Spotlight

Turning Down the Malaria Knob

In many parts of the world, a bite from a mosquito can mean the risk of contracting *Plasmodium*, a parasitic eukaryote that infects red blood cells and causes malaria. Deploying mosquito nets and anti-malarial drugs to at-risk populations is helping the cause, but millions are still losing their lives to this illness. During infection, the *Plasmodium* parasite causes red blood cells to become rigid and more adhesive than normal cells, and these properties cause catastrophic consequences in the narrow vasculature, where blood needs to flow smoothly. Under an electron micrograph, a normal cell shows a smooth morphology, but an infected erythrocyte displays tiny raised knobs. These knobs, which contribute to rigidity, are made up of the *Plasmodium*-encoded knob-associated histidine-rich protein, or KAHRP, while the adherence properties are mediated by the extracellular protein PfEMP1. But how do these proteins get from the *Plasmodium* cell to the outside of the infected host cell? A new screen (Maier *et al.*, *Cell* 2008, *134*, 48–61) takes aim at candidate genes from *Plasmodium falciparum* that might act as helpers for getting these proteins to the cell surface.

The list consisted of known exported proteins, proteins that encoded an export signal, and proteins whose messenger RNAs were transcribed during the blood-borne stage of the parasite. In an impressive brute-force approach, 53 of the 83 candidates were successfully knocked out by homologous recombination. The authors postulate that perhaps many of the other 30 are essential for blood-borne growth. With 53 knockout strains in hand, the authors then subjected them to a battery of tests during erythrocyte infection and found that the various genes clustered into a few different phenotypes. By looking for PfEMP1 on the surface, they found 10 genes that lowered the amount of this sticky protein that was trafficked to the cell's surface. Adhesion assays confirmed that many of these *Plasmodium* knockout-infected erythrocytes had lower adhesion properties. In another class of knockouts, a complete loss of the knob protrusions was observed under an electron microscope. The final class of gene knockouts altered the rigidity phenotype as measured by deforming the cells under sheer forces and using a technique known as laser-assisted optical rotation analysis. Just a handful of genes had a large effect on the rigidity, an indication that many genes probably make a small contribution and that the additive effect causes the stiff cells. This study, unprecedented in depth in the *Plasmodium* community, cast a wide net to determine the players involved in pathogenesis, and now the follow-up biology on many of these genes with unknown function can begin. Importantly, these dozens of genes represent new possible targets to help eradicate this deadly parasite in humans. **Jason G. Underwood, Ph.D.**

Image courtesy of Alan Cowman.

Probing Progerias

Progerias are rare genetic disorders, including Hutchinson–Gilford progeria syndrome (HGPS), in which the aging process is markedly accelerated. These disorders have been associated with the accumulation at the nuclear envelope of either farnesylated versions of the protein prelamin A or its truncated form, progerin. In progeroid mouse models, use of farnesyltransferase inhibitors (FTIs) has resulted in modest yet encouraging improvements in premature aging symptoms, intimating that this strategy has potential for the treatment of these devastating conditions. Varela *et al.* (*Nat. Med.* 2008, *14*, 767–772) probe the molecular basis for the limited activity of the FTIs and unveil a new strategy for treatment of progeroid syndromes.

The authors hypothesized that in the presence of FTIs, prelamin A might be alternatively

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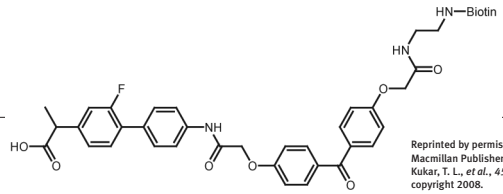
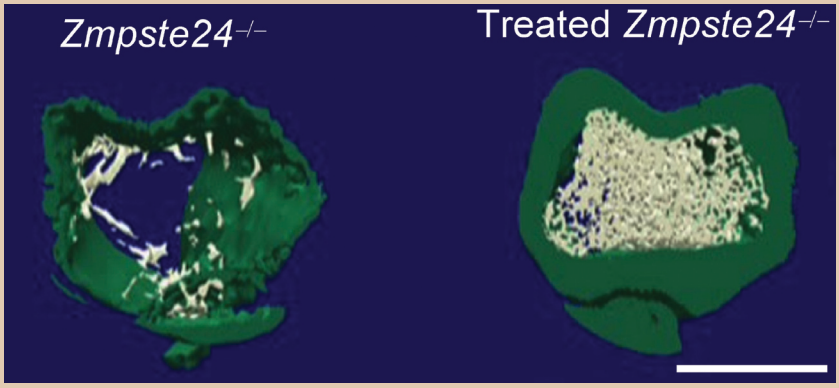


