In This Issue

Enzymatic Formation of a Disulfide Bond in Natural Products

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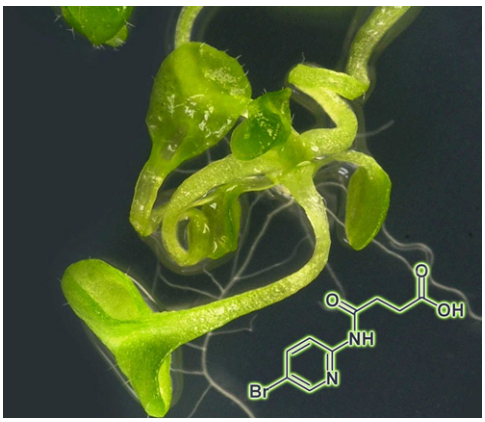
The biochemistry of disulfide bond formation in proteogenic products has been well studied. On the other hand, disulfide bonds are rarely present in nonribosomally produced natural products, although they can be found in some important anticancer agents. The enzymology of disulfide bond formation in those products is largely unknown. Wang et al. now address the mechanism of a disulfide bond formation in FK228, an epigenetically acting anticancer natural product made nonribosomally by a hybrid nonribosomal peptide synthetase/polyketide synthase pathway in *Chromobacterium violaceum* No. 968. Several lines of evidence suggest that DepH, identified in the gene cluster responsible for FK228 production, is an FAD-dependent pyridine nucleotide disulfide oxidoreductase, specifically and efficiently catalyzing a disulfide bond formation in FK228.

Bikinin Inhibits Plant GSK3-like Kinases, Brassinosteroids Respond!



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Steroid-derivative brassinosteroids are plant hormones that are involved in the regulation of plant growth and development. By using a chemical biology approach, De Rybel et al. identified bikinin, a nonsteroidal synthetic molecule that induces brassinosteroid responses by specifically inhibiting seven of the nine GSK3s in plants. The authors demonstrate that inhibition of GSK3s is the sole activation mode of brassinosteroid signaling and could indicate new GSK3s as potential regulators of the signaling cascade. Further insight into the identity of amino acids crucial for GSK3-bikinin interaction, reported here, provides a better understanding of GSK3 inhibition mechanisms and could potentially aid in the development of specific GSK3 inhibitors. (Figure credit: De Rybel et al.)

ClpXP Well-Oiled Translocation Machinery

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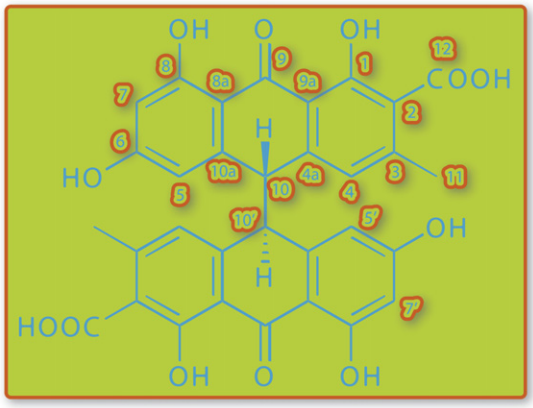
The AAA+ ClpXP protease is composed of ClpX, an ATP-dependent chaperone organized in a hexameric ring, and ClpP, a peptidase organized in a double heptameric ring, contributing 14 active sites. The protein substrates destined for degradation bind to ATPase, which leads to their unfolding and translocation to the protease chamber. Barkow et al. use synthetic peptides to investigate polypeptide chain features important for translocation and demonstrate that ClpXP machinery tolerates a diverse set of substrates with both natural and nonnatural amino acids, suggesting that ClpX hexamer must be highly adaptable but, at the same time, able to bind the substrate in a manner that allows coupling between ATP hydrolysis and peptide chain translocation.

β-Lactamase-type Thioesterase Discretion

PAGE 613

Fungal polyketides, most of which are synthesized by iterative type I polyketide synthases (PKSs), possess structural diversity and a variety of biological activities. Awakawa et al. now show that a thioesterase (TE)-less type I PKS, atrochrysone carboxylic acid synthase (ACAS), in *Aspergillus terreus,* produces atrochrysone carboxylic acid as a direct product, in collaboration with a discrete thioesterase, atrochrysone carboxyl ACP thioesterase (ACTE). ACTE, showing no similarity to the general TE domain of nonreducing PKSs, belongs to the β-lactamase superfamily, which is an exciting finding given that a β-lactamase-type thioesterase that releases the product from a PKS in polyketide synthesis has not been previously described.



