Stereochemistry-independent sterols

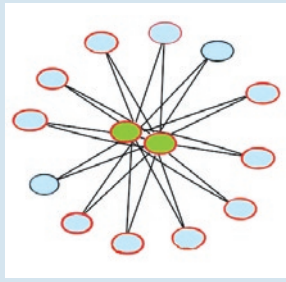
Oxygenated derivatives of cholesterol, or oxysterols, regulate cholesterol homeostasis by interacting directly with Insig proteins and promoting both endoplasmic reticulum (ER) retention of transcription factors that control expression of cholesterol synthesis genes and degradation of the cholesterol biosynthetic enzyme HMG CoA reductase (HMGR). Gale *et al.* used the side chain oxysterol 25-hydroxycholesterol (HC) and its enantiomer, *ent*-25-HC, to demonstrate that sterol regulatory element (SRE)-dependent transcription and HMGR degradation occur independently of HC stereochemistry. Because steroid-protein interactions are expected to be enantiospecific, these data are consistent with the oxysterols acting through a protein-independent pathway. Further *in vitro* experiments indicate that both oxysterol enantiomers disorder membranes in phospholipid monolayers and bilayers. Interestingly, this disordering effect is counteracted by higher concentrations of phospholipids with saturated acyl chains. Indeed, incubation of cultured mammalian cells with palmitate abrogates the effects of oxysterols on SRE-dependent transcription and HMGR degradation. Evaluation of cell homogenates and ER membrane fractions indicates that palmitate does not affect the partitioning of cholesterol between the plasma membrane and the ER membrane, but it does promote ER phospholipid remodeling. Based on these data, the authors propose that oxysterols interact directly with phospholipids in the ER membrane and that fatty acids affect sterol metabolism by modulating the activity of oxysterols. (*J. Biol. Chem.*, published online 6 November 2008, doi:10.1074/jbc.M807210200) *AD*

Coats for fat flies

Energy in the form of lipids is taken up by cells as free fatty acids and stored as triglycerides in lipid droplets—specialized organelles bound by a phospholipid monolayer that are thought to originate from the ER membrane. Though the details of their formation and dynamics are scarce, trafficking between lipid droplets and other cellular compartments is assumed based on the identities of lipid droplet coat proteins. Beller *et al.* have conducted an RNAi-based screen for additional *Drosophila melanogaster* genes involved in lipid storage or use in cell lines. Data from a microscopy-based primary screen were analyzed in light of the Gene Ontology database, which groups genes with pathway-related functions, and further validated by an independent lipid storage gene screen, as well as data from *D. melanogaster* subproteomes. Surprisingly, of the hits not already known to be directly involved in lipid utilization several have known cellular transport functions, including COPI, the coat protein involved in Golgi-to-ER retrograde transport. Based on an epistasis analysis with small compound regulators of this pathway, as well as experiments in mouse models, the authors concluded that lipid overstorage due to COPI knockdown is not a general consequence of disrupted ER-to-Golgi traffic, and it may be part of a Golgi-independent traffic and lipolysis pathway. (*PLoS Biol.* **6**, e292, 2008) *MB*

