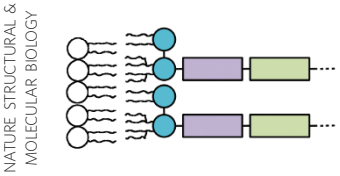
MICROBIOLOGY

Twistin' the fat away

Nat. Struct. Mol. Biol., published online 7 October 2012; doi:10.1038/nsmb.2393



Lipid A is the innermost component of lipopolysaccharides, which are found at the outer leaflet of the outer membrane of Gram-negative bacteria. The long fatty acyl chains of lipid A help anchor the lipopolysaccharides, which protect the bacteria from various environmental stressors, to the outer membrane. During lipid A biosynthesis, LpxI hydrolyzes the β-phosphate group of UDP-2,3-diacylglucosamine, forming lipid X, which is then converted to lipid A by several other enzymes. Metzger *et al.* now report two X-ray crystal structures of LpxI: a D225A mutant of the enzyme bound to UDP-2,3-diacylglucosamine and the wild-type protein bound to lipid X. The structures revealed that LpxI is a homodimer, and each monomer is made up of an N-terminal lipid-X–binding domain and a C-terminal catalytic domain. The authors determined that the homodimer observed in the crystal lattice also occurs in solution. Comparison of the two structures revealed that the lipid X–binding domains, which envelop the substrate in the substrate-bound state, 'open up' and rotate to generate the elongated conformation seen in the product-bound form of the enzyme. Biophysical experiments confirm that the substrate-bound state is more compact and spherical than the product-bound state in solution. The authors speculate that LpxI opens up after the lipid X product is formed so that the phosphodiester hydrolase can more easily transfer lipid X to the next enzyme in the lipid A biosynthetic pathway or to facilitate its release into the lipid bilayer. *JMF*

PLANT DEFENSE

Tainted LOV1

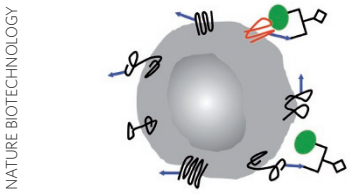
Science, published online 18 October 2012; doi:10.1126/science.1226743

Effector-triggered immunity (ETI) in plants is initiated when pathogens produce effector molecules that target the plant's defense pathways and the plant responds with resistance proteins (R-proteins) that 'guard' the target from the effector. However, in the case of *Cochliobolus victoriae*, a fungus that infects *Arabidopsis thaliana*, the small-molecule effector victorin collaborates with an R-protein to enhance rather than reduce susceptibility to infection. Previous studies had shown that thioredoxin TRX-h5 and the R-protein LOV1 are required for victorin-mediated susceptibility. Lorang *et al.* now show that victorin acts by covalently modifying TRX-h5 to hijack ETI. Mutational analysis and MS revealed that Cys39 of TRX-h5, a residue previously linked to LOV1 activation, becomes modified with the natural product in victorin-treated *Arabidopsis*. Victorin modification inhibited TRX-h5 activity and dampened plant defense gene activation mediated by one of its substrates, the NPR1 transcription factor. In contrast, LOV1 activation was dependent on the presence of TRX-h5 and victorin, and imaging and yeast two-hybrid experiments confirmed a physical interaction between LOV1 and TRX-h5 in the plasma membrane. Together, these data may support a general pathway in which an R-protein, tasked with guarding a particular defense protein, can be redirected by effectors to aid pathogenic infection. *TLS*

CHEMOPROTEOMICS

Flex your TRICEPS

Nat. Biotechnol., published online 16 September 2012; doi:10.1038/nbt.2354



Identifying ligands for receptors in live cells can provide valuable information, providing insight into signaling pathways as well as drug targets, but these interactions can be difficult to detect and quantify. Frei *et al.* now report a trifunctional chemoproteomic reagent called TRICEPS that binds glycosylated receptors on live cells and allows for the purification of the ligand-receptor complex and identification of receptor-derived peptides by MS. TRICEPS contains three functional groups: an *N*-hydroxysuccinimide ester for ligand conjugation, a protected hydrazine that reacts with aldehydes introduced into the carbohydrates of cell-surface glycoproteins and biotin for the affinity capture of glycopeptides. The authors validated their approach by using insulin-coupled TRICEPS to isolate the insulin receptor from adipocytes. The authors then used their reagent to compare receptor interactions of EGF and trastuzumab, a therapeutic antibody that binds ErbB2, a member of the EGF receptor superfamily. The antibody bound selectively to ErbB2, whereas EGF bound EGFR; the preferential identification of only two glycosylated peptides in the EGF-EGFR samples provided insight into the ligand-binding site. Application of TRICEPS also revealed binding sites for DARPins, ankyrin repeat proteins, on ErbB2. Evaluation of trastuzumab-receptor interactions in primary cancer tissue revealed binding to ErbB2 as well as to immunoglobulin Fcγ receptors, indicating that trastuzumab can bind multiple targets in complex tissue. Taken together, these data indicate that TRICEPS allows ligand-based receptor capture from live cells and provides insight into the approximate binding site on receptors. *AD*

BIOSYNTHESIS

Crowdsourcing clusters

Nucl. Acids Res., published online 26 October 2012; doi:10.1093/nar/gks993

Bacterial sequencing has led to a wealth of genetic information regarding natural product biosynthesis pathways, but accessing that data in a robust and straightforward way has been more challenging. Several databases focusing on aspects of polyketide synthase or nonribosomal peptide synthetase function have been developed, but Conway and Boddy hoped to create a more comprehensive and current platform by enabling the biosynthesis community to directly contribute. The result is ClusterMine360, a new database that integrates existing genetic, chemical and bioinformatic tools to speed cluster deposition and analysis as well as ensures standardization and limits potential for user error. In particular, the database draws information from the NCBI nucleotide records to assign species, linked papers and annotations. Chemical structures are queried against ChemSpider and PubChem to collect synonyms, avoiding duplicate entries, and compared across database entries to create linked compound families. AntiSMASH provides a bioinformatic analysis of each cluster, with more than 10,000 individual domains currently documented in the repository, and enables fast retrieval of relevant sequences for homology comparisons and bioprospecting. For example, the authors were able to quickly extract and analyze 106 heterocyclization domains, showing phylogenetic clustering of the enzymes according to the identity of amino acid involved in cyclization. This cluster of technologies should lead to new opportunities in biosynthetic research. *CG*