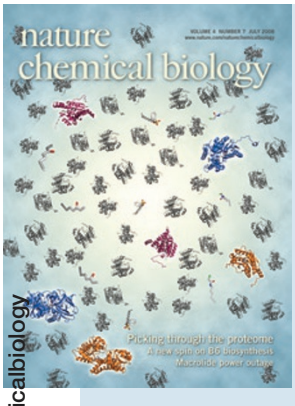

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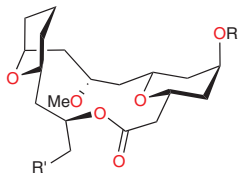
Electrophiles on target

Activity-based probes have proven to be a powerful tool for characterizing enzymatic activity in cells. However, the number of protein classes that can currently be targeted by this approach is limited to enzymes with known irreversible mechanism-based inhibitors. To expand the breadth of proteomic coverage, Weerapana *et al.* have profiled the reactivities of five carbon electrophiles. An α,β-unsaturated ketone had the highest reactivity, whereas two epoxides had little to no reactivity. The α,β-unsaturated ketone and an α-chloroacetamide reacted exclusively with cysteines, while a phenylsulfonate ester labeled a range of nucleophilic amino acids. Surprisingly, the reactivity profiles of the electrophiles in solution did not match their reactivities in proteomes, which emphasizes the importance of protein microenvironment in modulating chemical reactivity. The varied levels of reactivities and specificities observed with these electrophiles suggest that they will be flexible scaffolds for designing the next generation of activity-based probes. [**Brief Communications, p. 405; News & Views, p. 387**] *JK*

News on nitrate

In mammals, nitrite (NO_2^-) can be reduced to nitric oxide (NO), a key regulator of cardiovascular homeostasis, and may also function on its own as a physiological signaling molecule. Bacteria form nitrite by reducing nitrate (NO_3^-), but whether nitrate reductase activity is present in mammalian tissues has remained uncertain. Using the small-molecule inhibitor allopurinol, Jansson *et al.* now provide evidence that, at normal oxygen levels, rodent and human xanthine oxidoreductase reduce nitrate to nitrite *in vitro* and *in vivo*. Nitrate administration resulted in higher levels of nitrite in germ-free mice, which indicates that nitrate reduction was not achieved by commensal bacteria. Nitrate also decreased blood pressure and increased post-ischemic blood flow, which suggests a role for nitrate in mammalian nitrite and NO homeostasis. [**Articles, p. 411**] *KS*

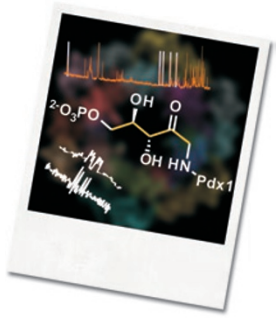
Circling cytochromes



Identifying the mode of action of natural products is an important endeavor, but this process can be hampered by the limited quantities of molecules from natural sources or by necessarily complex synthetic pathways. Ulanovskaya *et al.* now bypass this problem by identifying a synthetically tractable but still potent analog of the antiproliferative natural product leucascandrolide A. The authors developed a general synthetic strategy to access this analog and a structurally related macrolide, neopeltolide, in which elaboration and condensation of constrained ketones provided the initial macrocycle scaffold. Detective work utilizing cell cycle assays, gene deletion screens and other small-molecule probes pinpointed the cytochrome bc_1 complex as the molecular target of these compounds. Leucascandrolide A and neopeltolide are structurally unrelated to prior inhibitors of this complex and thus may serve as important new tools in deciphering the role of the cytochrome bc_1 complex in mitochondrial ATP production. [Articles, p. 418; News & Views, p. 388] *CG*

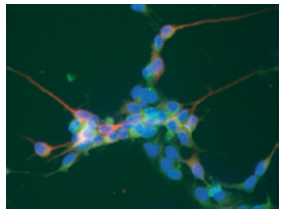
Written by Catherine Goodman, Joanne Kotz & Kenneth Sercy

Snapshots of B6 biosynthesis



Vitamins are well known for their requisite roles as enzyme cofactors, but the biosynthetic routes to these multifunctional molecules are not always as clear. Pyridoxal 5′-phosphate, the coenzyme form of vitamin B6, can be synthesized by the enzyme pair of Pdx1 and Pdx2 by the condensation of three substrates: D-ribose 5-phosphate or D-ribulose 5-phosphate, glutamine and D-glyceraldehyde 3-phosphate. While earlier studies raised mechanistic proposals for this complicated conversion, definitive support for intermediates in the process has remained elusive. Hanes *et al.* therefore used NMR of isotopically labeled substrates and Pdx1 to gain a better picture of the biosynthetic path in this ~400 kDa protein. By denaturing the protein after covalently trapping imine intermediates, the authors were able to visualize several proposed intermediates, thereby confirming a unique 1,5 migration during the reaction mechanism. The successful use of NMR in this case reestablishes the technique as a tool for biochemical studies of very large proteins. [Articles, p. 425; News & Views, p. 390] *CG*

NSCs grow up



Neural stem cells (NSCs) are known to be receptive to extracellular signals regulating growth and differentiation, but the signaling pathways that transduce these signals to affect NSC gene expression are poorly understood. Schneider *et al.* now report that a group of isoxazole small molecules activate a neuronal gene program in adult rat NSCs that includes *neuroD*, *gluR2* and *NR1* and leads to neurogenesis. In a series of biochemical studies, the authors showed that the isoxazoles trigger a Ca^{2+} influx that activates CaM kinase, which in turn phosphorylates the histone deacetylase HDAC5, thereby de-repressing the transcription factor MEF2 and activating the neuronal gene program. This study identifies a molecular pathway for neuronal differentiation and provides *in vitro* evidence that the HDAC-MEF2 epigenetic regulatory network has a role not only in adult neurons but also in NSCs. [**Brief Communications, p. 408**] *KS*

GPCR structure raises the BAR

The structure of the human β_2-adrenergic receptor offered the first molecular glimpse of a G protein–coupled receptor that binds small-molecule ligands. In a Perspective in this issue, Audet and Bouvier provide a functional analysis of the receptor structure and, through insights from the structure and virtual ligand docking, suggest a molecular basis for how ligands can differentially regulate adenylate cyclase and mitogen-activated protein kinase signaling pathways through this single receptor. [Perspective, p. 397] *JK*