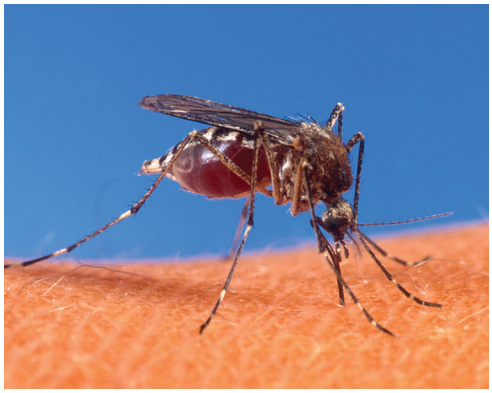
Spotlight

Turning Up the DEET

The vampires of the insect world, mosquitoes, not only represent a risk to one's camping fun but, more importantly, put millions at risk in parts of the world where malaria or other blood-borne pathogens are prevalent. Over the last 50 years, humans have fought back with a chemical repellent called *N*,*N*-diethyl-*m*-toluamide, better known as DEET. A complete mechanism for how DEET affects the mosquito sensory system has remained elusive, but in a recent study, Ditzen *et al.* (*Science* 2008, *319*, 1838–1842) take on the problem by combining electrophysiology and a clever, yet simple, behavioral assay.



Picture from the USDA website at http://www.ars.usda.gov/is/graphics/photos/aug00/k4705-9.htm. (Image accessed at http://commons.wikimedia.org/wiki/Image:Aedes_aegypti_biting_human.jpg)

Previous work demonstrated that certain olfactory sensory neurons in mosquitoes are key to sensing carbon dioxide (CO_2) and 1-octen-3-ol in human breath. Mosquitoes house neurons that are sensitive to these and other attractants in a structure known as the maxillary palp, an antenna-like structure near their mouthparts. Using these sensilla neurons, the authors made electrophysiological recordings in the presence of CO_2 and 1-octen-3-ol. They found that while CO_2 responses were not affected by the insect repellent, the 1-octen-3-ol-induced responses were strongly inhibited by DEET in a family of cells known as cpB neurons. These neurons are known to express a receptor system that is sensitive to the octenol compound. In this system is a key co-receptor protein, GPROR7, a mosquito ortholog of the *Drosophila* protein Or83b, so the study then asked whether fruit flies would mind a little DEET in their way.

A behavioral test was constructed in which flies could chose two paths to food, but one of the paths was treated with DEET. Indeed, the flies gathered in the DEET-less chamber at far higher rates, and this behavior required antennae and was completely absent in a $Or83b^{-/-}$ mutant. After testing a number of antennal olfactory neurons and their responses to food during DEET exposure, the authors honed in on several subsets of neurons. The ab5 family of olfactory neurons was especially sensitive to DEET, and the authors went on to look at the olfactory receptors (ORs) found in those cell types by electrophysiology and chemoattractant assays. Also using a heterologous system with receptors expressed in frog oocytes, electrophysiology showed that some ion currents usually mediated by ORs were down in response to DEET. This study is by no means the end of the story, but it gives interesting molecular clues as to how this simple molecule throws a monkey wrench into the mosquito's sensory computer. **Jason G. Underwood, Ph.D.**

Taking Charge of Cell Membranes

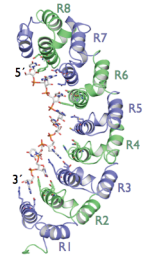
Bacterial resistance to antimicrobials is a widespread concern in the treatment of infectious diseases. In many bacteria, resistance is conferred by decreasing cellular permeability to cationic antibiotics and antimicrobial peptides. This is achieved, in part, by increasing the net positive charge of the (mostly) anionic cell membrane by attaching positively charged and neutral amino acids to the phosphatidylglycerol (PG) molecules in these membranes. In the pathogen *Staphylococcus aureus*, the multiple peptide resistance factor (MprF) catalyzes the transfer of lysine from tRNA charged with this amino acid to a hydroxyl of the glycerol moiety of PG. Now, Roy and Ibba (*Proc. Natl. Acad. Sci. U.S.A.* 2008, *105*, 4667–4672) characterize a second MprF enzyme from the pathogen, *Clostridium perfringens*, with altered substrate specificity.

