor Fms leads to formation of a complex between the receptor, Fms, and adaptor proteins Grb2 and Shc plus a tyrosine-phosphorylated protein of 150 kD designated p150^{Shp} (SH2-containing inositol phosphatase). p150^{Shp} encodes a protein that contains two conserved motifs that define IP5Pases (Jefferson et al. 1995). These are GDXN(Y/F)R and P(S/A)W(C/T)DRIL. These conserved residues appear to function directly in catalysis as substitution of any of them with alanine destroys enzyme activity (A.B. Jefferson and P.W. Majerus 1996). p150^{Shp} also contains other motifs characteristic of intracellular signaling molecules, including an SH2 domain, two protein tyrosine-binding (PTB) sites, and several proline-rich regions that bind to SH3 domains. p150^{Shp} was isolated in a yeast two-hybrid screen based on its ability to be phosphorylated on tyrosine at its PTB-binding site and thereby bind to a bait that contained the PTB domain of Shc. Expression of p150^{Shp} in FD-Fms cells inhibits growth in response to both M-CSF and IL-3 suggesting that p150^{Shp} negatively regulates growth in this system. p150^{Shp} is an IP5Pase, as Kavanaugh et al. (1996) and Damen and co-workers (1996) have isolated the same protein as one that forms complexes with Grb2 in response to activation by platelet-derived growth factor (PDGF) or by multiple hematopoietic cytokine receptors. This protein was shown

to have IP5Pase activity with a unique substrate specificity in that it only hydrolyzed IP5Pase substrates containing phosphate in the 3 position of the inositol ring, that is, $Ins(1,3,4,5)$ -tetrakis phosphate [$Ins(1,3,4,5)P_4$] and the lipid substrate $PtdIns(3,4,5)P_3$. Another IP5Pase with this same substrate specificity is a 110-kD protein that forms complexes with Grb2 in PDGF-stimulated cells (Jefferson et al. 1996; Kavanaugh et al. 1996). This protein designated Ga-IP5Pase (or SIP-110) is 87.5% identical to p150^{Shp} but lacks the amino-terminal SH2 domain as shown in Figure 1. The amino acid differences between the two proteins can be explained by the fact that p150^{Shp} is a murine gene and Ga-IP5Pase is human. A partial sequence corresponding to the human version of p150^{Shp} indicates that these proteins are alternatively spliced versions of the same gene (Kavanaugh et al. 1996). The association of these proteins with Grb2 and Shc suggests that they may function in the Ras pathway, although a number of questions remain to be resolved. Is the active signaling molecule the substrate lipid $PtdIns(3,4,5)P_3$ or its product $PtdIns(3,4)P_2$? Alternatively, the soluble substrate $Ins(1,3,4,5)P_4$ could play a role in signaling. Cullen et al. (1995) have reported that $Ins(1,3,4,5)P_4$ binds to and activates a member of the GAP 1 family. Thus, hydrolysis of $Ins(1,3,4,5)P_4$ could reduce Ras GAP activity and thereby stimulate the Ras pathway. Another question to be resolved is whether the binding of IP5Pases to Grb2 or Shc serves to localize them or to alter their enzymatic activity. The former seems to be the case for Ga-IP5Pases, as recombinant protein has been found to form a 1:1 complex with Grb2 without any effect on enzyme activity (Jefferson et al. 1996).

There are six mammalian IP5Pases that have been characterized at the enzymatic level. Furthermore, IP5Pase homologs are found in *Arabidopsis*, *Caenorhabditis elegans*, and yeast (Jefferson et al. 1995). There are three IP5Pase genes in yeast (J.D. York, pers. comm.).

The IP5Pases have been grouped according to their substrate specificity (Jefferson et al. 1996). Group 1 includes the type I IP5Pases that have molecular masses of 32–43 kD and hydrolyze both $Ins(1,4,5)P_3$ and $Ins(1,3,4,5)P_4$ but neither lipid substrate. They most likely represent the enzymes that serve a "signal terminating" function in calcium ion mobilization. Group 2 includes enzymes that hydrolyze four IP5Pase substrates, the soluble phosphates mentioned above, and the

Figure 1. The structures of the known IP5Pases are shown with the two motifs that define the family noted as solid lines. The spacing between these two motifs is fairly constant, ranging from 69 to 112 amino acids. The shaded areas show regions of similarity to 5-ptase II, the open boxes are regions similar to the yeast *SacI* domain. The cDNA molecules depicted include 5-ptase I (GenBank accession no. X75094), 5-ptase II (5 ptII; M74161), OCRL (M88162), INPP5B (L36818), p150^{SHIP} (U51742), Ga-IP5Pase (synaptojanin; U50040), Yeast I (Z38062), Yeast 2 (Z47047), Yeast 3 (X79743).

lipids PtdIns(3,4,5)P₃ and PtdIns(4,5)P₂. The original member of this group, inositol polyphosphate-5-phosphatase type II, was isolated from platelets as a 75-kD protein that is presumably processed from a much larger precursor predicted from the cDNA. A second enzyme in this group is the IP5Pase mutated in Lowe's oculocerebrorenal syndrome gene (OCRL). This is an X-linked disorder characterized by mental retardation, eye abnormalities, and renal tubular absorption defects (Attree et al. 1992). OCRL is a group 2 enzyme, although it shows a clear preference for the lipid substrates. It is most closely related to inositol polyphosphate-5-phosphatase type II with 51% amino acid identity over the entire 741 amino acids of the mature enzyme (Zhang et al. 1995). A third member of this family, synaptojanin, was recently isolated (McPherson et al. 1996). This IP5Pase is proposed to function in synaptic vesicle trafficking. It is most similar to the yeast IP5Pases, as it also forms complexes with Grb2. Group 3 includes two IP5Pases that form complexes with hematopoietic cytokin receptors and activate the Ras pathway. These enzymes hydrolyze only the 3-phosphate-containing substrates PtdIns(3,4,5)P₃ and Ins(1,3,4,5)P₄.

Additionally, there are several other putative IP5Pases that have not been characterized as yet. These include INPLP1, which was isolated by its ability to complement the defect in DNA repair in cells from patients with Fanconi's anemia (FA-A) (Hejna et al. 1995), and several expressed sequence tags (ESTs) sequences that are in GenBank but not yet fully cloned and expressed. Thus, much remains to be learned about this expanding family of genes.

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