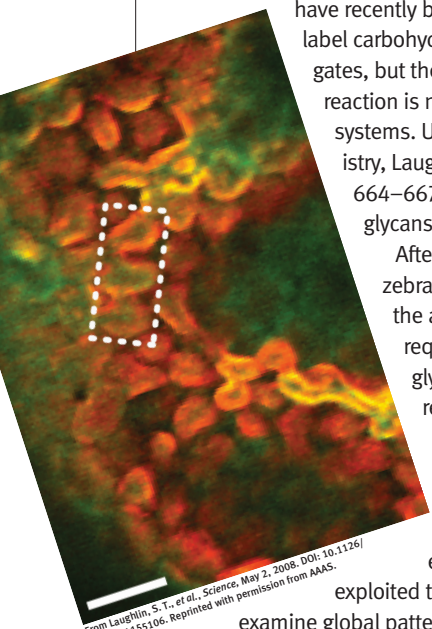
Spotlight

The Sweeter Side of Zebrafish

In vivo imaging is a powerful method for investigating dynamic cellular processes, such as protein trafficking and metabolic activity. Though genetic methods are often exploited to generate fluorescent proteins for *in vivo* imaging, glycans are not amenable to this labeling strategy. Chemical reporter methods have recently been developed to fluorescently label carbohydrates on cell surface glycoconjugates, but the copper catalyst required for the reaction is not compatible with use in living systems. Using newly developed chemistry, Laughlin *et al.* (*Science* 2008, *320*, 664–667) now present *in vivo* imaging of glycans in developing zebrafish.



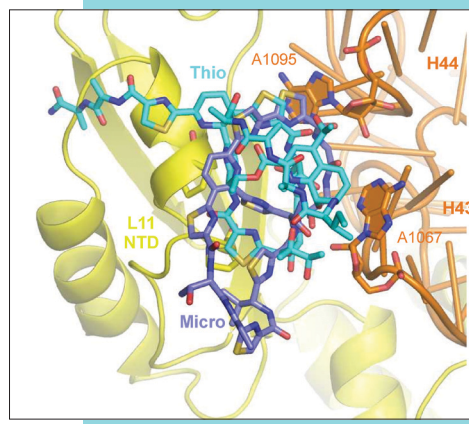
From Laughlin, S. T., *et al.*, Science, May 2, 2008. DOI: 10.1126/science.1155106. Reprinted with permission from AAAS.

After it was demonstrated that zebrafish could indeed incorporate the azide-containing carbohydrates required for glycan labeling into glycoproteins, a copper-free click reaction using difluorinated cyclooctyne reagents enabled covalent attachment of a fluorophore to the azide-bearing glycoproteins in zebrafish embryos. Subsequent studies exploited these labeled glycoproteins to examine global patterns of glycosylation over a 5-day period. Remarkably, labeled glycans appeared as early as 24 h after fertilization and were observed in the jaw, pectoral fins, and olfactory organs by 72 h. Next, multicolor detection experiments were used to investigate the temporal regulation of glycan expression in more detail. Conveniently, the fluorescent reagents used to label the glycoproteins are not cell-permeable. Thus, embryos could be labeled with one fluorophore, unreacted azides could be chemically capped, and then a second reagent containing an orthogonal fluorophore could be added after a desired amount of time. These studies revealed intriguing time-dependent patterns of glycan expression in various locations such as the jaw region, the hair cells surrounding the head of the embryo, and the olfactory organ. This innovative approach for exploring differences in the dynamics of cell-surface expression, intracellular trafficking, and tissue distribution of glycans in zebrafish embryogenesis can be extended to the examination of other glycans during zebrafish development as well as the investigation of glycan expression in other organisms. **Eva J. Gordon, Ph.D.**

Antibiotics Flip a Switch

By throwing a wrench in the ribosome, many antibiotics, old and new, shut down microbial growth. When the first high-resolution structures of the prokaryotic ribosome were published 8 years ago, a new emphasis on structural characterization of antibiotics complexed with the protein synthesis machine emerged. Previous biochemical footprinting experiments often showed where an antibiotic bound the ribosome, but a structural picture helps with understanding the drug's molecular contacts and how resistant bacterial strains might escape demise. A new set of X-ray structures by Harms *et al.* (*Mol. Cell* 2008, *30*, 26–38) takes a look at the thiopeptide antibiotics and finds, as expected, that these molecules function away from the peptidyl transfer active site.