The authors discovered enzymes that catalyze the synthesis of alanine-PG and lysine-PG using tRNAs charged with alanine and lysine, respectively. Interestingly, whereas aminoacyl-PG formation is RNA-dependent, the overall shape and sequence of the tRNA are not critical for catalysis. Instead, the aminoacyl moiety is the primary determinant of recognition by the two MprFs. This poses interesting questions: what is the specific role of tRNA in MprFs catalyzed aminoacyl-PG formation, and what are the recognition elements that confer substrate specificity?

Published online May 16, 2008 • 10.1021/cb800093f CCC: $40.75

PUF, the Magic Protein?

Crystal Structure of the Puf4 RNA-binding Domain



Puf4:RNA
UGUAUAUUA

2.7 Å resolution, R/Rfree=20.0/27.2

In eukaryotes, gene expression is regulated from the birth of the pre-mRNA to the destruction of the message. In between, RNA-binding proteins play active roles in RNA processing, transport, localization, translation, and destabilization. Some RNA-binding proteins possess exquisite sequence specificity, and understanding these properties has kept both biochemists and structural biologists busy for many years. One mystery that made biochemical data difficult to reconcile with structural predictions was the **PUF** family of RNA-binding proteins. A crystal structure of one PUF protein, human **PUM1**, displayed a repeat structure with eight separate but similar α-helical motifs, and each of these motifs recognized one RNA base. But, a separate study found that although all 6 yeast PUF proteins have 8 α-helical repeats, some preferentially bound to 9 or 10 nucleotide motifs instead. Now, a study by Miller *et al.* (*Nat. Struct. Mol. Biol.* 2008 *15*, 397–402) tackles this problem by co-crystallizing the Puf4p protein with its favorite nine-nucleotide site from a yeast 3′ untranslated region.

The results were as expected for the protein structure. It adopted a similar fold to the previously solved PUM1 structure with eight curly helical repeats. When the authors looked at the RNA, however, the story was quite novel. The repeats on the end of the crescent-shaped domain bound to single nucleotides, but in the middle, two bases were not in contact with the protein repeats. One of these nucleotides, U5, was coaxially stacking with the adjacent base. In PUM, these two bases were spaced apart by sandwiching around an arginine, but with Puf4p possessing a cysteine at this position, this spacer was lost. In addition, an extra nucleotide was accommodated by flipping away from the binding interface. Most impressively, the authors then went on to perform a convincing swap experiment. Keeping an eye on the Puf4p structure, they scanned the amino acid sequence of the Puf3p protein, which prefers eight-nucleotide binding sites. Though the two yeast proteins possessed an overall homology of just 41%, the residues contacting the RNA in the structure were identical in 21 out of 24 cases. Using mutagenesis, they engineered a Puf4p protein with that key cysteine changed to an arginine, and a threonine changed to the cognate Puf3p cysteine. The result was a Puf4p that now bound preferentially to an eight-nucleotide site. With this knowledge in hand, one can envision chimeric PUF proteins engineered into the amenable model organism, yeast, to aid dissection of the cellular roles for this interesting fold. **Jason G. Underwood, Ph.D.**

