In This Issue

Myxobacterial Secondary Metabolism Mixology

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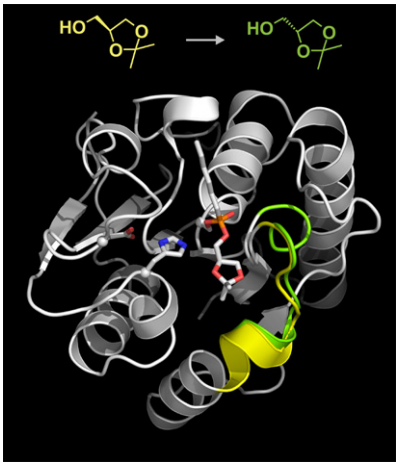
Many myxobacterial species engage in "diversity oriented biosynthesis"—assembling sometimes very large families of related and often bioactive natural products using a single biosynthetic pathway. A study by Meiser et al. on the biosynthesis of the yellow pigments DKxanthenes in the myxobacteria revealed several mechanisms underlying this molecular promiscuity: the variable iteration of one or more modules of enzymatic activities within the multienzyme polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS) assembly line and the presence of an inherently broad-specificity acyl transferase domain. These insights are relevant to attempts to expand the diversity of natural products by genetic engineering.

(R)- or (S)- : Take a Guess



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Bacillus subtilis lipase A (LipA) has excellent properties with regard to its kinetics for application in industrial biocatalysis of enantiopure β-adrenergic receptor antagonists and in particular of their chiral building block 1,2-O-isopropylidene-*sn*-glycerol (IPG). Nevertheless, its enantioselectivity towards these substrates is modest and, moreover, directed towards the unwanted enantiomer of IPG. Drastic changes in amino acid sequence and active-site architecture can lead to altered enzyme function. To this purpose, Boersma et al. exchanged a loop lining the active site cleft of LipA with loops from homologous enzymes. The resulting loop-grafted hybrids showed an inverted and improved enantioselectivity towards IPG. (Figure credits: Boersma et al.)

Deazapurine Biosynthesis Pathway Exposé

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Deazapurine-containing compounds are utilized in biological niches as diverse as the hypermodified tRNA base queuosine, which is found in nearly all organisms, to secondary metabolites that are produced by various strains of *Streptomyces*. Deazapurine-containing secondary metabolites and their chemically modified derivatives are of importance clinically, as they have long been known to partition into nucleic acid pools. McCarty and Bandarian now describe isolation and characterization of a cluster of genes in *Streptomyces rimosus,* which is involved in the biosynthesis of two deazapurine nucleoside antibiotics, toyocamycin and sangivamycin. This cluster serves as a paradigm for biosynthesis of deazapurine containing-molecules.

STZ is a General Cytotoxic Compound

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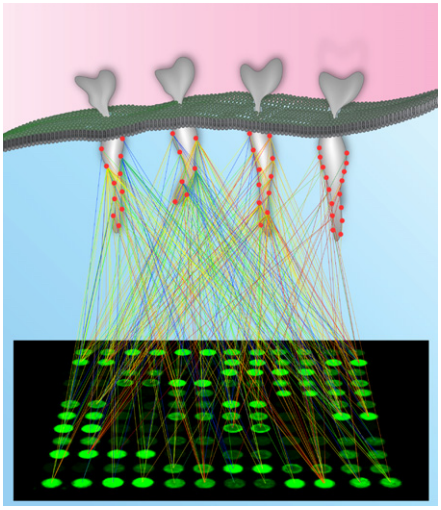
Streptozotocin (STZ) is widely used to generate mouse models of diabetes or to treat pancreatic tumors. It has been proposed that STZ toxicity is caused by its ability to inhibit *O*-GlcNAcse, thereby raising levels of the intracellular *O*-GlcNAc modification to lethal levels. Pathak et al. further study the mode of action of this drug, by synthesizing a *galacto*-configured isomer of STZ. Using X-ray crystallography, enzymology, and cell biological studies on an insulinoma cell line, they show that while streptozotocin competitively inhibits *O*-GlcNAcase and induces apoptosis, its *galacto*-configured derivative no longer inhibits *O*-GlcNAcase, yet still induces apoptosis. This strengthens the hypothesis that STZ is not a specific inhibitor of *O*-GlcNAcase; rather, it is a general cytotoxic compound.



Casting a System-Wide Net on ErbB4

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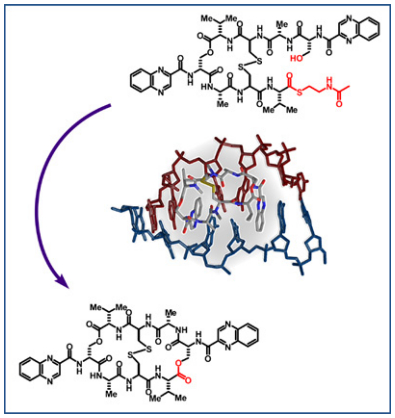
Although the first three members of the ErbB family of receptor tyrosine kinases have been well studied, much less is known about ErbB4. Kaushansky et al. used tandem mass spectrometry to identify sites of tyrosine phosphorylation on ErbB4 and protein microarrays to quantify interactions between these sites and virtually every human SH2 and PTB domain. Their approach highlighted several new interactions and led to the finding that ErbB4 can recruit and activate STAT1. At a broader level, they found that ErbB4 is much more selective than the other ErbB receptors, providing a possible explanation for the protective properties of ErbB4 in cancer. (Figure adopted from file provided by Kaushansky et al.)