Substrate Targeting

Drug discovery strategies often revolve around targeting enzymes that play a key role in the pathogenesis of a disease. For example, in Alzheimer's disease research, much effort has focused on inhibiting the enzymes β- and γ-secretase, which are involved in production of the amyloidogenic peptide Aβ. Exploring the mechanism of action of a group of small molecules, termed GSMs, known to modulate the activity of γ-secretase, Kukar *et al.* (*Nature* 2008, *453*, 925–929) discover that these compounds actually target γ-secretase's substrate rather than the enzyme itself.

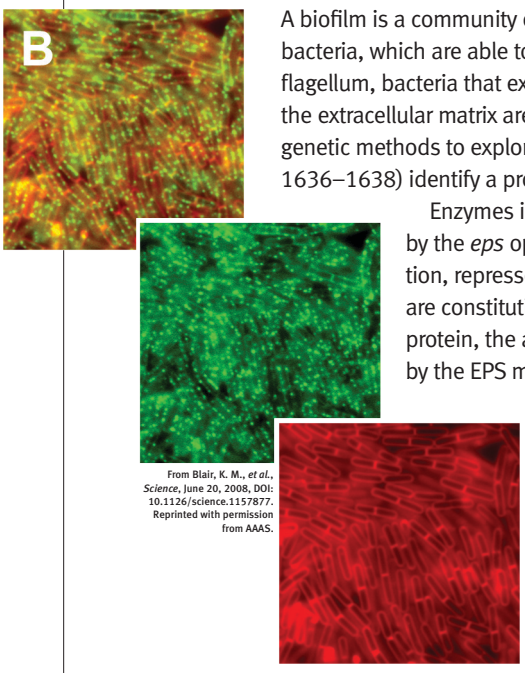
Two GSM derivatives, each containing a benzophenone moiety for photoaffinity protein labeling and a biotin group for detection and purification, were synthesized to determine the molecular target of the GSMs. Surprisingly, incubation with human neuroglioma cells followed by photolysis and precipitation with streptavidin revealed that the only component of the γ-secretase complex labeled by the GSM derivatives was the substrate, β-amyloid precursor protein (APP). Subsequent experiments with truncated APP fragments suggested that the GSMs bind to specific residues in APP that are found in the Aβ product and have been implicated in aggregation of Aβ. On the basis of this finding, other small molecules known to bind to Aβ were shown to have GSM activity, including the amyloid dye X-34. Indeed, oral administration of X-34 into APP transgenic mice resulted in decreased levels of soluble Aβ42 in the brain. The authors propose that these substrate-targeting GSMs may shift cleavage of APP by changing its position in the membrane such that alternative APP fragments are produced. Taken together, the data suggest that the substrate-targeting GSMs function by two mechanisms: altering Aβ production levels and preventing Aβ aggregation. These dual-action compounds thus point to a promising new therapeutic approach for creating drugs against Alzheimer's disease. **Eva J. Gordon, Ph.D.**

prenylated by geranylgeranyltransferase. Indeed, Western blot and mass spectrometry analysis of experiments using inhibitors of both farnesyltransferase and geranylgeranyltransferase I revealed that both prelamin A and progerin can be prenylated by geranylgeranyltransferase in the absence of farnesyltransferase activity. Thus, targeting the metabolic pathway responsible for both farnesylation and geranylgeranylation might be a more effective treatment strategy than preventing farnesylation alone. Because statins and aminobisphosphonates block two different stages of the biosynthesis of farnesyl pyrophosphate, which is a precursor for both farnesylation and geranylgeranylation, a combination of a drug from each class was tested for its effect on HGPS cells and progeroid mouse models. In cells, the drug combination, but not either drug alone, effectively inhibited farnesylation and geranylgeranylation and led to clear improvements in nuclear localization of prelamin A and nuclear morphology. In progeroid mouse models, treatment with the drug combination led to substantial recovery of several aging-like phenotypes, including improved body weight and bone density, reduced hair loss, and increased lifespan. These exciting results point to a promising new therapeutic approach for treating individuals with progeria syndromes. Eva J. Gordon, Ph.D.



