MOLECULAR RECOGNITION

Sphingomyelin in the groove

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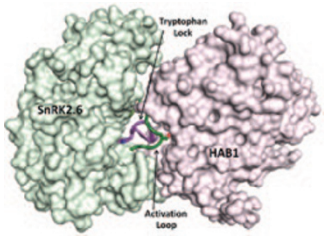
Sphingolipids are best known as lipids enriched in membrane microdomains and as signaling mediators. Contreras *et al*. now ascribe a cofactor function to the sphingolipid SM 18. Starting with the observation that Golgi-derived vesicles enrich a sphingomyelin species with a carbon chain length of 18, the authors used biochemistry and a newly established fluorescence resonance energy transfer (FRET)-based assay to show that SM 18 as well as other sphingomyelins of 18 carbons bind the transmembrane domain of the Golgi vesicle component p24. A mutational analysis identified amino acid residues in the C-terminal motif of the transmembrane domain that form a binding groove for interaction with SM 18, allowing the authors to define a nine-residue binding signature that was present in 48 candidate mammalian membrane proteins, some of which were confirmed to bind sphingolipids. Molecular dynamics simulations suggest that only sphingomyelin species within a narrow range of dynamic volumes can be accommodated within the cavity formed by these residues. The interaction between SM 18 and p24 is functionally important as it induces dimerization of p24, which the authors found to be important for Golgi vesicle trafficking. These results suggest that one source of complexity in membranes is derived from specific interactions between individual lipids and membrane proteins. *MB*

STRUCTURAL BIOLOGY

Kinase-phosphatase mashup

Science **335**, 85–88 (2012)
Proc. Natl. Acad. Sci. USA **108**, 21259–21264 (2011)



X. EDWARD ZHOU

Abscisic acid (ABA) is a hormone that regulates plant responses to environmental stressors. Formation of an ABA–ABA receptor complex inactivates type 2C protein phosphatases (PP2Cs) and leads to activation of Snf1-related kinases (SnRK2s) and engagement of ABA signaling pathways. A three-dimensional structure of a ternary complex of ABA–ABA receptor–PP2C previously allowed the identification of a 'gate-latch-lock' mechanism for binding the PP2C phosphatase in an inactive form. Now Soon *et al.* provide complementary structural insights into how PP2C phosphatases inactivate SnRK2 in the absence of ABA, and, in a related study, Ng *et al.* show how SnRK2s autoactivate when PP2C is inhibited by ABA-bound receptors. The authors solved two structures of SnRK2 and a structure of SnRK2.6 in complex with PP2C HAB1. The binding interface of the SnRK2.6 protein structurally mimics that seen in the ABA-receptor complex; SnRK2.6 presents its kinase activation loop as a gate mimic that inserts into the phosphatase active cleft, whereas HAB1 adopts a rigid geometry and inserts the lock into the kinase active site. The structural studies, along with additional biochemical experiments, established that HAB1 inactivates SnRK2.6 in two ways: by dephosphorylation of a critical serine in its activation loop and by disruption of its kinase domain at the binding interface. In addition to providing insight into ABA signaling, the authors hypothesized that inhibitory kinase-phosphatase interactions may serve as a general mechanism for regulation of other signaling pathways. *TLS*

ANTIMICROBIALS

Channel closure

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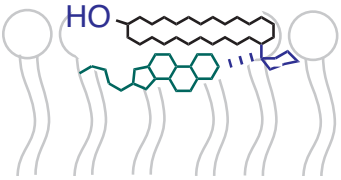

Amphotericin B is an antifungal natural product that forms channels in cell membranes, a function long assumed to be intimately linked to its ability to kill microbes. A recent study of a deglycosylated amphotericin B that was unable to form a direct interaction with ergosterol in the membrane also defined this interaction as having a role in amphotericin B's activity, but the relative importance of these two functions was unknown. To disentangle these effects, Gray *et al.* designed a new analog of amphotericin B, lacking a hydroxyl group critical for channel formation, that could be compared with the deglycosylated analog. To prepare the new compound, the authors used iterative boronic acid coupling mediated by an *N*-methyliminodiacetic acid ligand to prepare and combine three building blocks; macrocyclization and deprotection of this carbon skeleton led to the final dehydroxy compound. To validate the function of the new compound, the authors tested its ability to bind ergosterol and form channels: as expected, the natural product and the new dehydroxylated compound were able to bind membrane-embedded ergosterol, as monitored by ITC, but the deglycosylated analog was not. Lipid permeability tests showed that only the natural amphotericin B, and not the two synthetic analogs, was able to form channels. Surprisingly, however, antifungal studies showed that the dehydroxylated compound was comparable in activity against *Saccharomyces cerevisiae* and *Candida albicans* to the natural product, whereas the deglycosylated analog showed no activity. These results suggest that future drug discovery efforts should aim toward ergosterol sequestration. *CG*

VIROLOGY

Cholesterol pass for HCV

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The hepatitis C virus (HCV) hijacks human hepatocytes, causing serious liver disease. The HCV membrane consists of viral glycoproteins, cellular lipoproteins and cholesterol, with the latter being necessary for the infection process. Now, Sainz Jr. *et al.* provide evidence that HCV enters the host cells through the cell-surface Niemann-Pick C1-like 1 (NPC1L1) cholesterol absorption receptor. The infection capacity of the HCV particles declined in host cells with lower amounts of NPC1L1 compared to wild-type cells. Likewise, the authors observed a decrease in viral infection after treating the cells either with antibodies targeting the extracellular surface of NPC1L1 receptor—and specifically the large extracellular loop LEL1—or with the cholesterol-lowering drug ezetimibe, which inhibits the internalization of NPC1L1. Using a fluorescence-based method to monitor the viral entry process, they observed that NPC1L1 receptor activity is necessary for infection before viral cell membrane fusion. Also, viruses engineered to contain less or more cholesterol presented lower or higher dependence on the NPC1L1 receptor, respectively. Ezetimibe treatment of a mouse model of HCV infection significantly delayed the viral infection, providing *in vivo* validation of the role of the cholesterol receptor in HCV infection. Further elucidation of the recognition mechanism of HCV by the NPC1L1 receptor can provide the basis for the development of effective antivirals. *AC*