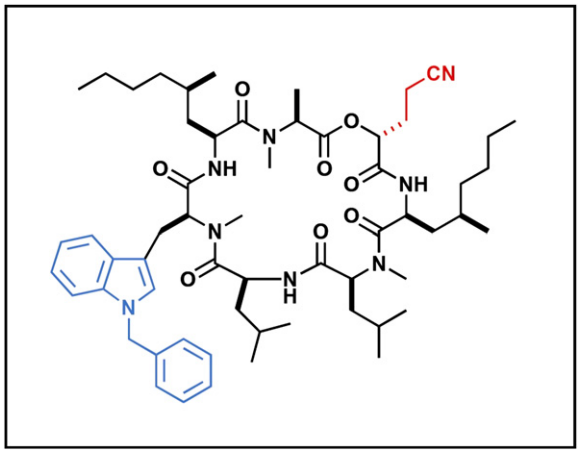
In This Issue

Opening PKSs' Black Box

PAGE 1075

Polyketide synthases (PKSs) are biosynthetic assembly lines for production of powerful natural products with diverse biological activities. To understand PKSs' key steps of acyl-CoA extender unit selection, Bonnett et al. analyze a PikAIV PKS model system using fluorescent-probe assay for steady state kinetic analysis and Fourier transform ion cyclotron resonance-mass spectrometry (FTICR-MS) to monitor active site occupancy. Findings from different enzyme variants and model substrates suggest a mechanism based on acyl-CoA substrate loading followed by differential rates of hydrolysis, which might help fine tune future pathway engineering efforts.



Modulating Sec61

PAGE 1082

Cotransins are cyclic heptadepsipeptides that bind the Sec61 translocon and inhibit cotranslational translocation of a subset of secreted and transmembrane proteins. Now Maifeld et al. show that subtle structural differences among cotransins can profoundly affect the extent to which different secretory proteins are inhibited. Secretory protein profiling revealed tumor necrosis factor alpha (TNFα), a major drug target for inflammatory diseases, as the most cotransin-sensitive protein discovered to date.

Getting Hooks into a Hookworm

PAGE 1089

Macrophage migration inhibitory factor (MIF) is a cytokine-like molecule that is involved in proinflammatory responses and cell homeostasis. MIF is also expressed by parasitic organisms. Cho et al. previously proposed that hookworm MIF (AceMIF) functions to evade the immune response. In this paper, the authors report the identification of small molecule hookworm MIF inhibitors. A promising inhibitor turned out to be a well-known diuretic, furosemide. Through structure-activity relationship studies, a nondiuretic furosemide analog was found and cocrystallized with AceMIF, providing a molecular probe for further investigation of the host-parasite relationship.

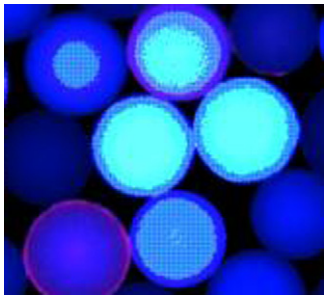
Genome-Mining Expedition towards Dihydrophenylalanine

PAGE 1102

As microbial genome sequence information is generated at an exponential rate, we continue to observe a growing biosynthetic potential for the construction of structurally diverse small molecules, some of which may regulate complex symbiotic interactions or have therapeutic applications. Genome-mining using recently discovered prephenate decarboxylase enzyme sequences has led Crawford et al. to the discovery of a previously orphaned eight gene cluster in the genomes of the plant pathogen *E. amylovora* and the insect pathogen *P. luminescens.* Genetic and biochemical work presented herein has connected this cluster to the synthesis of dihydrophenylalanine and onward to a dihydrostilbene derivative.

Peptoid Holds a Promise for Huntington's Disease

PAGE 1113



Huntington's disease (HD) is a neurodegenerative disorder caused by a polyglutamine expansion within Huntingtin (Htt) protein. In the unbiased screen of peptoid library, Chen et al. identified a HQP09 peptoid that specifically binds to mHtt. The authors demonstrate that HQP09 peptoid inhibited aggregation of mHtt and exerted calcium-stabilizing and neuroprotective effects in primary striatal neuronal cultures derived from HD mice. They further establish that intracerebroventricular delivery of HQP09 to an HD mouse model resulted in reduced accumulation of mHtt brain aggregates and improved motor perfromance. These results suggest that HQP09 peptoid hold promise for developing treatments for HD and other polyglutamine expansion disorders.

Switching Single G-protein Subunits

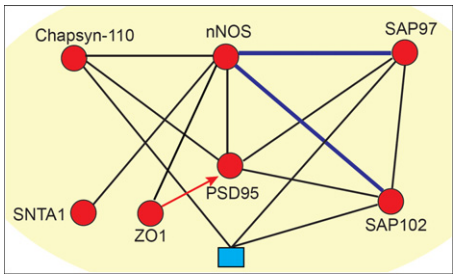
PAGE 1126

Trimeric G-proteins are crucial switching platforms for receptor-mediated signal transduction networks. Here, Putyrski et al. introduce an artificial switch technique based on the use of a chemical dimerizer to independently activate single G-protein subunits. This permits downstream response patterns such as calcium oscillations to be assigned to the activity of a single G-protein subunit. This method will largely facilitate the analysis of G-protein-coupled receptor signaling in the future.

