MICROBIOLOGY

Membranes get the gold

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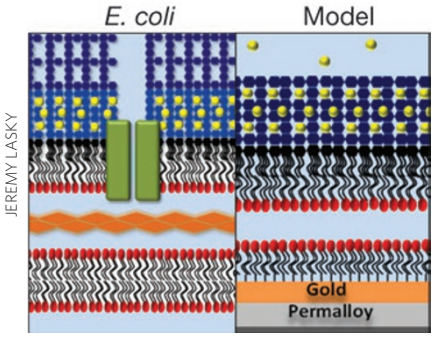

JEREMY LASKY

Gram-negative bacteria have a cellular envelope consisting of an asymmetric outer lipid bilayer (OM) and an inner bilayer (IM). The OM comprises cation-crosslinked lipopolysaccharide (LPS) molecules in its outer leaflet that create a physical barrier to penetration by antimicrobial molecules. The OM and IM also both provide an additional hydrophobic barrier. Although the chemical composition of the envelope is well defined, its physical and dynamic properties have been difficult to study because few *in vitro* models recapitulate the intact system. Clifton *et al.* have now added to this toolbox by generating an asymmetric model of the complete *E. coli* envelope. To do this, they coated a gold surface with a self-assembled monolayer, and sequentially modified this to create a floating supported bilayer (FSB) consisting of a deuterium-labeled phospholipid, DPPC, and unlabeled LPS. The authors were then able to monitor formation of the FSB and verify its asymmetry by deuterium-sensitive neutron scattering. They found that the FSB thickness and stability agreed with measurements from simpler model membranes. In addition, the response of the model to the removal of stabilizing cations was consistent with *in vivo* OM behavior. The authors were also able to use the OM model to probe the structural consequences of the antimicrobial protein (AMP) lactoferrin for the first time. Lactoferrin is thought to disrupt LPS crosslinks, and the authors saw large perturbations in the asymmetry and LPS thickness when it was added to the model membrane. Similar experiments with another AMP, lysozyme, demonstrated its ability to bind electrostatically to the anionic outer leaflet, in agreement with its previously demonstrated more limited OM-disruptive effects. These results suggest that the OM model recapitulates the intact OM system and enables studies of OM interactions with other antimicrobials. As well, the model should be amenable to techniques such as AFM and FRET to monitor further biophysical and chemical properties of the complex cellular envelope. *MB*

GLYCOBIOLOGY

Interior decorating

Glycobiology doi:10.1093/glycob/cwv078

Glycosylphosphatidylinositols (GPIs) are used in eukaryotic species to tether proteins to the plasma membrane, and in fungi, a truncated version is used to attach proteins to the cell wall. These processes require a minimal GPI construct of four or five glycans and a lipid. However, GPIs can be further functionalized with additional glycans, with the final structure varying across organisms and proteins, and the biological role of these 'decorations' is unclear. Although the enzymes responsible for the biosynthesis and transfer of the conserved GPI core are known, very few enzymes involved in the latter stages have been identified. Krüger *et al.* now search for an enzyme capable of adding a mannose group to the end of the GPI core through an α-1,3 glycosidic linkage, a known modification in *Aspergillus fumigatus*. The authors first identified three enzymes in the *A. fumigatus* genome annotated as belonging to CAZy family 69, known as α-1,3-mannosyltransferases. Deletion of one of these genes, *clpA*, followed by isolation of the glycan sequences from the GPI anchored proteins and their analysis by chromatography, mass spectrometry and enzymatic digestion, revealed the influence of *clpA*: loss of the gene led to shorter saccharide sequences sensitive to degradation by an enzyme specific for bonds internal to the minimal motif, whereas the wild-type strain and a variant of the deletion strain with *clpA* re-introduced had longer sequences that were protected from digestion. Introduction of *clpA* into *Saccharomyces cerevisiae*, which does not normally contain an α-1,3 linked sugar, led to the production of glycans protected from digestion, consistent with the introduction of the linkage. Finally, the authors confirmed that the enzyme is present in the Golgi apparatus, as expected, and requires more than one mannose group in the substrate. This discovery provides new opportunities to examine the role of these modifications in GPI functions. *CG*

PLANT TOXICOLOGY

Defusing the explosive

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ELIZABETH RYLOTT

2,4,6-Trinitrotoluene (TNT) is a known explosive and environmental pollutant that inhibits plant root growth and development. Plants can partially detoxify TNT through the upregulation of glutathione transferases U24 and U25, which mediate the denitration of TNT into 2-glutathionyl-4,6-dinitrotoluene, a metabolite more amenable to biodegradation. Although plants have the potential to manage TNT toxicity, the identity of the enzymes that mediate this process have not been characterized. Johnston *et al.* performed a genetic screen for *Arabidopsis thaliana* mutants that were capable of growing in the presence of TNT. The sequencing of one TNT resistant mutant strain revealed a deletion that introduces a stop codon in *mdhar6-1*, which encodes a member of the monodehydroascorbate reductase (MDHAR6) family. MDHARs are flavin adenine dinucleotide–dependent oxidoreductases that act as antioxidants through the regeneration of ascorbate. A majority of the MDHARs are expressed in the cytosol or peroxisome, whereas MDHAR6 was expressed specifically in the mitochondria and plastids. Electron paramagnetic resonance (EPR) spectral analysis using MDHAR6 and TNT with a 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) spin trap revealed that TNT is reduced to a nitro radical that reacts with oxygen to produce superoxide. The authors verified that wild-type plants exposed to TNT showed elevated levels of hydrogen peroxide, whereas *mdhar6-1* mutants contained reduced levels. Although it is unclear how MDHAR6 is able to mediate both pro-oxidant and antioxidant activities, these findings may inspire new strategies to engineer bacteria and other organisms to stimulate TNT degradation. *GM*

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