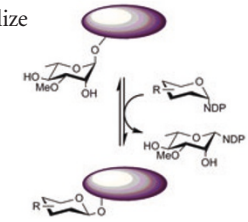
Cutting caspase-7

In response to death signals, apoptotic initiator caspases cleave and activate the executioner caspases, caspase-3 and caspase-7. The proteolytic processing of the executioner caspases from inactive zymogen homodimers to active proteases is generally believed to be a critical step in the apoptotic cascade. However, two recent papers now identify a new, uncleaved intermediate in caspase-7 activation. Berger *et al.* first developed caspase-specific activity-based probes that selectively label active enzymes. After inducing apoptosis, the authors used a probe specific for caspase-3 and caspase-7 and unexpectedly observed labeling of uncleaved caspase-7. This full-length, active caspase-7 intermediate was observed shortly after initiation of apoptosis and was later cleaved by executioner, rather than initiator, caspases. In an accompanying paper, Denault *et al.* generated caspase-7 heterodimers by coexpressing differently tagged caspase-7 constructs in *Escherichia coli*. Using combinations of site-directed mutants, the authors found that cleavage of a single subunit is sufficient to generate active caspase-7. Based on the combined results of the two papers, the authors have proposed a model in which cleavage of one subunit induces an allosteric change in the second subunit that results in full activity. Intriguingly, full-length caspase-7 had different inhibitor binding specificity from the cleaved enzyme, which suggests that the enzyme may have altered substrate specificity. For the moment it is not known whether this new caspase-7 intermediate has a unique role in apoptosis. (*Mol. Cell* **23**, 509–521, 2006; *Mol. Cell* **23**, 523–533, 2006) *JK*

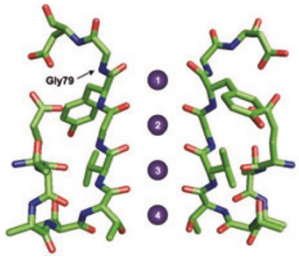
Sugar exchange



Glycosyltransferases (GTs) serve to functionalize natural products, proteins and other biological molecules with carbohydrates in a diverse and selective manner that has long been the envy of synthetic chemists. These reactions were thought to proceed unidirectionally, with the GT functioning only to conjugate the desired sugar to the aglycone scaffold. Now Zhang *et al.* prove otherwise: in their efforts to confirm the identity of CalG1 as the requisite rhamnosyltransferase in calicheamicin (CLM) biosynthesis, they observed that sugar attachments to CLM are reversible. Indeed, combinations of the product, free nucleotide diphosphate and enzyme that were anticipated to result in no reaction instead generated the nucleotide diphosphate sugar and aglycone starting materials. The authors were also able to substitute CLM with alternate sugars immediately after removal of rhamnose. Using combinations of various incoming sugars and modified aglycone units, they created a 70-member library, thereby illustrating the relaxed substrate specificity of CalG1. Zhang *et al.* further demonstrated the generality of GT reversibility by testing other enzymes involved in CLM (CalG4) and vancomycin (GtfD and GtfE) biosynthesis. Surprisingly, they even observed that mixing CalG1 and GtfE causes sugar transfer from one scaffold to another. This insight not only opens the door for improved synthetic methods and biosynthetic engineering efforts but also reverses the dogma that GTs are unidirectional. It remains to be seen whether this reversibility has a role *in vivo*. (*Science* **313**, 1291–1294, 2006) *CG*

Research Highlights written by Mirella Bucci, Catherine Goodman, Joanne Kotz and Terry L. Sheppard

Oxygen cools a channel



Tom Muir

The recent three-dimensional structure of the KcsA potassium channel has provided new insights into the mechanisms of ion transport across membranes. A constriction in the KcsA channel maintains selectivity for K^+ over other ions; this 'selectivity filter' has four K^+ binding sites, S1 through S4, and typically binds two K^+ ions spaced across two of the sites. Using protein semisynthesis, electrophysiology and structural biology, Valiyaveetil *et al.* showed that electronic perturbations of the outermost S1 binding site have dramatic effects on ion occupancy and channel conductance in KcsA. Using expressed protein ligation, the authors constructed a KcsA mutant ($KcsA^{ester}$) in which the peptide bond between Tyr78 and Gly79 in S1 is mutated to an ester linkage. This transformation converted an important hydrogen bond donor (NH) into a hydrogen bond acceptor (O) and decreased the polarity of the carbonyl group at this site. $KcsA^{ester}$ conductance was 50% lower than that of the wild-type channel and had an atypical dependence on K^+ concentration. Crystallographic data for $KcsA^{ester}$ in the presence of K^+, Rb^+ and Cs^+ showed that the structure of the selectivity filter was unperturbed relative to the native channel. However, the NH-to-O substitution altered the occupancy of metal ions across the S1–S4 sites and evicted a functionally important water molecule from behind the S1 site. These subtle effects underscore the fine tuning of the protein architecture that governs channel selectivity and illustrate the utility of chemical synthesis for answering precise questions about biological mechanisms. (*J. Am. Chem. Soc.* 128, 11591–11599, 2006) *TLS*

A mode-of-chemical-action map

Linking bioactive compounds to their target pathways is an important challenge in chemical biology. Parsons *et al.* have used a chemical genetics approach to begin to provide a map that should facilitate the determination of the mode of action of any compound. They screened a collection of ~5,000 viable yeast deletion mutants with 82 different synthetic compounds, natural products and natural-product extracts. To generate their map, the authors used two-dimensional hierarchical clustering analysis, which groups the compounds based on their chemical-genetic profiles and the genes based on their overlapping patterns of cell sensitivities to the compounds. Genes that cluster together are functionally related. Compounds with similar biological effects have similar chemical-genetic profiles, so compounds that cluster together have similar cellular effects, sometimes even inhibiting the same pathway or target. For instance, latrunculin B and cytochalasin A are known effectors of the actin cytoskeleton, and clustered together. Using a different technique called factorization analysis, the authors discovered even more connections by grouping related compounds that affect groups of related mutants, thereby allowing for any one mutant or compound to be linked to more than one cellular function. As a result, the authors were able to conclude that the anti-HIV compound papuamide B interacts with phosphatidylserine by first noting that papuamide B affects genes involved in cell wall organization and biogenesis in their map, and by then comparing it in a hierarchical analysis to data from numerous other genome-wide genetic screens. Thus, the integration of genetic interaction data from chemical-genetic data and genome-wide screens provides a map for linking compounds to their target pathways. (*Cell* **126**, 611–625, 2006) *MB*