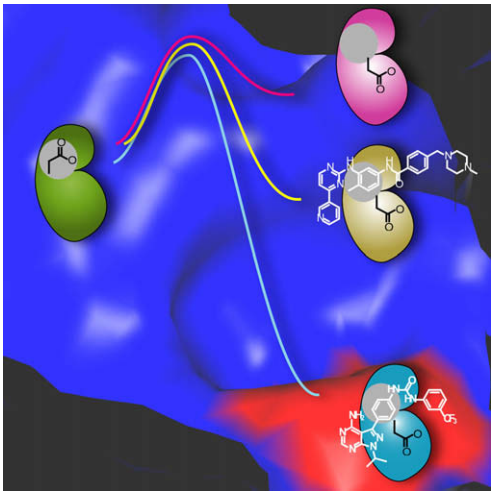
In This Issue

c-Src Reveals Its Imatinib-Binding Conformation



PAGE 1015

Cancer drug Imatinib, a 2-phenylaminopyrimidine derivative by chemical nature, is a selective Abl tyrosine kinase inhibitor that does not inhibit the closely related tyrosine kinase c-Src. To explain this observation, it was suggested that Abl can adopt an inactive conformation that binds Imatinib without paying a high energy penalty, whereas c-Src can't. From this, it can be extrapolated that a design of an inhibitor that binds to the inactive conformation of c-Src with high affinity would not be feasible. Dar et al. now report the exciting discovery of a series of such inhibitors that are capable of inducing the generally unfavorable conformation in c-Src. (Figure credits: Dar et al.)

ABC of Haloduracin Rings

PAGE 1035

Halα and Halβ are the two peptide components of a lantibiotic, haloduracin, formed from two posttranslationally modified precursor peptides that act synergistically to kill bacteria. Cooper et al. now investigate the role of the individual thioether rings that form unique haloduracin structure using an in vitro biosynthetic system. Their systematic study reveals interesting complexity of the different ring roles, demonstrating that both Halα and Halβ rings have varying degrees of essentiality for synergistic activity. Additionally, the study suggests that one or more secreted proteases are responsible for proteolytic processing of haloduracin, thus providing valuable insight into this process.

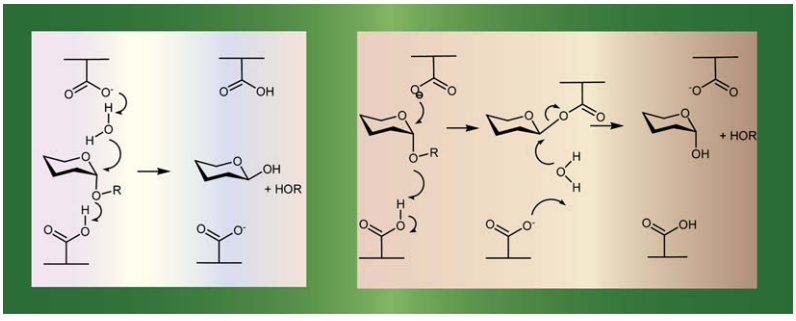
Secrets of the Alnumycin Gene Cluster

PAGE 1046

Alnumycin is an aromatic polyketide antibiotic closely related to benzoisochromanequinone (BIQ) compounds like actinorhodin. The complete alnumycin (*aln*) gene cluster from *Streptomyces* sp. CM020 was cloned, sequenced, and expressed in a heterologous host, and the results are reported here by Oja et al. Inactivation of the genes *aln4* and *aln5* and chemical characterization of the mutant strains revealed the involvement of these genes in pyran ring formation and suggests that Aln4 functions as a C-15 ketoreductase. Deletion of the genes *alnA* and *alnB* resulted in the production of a novel polyketide, prealnumycin, indicating that these genes are involved in synthesis and attachment of an unusual dioxan moiety.

Evolution of Carbohydrate Metabolism in Human Gut Flora

PAGE 1058



Glycosidic bond cleavage, catalysed by glycoside hydrolases, yields one of two stereochemical outcomes where configuration of the reactive center is either retained or inverted. The difference in mechanism reflects the dispositions of the functional groups within the active site of the enzyme, which is conserved within 112 sequence-based families. Gloster et al. demonstrate that family 97 enzymes have diverged from this general principle as the family contains both inverting and retaining enzymes. Evolutionary pressures may have encouraged these subtle differences between closely related enzymes, enabling them to acquire two different reaction mechanisms and thus generate stereochemically distinct products. (Figure adapted from Gloster et al.)

NAD Utilizing Enzyme Juggling Act

PAGE 1068

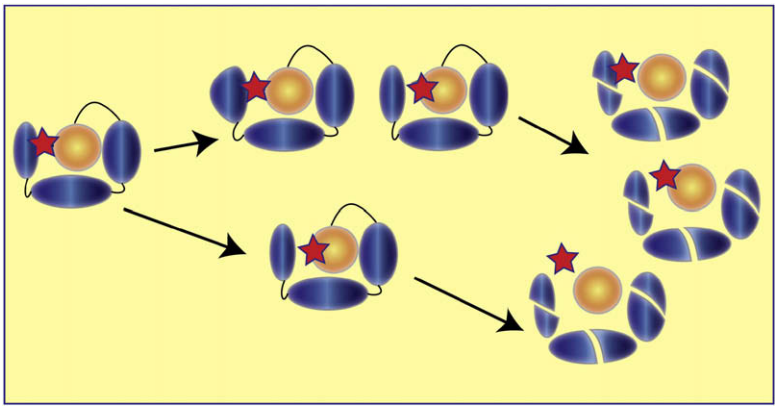
Enzymatic utilization of NAD requires the cleavage of the nicotinamide moiety from the substrate and the formation of a reactive intermediate. Liu et al. show that human CD38, an NAD utilizing enzyme, is capable of catalyzing such cleavage reactions through both covalent and noncovalent intermediates. The covalent intermediate results in mechanism-based enzyme inactivation, while the noncovalent intermediate appears to remain reactive. The structural results favor the proposal of a noncovalent intermediate during NAD catalysis by human CD38 and provide insights into the design of covalent and noncovalent inhibitors targeting NAD utilization pathways.

