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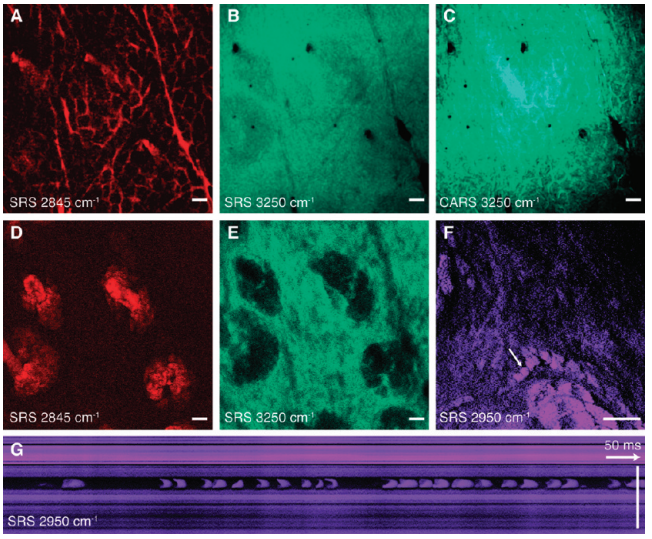
Stimulating Optical Imaging

Advances in diagnostic and therapeutic methods increasingly rely on imaging techniques with exquisite spatial and temporal resolution. Optical imaging methods offer superior spatiotemporal resolution when compared with magnetic resonance imaging and do not require the use of potentially toxic or disruptive dyes inherent in many other approaches. However, common optical imaging methods such as two-photon autofluorescence microscopy, second harmonic generation microendoscopy, and Raman spectroscopy present a host of other challenges for imaging in live animals, including inadequate sensitivity, small scope of molecular signatures that can be visualized, and the presence of artifacts. By creatively addressing two challenges associated with stimulated Raman scattering (SRS) microscopy, Saar *et al.* (*Science* 2010, *330,* 1368−1370) now demonstrate its use for the *in vivo* imaging of both mouse and human skin.

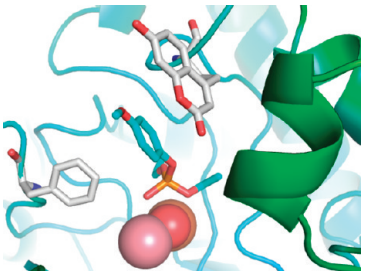


From Saar, B. G., *et al., Science,* 2010, *330,* 1368. Reprinted with permission from AAAS.

SRS is based on vibrational spectroscopy, but in order for it to be used for *in vivo* applications, methods to increase the imaging speed and collect light through thick tissues associated with animals needed to be developed. To improve the imaging speed, the authors developed a home-built, all-analog lock-in amplifier with a response time of ∼100 ns, 3 orders of magnitude faster than that available from commercial lock-in amplifiers. To overcome the light collection issue, the authors devised a method for substantially enhancing the collection of backscattered signal that is generated as the forward-going signal penetrates the tissue. With this improved SRS system in place, mouse and human skin were imaged *in vivo*. Images highlighting lipids, water, or proteins were generated, enabling novel visualization of various processes including red blood cells traveling through a capillary and penetration of the small molecules *trans*-retinol or dimethyl sulfoxide through the skin. The approaches used to adapt SRS for *in vivo* imaging can be applied to other optical imaging methods, expanding the scope of vibrational imaging to various additional *in vivo* applications for diagnostic and therapeutic benefit. **Eva J. Gordon, Ph.D.**

An Unnaturally Better Enzyme in Pesticide Degradation

Incorporation of unnatural amino acids into proteins has revolutionized protein engineering. Designer amino acids can lend unique structural and functional versatility to the peptides in which they are incorporated. Several different methodologies have been developed to insert unnatural amino acids into protein, the most common of which exploits an amber suppressor tRNA. Ugwumba *et al.* (*J. Am. Chem. Soc.* 2011, *133,* 326−333) demonstrate the power of this approach by significantly improving the efficiency of bacterial phosphotriesterase (arPTE) in the hydrolysis of pesticides.