The antibiotics thiostrepton (Thio) and the related nosiheptide (Nosi) and micrococcin (Micro) use mostly hydrophobic interactions and make specific contacts with 23S ribosomal RNA and proline residues on the ribosomal protein L11. These sites are located in the GTPase-associated center (GAC), which is responsible for binding to critical translation factors during elongation. Using the structures and a previous cryo-EM structure of the ribosome bound to elongation factor G (EF-G), the authors demonstrate why the antibiotics muck with the GAC. The protein L11 contains two structured regions separated by a flexible hinge, and the antibiotics Thio and Nosi can interact with the N-terminal domain to restrict its conformational flexibility. This flexibility is an on–off switch to accommodate binding of EF-G at the GAC. In contrast, Micro binds at the similar site but promotes an on-switch-like state where L11 and L7 are brought together. This resembles the activated conformational state for L11, but the loss in flexibility uncouples the GTP hydrolysis power of EF-G during the ribosomal elongation cycle. In all kingdoms of life, the protein L11 displays a two-domain flexibility, so the multiple-state model is probably a feature of eukaryotic translation as well. It appears that eukaryotes avoid ribosomal shutdown by thiopeptides because both a critical rRNA adenosine and the critical L11 prolines are not present in eukaryotic ribosomes.



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Resistant bacterial strains use rRNA mutation or methylation to reduce the drug's binding. This study contributes to our general knowledge of the ribosome's workings, but it may also aid in designing better thiopeptides or ones with simpler architecture. Jason G. Underwood, Ph.D.

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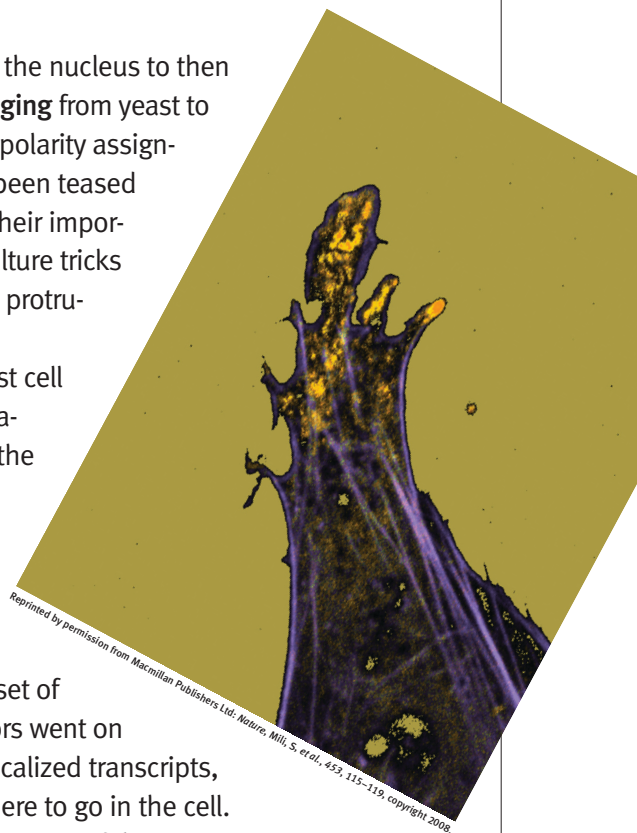
RNA Points the Way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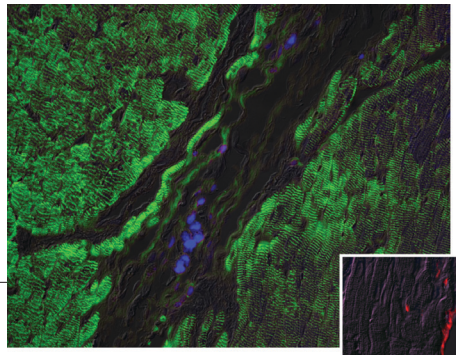
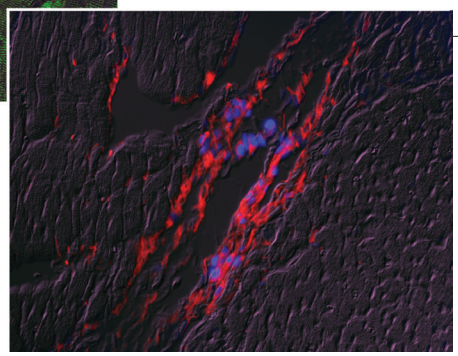
A growing body of work suggests that many eukaryotic mRNAs leave the nucleus to then be shuttled off to a specific location in the cytoplasm. In species **ranging** from yeast to humans, localized messages play various important functions, from polarity assignment to receptor abundance. Although a handful of examples have been teased apart in mammalian cells, a full landscape of localized mRNAs and their importance to the cell remain a challenge. A recent study combined cell culture tricks with microarray detection to identify mRNAs enriched in cytoplasmic protrusions.

