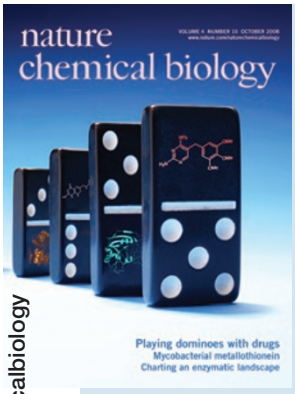
IN THIS ISSUE



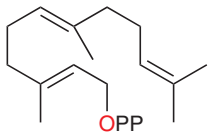
Antifolate drug in action

Trimethoprim is an antibiotic that inhibits dihydrofolate reductase (DHFR). Using mass spectrometry, Kwon *et al.* profiled the effects of trimethoprim on 31 intermediates in the folate metabolic pathway. In addition to DHFR inhibition, the authors found that folylpoly-γ-glutamate synthetase (FP-γ-GS), another enzyme in folate metabolism, was also inhibited. Trimethoprim was not a direct inhibitor of FP-γ-GS. Instead, DHFR inhibition led to accumulation of the enzyme substrate, dihydrofolate, which was shown to be a potent inhibitor of FP-γ-GS. A mathematical model confirmed that the observed dynamics of folate metabolites could be fully accounted for by inhibition of these two enzymes. These results illustrate the power of metabolomics for uncovering unexpected off-target drug effects. [**Articles, p. 602; News & Views, p. 581**] *JK*

CcO two-step

Cytochrome *c* oxidase (CcO), an essential enzyme for cellular respiration, contains a dinuclear copper site called Cu_A. Although several proteins have been suggested to play a role in Cu_A assembly, the molecular mechanism for delivering copper to CcO has not been elucidated. Using NMR spectroscopy, Abriata *et al.* demonstrate that a new periplasmic protein, pCu_AC, can directly transfer two Cu(I) ions to CcO to generate the Cu_A site. In contrast, Sco1, which had previously been suggested to be a metallochaperone, functioned as a reductase of the Cu_A cysteine ligands. These results led the authors to propose a two-step model for Cu_A assembly in which Sco1 reduces the Cu_A cysteines, thereby facilitating the sequential insertion of two Cu(I) ions by pCu_AC. [**Brief Communication, p. 599**] *JK*

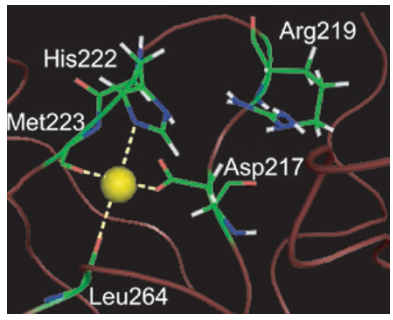
Charting an enzymatic course



Enzyme catalysis usually relies on the strict positioning of a few key residues, with related enzymes differing in either the placement or identity of these catalytic groups. Though several studies have shown that it is possible to convert between enzymes by altering the identity of a single or a few amino acids, it was not clear how additional permutations of likely or obligate mutations between related enzymes would affect product outcome. By constructing an enzyme library exploring nine point mutations that have been shown to functionally interconvert two sesquiterpene synthases, O'Maille *et al.* were able to create a biosynthetic 'landscape', thereby quantifying an unexpected level of catalytic promiscuity in the new enzymes. Further analysis demonstrated the importance of context in determining the impact of single mutations on function and highlighted cases of both small and large jumps in product specificity based on single amino acid changes. These studies provide a quantitative foundation for understanding enzyme evolution and engineering. [Articles, p. 617] *CG*

Written by Catherine Goodman & Joanne Kotz

A sodium bait-and-switch



Inwardly rectifying potassium (Kir) channels serve an important role in setting membrane potential and regulating excitability. These channels are gated by the phospholipid phosphatidylinositol-4,5-bisphosphate ($PtdIns(4,5)P_2$), but Kir3 channels require an additional gating molecule, such as sodium, for activity. Rosenhouse-Dantsker *et al.* now use a combined modeling and experimental approach to demonstrate the molecular basis of this dual gating. They observe that, in the inactive state, the arginine that is necessary for $PtdIns(4,5)P_2$ sensitivity is sequestered by an interaction with a neighboring aspartate. Sodium binding induces a rearrangement of this aspartate, thus interrupting the aspartate-arginine contact and restoring arginine's potential for phospholipid binding. Understanding the specific residues necessary for sodium sensitivity allowed the authors to identify Kir5.1 as sodium-sensitive and provides a deeper understanding of the mechanics of dual-gated channels. [**Articles, p. 624**] *CG*

Mycobacterial metallothionein

Metallothioneins are proteins commonly found in eukaryotic cells that bind physiological and heavy metals and are believed to offer protection from metal toxicity. Through a chemical screen, Gold *et al.* discovered a gene that confers copper toxicity resistance to *Mycobacterium tuberculosis*. The gene encodes a small, cysteine-rich protein, MymT, that can bind six Cu(I) ions. The unusual luminescence properties of the copper-bound protein suggested that MymT forms a tight Cu(I)-thiolate core. Taking advantage of this luminescence, the authors demonstrated that MymT binds Cu(I) *in vivo* and that nitric oxide can induce the release of MymT-bound copper *in vitro* and *in vivo*. These results identify MymT as a previously unrecognized metallothionein and suggest that these metal-sequestering proteins may be widespread, but often overlooked, in bacteria. [Articles, p. 609; News & Views, p. 582] *JK*

Probing histone modifications

Reversible acetylation and methylation of histone lysyl residues are important epigenetic regulators of chromatin structure. As a result of their central biological role, the enzymes that catalyze the modifications have emerged as important drug targets. In a review in this issue, Cole describes chemical probes that have been developed for each of these histone-modifying enzymes and the mechanistic and biological insights that these chemical tools have facilitated. Other emerging chemical biology approaches to investigating the structure and function of chromatin are also highlighted. [**Review, p. 590**] *JK*