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Bacterial phosphotriesterases catalyze rapid hydrolysis of organophosphate phosphotriester pesticides, such as paraoxon. The potential of these enzymes to detoxify pesticides with serious health and environmental implications is of particular interest. As a result, several studies have attempted to improve the efficiency of this class of enzymes, with limited success. Screening of hundreds of thousands of variants of arPTE by targeted and random mutagenesis resulted in modest improvements in activity of this already efficient enzyme. This study describes the incorporation of unnatural 7-methyl- and 7-hydroxycoumarinyl amino acids in place of a naturally occurring tyrosine residue. An engineered tRNA synthetase and a mutant amber suppressor tyrosyl-tRNA pair directed incorporation of the unnatural amino acids. Kinetic analysis of these variant proteins showed the 7-hydroxycoumarinyl-arPTE had an approximately 10-fold higher catalytic efficiency. This study emphasizes the utility of unnatural amino acids for the engineering and directed evolution of enzymes. **Jitesh A. Soares, Ph.D.**

Cleaner Tumor Tagging

To improve cancer diagnosis and treatment, clinicians and researchers would like to have better tools for accurately visualizing tumors and metastases. However, cancer researchers have faced challenges in finding a gene-based reporter system that

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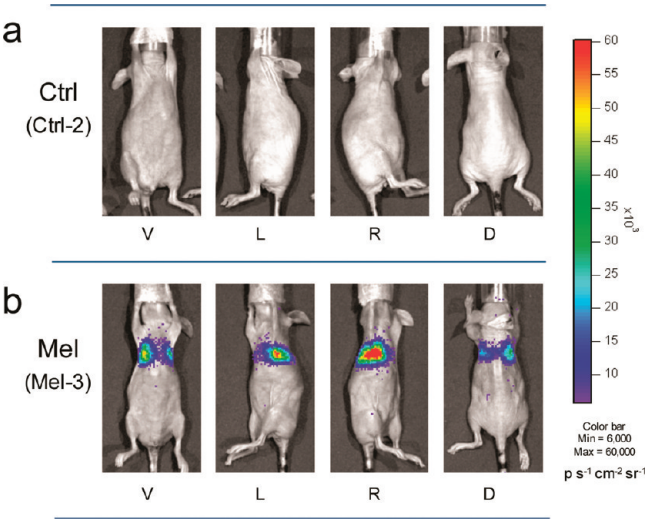
meets three important clinical criteria: sufficient signal, cancer cell specificity, and nonviral delivery. Now, Bhang *et al.* (*Nat. Med.*, 2011, *17*, 123−129) report a promising new gene-promoter-based imaging system that avoids these obstacles.



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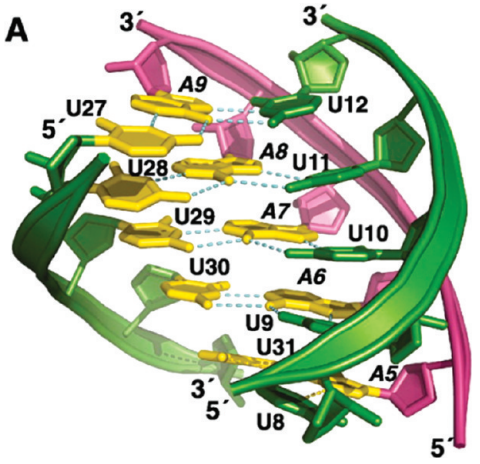
For the promoter, the researchers selected a minimal promoter region of progression elevated gene-3 (PEG-3), a rodent gene involved in tumor progression and metastasis. As an initial test, they combined PEG-3 promoter with the bioluminescent reporter gene, firefly luciferase and tested the specificity of this promoter in mouse models of metastatic human melanoma and breast cancer. They systemically delivered the genes using *in vivo*-jet PEI, which is based on a cationic polymer, linear polyethyleneimine (PEI), and looked for bioluminescence in the experimental metastasis mouse models and control mice. After 48 h, mice with both melanoma and breast cancer metastases glowed at specific cancer sites. Histological analysis of the tissues confirmed that the bioluminescence occurred in the metastatic cancer sites. Further studies of transfection efficiency indicated that tumor-selective expression of firefly luciferase was truly based on the cancer-specific activity of PEG-3 promoter, not on different transfection efficiency between normal and tumor tissues.