Mili *et al.* (*Nature* 2008, *453*, 115–119) chose the mouse fibroblast cell line, NIH 3T3, which under specific growth conditions, shows a migratory phenotype with extension of pseudopodial protrusions. Plating the cells on a porous growth surface over a secondary reservoir, which contained a chemoattractant or an extracellular matrix component, caused cells to extend protrusions through the pores and into the lower chamber. Then the cell bodies above or the protrusions below were isolated, and RNAs were prepared from the fractions. Hybridization of complementary DNA to microarrays revealed that a set of 50 mRNAs was significantly enriched in the pseudopodia. The authors went on to test a subset of these transcripts and showed that, like in other localized transcripts, the 3′ untranslated region often acts as a zip code to tell the RNA where to go in the cell. Hooking this region to a β-globin mRNA was sufficient to cause localization of the synthetic transcript to cell protrusions. Using a clever trick, they then fused this synthetic gene to tandem MS2 phage RNA-binding sites. A coexpressed GFP–MS2 fusion protein bound to the transcript, enabling visualization of the RNA and revealing its accumulation in small granules at the edge of the cytoplasmic extensions.

With this assay in hand, the authors used several complementary techniques to investigate the nature of these interesting granules. Drug treatments that unraveled the microtubules known to exist in protrusions or antibody co-stains to known tubulin types found that the mRNA granules anchored onto the plus end of detyrosinated microtubules, also known as Glu-microtubules. A candidate gene, APC, emerged because it was among the few tubulin-binding proteins known to bind near this end of Glu-microtubules in leading-edge protrusions. Using immunoprecipitation, colocalization with the MS2 granules, and RNA interference knockdown of APC, the authors make a convincing argument for APC's involvement in binding localized RNA complexes and anchoring them at the cell protrusion. This study not only breaks exciting new ground in identifying localized mRNAs but also indicates a novel function for APC, a protein with a well-established role as a tumor suppressor. **Jason G. Underwood, Ph.D.**





Sadek, H., *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, *105*, 6063–6068.
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Taking Screening to Heart

Among the many potential clinical applications of stem cell therapy, diseases that affect the heart are prime candidates for treatment with stem cells committed to becoming cardiac cells. Delineating the pathways that govern cardiomyogenesis is critical to the successful implementation of cardiovascular stem cell therapy, but the molecular mechanisms involved are not well understood. Sadek *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2008, *105*, 6063–6068) strive to get at the heart of cardiomyogenesis and possibly find new drugs in the process, by screening small molecules for their ability to direct pluripotent stem cells toward a cardiac fate.

The screen was designed to find activators of *Nkx2.5,* one of the earliest genes expressed in cardiovascular progenitor cells. From nearly 150,000 compounds screened, a family of sulfonylhydrazones was found to promote *Nkx2.5* expression in pluripotent mouse embryonic stem cells. Additional gene expression studies revealed that this class of compounds also activated three other key cardiac markers, *brachyury-T*, *myocardin*, and sarcomeric α-tropomyosin. Investigation into the mechanism by which the sulfonylhydrazones promote cardiogenic differentiation ruled out several candidate pathways known to be involved in stem cell differentiation, an indication that these molecules may use an as-yet undiscovered pathway for activating the cardiac genome. Despite their somewhat mysterious mechanism of action, sulfonylhydrazone treatment of human adult stem cells induced expression of several key cardiac markers, prompting investigation of the effects of these compounds in an animal model of heart disease. Indeed, use of sulfonylhydrazone-treated cells as xenografts in a rat heart cryoinjury model resulted in significant improvement in cardiac function that was correlated with increased expression of human cardiac mRNAs in the xenografts. These compounds are promising leads for drugs to treat various types of heart disease, as well as valuable new tools in the continuing quest to understand cardiac cell differentiation. **Eva J. Gordon, Ph.D.**

Micromanaging microRNAs

microRNAs (miRNAs) are small naturally occurring RNAs that post-transcriptionally modulate the expression of protein-coding genes. These noncoding RNAs are a major area of study in part because many have been implicated in the onset and progression of human diseases. To date, however, targeting miRNAs for therapeutic purposes has been problematic. Now, Elmén *et al.* (*Nature* 2008, *452*, 896–899) demonstrate that the systemic delivery of locked-nucleic-acid (LNA) oligonucleotides potently antagonizes a liver-expressed miRNA in nonhuman primates.

