PLANT HORMONES

Phyto-antiperspirants

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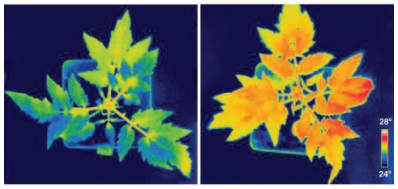

Changes in global water supply present agricultural challenges that have prompted research to enhance plant drought tolerance. Abscisic acid (ABA), a plant hormone produced under drought conditions, regulates aperture closure in stomatal guard cells, thereby reducing plant transpiration. This process is initiated when ABA binds the PYRABACTIN RESISTANCE 1 (PYR1) ABA receptor, which organizes PYR1 to bind type 2C protein phosphatases (PP2Cs); sequestration of inhibitory PP2Cs activates SNF1-related protein kinases, which are positive regulators of stomatal closure and other ABA-mediated pathways. Equipped with this biochemical insight, Park *et al.* hypothesized that engineering of an orthogonal PYR1-ligand interaction could be used to control plant water usage with synthetic small molecules. Starting with a library of PYR1 mutants, the authors used a yeast two-hybrid screen of agrochemicals to identify PYR1 mutant-compound pairs that enhanced PP2C-PYR1 binding and reduced PP2C activity. Optimization of the initial hits by further mutagenesis and selection resulted in the identification of a hextuple mutant of PYR1 ($PYR1^{M}$) that was selectively activated by nanomolar concentrations of the fungicide mandipropamid (MDP) in yeast, tobacco, tomato and *Arabidopsis thaliana*. X-ray crystallography of a PYR1 mutant–PP2C–MDP complex revealed how PYR1 was remodeled to generate a pocket with high affinity and specificity for MDP. RNA-seq analysis demonstrated that transgenic $PYR1^{M}$ *A. thaliana* plants treated with MDP exhibited transcriptional changes similar to those of ABA-treated wild-type plants. Similarly, the $PYR1^{M}$-MDP system induced physiological responses, such as inhibition of germination and root growth, and enhanced drought survival in transgenic *Arabidopsis*. Leaf thermography analysis in transgenic tomato and *Arabidopsis* plants revealed that MDP treatment induced stomatal closure, establishing that orthogonal chemical regulation of ABA signaling may offer a method for regulating water usage by plants. More broadly, the study suggests that plant hormone receptor engineering may be a general strategy to manipulate diverse plant signaling pathways with synthetic molecules. *TLS*

VIRAL MECHANISMS

Tat modulates DAT

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HIV-associated neurocognitive disorders (HANDs) are a set of neurological disorders including HIV encephalopathy and HIV dementia, which are associated with HIV-1 infection, and specifically with the HIV Tat protein, which is present in the brain of HIV-1–infected patients. Tat is thought to inhibit neuronal communication by acting directly on neurotransmitter transporters such as the human dopamine transporter, hDAT. hDAT transports dopamine from the presynaptic cleft into presynaptic neurons during neurotransmission. Curiously, cocaine blocks this clearance of dopamine by acting directly on hDAT, so HANDs are further exacerbated in HIV-1–positive patients who abuse cocaine. To probe the mechanism of Tat action on hDAT, Yuan *et al.* used a hDAT homology model to dock Tat onto the transporter and performed MD simulations to probe the conformational state of hDAT bound to Tat. They found that of the three known conformational states of hDAT, Tat binds only the outward-open structure with favorable binding energies. The authors predicted that Tat binding would block the entry pathway of the dopamine substrate, thereby inhibiting dopamine clearance from the presynaptic cleft. As well, the authors were able to identify key intramolecular interactions between Tat and key hDAT residues, including a cation-π interaction involving Y470 of hDAT and two hydrogen-bonding interactions involving hDAT residues Y88 and K92. These interactions persisted throughout the MD simulations. To obtain experimental evidence for the mode of binding predicted by the computational experiments, the authors monitored the effect of Tat on dopamine uptake by hDAT bearing mutations of the key residues they defined. This mutational analysis verified the role of the aromatic ring of hDAT Y470 and therefore the existence of a cation-π interaction with Tat and the role of hydrogen bonding at Y88 and K92. These results suggest a mechanism for how infection by HIV-1 leads to HANDs. *MB*

BIOSYNTHESIS

The X factor

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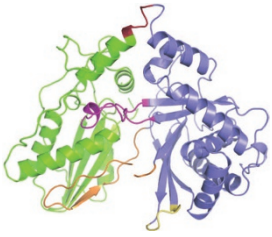
NATURE



Glycopeptide antibiotics such as vancomycin and teicoplanin are produced by nonribosomal peptide synthetases (NRPSs), with the initial peptide backbones further cross-linked by cytochrome P450 enzymes to create the three-dimensional shape needed to bind the antibiotics' cellular target, lipid II. Prior work demonstrated that the compounds remain on the NRPS while the modifications are introduced, but the mechanism by which the P450s are recruited to the NRPS was unclear. Haslinger *et al.* took interest in an uncharacterized 'X' domain that is near the end of all glycopeptide antibiotic NRPSs and phylogenetically related to condensation (C) domains yet missing residues known to be crucial for C-domain activity. The authors' crystal structure confirmed a typical C-domain structure and provided more support that the domain has an alternate function, as the side chains of two mutated residues block the canonical cofactor binding site. The authors thus tested for protein-protein interactions between the teicoplanin OxyB and truncated fragments of the NRPS. Gel filtration and mobility shift assays demonstrated a strong (low μM) interaction dependent only on the X domain for both the teicoplanin and vancomycin pathways. A crystal structure of the complex identified the interaction site and confirmed that both the P450's reductase partner and the antibiotic carrier protein would be able to access the appropriate sites on the P450 for catalysis. The authors further tested whether the other P450s might be recruited by the same mechanism: OxyA and OxyC, which act after OxyB, did bind the X domain, though with lower affinities, suggesting that interactions with the appropriate substrate could be driving sequential activity. Finally, *in vitro* assays confirmed that the activity of OxyBs from four different clusters was substantially increased by the X domain. These findings improve our understanding of NRPS machines and offer new opportunities to exploit these oxygenases for extended *in vitro* cyclization towards antibiotic discovery. *CG*