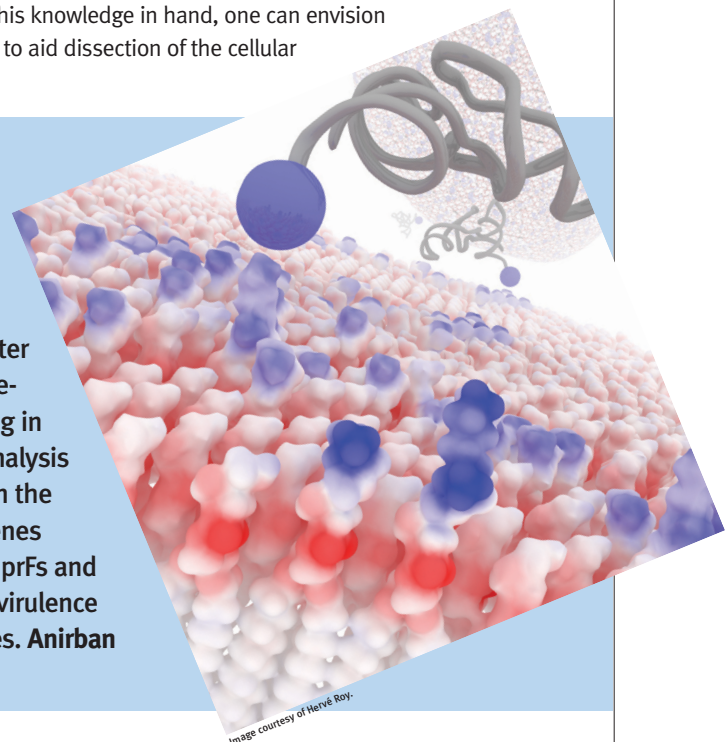


Image courtesy of Hervé Roy.

In translation, elongation factor Tu (Ef-Tu) forms a tightly bound ternary complex with aminoacyl-tRNA and GTP. Remarkably, the authors demonstrated that MprFs and Ef-Tu have similar binding affinities for aminoacyl-tRNAs. Although the exact details are yet to be elucidated, this suggested the tantalizing prospect of a ternary complex being involved in these metabolic processes.

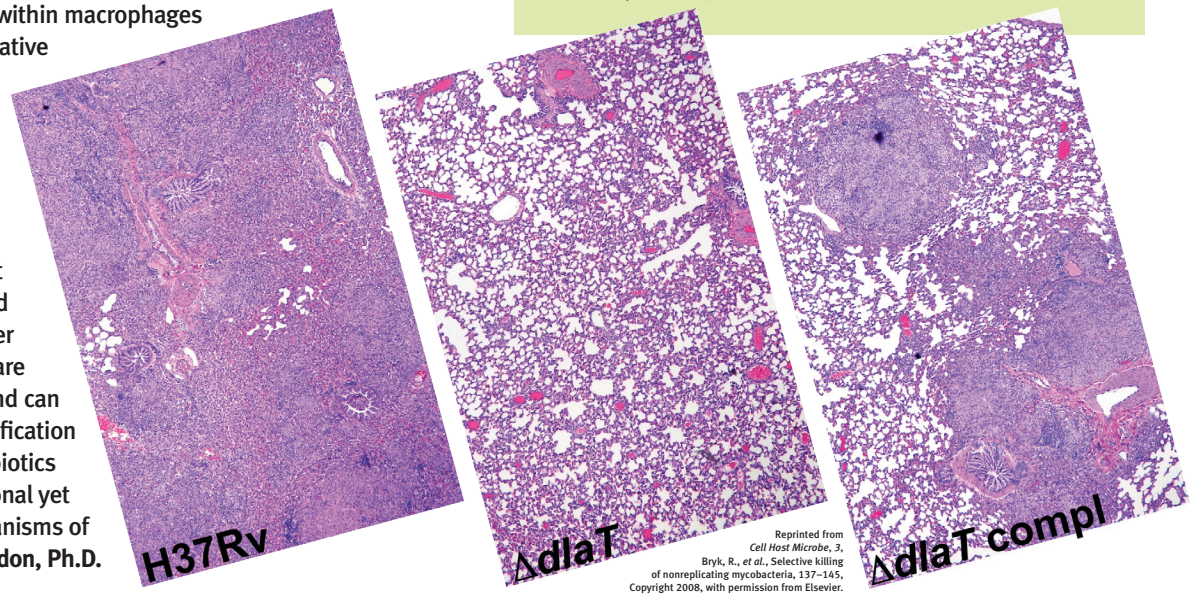
An unanswered question is why *C. perfringens* possesses two different MprFs while other studied organisms possess only one. As the authors note, studying how these genes are regulated might facilitate better understanding of the fine-tuning of lipid remodeling in this organism. Further analysis should also shed light on the transmissibility of the genes encoding the different MprFs and possible involvement in virulence in a wide range of species. **Anirban Mahapatra, Ph.D.**