DNA-Assisted Enzymatic Macrolactonization



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Although enzymatic cyclization is advantageous as it allows site-selective ring closure of functionalized precursors, its synthetic use is often precluded due to hydrolysis and product inhibition, especially when used with nonnatural substrates. To circumvent these obstacles, Koketsu et al. focused on the DNA-binding properties of triostin A analogs acting as synthetic precursors and devised an interesting synthetic strategy: enzymatic synthesis employing DNA to capture the cyclic product, thereby excluding it from the active site of the enzyme. This coincubation with DNA successfully suppressed undesired hydrolysis and prevented product inhibition and resulted in a 3-fold improvement in the yield of cyclized products. (Figure credits: Koketsu et al.)

PIPs: Fruitful Strategy for Gene Expression Regulation

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Pyrrole-imidazole polyamides (PIPs) are nuclease-resistant compounds that bind to the minor groove of DNA and inhibit gene expression. In this study, Takahashi et al. design and synthesize two PIPs (PIP-A and PIP-B) that specifically target Aurora kinase A and B (AURKA and B) promoter regions, respectively. PIP-A and -B inhibited the promoter activities of AURKA and B, leading to a decrease in mRNA expressions and protein levels. Additionally, simultaneous use of developed PIPs revealed potent antiproliferative synergy in tumor cell lines, inducing apoptosis-mediated severe catastrophe of cell-cycle progression. These results suggest that PIP-A and -B may exhibit selective toxicity towards proliferating tumor cells.

Completing Calicheamicin Biosynthetic Puzzle

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A study by Zhang et al. now completes the functional assignment of all four calicheamicin (CLM) glycosyltransferases (GTs), extends the concept of reversibility of enediyne GT-catalyzed reactions, and provides missing structural information on an enediyne GT, thus extending the common GT-B structural fold to include enediyne GTs. Given the notable architectural distinctions of enediynes, this work adds to the structural blueprints for engineering and/or evolving novel glycosylation catalysts. From a biosynthetic perspective, this work completes the functional annotation of the four calicheamicin GTs and presents conclusive evidence of a sequential glycosylation pathway in CLM biosynthesis.

Running LAPS in MGL Modulation

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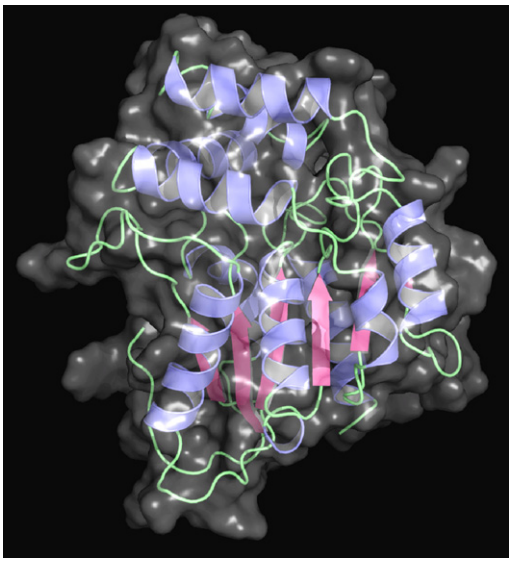

The enzyme monoacylglyerol lipase (MGL) is a component of the endocannabinoid signaling system responsible for deactivation of the lipid mediator 2-arachionoylglycerol (2-AG). Due to the neuroprotective property of 2-AG, selective MGL inhibitors hold promise as treatments for progressive neurodegenerative diseases, amyotrophic lateral sclerosis, multiple sclerosis, and HIV-associated dementia. However, lack of molecular-level understanding of how MGL operates to inactivate 2-AG has hampered the design of selective MGL inhibitors. By exploiting the advantages of covalent probes and site-directed mutagenesis in an experimental paradigm Zvonok et al. termed "ligand-assisted protein structure" (LAPS), the authors successfully identify amino acids critical for human MGL function and pharmacological modulation, opening the door to future design of selective MGL inhibitors. (Figure credits: Zvonok et al.)

N-Glycosyltransferase of Ansamitocins

PAGE 863

Glycosylations are nucleophilic replacement reactions between C-1 of nucleotide-activated sugars and aglycones carrying hydroxyl substituents or, less frequently, amines or nucleophilic carbons. In this issue, Zhao et al. identify *asm25* of *Actinosynnema pretiosum* as the *N*-glycosyltransferase gene responsible for the macrolactam amide *N*-glycosylation of ansamitocins to form ansamitocinosides. The team obtained a recombinant antibiotic *N*-glycosyltransferase in soluble, enzymatically active form by expression of *asm25* in *E. coli* and solubilization of the protein from inclusion bodies. This allowed in vitro characterization of the enzyme and delineation of its catalytic mechanism.