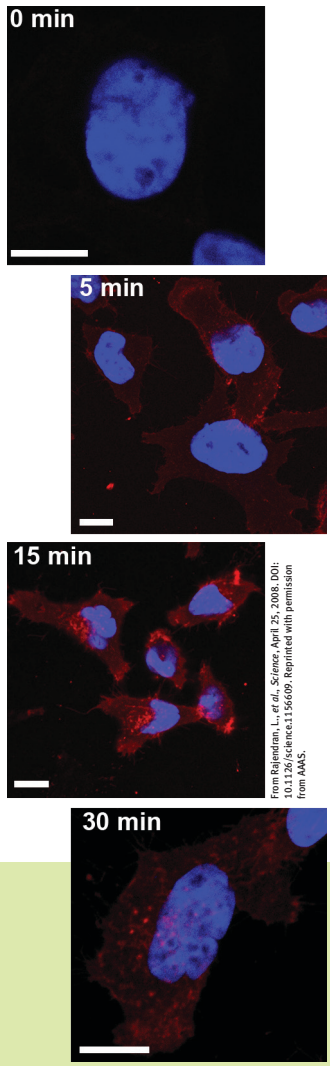
First, the authors tested a number of different LNA-modified DNA oligonucleotides for antagonism of miR-122 in a cultured cell line. The target miRNA, miR-122, is clinically relevant because it is associated with cholesterol and lipid metabolism and also with hepatitis C virus replication. The authors noted that the level of inhibition depended on the affinity of the antagonist to miR-122. In their screen, they found one high-affinity oligonucleotide that was more potent than others tested. In addition, the authors found that delivery of LNA-modified oligonucleotides with a complete phosphorothioate backbone enabled potent miR-122 antagonism compared with those with a mixed phosphorothioate–phosphodiester or an unmodified phosphodiester backbone.

Next, the authors tested the LNA-modified oligonucleotide in an obesity mouse model and found specific and marked derepression of miR-122 targets as well as a decrease in total cholesterol. Having tested the antagonist in cell lines and in mice, the authors then performed a thorough analysis in primates. African green monkeys

Unlocking a β-Secretase Secret

The molecular basis of Alzheimer's disease revolves around production of the toxic peptide Aβ, which is derived from the β-amyloid precursor protein (APP). The membrane protein β-secretase is a key enzyme involved in Aβ production and is known to cleave APP in endosomes. Many β-secretase inhibitors have been found to work well against the enzyme *in vitro*, only to fail in cells. Speculating that the secret behind such failures could be a lack of sufficient inhibitor concentration in endosomes, Rajendran *et al.* (*Science* 2008, *320*, 520–523) devise a strategy to direct an otherwise soluble β-secretase inhibitor to the plasma membrane so that it can undergo endocytosis and consequent relocation to endosomes.

The β-secretase inhibitor was cleverly composed of a transition state analog of a β-secretase substrate, which specifically inhibits enzyme activity, and a sterol moiety, which targets the compound to the cell membrane. Indeed, when tested in cells, the sterol-linked inhibitor was more effective at preventing Aβ production than the free inhibitor. Moreover, use of a fluorescent derivative of the sterol-linked inhibitor demonstrated that the compound was in fact localized in endosomes. In addition to simply guiding the inhibitor to the location of β-secretase in the cell, scanning fluorescence correlation spectroscopy and avalanche photodiode imaging experiments showed that the presence of the sterol also appeared to help enrich the inhibitor in the part of the membrane where β-secretase activity takes place. Finally, the sterol-linked inhibitor reduced Aβ toxicity *in vivo* in both *Drosophila* and mouse models of Alzheimer's disease. These exciting results expose a new strategy for creating effective β-secretase inhibitors, an approach that could be applied to the design of inhibitors against other membrane proteins as well. **Eva J. Gordon, Ph.D.**



