In This Issue

Worms in Drops

PAGE 427

How small can things get? Pushing the boundaries in miniaturization means going from milli to micro and from micro to nano. In designing microfluidic platforms for use in biological research, Clausell-Tormos et al. now develop a system with microcompartments with volume over 1,000-fold smaller than the smallest volumes used in microtiter-plate based assays. They also show that single or multiple human cells, as well as multicellular organisms such as *C. elegans*, can be compartmentalized and replicate in these systems and remain fully viable for several days. The decrease in size and amount of reagents required, accompanied by the increase in rate of readout available, makes this a very attractive set up for development of high-throughput, cell-based assays. (Figure credits Clausell-Tormos et al.)

Plants Beware of Bugs with Peptidoglycans

PAGE 438

In this issue, Erbs et al. show that peptidoglycan (PGN) from two Gram-negative plant pathogenic bacteria, *Xanthomonas campestris* pv. *campestris* and *Agrobacterium tumefaciens*, elicit the innate immune response in *Arabidopsis thaliana*, in particular transcription of the defence gene *PR1*, oxidative burst, medium alkalinization, and formation of callose. Highly purified muropeptides from PGNs are more effective elicitors of early defense responses than native PGN. Therefore, PGN and its constituents represent a new microbe-associated molecular pattern (MAMP) in plant-bacterial interactions. Based on the structure of muropeptide components described, differing defense-eliciting abilities appear to depend on subtle structural differences in either carbohydrate or peptide groups.

When an Enzyme Just Won't Let Go

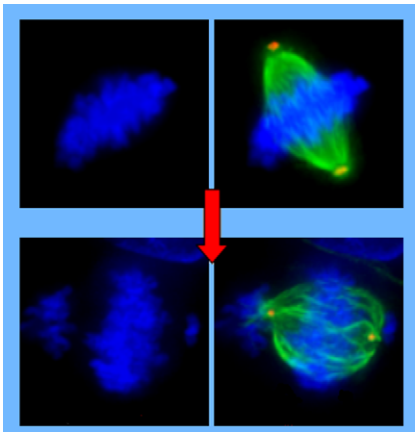
PAGE 449

Polyether ionophore antibiotics such as nanchangmycin are widely used in veterinary medicine and in animal husbandry for the treatment of diseases such as coccidiosis in poultry and as growth promotants. Although the basic outlines of polyether biosynthesis have been known for about 25 years, only recently has it become possible to investigate the biological formation of these complex polyketides at the enzymatic and molecular genetic level. Although nanchangmycin is apparently generated by a polyfunctional, modular polyketide synthase, Liu et al. have now established that all the intermediates of the pathway remain enzyme-bound until they are finally hydrolytically released by the dedicated thioesterase encoded by the *nanE* gene.

Watch Out Polo-Box Domain, Here Comes Poloxin

PAGE 459

Polo-like kinase 1 (Plk1) is a key player for multiple stages of mitosis and has been established as a target for anticancer therapy. Usually, inhibitors of Plk1 target the conserved ATP-binding site. Reindl et al. now provide proof-of-principle that Plk1 can alternatively be targeted by small-molecules that inhibit the protein-protein interactions required for correct intracellular localization of Plk1. Authors identified the natural product Thymoquinone and a synthetic derivative, dubbed ''Poloxin,'' as the first known inhibitors of the polo-box domain (PBD), a recently discovered protein domain which mediates intracellular localization of Plk1. (Figure credits Reindl et al.)



Down Which Metabolic Pathway Intermediate Went

PAGE 467

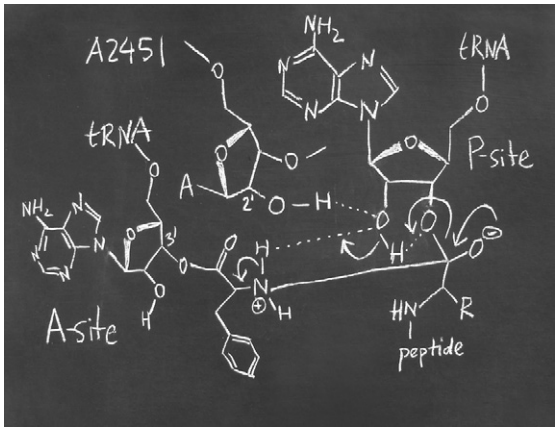
One plausible mechanism of enzyme evolution is optimization of a single activity from a catalytically promiscuous ancestor. Linsky et al. have used a mutant hydrolase and a nonnatural substrate to trap and crystallize a covalent intermediate common to many enzymes in the pentein superfamily. Chemical rescue experiments either restore the original activity or trigger an alternative activity. The ability to start with a common intermediate and then partition between two related pathways is consistent with the proposal that these enzymes may have derived from an ancestor with a reaction intermediate capable of promiscuous partitioning.