Written by Mirella Bucci, Amy Donner, Catherine Goodman & Joanne Kotz

Antibiotics do double time

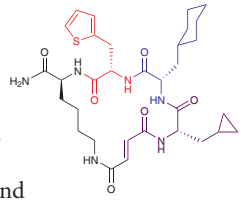


Bacterial two-component systems serve to instruct the cell's transcriptional machinery and other cellular systems about extracellular changes, and to solicit appropriate responses. For instance, in response to changing redox conditions, the Arc system modulates elements of the electron transport chain, the TCA cycle and respiration. Using a systems-based approach, along with extensive mutational screening and analysis, Kohanski *et al.* have linked the Arc system to another two-component system: the protein fidelity-sensing Cpx system. In trying to understand how the aminoglycoside gentamicin mediates bacterial cell killing after targeting the protein translation machinery, they compared expression profiles of treated cells to a panel of previously collected profiles. Consistent with known roles of metabolism and respiration in antibiotic-induced hydroxyl radical formation and cell death, the systems-level analysis found the Arc system, a network related to protein mistranslation, and a network of transport protein–related genes all to be linked to aminoglycoside lethality. From an analysis of cell death, membrane depolarization and radical formation of mutants in genes with known roles in protein translocation, membrane protein degradation and the Cpx system, the authors concluded that mistranslation and subsequent misfolding of membrane proteins induced by the antibiotic impact the electrochemical potential and integrity of the cell membrane via Arc-mediated induction of the Cpx system. Their studies suggest that aminoglycosides use multiple strategies to achieve bacterial cell death and that two-component systems have broad roles in bactericidal antibiotic-mediated death. (*Cell* **135**, 679–690, 2008) *MB*

Permuted proteins report on binding

Assaying enzyme function usually relies on a spectroscopic handle that is turned on or off by the action of the enzyme. However, assaying proteins that simply bind a ligand—particularly those that do not undergo any major conformational change upon ligand binding—poses a challenge. Stratton *et al.* now introduce a new method for monitoring binding proteins that relies on domain duplication and conformational dynamics to generate a FRET signal. This method, called 'alternate frame folding', first requires that a protein can be circularly permuted, or engineered to link the known N and C termini with concomitant generation of new termini, and still retain function. Then the wild-type and permuted sequences are merged to generate a longer protein, with the requirement that the now duplicated portion of the protein must contain some of the residues that make up the binding site. By setting up an enforced conformational dynamic with a mutation in the native binding site, and installing fluorophores at appropriate sites, the authors were able to monitor Ca^{2+} binding by the calcium-binding protein calbindin D_{9k}. Extending the method to more intricately woven proteins or to proteins that cannot fold reversibly may be difficult, but for many proteins, alternate frame folding will provide an important new way to assay function. (*ACS Chem. Biol.*, **3**, 723–732, 2008) *CG*

Maximizing macrocycles

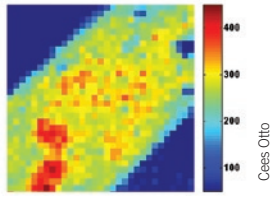


In DNA-templated synthesis, DNA base pairing is used to direct sequential reactions of tethered small molecules. In previous work, Liu and colleagues used this technique to synthesize a 65-member macrocycle library. The initial synthetic method involved three sequential DNA-templated amine acylation steps followed by macrocyclization. For the first two reactions, bond formation was followed by bead-based purification, washing and product elution. Following the third amine acylation step and bead capture, cyclization resulted in the self-elution of final products. Tse *et al.* have now modified this synthetic route to create a significantly larger and more chemically diverse library. First, the oligonucleotide coding sequences were designed so that they did not need to be removed before subsequent DNA-templated steps. Second, using the peptide synthesis technique of acylating unreacted intermediates at each step prevented them from participating in subsequent reactions. Finally, 36 coding sequences were designed such that 12 different building blocks could be used in each step with minimal cross-reactivity. These advances enabled all three DNA-templated reactions to be performed in a single reaction vessel without intermediate purification or washing steps. With this optimized synthetic approach, the authors used 4 different starting scaffolds and 36 chemically diverse building blocks to synthesize a 13,824-membered DNA-templated library that enables large-scale *in vitro* selections on synthetic macrocycles. (*J. Am. Chem. Soc.* **130**, 15611–15626, 2008) *JK*