Spotlight

A Clutched Community



From Blair, K. M., *et al.*, *Science*, June 20, 2008, DOI: 10.1126/science.1157877. Reprinted with permission from AAAS.

A biofilm is a community of microorganisms that is encapsulated in an extracellular matrix. In contrast to individual bacteria, which are able to roam the environment thanks to tiny molecular motors located at the base of each flagellum, bacteria that exist in biofilms are nonmotile. While it has been observed that motility and synthesis of the extracellular matrix are often oppositely regulated, the factors that dictate motility are not well defined. Using genetic methods to explore the regulation of motility in the bacteria *Bacillus subtilis*, Blair *et al.* (*Science* 2008, *320*, 1636–1638) identify a protein that functions like a molecular clutch.

Enzymes involved in the biosynthesis of the matrix extracellular polysaccharide (EPS) are encoded by the *eps* operon. The transcription factor SinR, which alternately controls motility and biofilm formation, represses transcription of genes in the *eps* operon. Interestingly, *sinR* mutants in which *eps* genes are constitutively derepressed are also nonmotile. Using bacteria containing a fluorescent flagellar protein, the authors demonstrated that the *sinR* mutants produce nonfunctional flagella concealed by the EPS matrix. Further genetic studies revealed that the *sinR* mutants are nonmotile specifically because of the constitutive derepression of EpsE, a putative family II glycosyltransferase. By selecting for mutants that could render motility insusceptible to EpsE inhibition, they found that EpsE arrests flagellar rotation through interaction with FliG, a protein similar to the flagellar motor component of *Escherichia coli*. By tethering cells by a single flagellum and measuring flagellar rotation by the counter-rotation of the cell body, they determined that EpsE acts like a clutch; it causes the flagella to behave as though they are unpowered but not immobilized. The authors hypothesize that the clutch helps stabilize the biofilm and ensures that flagella do not rotate while the cells are encapsulated in the EPS matrix. **Eva J. Gordon, Ph.D.**

Counterpoint: Minor Splicing

At the end of 2007, König *et al.* reported a dogma-breaking hypothesis to the scientific community: pre-mRNAs in the cytoplasm (*Cell* 2007, *131*, 718–729). The study focused on the minor spliceosome, a macromolecular RNA and protein machine responsible for removing a small subset of introns in plants and animals. These introns contain splice signals different from those present in canonical major spliceosome introns. The data indicated that two key components of the minor spliceosome, U12 and U6atac small nuclear RNAs (snRNAs), were localized to the cytoplasm both in zebrafish and in mouse tissue culture cells. In addition, data were presented wherein minor introns were found unspliced both in the nucleus and the cytoplasm, so somehow unspliced messages were being exported before splicing was complete. Because capped RNAs in the cytoplasm should be read by the ribosome and intron-containing RNAs would likely be degraded by non-sense-mediated decay, a number of questions immediately arose from this new hypothesis.

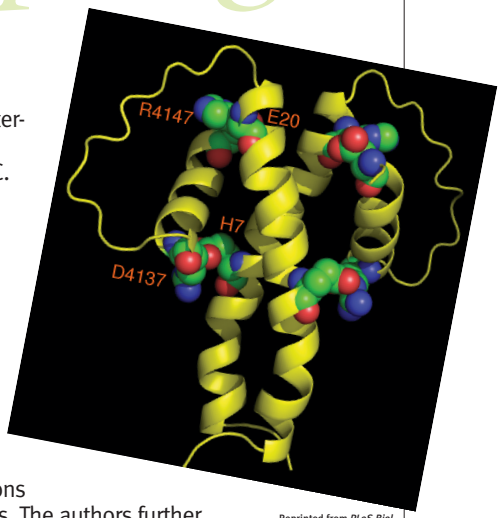
Now, the other side of the coin has been published in two peer-reviewed journals, and the release of these publications coincides with a critical essay and literature review on the subject (Steitz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 2008, *105*, 8485–8486). The last is coauthored by many principal investigators who have made the minor spliceosome a part of their scientific careers. The group that published the cytoplasmic spliceosome data was also given space for a counterpoint to the essay by Steitz *et al.*