Spotlight

Nonreplication of Replication Screens

In the search for desperately needed new antibiotics, assays are often designed to find molecules that prevent bacterial growth. Consequently, hits from these screens frequently act on protei ns involved in cell replication. However, many bacteria, such as *Mycobacterium tuberculosis* (Mtb), can persist as nonreplicating subpopulations that cause relapse or outbreaks during periods when the immune system is compromised. Thus, strategies to identify compounds capable of eradicating bacteria in a nonreplicating state are an appealing approach to the discovery of new antibiotics. Bryk *et al.* (*Cell Host Microbe* 2008, *3*, 137–145) report the identification of promising new drug leads from a screen designed to target nonreplicating mycobacteria.

The mycobacterial enzyme dihydrolipoamide acyltransferase (DlaT) is required for mycobacterial virulence and helps Mtb resist the stress caused by nitric oxide-derived reactive nitrogen intermediates, compounds produced by the host's immune system that can be toxic to Mtb. Using a colorimetric assay, the authors screened 15,000 small molecules for their ability to inhibit DlaT activity *in vitro*. Rhodanines, which contain a 2-thioxo-thiazolidi-4-one functionality, were found to be the most potent, and subsequent structure–activity and mechanistic studies led to the identification of the micromolar, rhodanine-containing DlaT inhibitor D157070. D157070 selectively killed Mtb under conditions where replication was halted, including low pH, the presence of nitric oxide, and decreased oxygen levels. Moreover, D157070 was effective against bacteria within macrophages as well, an imperative finding because nonreplicating mycobacteria typically reside in macrophages. These findings demonstrate that enzymes involved in processes other than replication are worthy targets and can lead to the identification of potential antibiotics with unconventional yet promising mechanisms of action. **Eva J. Gordon, Ph.D.**



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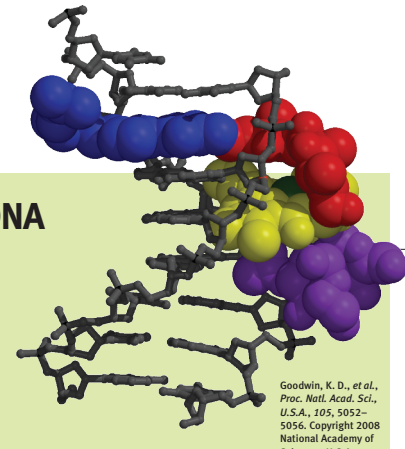
Crystallizing DNA Cleavage



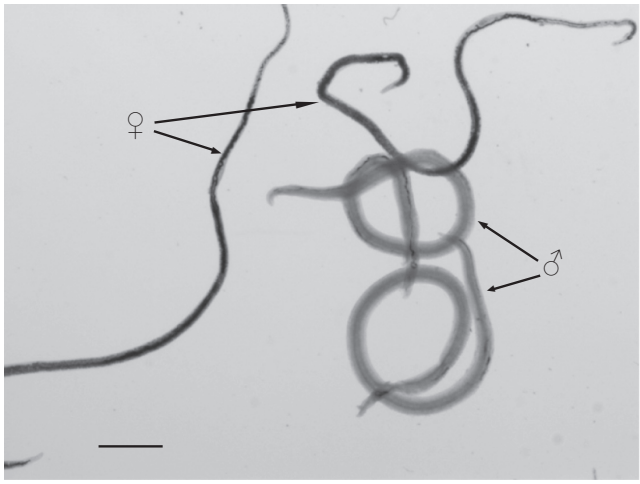
Goodwin, K. D., *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, *105*, 5052–5056. Copyright 2008 National Academy of Sciences, U.S.A.

