In This Issue

Sweet Recognition

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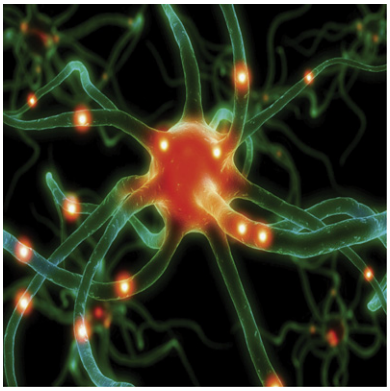
Protein *O*-GlcNAcylation is an essential and reversible glycosylation event in higher eukaryotes. *O*-GlcNAc addition and removal is catalyzed by *O*-GlcNAc transferase and *O*-GlcNAcase, respectively. Schimpl et al. report the molecular details of the interaction of a bacterial *O*-GlcNAcase homolog with its substrates, using three synthetic glycopeptides matching established *O*-GlcNAc sites in the human proteome. The findings elucidate molecular basis of *O*-GlcNAcase substrate specificity, explaining how a single enzyme achieves cycling of the complete *O*-GlcNAc proteome.



Protecting the Axons

PAGE 179

The degeneration of axons is a major contributor to the pathology of various neurodegenerative diseases. The Wld^S protein exhibits remarkable axon protective effects in mouse models of these diseases. Wld^S is predominately expressed in the nucleus and is thought to function in this compartment to protect axons; however, recent evidence suggests that trace amounts of extranuclear Wld^S is the functionally relevant pool. Using a combined chemical genetic and microfluidic compartmentalization approach, Cohen et al. show that the axonal pool of Wld^S is necessary for protection from axon degeneration and implicate an axonal pathway regulated by Wld^S as the key control route.

A Tale of Two NRPS Domains

PAGE 188

Nonribosomal peptide synthetases (NRPSs) are modular enzymes that use multiple catalytic domains and peptidyl carrier domains for the assembly line synthesis of important peptides. Sundlov et al. describe a crystal structure of a complex between NRPS adenylation and carrier protein, defining the protein-protein interface in a catalytic state. The structure was stabilized by genetically fusing the two domains and by the use of a mechanism based inhibitor. The structure of the interface enabled engineering of an adenylation domain homolog to better recognize a noncognate carrier protein partner.

Tethered Peptides Like the Membranes

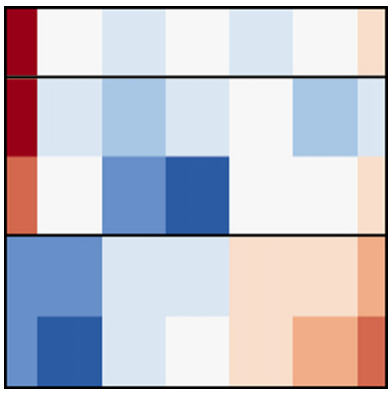
PAGE 199

The availability of biocompatible anti-infective surfaces with broad spectrum antimicrobial activity would significantly decrease the infection rates associated with biomedical devices and implants. Polymer brush tethered host defence peptides (HDPs) offer an excellent opportunity in this direction. However, there is a paucity of mechanistic information on the action of tethered HDPs. In this study, Gao et al. compare the biomembrane interactions of surface tethered HDPs with their soluble versions and demonstrate that polymer brush tethered HDPs exhibit enhanced interactions with lipid biomembranes. Thus, the mechanism of action of surface tethered HDPs is different from soluble HDPs.

Signaling Nodes under Interrogation

PAGE 210

There is a pressing need for robust technologies to enable direct, quantitative protein kinase activity profiling in basic cell biology, medical diagnostics, and therapeutic agent development. Here, Stains et al. demonstrate the power of fluorescence-based kinetic analysis, using the CSox amino acid coupled with kinase-selective substrates, to provide direct measurements of kinase enzymatic activity. The study demonstrates that CSox-based probes are capable of providing direct, quantitative readouts of kinase enzymatic activity which clarify the biochemistry of cellular differentiation and individual human tumors.



Scavenging for Cholesterol

PAGE 218

Intracellular pathogens, such as *Mycobacterium tuberculosis,* must subsist on nutrients available within the host cell. Griffin et al. use metabolite profiling to identify the biochemical pathways utilized by this organism during growth on cholesterol, an important carbon source during infection. The authors find that growth in cholesterol required the propionyl-CoA assimilating methylcitrate cycle (MCC). They show that the transcriptional induction of these enzymes is required for intracellular growth in macrophages and that the growth defect of MCC mutants is largely attributable to the degradation of cholesterol. Together, these observations define a coordinated transcriptional and metabolic adaptation that is required for scavenging carbon during intracellular growth.