The two peer-reviewed publications take on the controversy with different approaches, but both conclude that the minor spliceosome is localized in the nucleus and that it probably carries out its catalytic function there. Friend *et al.* (*RNA* 2008, *14*, 1459–1462) appropriately utilize the same *Xenopus* oocyte injection system used by Mattaj and DeRobertis >20 years ago to show how snRNAs are trafficked and how snRNPs mature. They found that the common minor splicing substrate could be spliced in the injected nuclei, albeit inefficiently, as is usually the case for minor splicing reactions in nuclear extract. They detected no splicing of the pre-mRNA when the cytoplasm was injected, nor did they find spliced RNA in the cytoplasm when the nucleus had been injected with the radiolabeled pre-mRNA. Further, they showed that by sequentially adding a specific oligonucleotide that destroys

Modularly Iterative

Polyketide synthases (PKSs) are fascinating multifunctional enzymes responsible for the biosynthesis of various complex metabolites. Type I PKSs are composed of modules containing several catalytic domains on a single polypeptide chain and generally come in two flavors, modular and iterative. Modular type I PKSs synthesize their products using each domain only once, whereas iterative type I PKSs repeatedly use the same active sites to generate their polyketides. In *Mycobacterium tuberculosis*, PKS12 is thought to be responsible for the biosynthesis of the phosphoglycolipid mannosyl-β-1-phosphomycoketide (MPM). The structure of MPM hints that an iterative mechanism may be employed. However, PKS12 is composed of two modules, and for iterative catalysis to take place, an unprecedented long-distance chain transfer between the two modules would have to occur. Using a combination of biochemical, computational, and biophysical methods, Chopra *et al.* (*PLoS Biol.*, published online July 8, 2008; DOI:10.1371/journal.pbio.0060163) propose that PKS12 uses a novel "modularly iterative" mechanism for MPM biosynthesis.

To deconstruct the MPM biosynthetic mechanism, engineered PKS12 constructs were created and cell-free reconstitution studies were performed. The substrate specificity and synthetic capabilities of each PKS12 module were established by characterizing the products with radioactive thin-layer chromatography and HPLC. However, how the long-distance chain transfer event required for an iterative biosynthetic mechanism to occur was still not clear. Thus, the structural characteristics of PKS12 were explored using computational models, analytical ultracentrifugation, mutagenesis, and atomic force microscopy. These studies suggested that PKS12 forms a large supramolecular assembly through intermolecular interactions between the N- and C-termini helices. The authors further demonstrated that an acyl chain could be directly transferred from one PKS12 protein to another, clearly indicating that an intermolecular interaction can transpire. Thus, the authors propose that PKS12 uses a hybrid "modularly iterative" mechanism to achieve the repetitive condensations required to generate polyketides like MPM. These findings highlight the incredible versatility of PKSs and offer a new perspective on how PKSs create their diverse repertoire of unique polyketide structures. **Eva J. Gordon, Ph.D.**



Reprinted from *PLoS Biol.* Chopra, T., *et al.* Novel intermolecular iterative mechanism for biosynthesis of mycoketide catalyzed by a bimodular polyketide synthase, DOI: 10.1371/journal.pbio.0060163.

the minor spliceosome's catalytic power and then the pre-mRNA, all minor splicing could be abolished. The cytoplasmic spliceosome study had reported that minor splicing is not inhibited during mitosis, so the Friend *et al.* study took a look at this by inducing nuclear membrane breakdown of the oocyte with progesterone. This event was indeed accompanied by a loss in splicing efficiency. Finally, subcellular fractionation was used to determine that the snRNAs from both the minor and major spliceosomes were predominantly nuclear.