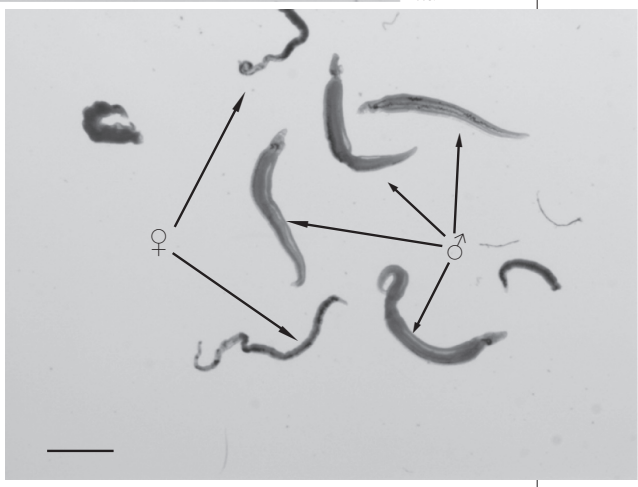
Isolated >40 years ago, the bleomycins are glycopeptide natural products that have found clinical utility in the treatment of various types of cancer. Their anticancer activity is linked to their ability to cleave DNA, but the molecular details behind their mechanism of action are not totally defined. Goodwin *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2008, *105*, 5052–5056) find new insight into how bleomycin interacts with DNA by solving the crystal structures of two oligonucleotides with and without a stable, cobalt-containing bleomycin analog.

Key functionalities in bleomycin that interact with DNA include a bithiazole moiety, a metal binding domain, and a disaccharide unit, and the crystal structures provided valuable structural details for each of these interactions. First, the bithiazole moiety of bleomycin was found to intercalate DNA in two different modes, and in contrast to other findings, it did not appear to interact with the minor groove. Rather, minor groove binding involved several hydrogen bonding interactions of the metal binding domain and the α-D-mannose of the disaccharide unit. Notably, NMR-derived models had suggested that the disaccharide did not make any direct contact with the DNA. In addition, the metal binding domain was embraced in a distorted square pyramidal arrangement, with the imidazole, the histidine amide, the pyrimidine, and the β-aminoalanine secondary amine forming the equatorial ligands and the β-aminoalanine primary amine providing an axial ligand. Finally, modeling studies indicate that the distal oxygen of an axially bound hydroperoxide ligand is ideally situated for C4′ hydrogen atom abstraction, a key step in the DNA cleavage reaction. Bleomycins have served as a model for DNA-cleaving agents as drugs, and these findings contribute additional insight into this important research area. **Eva J. Gordon, Ph.D.**

Flattening the Flatworm

Parasitic flatworms of the genus *Schistosoma* are responsible for schistosomiasis, a chronic disease that affects 200 million people worldwide. Although the drug praziquantel is an effective treatment for schistosomiasis, its extensive use could accelerate evolution of resistant strains, and no effective alternative therapeutics exist. Now Sayed *et al.* (*Nat. Med.* 2008, *14*, 407–412) report the identification of oxadiazole 2-oxides as potential new drugs for schistosomiasis treatment.





