In This Issue

Cannabinoid Receptor Imaging in the Brain

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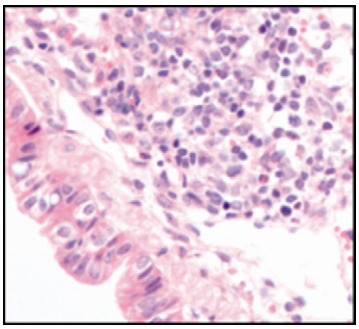
Cannabinoid CB_2 receptors are expressed by immune cells and almost absent in healthy brain but under pathological conditions, their expression in the brain increases. This specific increase in CB_2 expression makes this protein an ideal target for drugs aimed at regulating neuropathologies, including chronic brain inflammations and tumors. Here, Sexton et al. developed a novel molecular imaging agent, NIR-mbc94, which selectively binds to CB_2 receptors. Upon interaction with CB_2 receptors, it emits a signal that is easily detected and thus allows for unbiased high-throughput screening of compounds interacting with this therapeutic target.



Attacking Airway Inflammation with Bisdionin F

PAGE 569

Acidic mammalian chitinase (AMCase) has been identified as a mediator of allergic inflammation and asthma. In this study, a rational approach was used by Sutherland et al. to design a selective AMCase inhibitor, bisdionin F, which was effective *in vivo* and attenuated several aspects of allergic inflammation. Unexpectedly, bisdionin F treatment led to a striking enhancement of inflammatory neutrophils in allergic animals, revealing new functions for AMCase and also raising questions about the therapeutic potential of chitinase inhibition. However, this inhibitor class could potentially be developed to be active against chitinase-like proteins, which have also been implicated in asthma.

Vaccine Candidate for *Clostridium difficile*

PAGE 580

Nosocomial infections with the Gram-positive pathogen *Clostridium difficile* pose a major risk for hospitalized patients and result in significant costs to health care systems. In this study, a hexasaccharide-hapten of the *Clostridium difficile* PS-II cell surface polysaccharide was synthesized and microarrays were manufactured, which were used by Oberli et al. to identify antibodies recognizing the synthetic structure in patient stool samples. Mice immunized with the corresponding neoglycoconjugate produced antibodies, thus indicating that the PS-II glycopolymer is recognized by the immune system upon infection and constitutes a viable vaccine candidate against *C. difficile*.

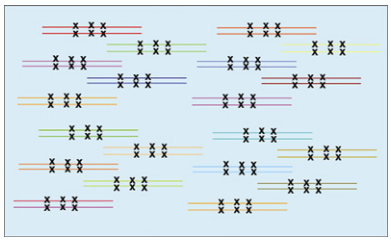
Deciphering Antibiotic Inhibition

PAGE 589

The ribosome is a major target in the bacterial cell for antibiotics. Therefore, insight into how antibiotics inhibit ribosome function can provide not only information regarding the fundamental process of translation but is also important for the development of new antimicrobial agents to overcome the rise in multidrug-resistant bacteria. Among the current clinically used antibiotics, the thiopeptide and orthosomycin antibiotics have unique binding sites on the large ribosomal subunit. Here, Mikolajka et al. show that these thiopeptide and orthosomycin antibiotics exhibit differential effects on translational GTPases, thereby providing mechanistic insights into the antibiotic inhibition of distinct steps during protein synthesis.

Diversifying the Antibiotic Biosynthesis Repertoire

PAGE 601



In this study, Evans et al. reengineer an antibiotic assembly line to produce new derivatives of the antibacterial agent andrimid. Previous antibiotic synthetase reengineering studies have primarily relied on domain swapping strategies, in which nearly intact enzymes are swapped between pathways to yield a change in the final product. This study uses directed evolution in order to effect a change in the final product, with the advantage that multiple "unnatural" natural products can be generated at once. This advance in diversifying antibiotic biosynthetic pathways was accomplished by Evans et al. with the development of high-throughput LC-MS/MS for screening mutant libraries.