Getting in between a Small GTPase and Its Effector

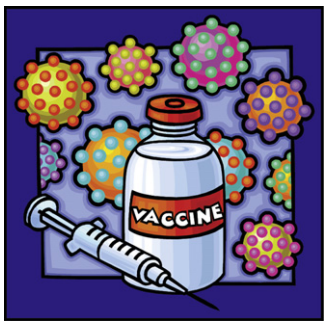
PAGE 228

The study by Bosco et al. aims to use structure-function information to rationally design inhibitors of a unique downstream effector of Rac GTPases, $p67^{phox}$, of the NOX2 NADPH oxidase complex involved in inflammatory diseases. The Phox-I1/Phox-I2 lead inhibitors identified in this work specifically compete with Rac binding to $p67^{phox}$ and are capable of abrogating superoxide production in neutrophils. The study presents evidence that structure-function based rational design can be a useful means of identifying inhibitors targeting the small GTPase–effector interface downstream of small GTPase signaling.

Unique α-Pyridone Ring Formation

PAGE 243

Piericidins are a class of α-pyridone antibiotics with antimicrobial, antifungal, and antitumor activities. Here, Liu et al. have sequenced and identified biosynthetic gene cluster of piericidin A1 from a *Streptomyces* strain. Gene functional analysis, deletion results, and in vitro biochemical characterization of the PKS terminal TE revealed a unique pathway for α-pyridone ring formation: hydrolysis of the carboxylic acid product followed by amidation and cyclization. These findings provide a foundation for genome mining of new α-pyridone antibiotics and generation of novel analogs by molecular engineering.



Using Bacteria to Fight a Virus

PAGE 254

Antibodies capable of neutralizing infectivity of a wide variety of HIV-1 strains serve to pinpoint conserved sites on HIV that are targets for vaccine design. The broadly neutralizing antibody 2G12 recognizes a patch of sugar molecules on the HIV surface glycoprotein gp120. Strategies to elicit neutralizing antibodies to these sugars have had limited success so far. Here, Clark et al. describe a bacterial oligosaccharide of which a segment is analogous to the segment recognized by 2G12 on HIV. Antibodies that bind gp120 were raised upon injecting mice with heat-killed bacteria, suggesting utility of the bacterial oligosaccharide for HIV vaccine design.

Tracking Soluble and Aggregated Protein Species In Vivo

PAGE 264

Quantification of soluble and insoluble protein conformers represents a crucial readout in the field of proteostasis disorders such as Huntington's disease or Parkinson's disease. Baldo et al. describe an exciting time-resolved FRET assay that is able to simultaneously monitor endogenous levels of soluble and aggregated protein forms. Using the method, the authors found a decrease in soluble mutant huntingtin protein that is tightly correlated with an increase in the aggregated form in aging HD animal models. This newly identified relationship of two disease relevant protein conformers contributes to further insights into diseases characterized by protein accumulation.

Damage Control

PAGE 276

The bacterial DNA glycosylase MutY and its human counterpart MUTYH seek out damaged bases and excise them from DNA. Inherited MUTYH variations are associated with colorectal cancer and knowledge on how alterations in MUTYH lead to dysfunction and disease is sorely needed. Using a combination of in vivo enzymology and cellular assays of MutY variants, Brinkmeyer et al. reveal the role of key residues involved in damaged base removal process. Moreover, the impact of specific changes on cellular repair and prevention of DNA mutations allows for a prediction of the types of mutations that are most likely to lead to deleterious MUTYH function.

Autotransporting Virulence

PAGE 287

Autotransporters are the largest class of virulence proteins secreted from Gram-negative bacterial pathogens. It was long suspected that autotransporters do not exploit a conventional energetic mechanism to transport their passenger domain across the bacterial outer membrane, as neither ATP nor a proton gradient is available. Now, Renn et al. report that regionalized stability within the passenger domain drives its secretion across the outer membrane, defining a novel Brownian ratchet mechanism for protein secretion. Secretion efficiency increases when either the C terminus is stabilized or the N terminus is destabilized. Conversely, secretion efficiency decreases when the C terminus is destabilized or the N terminus is stabilized.

Take Five

PAGE 297

Two NRPS enzymes comprising 5 modules, NocA and NocB, act in the biosynthesis of the nocardicin monocyclic β-lactam antibiotics. Davidsen et al. report a double replacement gene strategy in which point mutations were engineered in the two encoding NRPS genes without disruption of the *nocABC* operon by placing selective markers in adjacent genes. A series of mutants was constructed to inactivate the thiolation (T) domain of each module. The loss of nocardicin A production in each of these mutants indicates all five modules are required for biosynthesis of the tripeptide core of nocardicin G, and these unusually modified proteins, in fact, appear to function normally.

