In This Issue

Protein Misfolded Oligomers

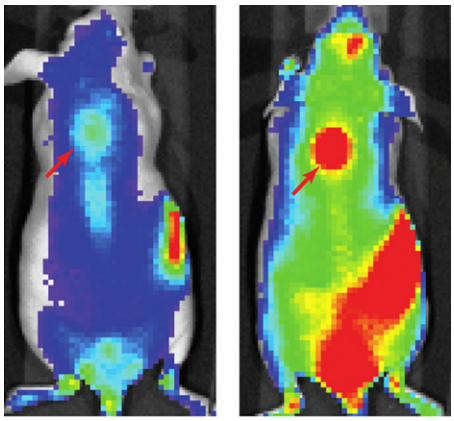
PAGE 315

Formation of protein misfolded oligomers is associated with many human diseases, including Alzheimer's disease and Parkinson's disease. Understanding the mechanisms by which such oligomers form and the relationships between their structure and toxicity is thought to be a crucial step in the search for new therapies for these devastating diseases. It is becoming increasingly clear that oligomers are best described as ensembles of many different aggregates differing in dimension and structure. However, recent efforts, reviewed here by Bemporad and Chiti, are now starting to unveil that highly dynamic, small oligomers that expose hydrophobic clusters are the most deleterious species formed along the aggregation pathway.

Proofreading by an Acyltransferase-like Enzyme

PAGE 329

Bacterial polyketide synthases of the trans-acyltransferase family (trans-AT PKSs) produce a wide range of bioactive natural products. Many trans-AT PKS gene clusters encode an AT-like component with a previously unknown role in polyketide biosynthesis. Now, Jensen et al. provide insights into the function of these enzymes by studying PedC, a homolog of the pederin pathway from an uncultivated beetle symbiont. As opposed to standard ATs that load PKSs with acyl building blocks, PedC acts as hydrolase on various acyl thioesters, suggesting a role in the removal of stalled PKS intermediates.



Executioners in Special Light

PAGE 340

Apoptosis, or programmed cell death, is a process mediated by a family of cysteine proteases termed caspases. Here, Edgington et al. describe a caspase probe with optimized in vivo properties that targets caspase-6, -3 and -7, allowing direct monitoring of all executioner caspases simultaneously. Using the probe, the authors find that caspase-6 has an unusual activation mechanism involving the production of partially cleaved complexes that can be generated in the absence of active caspase-3 and -7. These intermediates in the activation process seem to be associated with conformational changes in the dimeric complex, suggesting that caspase-6 has a unique activation mechanism compared to the other executioner caspases.

Red Fluorescent Heterodimer

PAGE 353

To facilitate the imaging of multiple fluorescent protein (FP)-based biosensors in a single cell, Alford et al. have engineered a dimerization-dependent red FP (ddRFP) that exhibits a 10-fold increase in fluorescence upon heterodimer formation. The dimerization-dependent fluorescence of ddRFP can be used for detection of a protein-protein interaction in vitro, imaging of the reversible Ca^{2+}-dependent association of calmodulin and M13 in live cells, and imaging of caspase-3 activity during apoptosis.

Tubulysin Biosynthetic Gene Cluster

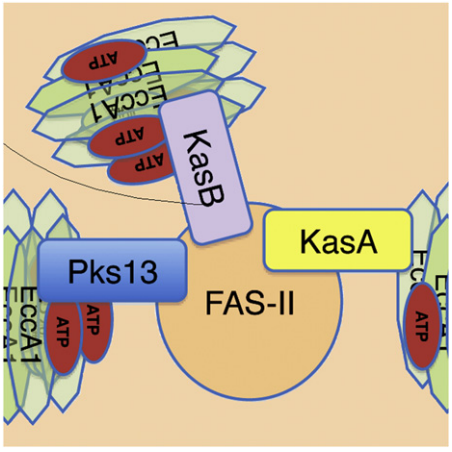
PAGE 361

The gene cluster responsible for biosynthesis of the antitumor natural product tubulysin was reconstituted by Red/ET recombineering and heterologously expressed in the host strains *Pseudomonas putida* and the myxobacterium *Myxococcus xanthus*, resulting in the production of pretubulysin A and tyrosine pretubulysin A. Chai et al. also exploit this expression system to investigate the function of several genes whose role in the biosynthesis had previously been unclear. The authors identified a candidate in the SBCb004 genome for one of the missing tubulysin oxygenases, setting the stage for the complete reconstitution of tubulysin biosynthesis.