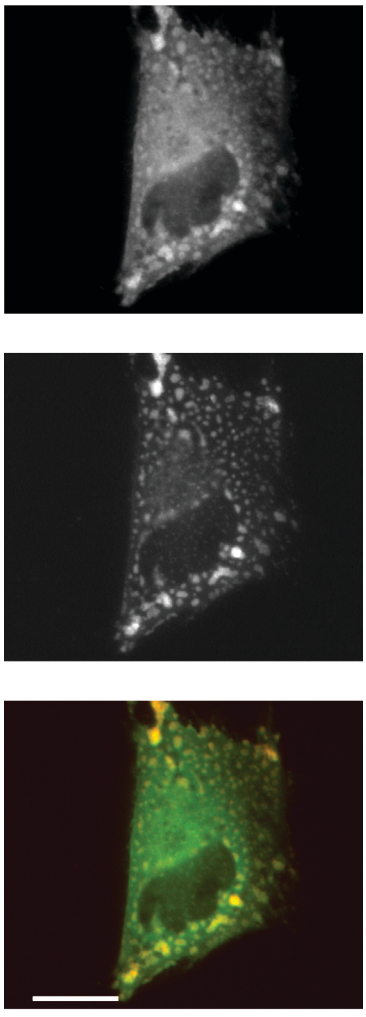
Ideally, a new drug for schistosomiasis would act on a drug target different from that of praziquantel to increase the efficacy of the drug alone and in combination should resistant strains emerge. The parasitic enzyme thioredoxin-glutathione reductase (TGR) is essential for parasite survival and helps the parasite manage exposure to reactive oxygen species while residing in its host. Previous screening studies revealed that oxadiazole 2-oxides and phosphinic amides were effective inhibitors of TGR, and thus these compounds were evaluated for their ability to inhibit TGR activity *in vitro*, their effect on live cultured worms, and their activity in parasite-infected mice. Although both classes of compounds could effectively inhibit recombinant TGR, the oxadiazoles were much more potent against live worms, with low micromolar concentrations resulting in death within days of exposure in several species of worms and in all developmental stages. Investigation of the mechanism of action of the most effective oxadiazole, compound *9*, suggested that its activity is linked to its ability to inhibit TGR and function as a nitric oxide donor. Finally, mice treated with compound *9* either 1, 23, or 37 days after infection (corresponding to different stages in the life cycle of the parasites) were protected from the effects of infection. On the basis of these encouraging results, further development of oxadiazoles as schistosomiasis drugs is underway. **Eva J. Gordon, Ph.D.**

Spotlight

The Quest for X

A cross-linking agent has the unique ability to find sites, either within a single protein or between two proteins, that are close to each other in space. The agent acts as a small molecular stapler that fastens the pieces together, providing clues to the conformation of single proteins or interaction sites between multiple proteins. Although this method has proven valuable for studying one protein or a small protein complex, identification of cross-linked proteins in larger samples has faced both experimental and computational challenges. It can be difficult to find the relatively small number of cross-linked peptides present in a complex peptide mixture, and no cross-link analysis programs are available that can manage the large number of possible peptide–peptide combinations. Tackling both the experimental and analytical challenges, Rinner *et al.* (*Nat. Methods* 2008, *5*, 315–318) present a method for identifying cross-linked peptides from complex samples and large protein sequence databases.

Software termed xQuest was developed to function as a search engine for cross-linked peptides. The software is designed to reduce the search space by first performing a low-stringency search for candidate peptides, followed by a stringent spectrum-matching recombination step. In addition, several tactics were developed to enrich both the spectra and the sample

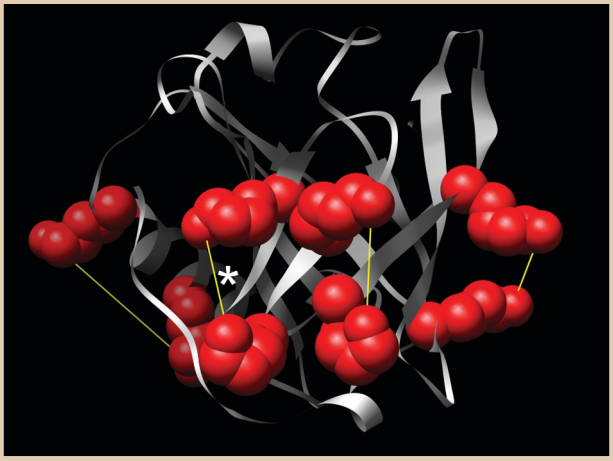


From An, S., *et al.*, *Science*, April 4 2008, DOI: 10.1126/science.1152241. Reprinted with permission from AAAS.

A Purine Machine

Purines, such as adenine and guanine, are essential components of many important biomolecules, including of course DNA and RNA. In humans, the biosynthesis of purine-containing nucleotides consists of 10 chemical steps performed by 6 enzymes, but little is known about how these enzymes work together in the cell. An *et al.* (*Science* 2008, *320*, 103–106) now report compelling evidence of a cellular purine machine, or "purinosome", that consists of a complex of all six enzymes in the cytoplasm of human cells.