Because bioluminescent reporters cannot be used in clinical studies, Bhang e*t al.* set up a PEG-3 promoter-driven imaging system with HSV1 thymidine kinase, a reporter protein that has already been translated to clinical studies and can be detected using radionuclides. They injected the mice with plasmid DNA using the PEI polymer and 46 h later administered the radiotracer molecule (2′-fluoro-2′-deoxy-β-D-5-[^{125}I]iodoracil-arabinofuranoside). The tracer molecule built up in the lungs of melanoma mice at levels more than 30-fold higher than controls and in a tumor-specific fashion. The researchers are tweaking the delivery polymer to improve transfection efficiency and are studying this gene-delivery imaging system in other tissues. **Sarah A. Webb, Ph.D.**

A Viral RNA Hits a Triple

A viral infection can spark a wide variety of perturbations in a mammalian cell. In the prototypical scenario, the virus might hijack cellular machinery to replicate its own nucleic acids and produce encoded proteins. While churning out the viral genome and proteome, important host pathways and expression programs can fall out of line, leading to cell death or even cancer. One such virus which infects humans is Kaposi's sarcoma-associated herpes virus, or KSHV. This double-stranded DNA virus can cause cancer, especially in immunocompromised patients. Among the RNA products of KSHV is the PAN RNA, which displays the features of a typical mRNA such as the 5′ cap and the 3′ poly(A) tail. Though it is very highly expressed upon viral infection, interestingly, PAN RNA does not code for a protein and instead remains in the nucleus as a stable transcript. The key to its stability is a small RNA element near the poly(A) tail known as the ENE, or expression and nuclear retention element. Recently, a new study by Mitton-Fry *et al.* (*Science* 2010, *330*,1244−1247) combined crystallography and biochemical experiments to answer how the ENE element contributes to PAN RNA's remarkable stability.

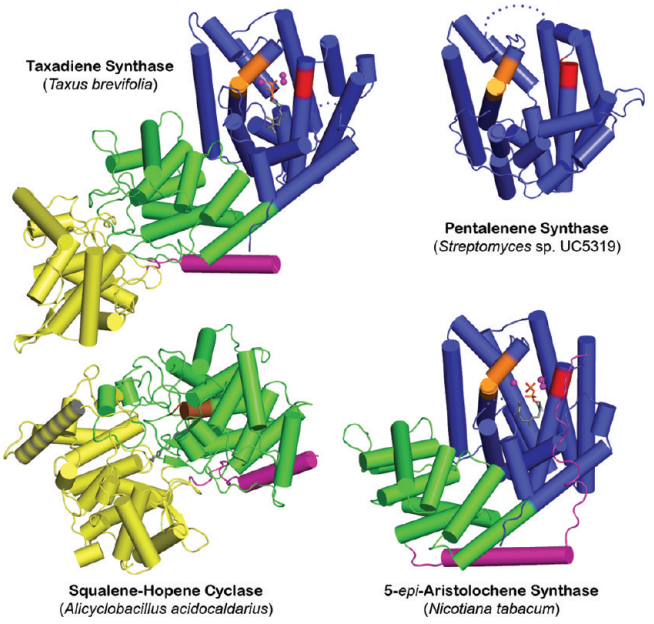


From Mitton-Fry, R. M., *et al.*, *Science*, 2010, *330*,1244. Reprinted with permission from AAAS.

It was previously shown that the ENE binds to the poly(A) tail, but the crystal structure of a minimalist ENE complexed with an A(9) oligonucleotide literally unveiled a new twist. Since the ENE has two U-rich loop sequences, the previous model brought these regions together with the poly(A) tail by Watson−Crick base pairing. The structure revealed that both U-rich loops interact with the same 5 A bases to form a major-groove triple helix. The binding is further augmented by three more A bases forming specific interactions with G-C base pairs present in the ENE stem flanking the U-rich loops. With these static snapshots of the interaction in hand, the authors went back to the lab to connect the relevance of the triple helix to the biology of PAN RNA. They used site-directed mutagenesis of ENE or the poly(A) tail to promote or destabilize the triple helix. Then, they used these mutants in a deadenylation assay in human nuclear extract. In this assay, RNAs that have a wild type ENE and a 3′ poly(A) tail are extremely stable, as is observed *in vivo*. Point mutations in the ENE or poly(A) tail that destabilized the triple helix also destabilized the transcript. Compensatory mutations aimed at restoring the triple helix also restored the transcript's stability. Specifically, proper placement of a single guanosine in the poly(A) tail showed that binding to a mutant ENE can be exquisitely specific. This study details another clever trick that viruses use and illustrates yet another way that noncoding RNAs might alter the expression program of a cell. **Jason G. Underwood, Ph.D.**