Unique Active Site of Type III Pentaketide Synthase

PAGE 1079

Enzymes in the chalcone synthase superfamily of type III polyketide synthases (PKSs) produce a wide variety of small molecules that have a range of biological function. *Neurospora crassa* 2′-oxoalkylresorcylic acid synthase (ORAS), a fungal type III PKS, produces pyrones, resorcinols, and resorcylic acid products. The structures of ORAS, reported by Rubin-Pitel et al., reveal an active site that is distinct from those of previously studied type III PKS and a gating mechanism that controls the production of polyketides. The function of individual amino acids in this altered active site has been further clarified using site-directed mutagenesis. With the structural and functional information available, future engineering experiments on ORAS aimed at generating novel metabolites can now be designed.

σ Factors and More σ Factors



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σ factors are prokaryotic transcription initiation factors that bind to RNA polymerase and guide its binding to specific promoter regions. While there is a wealth of structural data on σ factors, to date there has been no structure available of $\sigma_{1.1}$, until now (see Schwartz et al.) In addition to describing $\sigma_{1.1}$ structural features, the authors have synthesized and applied a new chemical crosslinker, which will also be useful for studying transient interactions in other systems. Finally, the authors have addressed a long-standing mechanistic issue in prokaryotic transcription; namely, how σ factors are autoinhibited by demonstrating that $\sigma_{1.1}$ interacts with the σ factor DNA binding domains, suggesting a compacted structure that is incompatible with DNA binding. (Figure adapted from Schwartz et al.)

One FlAsH and Two Cys-Xxx-Cys

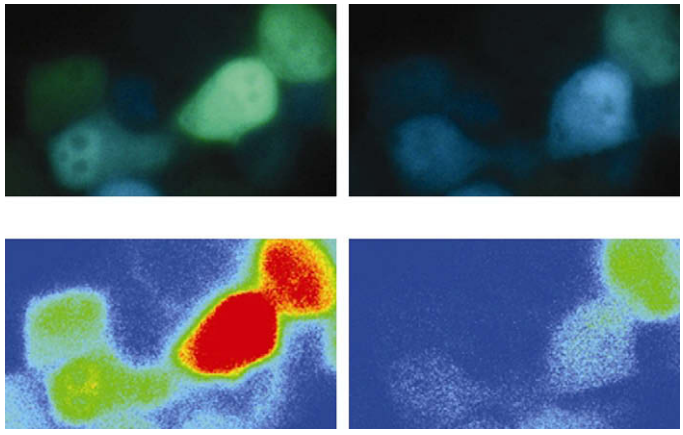
PAGE 1104

Successful engineering of genetically encoded, two-part dye-binding sequences across strands of a β sheet in a model protein is now reported by Krishnan and Gierasch. The two Cys-Xxx-Cys components of the binding sequence together make up a tetra-Cys motif, which binds the fluorophore FlAsH; thus, the cross-strand split tetra-Cys motifs would bind the reporter fluorophore in a structure-dependent manner. Although one motif bound FlAsH only in native form, two others retained dye binding when denatured, indicating that some structures in the unfolded ensemble satisfy the spatial requirements for dye interaction. Additionally, the authors show that split tetra-Cys motifs can be used to assess cross-strand proximities in vivo.

Into the Bright Blue

PAGE 1116

Subach et al. now report a structure-based strategy to develop bright Blue Fluorescent Proteins (BFPs) by utilizing rational site-specific mutagenesis strategy and starting from Red Fluorescent Protein. The result, mTagBFP, contains a tyrosine residue uniquely positioned in the chromophore, providing mTagBFP with advantages when compared to currently available BFPs with a histidine residue in the chromophore. Those advantages include higher brightness, higher chromophore formation rate, extraordinary pH stability, distinctive fluorescence lifetime, and narrower fluorescence spectrum. Finally, the authors demonstrate that mTagBFP is a donor of choice for FRET applications with GFP, since it exhibits very low background FRET due to its true monomeric state and, thus, inability to dimerize with FRET partners. (Figure adapted from Subach et al.)



Turn on the Riboswitch

PAGE 1125

The tetracycline aptamer is an artificially evolved RNA that binds the antibiotic with a subnanomolar dissociation constant and high specificity. It is one of few aptamers that has been successfully employed as a small-molecule-responsive genetic switch in vivo. Xiao et al. now describe a structure of the aptamer bound to the antibiotic that reveals how the RNA binds to tetracycline with an affinity ~1000 times higher than that of the biological target, the ribosome. Moreover, the overall architecture of the aptamer and the local folding transition it undergoes upon tetracycline binding are both reminiscent of natural gene-regulatory RNAs (riboswitches).