The enzymes involved in purine synthesis include phosphoribosyl pyrophosphate amidotransferase, the trifunctional enzyme TrifGART, formylglycinamidine ribonucleotide synthase (FGAMS), the bifunctional enzyme PAICS, adenylosuccinate lyase, and the bifunctional enzyme ATIC. Green fluorescent protein fusion proteins were generated for each of these enzymes, and subsequently each was cotransfected into human cells with FGAMS tagged with orange fluorescent protein. Examination using fluorescence microscopy revealed that all six enzymes colocalized into clusters in the cytoplasm. Moreover, the formation of these clusters depends upon whether the cell must synthesize purines or whether it can obtain purines directly from the media surrounding the cell, at which time the clusters dissipate. This suggests that the multienzyme complex can respond dynamically to accommodate the needs of the cell. The authors propose that the enzyme clustering facilitates purine construction by physically linking the catalytic active sites and provides a cellular mechanism for regulating purine synthesis. Further examination of the function and regulation of the purinosome not only will enhance our understanding of purine biology but also could represent an intriguing new pharmacological target for diseases associated with purine biosynthesis. **Eva J. Gordon, Ph.D.**



for cross-linked peptides. First, isotopically labeled cross-linkers were used to incorporate a characteristic isotopic shift in cross-linked peptides. Second, the mass spectrometer was directed to highly charged ions upon the observation that the majority of identified cross-links had charge states >+3. Finally, also based on the highly charged state of many cross-linked peptides, strong cation exchange chromatography under acidic conditions was used to enrich the samples. The publicly available xQuest (www.xQuest.org), used in combination with these enrichment techniques, illuminates a much needed path for identifying cross-linked proteins in a high-throughput manner. **Eva J. Gordon, Ph.D.**

Mastering the Mitochondria

Mitochondria house the oxidative phosphorylation (OXPHOS) pathway that is responsible for ATP synthesis. Both the nuclear and mitochondrial genomes encode the ~90 proteins involved in OXPHOS, and transcriptional and translational regulation of these genes helps maintain energy homeostasis. In an effort to systematically examine the function and regulation of OXPHOS in intact cells, Wagner *et al.* (*Nat. Biotechnol.* 2008, *26*, 343–351) combine information from cell-based assays exploring OXPHOS physiology with nuclear and mitochondrial genomic profiling data.

In addition to a viability assay, four physiological assays, which examined mitochondrial membrane potential, mitochondrial dehydrogenase activity, cellular ATP levels, and reactive oxygen species (ROS) (a byproduct of OXPHOS), as well as genomic profiling of all 13 mitochondrial and 12 nuclear OXPHOS gene transcripts, were performed in cells treated with each of ~2500 small molecules. The resulting compendium was used to explore three aspects of OXPHOS as it relates to mitochondrial biology, drug toxicity, and drug discovery. It was determined that, although nuclear and mitochondrial OXPHOS gene expression is normally tightly coupled, small-molecule protein synthesis inhibitors disrupt this coordination by selectively increasing nuclear OXPHOS gene expression, and this increase ultimately led to a decline in cellular ATP levels. It was also observed that a subset of the statin family of cholesterol-lowering drugs causes a strong decrease in cellular ATP levels, and this decrease was even greater in the presence of the antihypertensive drug propranolol. The resulting mitochondrial toxicity points to a possible molecular explanation for the myopathic side effects associated with statin drugs. Finally, the compendium was searched for compounds that could have therapeutic value against type 2 diabetes and neurodegeneration, because these conditions are associated with a decrease in OXPHOS gene expression and an increase in ROS. Several microtubule modulators were found to increase OXPHOS gene expression while decreasing ROS, an indication that, in addition to the established role of many microtubule modulators as anticancer agents, these or related compounds could also have potential in the treatment of neurodegenerative disorders. **Eva J. Gordon, Ph.D.**