Dissecting a Diterpenoid Cyclase

Taxol, the best-selling cancer drug ever manufactured, is a polycyclic diterpenoid found in the Pacific yew tree. The first committed step in the biosynthesis of this powerful chemotherapeutic agent is the cyclization of the isoprenoid geranylgeranyl diphosphate to form taxa-4(5),11(12)diene. The enzyme taxadiene synthase catalyzes this cyclization, and despite the existence of X-ray crystal structures for monoterpene, sesquiterpene, and triterpene cyclases, the structure of a diterpene cyclase has thus far eluded researchers. Now, Köksal *et al.* (*Nature* 2011, *469*, 116−120) report the structure of a truncated variant of taxadiene synthase in complex with a diterpene substrate analogue.



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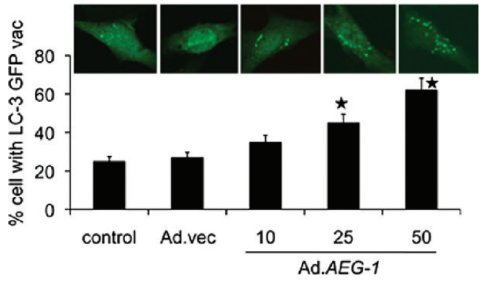
Prior structures of other terpenoid cyclases have revealed that the enzymes are modular and comprise one or two domains. The taxadiene synthase variant, referred to as TXS, is a new variation on this pattern and contains three α-helical domains. Furthermore, each domain in terpenoid cyclases adopts either what has been termed a class I fold, such as that found in farnesyl diphosphate synthase, or a class II fold, found in squalene-hopene cyclase. TXS was found to contain both folds; the C-terminal domain assumes the class I fold, and the N-terminal domain (along with the remaining domain) takes on the class II fold. The C-terminal domain contains the substrate binding site and the three-metal binding motif, which facilitates ionization of the substrate diphosphate group and carbocation formation that occurs during the initial step of catalysis. In contrast, the N-terminal domain is missing the general acid catalytic motif, which otherwise could also facilitate carbocation formation, and the active-site cavity normally associated with the class II fold. In other diterpenoid cyclases, either one or both of these domains can be catalytically active. The presence of both class I and II folds points to a fascinating evolutionary process likely exploited by terpenoid cyclases, no doubt to accommodate the versatility required for the synthesis of the over 55,000 unique terpenes that have been identified thus far. **Eva J. Gordon, Ph.D.**

Dealing with Stress

When cells get stressed out, for example, when nutrients are scarce, oxidative damage is rampant, or pathogens are lurking, they can catabolize their own components to provide energy and biosynthetic starting materials that may be needed to promote their survival. This normal, evolutionarily conserved process is called autophagy. Cancer cells, however, have devised methods to hijack autophagy for their own sinister purposes, though the mechanisms directing this process are unclear. Bhutia *et al.* (*Proc. Natl. Acad. Sci.* 2010, *107*, 22243−22248) now report that astrocyte-elevated gene-1 (AEG-1), a molecule already infamous for its role in oncogenesis, tumor progression, and metastasis, also mediates protective autophagy. Using an impressive suite of cellular and molecular techniques, they exquisitely delineate how this function of AEG-1 is associated with its cancer-promoting properties.



Bhutia, S. K., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, *107*, 22243−22248. Copyright 2010 National Academy of Sciences, U.S.A.

Initial experiments examined the role of AEG-1 in autophagy in normal cells. It was demonstrated that AEG-1 induces autophagy through a noncanonical pathway involving ATG5, an essential autophagy protein, and AMP kinase, an important mediator of cellular energy homeostasis. In addition, examination of serum-starved cells under conditions in which ATG5 activity was blocked suggested that AEG-1 can modulate both autophagy and apoptotic mechanisms to promote cell survival. In probing the role of AEG-1 in autophagy in cancer cells, it was observed that autophagy was indeed diminished in numerous cancer cell lines in which AEG-1 expression was knocked down. Moreover, chemoresistance to common chemotherapeutic agents increased in cancer cells lines overexpressing AEG-1, while chemosensitivity increased in cell lines deficient in ATG5. This aspect of AEG-1 function implicates it in yet another pro-cancer process and points to the AEG-1-mediated autophagy pathway as an intriguing anticancer target. **Eva J. Gordon, Ph.D.**

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