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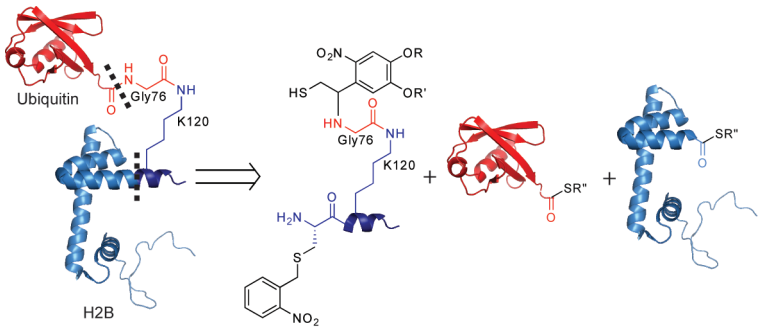
that received three intravenous injections of the LNA-modified antagonist (10 mg kg^{-1}) displayed a decrease in cholesterol of 40%. LNA-mediated antagonism was also shown to be dose-dependent and long-lasting. Finally, a wide array of tests over a short study period failed to detect abnormal levels of toxicity.

Chemically, LNAs are RNA analogs in which the furanose ring of the monomers is "locked" in the C3′-endo conformation. Because the conformational rigidity stabilizes binding with RNA strands, oligonucleotides containing these nucleotide analogs have rather high melting temperatures. For example, the oligonucleotide with the highest potency in this study contains >50% LNA monomers and has a melting temperature of 80 °C. In addition, previous studies have demonstrated the superior stability of LNAs against endogenous nucleases. As this study emphasizes, LNA-modified oligonucleotides are promising agents in designing antisense nucleic acid antagonists. Further studies over longer periods of time and using multiple dosing schemes should shed light on whether there are any adverse long-term effects due to either oligonucleotide toxicity or to off-target effects of antagonizing miRNAs. Even in the best-case scenario, there may be a long way to go before human trials are possible. However, this study is an excellent proof-of-principle that modified nucleotide-based antagonists can be used for potent antagonism of miRNAs. **Anirban Mahapatra, Ph.D.**

Cracking the Histone Code

Many mechanisms exist for regulating expression of the genome. The histone proteins that serve as a scaffold for chromosomal DNA participate in these regulatory mechanisms through post-translational modifications such as methylation, acetylation, and ubiquitylation. Evidence suggests that these modifications talk to each other, crafting a "histone code" for orchestrating gene expression. For example, ubiquitylation of histone H2B appears to enhance methylation of Lys79 on histone H3 (H3 K79). However, the mechanisms behind the cross-talk are not well understood. In an effort to help decipher the histone code, McGinty *et al.* (*Nature* 2008; DOI: 10.1038/nature06906) present a method for chemically ubiquitylating H2B and systematically probe its role in the methylation of H3 K79.



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Effective examination of the part that ubiquitylated H2B (uH2B) plays in H3 K79 methylation required access to pure, homogeneous uH2B. A semisynthetic approach was adopted using expressed protein ligation technology, which employed two traceless ligation reactions to unite three strategically designed polypeptides, two recombinant in origin and one created through chemical synthesis. The resulting uH2B was incorporated into core histone octamers, which were used to reconstitute mono- and dinucleosomes. H3K79 is methylated through the action of the methyltransferase Dot1, and when Dot1 was added to the reconstituted nucleosomes, only those containing uH2B were methylated on H3 K79. Biochemical analysis suggested that uH2B promotes Dot1 activity through an allosteric mechanism. Notably, though the ubiquitylated and methylated amino acids reside on different histones, they are in close proxmity to each other on the surface of the nucleosome, thus presenting a structural basis for cross-talk. This method for chemically ubiquitylating histone proteins can be extended to other histones as well as other proteins, facilitating future exploration of the mechanisms that govern the histone code and ubiquitin function in general. **Eva J. Gordon, Ph.D.**





Scalliet, G., *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, *105*, 5927–5932. Copyright 2008 National Academy of Sciences, U.S.A.