New Tools to Target the Proteasome

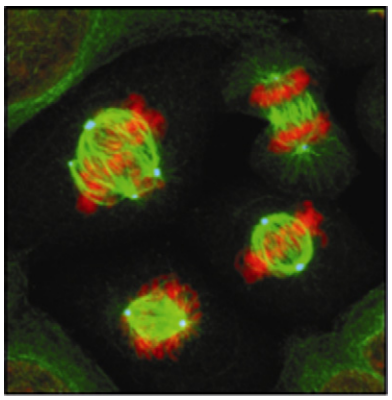
PAGE 608

Proteasome inhibitors have proven to be valuable tools to study proteasome biology and one particular compound, bortezomib, is used for the treatment of multiple myeloma. The majority of proteasome inhibitors primarily target chymotrypsin-like sites of these proteolytic complexes. Although highly specific cell-permeable inhibitors of caspase-like sites became available recently, the synthesis of cell-permeable inhibitors of trypsin-like sites has proven technically challenging. Here, the Kisselev and Overkleeft laboratories describe the development and characterization of such compounds as well as the development of a full palette of tools needed to manipulate individual proteasome active sites in living cells.

Second-Generation Inteins

PAGE 619

Small molecule-dependent inteins enable protein structure and function to be controlled posttranslationally in living cells. Starting with two previously evolved ligand-dependent inteins that splice in a manner that is dependent on 4-hydroxytamoxifen (4-HT), Peck et al. evolved second-generation inteins with substantially improved splicing efficiency in both yeast and mammalian cells. These newly evolved inteins typically resulted in 50%–90% of spliced protein in the presence of 4-HT and less than 5% splicing in the absence of 4-HT. These second-generation evolved inteins augment the promise of ligand-dependent protein splicing as an effective and general approach to probing protein function in mammalian cells.



UA62784: Arresting Cancer in Mitosis

PAGE 631

A recent screen for compounds that selectively targeted pancreatic cancer cells isolated UA62784. Here, Tcherniuk et al. found that UA62784 inhibits microtubule polymerization in vitro and interacts with tubulin dimers at or near the colchicine-binding site ten times more potently than colchicine, vinblastine, or nocodazole. Nanomolar doses of UA62784 promote the accumulation of mammalian cells in mitosis owing to aberrant mitotic spindles. They authors also show that effects of UA62784 and of some known microtubule-depolymerizing drugs are additive and that low doses of UA62784 and vinblastine potentiate each other to inhibit proliferation.

New Screen for Probing Oxygenases

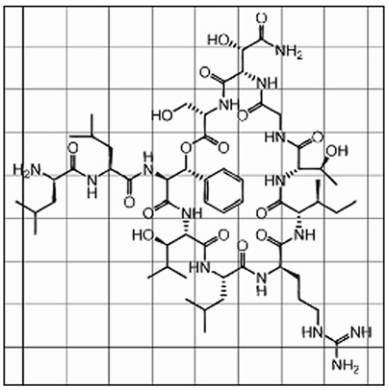
PAGE 642

2-Oxoglutarate (2-OG) dependent oxygenases catalyze hydroxylation and *N*-methyl demethylation reactions that are important in oxygen sensing and the control of gene expression. They constitute therapeutic targets for the treatment of anemia, ischemic diseases, and cancer. Here, Rotili et al. demonstrate the usefulness of a probe-based approach that employs photo cross-linking and affinity purification for the identification of 2-OG oxygenases and their interaction with inhibitors in cell extracts. The approach was validated using the therapeutically relevant oxygenases HIF-α hydroxylase and a histone demethylase. The authors also demonstrate that this probe approach has the potential to capture substrates.

Lysobactin Synthesis: Termination in Tandem

PAGE 655

Lysobactin is a macrocyclic depsipeptide that displays a very strong activity against pathogenic Gram-positive bacteria and is considered a potent agent for treatment of bacterial infections caused by resistant pathogens. In this work, Hou et al. have identified and characterized the entire lysobactin biosynthetic gene cluster, which reveals an NRPS-based assembly. The association between the synthetases and lysobactin was confirmed by investigating the adenylation domain specificities in vitro. Analysis of the unusual tandem thioesterase domain architecture revealed that the penultimate TE domain mediates the cyclization of lysobactin, whereas the final TE is suggested to govern regeneration of the assembly line.



Burkholderia's Antibiotic Treasure Trove Revealed

PAGE 665

To combat the emergence of multidrug-resistant bacteria, new antibiotics are urgently needed. Here, Mahenthiralingam et al. identified that the bacterium *Burkholderia ambifaria* produces a potent antibiotic called enacyloxin that kills a range of drug-resistant bacteria. They mapped the enacyloxin production genes and found that they encoded an unusual mixture of antibiotic-producing enzymes (polyketide synthases) that are normally associated with the *Streptomyces* group of bacteria, which produce most of our useful antibiotics. These findings suggest that *Burkholderia* bacteria are a promising resource for the discovery of new antibiotics, with unique production pathways and potent activity against drug-resistant bacteria.