Ancestral Catalytic Function Comes to Life

PAGE 476

The glycopeptide antibiotics are invaluable towards the treatment of multi-drug-resistant bacterial infections. An understanding of the biosynthesis of these natural products should facilitate the bioengineering of novel glycopeptide antibiotics. Here, Truman et al. revise the function of Cep15, a protein encoded in the gene cluster for the glycopeptide chloroeremomycin, from a nucleotidyltransferase to a redundant minimally deactivated deacetylase. A single point mutation has been shown to reactivate Cep15 into an *N*-acetylglucosaminyl deacetylase, its likely ancestral role. Such minimal mutagenesis to reactivate a bacterial gene is unprecedented and indicates that other minimally mutated genes may be commonplace in bacterial secondary metabolite gene clusters.

Ribosomal Peptide Bond Synthesis through Atomic Mutagenesis



PAGE 485

The means by which the ribosome catalyzes peptide bond formation has been a subject of intense discussions. By applying an "atomic mutagenesis" approach to active site rRNA residues, Lang et al. revealed that the ribose 2′-group of A2451 must have hydrogen donor capability. Authors propose that A2451 directly participates in an intricate hydrogen bonding network, thereby positioning the P-site tRNA substrate in its productive conformation to support amide synthesis via a proton shuttle mechanism. This model appreciates the concept of "substrate-assisted catalysis" and combines with it the strict functional necessity of the ribose 2′-group at A2451 to own hydrogen donor capacity. (Figure credits Lang et al.)

Ribosomal Peptide Bond Synthesis through Brønsted Coefficient Analysis

PAGE 493

The ribosome has an active site comprised of RNA that catalyzes peptide bond formation. To understand how RNA promotes this reaction requires a detailed understanding of the chemical transition state. Utilizing novel puromycin derivatives, Kingery et al. have measured the Brønsted coefficient of the α-amino nucleophile (β_{nuc}) for the peptidyl transferase reaction on both 50S and 70S ribosomes. This β_{nuc} value provides a clear and important constraint on the transition state of the ribosomal peptidyl transferase reaction and establishes that the catalyzed and uncatalyzed reactions proceed through a different transition state.

Neural Pathology Meets Proteasome Inhibitor

PAGE 501

Given the effectiveness of nerve growth factor (NGF) for treating neuronal pathologies, interest exists in developing therapeutics that mimic NGF and characterizes its mechanism of action. Herein, Hines et al. show that fellutamide B, isolated from *P. fellutanum*, potently induces production of NGF from cells by directly inhibiting the 20S proteasome. While the list of small molecules shown to directly cause neurite outgrowth or differentiation of preneuronal cells is growing, fellutamide B has the advantage of upregulating the naturally occuring neurotrophin. With the suitability of proteasome inhibitors as therapeutics already established (e.g., for cancer), the findings suggest they also could be developed for treating neurological disorders.

Heme + Virus = Electroactive Particle in Nanoscale

PAGE 513

Virus capsids are hollow particles composed of multiple copies of identical proteins arranged in highly symmetric patterns. When a genetic mutation is introduced into the coat protein sequence, the amino acid change is propagated around the particle, and the position of the mutation can be selected to cause interactions between the newly introduced residues. Prasuhn et al. describe installation of a histidine-containing, C-terminal extension on the hepatitis B coat protein that creates 80 sites on the particle with high local concentrations of imidazoles, which are each shown to tightly bind iron heme molecules. In this way, a polyvalent electroactive particle is easily assembled, laying the foundation for further construction of nanomaterials with catalytic, electron-transfer, or light-harvesting capabilities. (Figure adopted from Prasuhn et al.)