Prenylcysteine recycling program

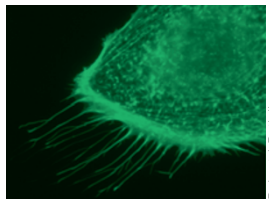
Protein prenylation, in which linear terpenes are covalently attached to the C-terminal cysteine residues of proteins, is a well-characterized post-translational modification that targets proteins to membranes or facilitates protein-protein interactions. The biosynthesis of prenylated proteins is well established; in contrast, the degradation pathway for plant proteins that have undergone prenylation is not well understood. Previous studies in mammalian cells suggested that proteolytic degradation of prenylated proteins generates lipidated amino acids, such as farnesylcysteine (FC), which are broken down further. Crowell *et al.* now extend our knowledge of prenyl recycling in plants by showing that FC lyase, an enzyme that fragments the thioether bond of FC, is found in *Arabidopsis thaliana*. FC lyase is a membrane-bound enzyme found in most *A. thaliana* tissues that catalyzes the oxidative conversion of FC into cysteine and the aldehyde farnesal. In contrast to mammalian systems, the FC lyase from *A. thaliana* seems to be specific for FC over other prenylated substrates, such as geranylgeranylcysteine, and shows affinity for the substrate analog *N*-acetylfarnesylcysteine, which suggests that FC lyase may also use prenylated proteins as substrates. The authors mapped the *fcly* gene to chromosome 5 of *A. thaliana* and showed that plants with mutant *fcly* accumulated FC. Taken together, these studies provide evidence that, in plant systems, FC lyase selectively degrades FC and provides an enzymatic gateway to salvage farnesyl diphosphate. (*Plant J.*, **50**, 839–847, 2007) *TLS*

A flavin reaction with a proton twist

Isoprenoid biosynthesis requires isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) as building blocks. In an essential step in many organisms, IPP isomerase (IDI) catalyzes the conversion of IPP to DMAPP. Type 1 IPP isomerase (IDI-1) catalyzes isomerization via a proton addition-elimination reaction in which the first mechanistic step is protonation of the IPP double bond. The recently identified type 2 IPP isomerase (IDI-2) contains a flavin cofactor that must be in the reduced form for the enzyme to be active. The presence of a redox-active cofactor suggests that IDI-2 could initiate the isomerization by hydrogen-atom transfer from the flavin to IPP. To distinguish between a reaction initiated by proton addition and one initiated by hydrogen-atom addition, Johnston *et al.* generated two substrate analogs: cyclopropyl IPP (cIPP) and epoxy IPP (oIPP). IDI-2 converted cIPP to the expected DMAPP analog with no evidence of enzyme inhibition, whereas incubation of IDI-2 with oIPP resulted in enzyme inactivation through formation of a covalent adduct between oIPP and the flavin. Solvent isotope experiments suggested that the mechanism of inhibition involves an initial protonation of the epoxide ring. Based on these results, along with the lack of any evidence for radical intermediates, the authors suggest that, analogous to IDI-1, proton transfer initiates IDI-2–catalyzed IPP isomerization. Although the direct reaction observed between oIPP and the flavin suggests close proximity between the substrate and the cofactor, the exact mechanistic role of the flavin remains to be determined. (*J. Am. Chem. Soc.*, published online 5 June 2007, doi:10.1021/ja072501r) *JK*

Research Highlights written by Mirella Bucci, Joanne Kotz and Terry L. Sheppard

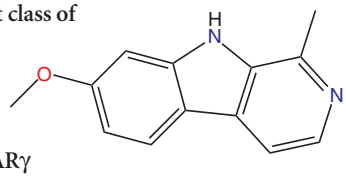
Modular and malleable GEFs



Robert J Rutigliano

Rho-family GTPases are central regulators of the actin cytoskeleton and therefore of cell shape and motility as well. For instance, the canonical GTPases cdc42 and Rac1 control the formation of filopodial and lamellipodial extensions, respectively. Guanine nucleotide exchange factors (GEFs) activate GTPases by catalyzing the exchange of GTPase-bound GDP for GTP. To investigate the plasticity in GEF signaling pathways, Yeh *et al.* created GEFs that respond to non-natural signaling inputs by substituting natural regulatory domains from Dbl-family GEFs with a PDZ domain fused to a peptide ligand that contains a protein kinase A (PKA) phosphorylation motif. By linking this artificial regulatory domain to natural Dbl catalytic domains, Yeh *et al.* created cdc42- and Rac1–activating GEFs that are controlled *in vitro* by PKA phosphorylation. Inside cells, these artificial GEFs linked forskolin concentration, a PKA activator, with the expected downstream cell motility phenotypes. In an effort to create a two-GEF signaling pathway, the authors generated a GEF that activates Rac1 in response to cdc42 activation. When this GEF was introduced into cells, along with a GEF linking PKA to cdc42 activation, forskolin induced lamellipodial formation. These results highlight the malleability of GEF signaling and provide a new approach for synthetic control of the actin cytoskeleton. (*Nature*, **447**, 596–600, 2007) *JK*

Harmine raises the PPAR



Thiazolidinediones are the largest class of antidiabetic drugs, and improve insulin sensitivity by activating the key adipogenic factor, peroxisome proliferator-activated receptor γ (PPARγ). PPARγ target genes include those encoding CD36 and adiponectin and are involved in lipid and glucose metabolism in adipocytes. The adverse effects associated with PPARγ agonists have left open the search for antidiabetic drugs. Waki *et al.* used a cell-based screen to identify compounds that affect adipocyte differentiation. They identified pro- and antiadipogenic compounds, including harmine, a β-carboline alkaloid from the *Peganum harmalla* plant. Harmine's proadipogenic effects were cell-type specific and were most potent in preadipocytes, but the compound was not a direct ligand for PPARγ. Harmine increased expression of PPARγ mRNA and downstream target genes *in vitro* and in white adipose tissue (WAT) of intact mice, thereby distinguishing its mechanism from that of induction by GW7845, a direct PPARγ agonist that does not alter PPARγ expression. In searching for differentiation pathways that harmine might directly affect, the authors found that several Wnt signaling pathway factors are suppressed by harmine. Wnt activation suppressed PPARγ expression, which suggests that harmine regulation of PPARγ is mediated through its ability to block Wnt signaling during preadipocyte differentiation. Harmine could improve insulin and glucose tolerance in diabetic mice and this effect correlated with increases in expression of metabolic genes and of genes involved in fatty acid oxidation. These results suggest that small molecules that promote adipocyte differentiation by mechanisms distinct from those of classical antidiabetics could make desirable lead compounds for diabetes drugs. (*Cell Metab.* **5**, 357–370, 2007) *MB*