Albumin and Hsp70: Driving Cytosolic Anandamide

PAGE 624

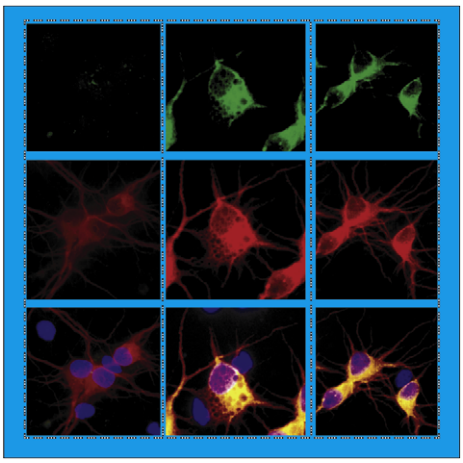
The endocannabinoid anandamide is a lipid messenger endowed with a wide variety of biological actions, both centrally and peripherally. A hot spot in the regulation of anandamide signaling is the identity of the machinery responsible for cellular uptake and intracellular trafficking of this lipophilic compound. The present study by Oddi et al. provides evidence for the existence of a constitutive chaperon system, composed of albumin and heat shock protein 70 (Hsp70), that can provide the rapid and efficient shuttling of anandamide within the cell.

Kallikrein-Related Peptidase Inhibitor – Sunflower Power

PAGE 633

The trypsin-like serine protease kallikrein-related peptidase 4 (KLK4/prostase) is a potential target for prostate cancer treatment as a consequence of its ability to activate many tumorigenic and metastatic pathways, including the protease activated receptors (PARs). Here, Swedberg et al. reengineer the naturally occurring sunflower trypsin inhibitor (SFTI) to selectively block the proteolytic activity of KLK4, thus preparing a small-molecule inhibitor able to selectively block KLK4 and via KLK4 inactivation of PAR-2 signaling. The applied approach combines molecular modeling and sparse matrix substrate screening to produce a SFTI variant with high potency and a highly restricted range of inhibitory activity towards KLK4.

Antidepressant with Potent Neurotrophic Activity



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Neurotrophins, the cognate ligands for the Trk receptors, are homodimers that exert the physiological functions through provoking neurotrophin receptors (TrkA, B, and C) homodimerization. Here, Jang et al. show that a tricyclic antidepressant drug, amitriptyline, provokes both TrkA and TrkB homo- and heterodimerization and activation. Amitriptyline acts as an agonist for both TrkA and TrkB. Strikingly, this small molecule (but not any other antidepressant drug) selectively stimulates endogenous TrkA/TrkB heterodimer formation in mouse brain. This finding provides a molecular mechanism for dimerization and activation of transmembrane receptor tyrosine kinase (RTK), establishing a proof-of-concept model for identifying small molecular agonists and antagonists for RTK.

Inhibitor of the Trio/RhoG/Rac1 Signaling Pathway

PAGE 657

Exchange factors of the Dbl family (RhoGEFs) are upstream regulators of Rho GTPases and, as such, they control cell adhesion and migration pathways. Bouquier et al. identify a small molecule they name Inhibitor of Trio eXchange 3 (ITX3) as a nontoxic inhibitor of the N-terminal RhoGEF domain of the Trio protein, a key mediator in nerve and muscle cell differentiation. ITX3 specifically inhibited the cellular effects mediated by endogenous Trio activity in various cell types, while having no effect on the activity of other GEFs of the Dbl family. The study validates the Dbl family as druggable targets and opens new possibilities for the development of anti-invasive inhibitors.

Fatty Acid Synthesis Initiation

PAGE 667

Metazoans synthesize palmitate in the cytosol using an ensemble of covalently linked enzymes but synthesize octanoyl moieties, the lipoyl precursor, in mitochondria using a suite of freestanding enzymes. The acyltransferases responsible for initiation of fatty acid biosynthesis in the two compartments are distinguished by their different substrate specificities: the cytosolic enzyme transfers both the acetyl primer and the malonyl chain extender, whereas the mitochondrial counterpart is responsible for translocation of only the malonyl substrate. Bunkoczi et al. combine X-ray crystallography with in silico substrate docking and mutagenesis experiments to reveal that although the two enzymes adopt a similar fold, subtle differences at their catalytic centers account for their different specificities.

Chimera for Hybrid Glycopeptides Production

PAGE 676

A promising approach to new drug leads is to engineer the biosynthesis of natural products to produce novel compounds. Truman et al. report the successful construction of highly active chimeric glycopeptide glycosyltransferases (GTs). Glycopeptides are clinically indispensable antibiotics active towards multi-drug-resistant bacteria, and the sugars that decorate them are important determinants of bioactivity. The authors show that the sugar donor specificity of glycosyltransferases can be controlled by fully swapping substrate binding domains. Since the vast majority of glycosyltransferases involved in natural product biosynthesis belong to the same structural superfamily, this may be a general strategy for directing the biosynthesis of clinically important natural products.

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