PLANTS

Making auxin

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Indole acetic acid (IAA) is the archetypal auxin, a class of hormones that regulate numerous growth and developmental pathways in plants. A major biosynthetic source of IAA is tryptophan and its metabolite, indole-3-pyruvic acid (IPA), which is thought to be converted to IAA in an oxidative decarboxylation reaction catalyzed by a YUCCA enzyme (YUC). Although the 11 *YUC* genes in *Arabidopsis thaliana* are members of the flavin-containing monooxygenase family and have roles in localized auxin biosynthesis in various tissues, the molecular mechanisms for YUC-mediated transformations remain unresolved. Because this lack of insight was due, in part, to the poor availability of biochemical samples, Dai *et al.* first developed a route to purify active *A. thaliana* YUC6 enzyme after expression in *Escherichia coli* and concluded that YUC6 contains an oxidized FAD cofactor and is not an FMN-containing enzyme. Spectroscopic and stopped-flow methods showed that the FAD cofactor is reduced in the presence of NADPH and reacts with molecular oxygen to form a metastable C4a-(hydro)peroxyflavin intermediate. Further spectroscopic and HPLC data revealed that activated YUC6 converts IPA to IAA and regenerates oxidized FAD and can also use other ketoacids, such as phenylpyruvic acid, as substrates. Taken together, the studies establish that YUC6, and perhaps other YUC enzymes in plants, share mechanistic similarities to known Baeyer-Villiger monooxygenases. *TLS*

ENZYME DESIGN

Going gluten-free

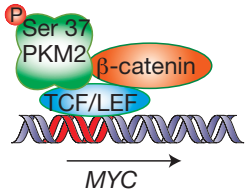
J. Am. Chem. Soc. **134**, 20513–20520 (2012)

Celiac disease is caused by an inflammatory response to oligopeptides that remain after the incomplete digestion of the gluten component α-gliadin. As a potential treatment strategy for this condition is to enhance degradation of these ProGln-rich peptides, previous research has focused on engineering proteases specific for ProGln dipeptides to function in the stomach, that is, under highly acidic conditions. Gordon *et al.* now describe an alternative approach: re-engineering the substrate specificity of an endopeptidase known to be active at low pH. To accomplish this, the authors first searched for enzymes with available crystal structures that functioned in a defined pH range at biologically relevant temperatures. They identified kumamolisin-As from *Alicyclobacillus sendaiensis* as a strong candidate, as the enzyme is specific for proline at the P2 site and does process substrates—though poorly—with glutamine at the P1 site. To enhance specificity for glutamine, the authors computationally varied residues within 8 Å of glutamine modeled in the active site to find energetically favorable solutions. Expression and activity assays of 261 of the resultant enzymes yielded an improved construct, KumaMax, which was ~120-fold more active than the wild-type protease. KumaMax was also proteolytically stable, specific for the ProGln motif compared to wild-type substrates and capable of degrading a longer biologically relevant oligopeptide with a half-life of 8.5 min, suggesting its potential utility in the further development of oral enzyme therapies for Celiac disease. *CG*

CANCER

Translocation, translocation, translocation

Nat. Cell Biol. **14**, 1295–1304 (2012)



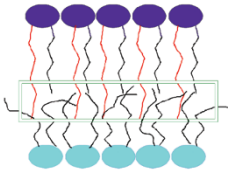
NATURE CELL BIOLOGY

The Warburg effect, or aerobic glycolysis, facilitates tumor cell growth. Pyruvate kinase M2 (PKM2) and PKM1 are glycolytic enzymes with overlapping but distinct roles in tumorigenesis that are only partially understood. Both enzymes are active in glucose metabolism, but PKM2 is also a transcriptional coactivator; the relationship between this latter activity of PKM2 and tumorigenesis is unclear. Yang *et al.* now show that PKM2 translocates to the nucleus in response to EGFR signaling. Small-molecule inhibition of kinases downstream of EGFR showed that only inhibition of MEK-ERK blocked nuclear translocation of PKM2. The authors showed that ERK phosphorylates PKM2 but not PKM1 and that phosphorylation by ERK at Ser37 is important for nuclear translocation of PKM2. Phosphorylation at Ser37 promoted the association of PKM2 with the peptidyl-prolyl isomerase PIN1, and catalytically active PIN1 was required for nuclear translocation of PKM2. In the nucleus, PKM2 promoted association of its cofactor β-catenin with the *MYC* promoter and affected c-Myc–dependent expression of downstream genes. Genetic depletion of PKM2 reduced glucose consumption and lactate production, indicators of the Warburg effect. Taken together, these data indicate that ERK-dependent phosphorylation, isomerization and nuclear translocation of PKM2 are necessary for the EGFR-dependent Warburg effect. *AD*

LIPIDS

Leaflets out of order

Biophys. J. **103**, 2311–2319 (2012)



SALVATORE CHIANTIA

The inner and outer leaflets of the plasma membrane maintain a compositional asymmetry, with sphingomyelin (SM) found mostly on the outer leaflet and lipids such as phosphatidylserine and phosphatidylethanolamine found mostly in the inner leaflet. Another difference between leaflets is in the propensity to form lipid rafts: inner-leaflet lipids form homogenous membranes that do not form rafts, but the outer-leaflet lipids can form rafts by themselves. However, the leaflets are coupled in some fashion, as outer-leaflet lipids can influence organization of inner-leaflet signaling proteins. To gain insight into the mechanisms of interleaflet coupling, Chiantia and London used fluorescence correlation spectroscopy to monitor the diffusion of lipids of various acyl chain length and saturation within the two leaflets of asymmetric giant unilamellar vesicles. They found that outer-leaflet SM enriched with acyl chains longer than the sphingoid base, which allows them to reach the opposing monolayer, slows the inner-leaflet lipids and increases coupling. The authors also observed that with a shorter acyl chain, SM could slow lipid dynamics in asymmetric membranes, with inner-leaflet lipids having one saturated and one unsaturated acyl chain. Although there was high coupling between the leaflets in terms of lipid diffusion, there was no coupling of structural order (that is, of raft-like domain formation) between the two leaflets. These results suggest that acyl chain interdigitation modulates coupling between leaflets of signaling membranes. *MB*

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