From Qs to As

PAGE 1134

Coenzyme Q (Q) is a redox-active lipid essential for respiration in eukaryotic cells. Q biosynthesis requires at least eleven proteins in *S. cerevisiae* but the precise function of several of them is not known. Ozeir et al. establish that the predicted monooxygenase Coq6 is involved in the C5-hydroxylation reaction. The authors demonstrate that synthetic analogs of 4-hydroxybenzoic acid (4-HB) such as 3,4-dihydroxybenzoic acid and vanillic acid bypass Coq6 deficiency in *S. cerevisiae*. This work suggests that hydrophilic analogs of 4-HB may restore Q biosynthesis in patients with some primary Q deficiencies by bypassing the altered biosynthetic step.



Systems View of PDZ-PDZ Interactions

PAGE 1143

PDZ domains are best known for mediating protein-protein interactions by binding the C termini of their target proteins. They have also been reported, however, to dimerize with other PDZ domains. To further characterize this alternative binding mode, Chang et al. undertook an unbiased, proteome-wide investigation of mammalian PDZ-PDZ interactions using protein microarrays, fluorescence polarization, and coaffinity purification. In total, the authors identified 37 PDZ-PDZ interactions involving ~30% of all PDZ domains tested, indicating that PDZ-PDZ dimerization occurs at a higher frequency than previously appreciated. This suggests that many PDZ domains evolved to form multiprotein complexes by simultaneously interacting with more than one ligand.

Flexing the Reprogramming Muscle

PAGE 1153

Muscle regeneration is profoundly impaired upon aging and in muscular dystrophies. One promising approach is to reprogram differentiated muscle to their progenitors that can be utilized for cell therapy. Previous attempts failed to unequivocally show that multinucleated muscle cells can undergo dedifferentiation and contribute to muscle regeneration. Here, Paliwal and Conboy irreversibly labeled differentiated skeletal muscle cells and reprogrammed them to muscle progenitors for in vivo muscle repair. This was accomplished using small molecules without gene overexpression, which is safe for therapies. This presents a novel paradigm of cell fate reversal and creates novel clinical approaches for improved muscle regeneration.

Activators of Cylindrical Proteases as Antimicrobials

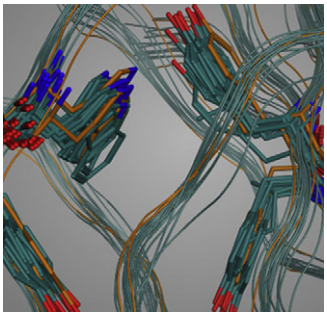
PAGE 1167

ClpP is a cylindrical protease whose ability to degrade proteins is regulated by ATP-dependent unfoldase chaperones. Leung et al. used ClpP as a target in a high-throughput screen for compounds which activate the protease and allow it to degrade larger proteins. The screen resulted in five structurally distinct compounds designated as activators of self-compartmentalizing proteases 1 to 5 (ACP1 to 5). The ACP1 chemical structure was considered to have drug-like characteristics and was further optimized to give analogs with bactericidal activity. Hence, the ACPs represent classes of compounds that can activate ClpP and that can be developed as potential novel antibiotics.

No More IgE Clustering on Mast Cells

PAGE 1179

Heterobivalent ligands (HBLs) were engineered by Handlogten et al. to inhibit the binding of allergens to mast cell-bound IgE antibodies, thus inhibiting IgE clustering, which leads to mast cell degranulation. The HBLs simultaneously bound to the antigen binding site and proximal "unconventional nucleotide binding site" on IgE Fab domain. Simultaneous bivalent binding provides HBLs with greater than 100-fold enhancement both in avidity for IgE^{DNP} and in inhibition of allergen binding to IgE^{DNP} compared to the monovalent allergen. Furthermore, in cellular assays HBLs were able to inhibit receptor signaling and thereby inhibit degranulation of mast cells, while monovalent allergen was not.



Peptide Nucleic Acids Block the *CCR5*

PAGE 1189

CCR5 is a chemokine receptor required for HIV-1 entry into human cells, making it an attractive target for gene therapy. Optimized triplex-forming peptide nucleic acids (PNAs) were developed by Schleifman et al. to stimulate recombination at the *CCR5* locus, with donor DNAs designed to introduce inactivating stop codons. Site-specific disruption of the *CCR5* gene was achieved in 2.46% of human cells in a single treatment, with extremely low off-target effects compared to alternative approaches based on zinc-finger nucleases. The targeted *CCR5* modification induced by the PNAs rendered cells resistant to HIV-1 infection, providing proof-of-principle for possible therapeutic application.