Secret Connections behind Mycobacterial Pathogenesis

PAGE 372

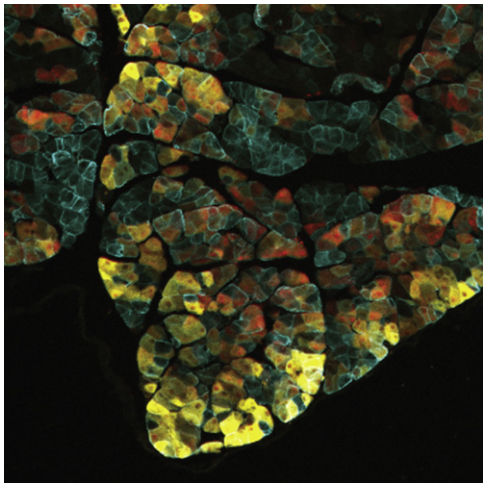
Mycobacteria, the agents of diseases like tuberculosis and leprosy, have highly hydrophobic cell walls containing mycolic acids, long-chain lipids that protect the cell from desiccation, host defenses, and antibiotics. ESX-1 is a mycobacterial virulence-related protein secretion system reported to export molecules through this waxy cell wall. Joshi et al. perform studies of the ATPase EccA1, a component of the ESX-1 system, which reveal ATP-dependent functional interactions between EccA1 and enzymes that make mycolic acids. This unexpectedly connects two of the best studied mycobacterial pathogenic mechanisms, virulence protein secretion and mycolic acid synthesis, and could present opportunities for the design of future therapies.



Antagonizing Bacterial Chitchat

PAGE 381

PqsR is the receptor of the pqs quorum sensing communication system of the pathogen *Pseudomonas aeruginosa*, which controls the expression of virulence factors and is involved in biofilm formation. Lu et al. consider PqsR as an attractive target for the development of antivirulence drugs. Following a ligand-based drug design approach, the authors identified PqsR antagonists that reduce the production of virulence factor pyocyanin in *P. aeruginosa* PA14. The finding provides valuable scientific tools for study of the ligand-receptor interaction and makes an important step towards further drug design targeting PqsR.



Targeted Regulation of Protein Stability in Mice

PAGE 391

User-defined chemical-genetic tools provide an opportunity to stringently define the role of enzymes in an in vivo context. Here, Rodriguez and Wolfgang combine mouse conditional transgenics and synthetic posttranslational protein stabilization to produce a broadly applicable strategy to regulate protein and pathway function in a cell autonomous manner in vivo. They targeted the expression of Malonyl-CoA Decarboxylase to specific tissues in transgenic mice and controlled this enzymes activity and downstream biochemical and physiological consequences by a small inert synthetic chemical. This technique provides a practical, specific, and reversible means of manipulating enzymes in adult mice, providing novel biological insight.

Bipyridyl Ring Formation

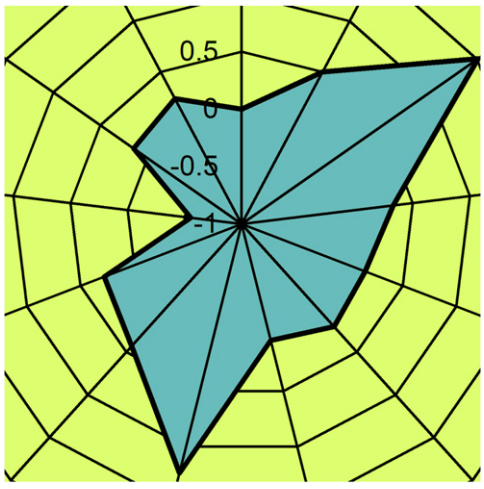
PAGE 399

Garcia et al. characterize a gene cluster for the bipyridyl collismycin A synthesis. The model for collismycin A biosynthesis they propose includes conversion of lysine into picolinic acid, participation of a hybrid PKS-NRPS, and an unusual NRPS-mediated incorporation of a cysteine residue followed by the extension of the peptide chain by a leucine incorporation and later removal by an amidohydrolase. This opens the way for future studies aimed at pathway engineering through combinatorial biosynthesis to generate collismycin A analogs with improved therapeutic activities.

GST A2-2 Grand Redesign

PAGE 414

Zhang et al. report on the successful structure-based redesign of GST A2-2 for enhanced activity with the prodrug azathioprine. The authors demonstrate an unusually high proportion of mutants with enhanced activity in the mutant library. The main effect of the mutations present in the best mutant is to change the topography for proper substrate binding rather than to provide novel functional groups for enhancing catalytic process, as originally planned.



Post-PKS Redox Tailoring Steps

PAGE 422

A complete gene cluster (xan) for the polycyclic xanthone antibiotic xantholipin from *Streptomyces flavogriseus* was cloned and sequenced, as described by Zhang et al. in this report. The gene cluster contains identified genes for xanthone, δ-lactam, methylenedioxy bridge formation and hydroxylation of the carbon backbone, and an unusual C11 ketoreductase. The order of the reactions for xantholipin biosynthesis was deduced from nine xantholipin analogs accumulated in engineered *S. flavogriseus* mutant strains. These findings may also apply to other polycyclic xanthone antibiotics, and they form the basis for genetic engineering of the xantholipin and similar biosynthetic gene clusters for the generation of compounds with improved antitumor activities.

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