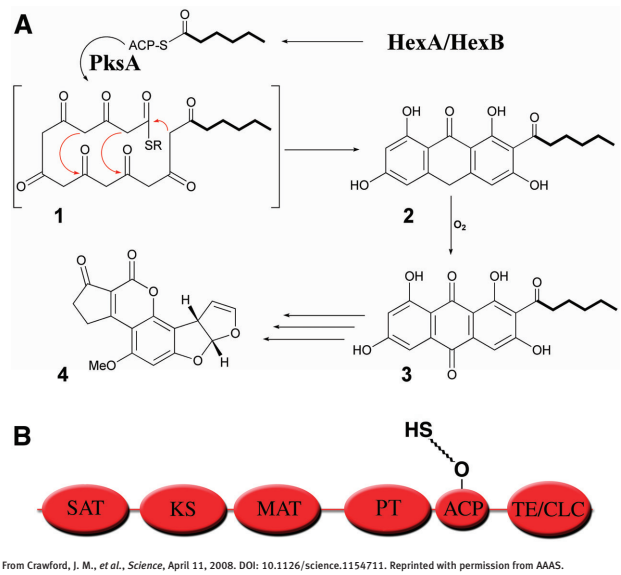
The Scent of a Rose

It is hard enough to find time to stop and smell the roses, much less attempt to figure out how they acquired their scent. However, the many varieties of modern roses, along with the scents they produce, have actually been bred from a relatively small number of progenitors of both Chinese and European descent. Recently, several genes involved in the biosynthesis of various scent components have been characterized, including the orcinol *O*-methyltransferases OOMT1 and OOMT2. Scalliet *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2008, *105*, 5927–5932) examine the substrate specificities and phylogenetic characteristics of these enzymes, revealing intriguing details about their role in the evolution of the scent of the tea rose.

OOMT1 and OOMT2 catalyze the final two steps, respectively, in the biosynthesis of phenolic methyl ethers, the compounds responsible for the scent associated with tea roses. Though their amino acid sequences are highly similar, molecular modeling and mutagenesis studies demonstrated that a difference in one amino acid, tyrosine-127 in OOMT1 and a phenylalanine-126 in OOMT2, is the basis for the substrate specificity of the enzymes. Interestingly, when OOMT-like genes in a selection of rose species were examined, all roses possessed OOMT2-like genes, but OOMT1-like sequences were found only in the genomes of the Chinese roses. Phylogenetic analysis of 73 OOMT-like genes suggested that OOMT1 arose by duplication of OOMT2 followed by functional diversification. In addition, the authors found that OOMT protein accumulated in the petals of Chinese roses but not those of Eurasiatic roses. Taken together, the data suggest that the unique scents associated with specific rose species have evolved around optimization of enzymes that can efficiently synthesize particular scent compounds as well as regulatory mechanisms that favor production of the necessary biosynthetic enzymes in the petals. **Eva J. Gordon, Ph.D.**

Constructive Deconstruction

Polyketides are a group of structurally diverse natural products possessing a wide range of biological activities. Understanding the prokaryotic and eukaryotic pathways for polyketide biosynthesis will help provide access to both natural polyketides and unnatural analogs with numerous biological applications, including potential as therapeutic agents. Toward constructing a comprehensive picture of the polyketide biosynthetic machinery in eukaryotic organisms, Crawford *et al*. (*Science* 2008, *320*, 243–246) deconstruct PksA, the fungal multidomain iterative polyketide synthase involved in production of the aflatoxin family of carcinogenic compounds.



From Crawford, J. M., *et al.*, *Science*, April 11, 2008. DOI: 10.1126/science.1154711. Reprinted with permission from AAAS.

PksA is composed of six fused catalytic domains: a starter unit-acyl carrier protein transacylase (SAT), a ketosynthase (KS), a malonyl-CoA:ACP transacylase (MAT), a product template (PT) domain, an acyl carrier protein (ACP), and a thioesterase/Claisen cyclase (TE/CLC). During polyketide synthesis, these domains are reused in multiple catalytic cycles, but it is unclear how the cycles are programmed to generate distinct products. The authors cleverly explored the function of each domain by generating individual domains, didomains, and tridomains and reassembling selected pieces in various compositions. Upon examination of the resulting products by mass spectrometry, UV–vis absorption spectroscopy, high-pressure liquid chromatography, and comparisons with authentic standards, several key features of PksA function were revealed. For example, the machinery encoding polyketide chain length was found to contain the SAT, KS, MAT, and ACP domains; the TE/CLC domain was found to be responsible for product release *via* an intramolecular Claisen reaction; the KS domain was implicated in the selection and extension of specific starter units tethered to the ACP domain; and the PT domain was revealed to be an cyclase/aromatase domain. The authors further determined that the PT domain works with the KS and TE/CLC domains to assemble specific building blocks and to mediate a cyclization cascade. Little was known about the function of the PT domain prior to this study, so this information should help decipher biosynthetic pathways used by other iterative polyketide synthases as well. **Eva J. Gordon, Ph.D.**