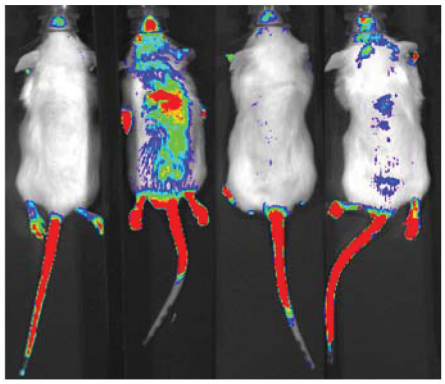
In an impressive showing of histology, the second publication (Pessa *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 2008, *105*, 8655–8660) scrutinized the *in situ* hybridization data of the cytoplasmic spliceosome study. They utilized brain and cartilage tissues from mouse embryos coupled with full-length RNA probes instead of shorter locked nucleic acids. They used a battery of probes against the major and minor class spliceosomes and a sense control probe to show that all of the snRNAs display an almost exclusively nuclear pattern. A more detailed look came from fixed HeLa cells with fluorescent probes against minor spliceosome snRNAs. These also showed the nuclear speckled pattern that is well known for the major snRNAs. Like the Friend *et al.* study, these researchers also turned to subcellular fractionation but took an extra step after finding the minor snRNAs in a similar, mostly nuclear distribution to their major snRNA counterparts. They performed immunoblots with antisera against several proteins that are found only in the minor spliceosome and showed that these too were found almost exclusively in the nuclear fractions.

These two papers make the burden of proof far more challenging for the cytoplasmic camp. The components for both major and minor splicing seem to all live together in the nucleus. With just a handful of introns utilizing the minor spliceosome, it may be that there is a kinetic race between nuclear export and minor splicing and this is why König *et al.* found introns in the cytoplasm. Other introns in a multiexonic pre-mRNA may be spliced by the major spliceosome and marked with the exon-junction complex that acts as a zip code to the cytoplasm. In a kinetic race scenario, this RNA leaves the nucleus before the splicing of the minor intron, and this unspliced intron arrives in the cytoplasm only to die under the sword of nonsense-mediated decay. Jason G. Underwood, Ph.D.

Spotlight

Innate Interference

Our innate immune system relies on intricate, highly regulated pathways to fight off invading pathogens. For example, Toll-like receptor 4 (TLR4) recognizes the lipopolysaccharide (LPS) present in the outer membrane of Gram-negative bacteria. This interaction leads to activation of the transcription factor NF-κB, which induces expression of several pro-inflammatory cytokines, including tumor necrosis factor α (TNF). Some pathogens, however, like the opportunistic bacteria *Pseudomonas aeruginosa*, manage to overcome this innate immune response and establish persistent infection. Kravchenko *et al.* (*Science* 2008, *321*, 259–263) explore how *P. aeruginosa* affects the response of immune cells and reveal how a small molecule can interfere with the innate immune system.



From Kravchenko, V. V., *et al.*, *Science*, July 11, 2008, DOI: 10.1126/science.1156499. Reprinted with permission from AAAS.

Activation of TLR pathways can result in numerous important events, such as degradation and resynthesis of the inhibitor of NF-κB alpha (IκBα); phosphorylation of several proteins, including p38, a subunit of IkB proteins termed RelA, and IκBα; and induction of NF-κB-responsive genes. In an initial experiment, a significant delay in IκBα resynthesis was observed in cells exposed to *P. aeruginosa* but not other pathogenic bacteria. *P. aeruginosa* synthesizes a small quorum sensing signaling molecule *N*-(3-oxo-dodecanoyl) homoserine lactone (C12), and the authors hypothesized that C12 may be responsible for the altered regulation of the NF-κB pathway. Indeed, subsequent experiments revealed that C12 selectively disrupts the activity of the IkB kinase complex, which is responsible for phosphorylating RelA and IκBα, and impairs the induction of NF-κB-responsive genes. Moreover, C12 significantly suppressed LPS-induced responses in transgenic mice harboring a luciferase reporter driven by NF-κB responsive promoter of the IκBα or TNF gene. Thus, by disrupting NF-κB signaling pathways, C12 effectively interferes with the innate immune response, to the benefit of the pathogen. **Eva J. Gordon, Ph.D.**

Small-Molecule Reprogrammers

Recent discoveries have demonstrated that expression of four transcription factors, Oct4, Klf4, Sox2, and c-Myc, leads to reprogramming of both mouse and human somatic cells to cells that closely resemble embryonic stem (ES) cells, albeit with low efficiency. These discoveries have diminished much of the ethical controversy involved in ES cell research, facilitating realization of its tremendous therapeutic potential. Huangfu *et al.* (*Nat. Biotechnol.* 2008, *26*, 795–797) now report that specific small molecules can improve the reprogramming efficiency of mouse embryonic fibroblasts (MEFs).