iPS cells take two

Differentiated somatic cells can be converted to pluripotent stem (iPS) cells by ectopic expression of three or four transcription factors. Because reprogrammed iPS cells are highly similar to embryonic stem cells, they should be useful for modeling diseases *in vitro* and in regenerative medicine. Two new studies identify small molecules that functionally replace exogenous transcription factors and thereby reduce the number of ectopic genes needed to achieve cellular reprogramming to two. In the first report, Huangfu *et al.* demonstrate that the histone deacetylase inhibitor valproic acid (VPA) increases the efficiency of iPS formation from human fibroblasts and enables reprogramming with *Oct4* and *Sox2*. The inclusion of VPA bypasses the need for the oncogenes *Klf4* and *c-Myc*, which can promote tumor formation in mice containing iPS cells. In the second report, Shi *et al.* discover through a high-throughput chemical screen that the combination of the G9a methyltransferase inhibitor BIX and the L-channel calcium agonist Bayk8644 enables mouse fibroblast reprogramming with *Klf4* and *Oct4*. The unexpected discovery of a channel agonist in the chemical screen links pluripotency with calcium signaling pathways. These exciting studies suggest that small molecules can overcome the need for virally transduced transcription factors and improve reprogramming efficiency. Future studies on Bayk8644 and the identification of additional pathway-specific small molecules that facilitate cellular reprogramming may ultimately shed light on the signals that govern the differentiation capacity of the cell. (*Nat. Biotechnol.* **26**, 1269–1275, 2008; *Cell Stem Cell* **3**, 568–574, 2008) *AD*

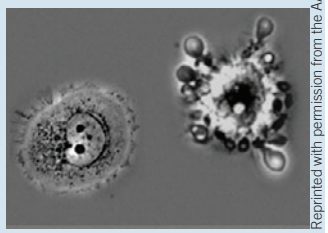
Seeing SILAC



Cees Otto

Stable isotope labeling by amino acids in cell culture (SILAC), or the introduction of deuterated or ^{13}C-labeled amino acids into proteins in intact cells, has enabled significant insights into protein synthesis and metabolism. However, standard analysis methods require cell lysis and proteolysis to generate mass spectrometry samples, which prevents analysis of protein localization and observation of cellular time courses. Van Manen *et al.* now combine Raman microspectroscopy with SILAC to monitor incorporation of three amino acids in intact HeLa cells. After incubation periods as short as 8 hours, labeled phenylalanine, tyrosine and methionine residues could be detected using characteristic Raman frequencies with incorporation levels as low as ~3.5%. Hierarchical cluster analysis of high-resolution Raman spectral images identified four distinct regions of the cell and allowed the authors to compare the locations of Phe-d_5 and Phe-h_5 incorporation. A pronounced lack of Phe-d_5 in lipid droplets extends previous studies suggesting that the proteins associated with these droplets are protected from degradation. With *in vivo* imaging established, extensions of the method such as the simultaneous introduction of multiple isotopically labeled residues should be forthcoming. (*Anal. Chem.*, published online 11 November 2008, doi:10.1021/ac801841y) *CG*

Dynamic drug response



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Little is known about how drugs affect the proteome of a cell and how this response might vary from cell to cell. Cohen *et al.* now look at the dynamic response of proteins to camptothecin, a topoisomerase-1 inhibitor. The authors first fluorescently tagged approximately 1,000 proteins at their endogenous chromosomal location in individual human lung carcinoma cells. Using a dual fluorescence tagging strategy to visualize cell boundaries and customized image analysis software, the authors tracked the concentration and location of each of these proteins in living cells for 48 hours following exposure to camptothecin. Although changes in cellular location were rare compared to changes in protein abundance, protein translocation events were often connected with the drug's mechanism of action. For instance, topoisomerase-1 was one of the first proteins to respond to the drug, with a shift in localization from the nucleolus to the nucleus. Additional translocation events revealed a drug response that included transcriptional inhibition within minutes, DNA damage around an hour and an oxidative stress response after several hours. Surprisingly, although the response of most proteins was consistent between cells, RNA helicase DDX5 showed significant cell-to-cell variability, with higher protein levels being correlated with cell survival. RNAi-mediated knockdown of the RNA helicase accelerated the cell death rate, which supports the functional significance of DDX5 in controlling cell fate. This approach provides a more global glimpse at the cellular drug response and highlights the potential functional importance of stochastic variation between cells. (*Science*, published online 20 November 2008, doi:10.1126/science.1160165) *JK*