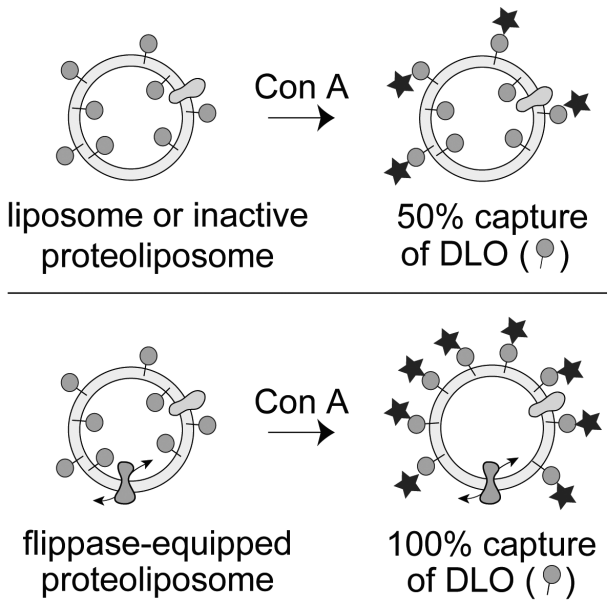
On the basis of recent evidence that inhibitors of histone deacetylase (HDAC) and DNA methylation moderately improved the induction efficiency of an alternative method for creating ES cells, somatic cell nuclear transfer, the authors hypothesized that small molecules known to affect chromatin modification may also enhance reprogramming by transcription factors. Upon surveying several compounds known to inhibit either DNA methylation or HDAC, they found that the HDAC inhibitor valproic acid (VPA) improved the induction efficiency of MEFs >100-fold. Moreover, they found that in the presence of VPA, efficient induction could be accomplished in the absence of c-Myc expression, an oncogene whose presence could be tumorigenic. Though treatment with VPA alone is not sufficient to reprogram MEFs, microarray analysis indicated that exposure to VPA does up-regulate ES-specific genes and down-regulate MEF-specific genes. The authors propose that chromatin modification is a key step in reprogramming fibroblasts to pluripotent cells. These exciting results suggest that additional small-molecule screening could lead to the identification of other compounds capable of inducing pluripotent stem cells, perhaps without the need for forced expression of transcription factors. **Eva J. Gordon, Ph.D.**

Finding the Flip-Floppers

Many important biological processes, including membrane biogenesis, protein glycosylation, and glycosylphosphatidylinositol (GPI) anchoring, rely on the transfer, or flip-flop, of lipids across the endoplasmic reticulum (ER). Specific flippases have been proposed to facilitate this process, but none have been definitively identified. Sanyal *et al.* (*Biochemistry* 2008, *47*, 7937–7946) report the development of an assay that will help identify the flippase responsible for transferring the glycolipid Man5GlcNAc2-PP-dolichol (M5-DLO), a precursor of the oligosaccharide donor for protein N-glycosylation, across the ER membrane.

M5-DLO is synthesized on the cytoplasmic face of the ER, but the remaining portion of the protein N-glycosylation oligosaccharide donor Glc3Man9GlcNAc2-PP-dolichol is synthesized on the lumen face. Toward identifying the mechanism responsible for the transfer of M5-DLO across the membrane, the authors developed an assay that uses the mannose binding protein concanavalin A (Con A) as an M5-DLO capture agent and proteoliposomes derived from Triton X-100 solubilized yeast ER membrane proteins as a source of the flippase. Because Con A can interact with M5-DLO present on the outer leaflet but not the inner leaflet of the proteoliposomes, most of the M5-DLO will be captured by Con A as it is transferred across the membrane, provided the flippase is present.

Using this assay, the authors demonstrated that the M5-DLO flippase does not require metabolic energy and is sensitive to trypsin; it is also specific for M5-DLO because it transports M7-DLO much more slowly. In addition, the M5-DLO flippase was shown to



be distinct from the flippase responsible for transferring glycerophospholipids across the membrane. These flippases could be separated using velocity sedimentation and dye resin chromatography, providing methods for their purification and identification. Notably, these flippases were also distinct from Rft1, a protein that has been proposed to be the M5-DLO flippase on the basis of genetic experiments. These studies provide innovative new tools for characterizing this intriguing class of transporters. **Eva J. Gordon, Ph.D.**