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SPOTLIGHT

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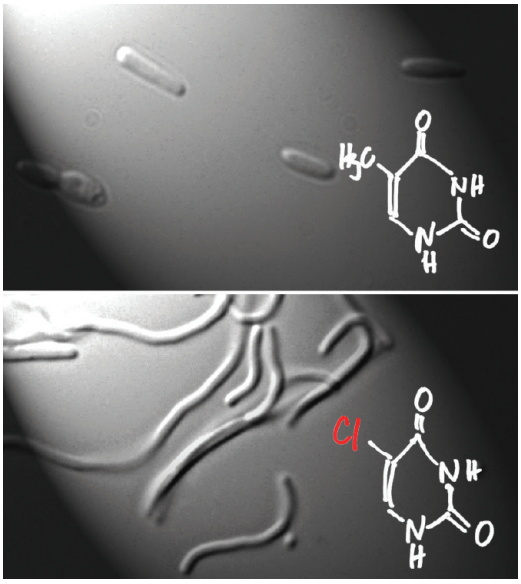
A Chemical Evolution

Manipulation of the chemical composition of RNA and DNA is a powerful method for investigating the biosynthetic and metabolic pathways that govern cell growth and reproduction. Noncanonical bases have been incorporated into RNA and DNA in some systems under defined conditions, but unlimited self-reproduction of an organism through complete genome or transcriptome substitution has not been achieved. Marlière *et al.* (*Angew. Chem., Int. Ed.*, Epub ahead of print June 27, 2011; DOI: 10.1002/anie.201100535) now report a general method for evolving the DNA composition of a bacterial population.



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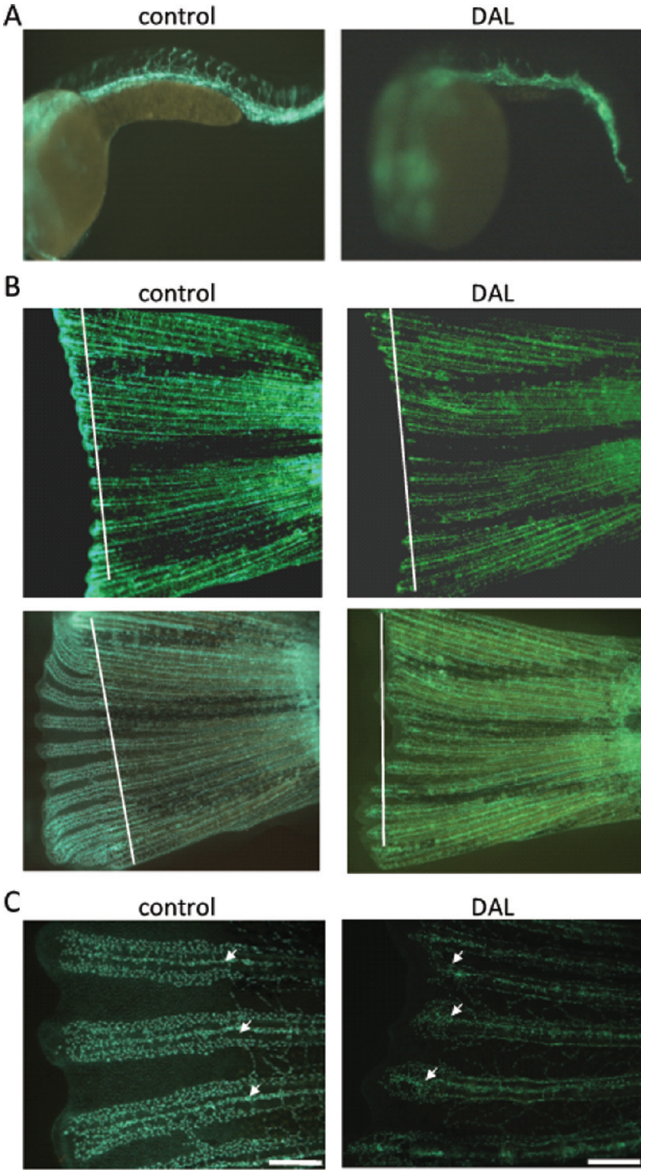
The success of their approach relied on a cultivation process referred to as the conditional pulse-feed regime. The design consisted of a cultivation device connected to two nutrient reservoirs, one containing the canonical base thymine and the other containing the noncanonical base 5-chlorouracil. Thymine was selected to be replaced because it is present only in DNA, its metabolism is separate from that of RNA, and its biosynthesis can be abolished through the disruption of a single gene. 5-Cholouracil was selected as the replacement due to its structural similarity to thymine, its compatibility as a substrate for enzymes involved in thymine biochemistry, and its biochemical stability. A genetically modified strain of *Escherichia coli* unable to grow without a supply of thymine was used for transliteration with 5-chlorouracil. Growth conditions were set up such that every 10 min the cultures received a pulse of either thymine or 5-chlorouracil. Over the course of approximately 25 weeks, the bacteria adapted to consume only 5-chlorouracil. Characterization of the adapted bacteria revealed that construction of DNA with 5-chlorouracil was inheritable; that 5-chlorouracil was largely encoded in the genomes of the adapted strains; and that numerous mutations including base substitutions or chromosome rearrangements were present, the relative amounts of which depended on the adaptation conditions. **Eva J. Gordon, Ph.D.**

Planting an Antivascular Agent

Angiogenesis, the growth of new blood vessels, is critically important in both normal and pathological processes. Drugs that target angiogenesis have found some success in the treatment of cancer, but agents developed thus far suffer from modest efficacy, cause adverse side effects, and often lose effectiveness as the disease develops resistance to their mode of action. As most antiangiogenic drugs target new blood vessel growth by inhibiting a protein called vascular endothelial growth factor, new antivascular agents that act by different mechanisms hold promise of improved performance. Now, Garkavtsev *et al.* (*Proc. Natl. Acad. Sci.* 2011, *108*, 11596−11601) report the discovery of a plant natural product that exhibits antivascular activity by targeting a different process, endothelial cell adhesion.



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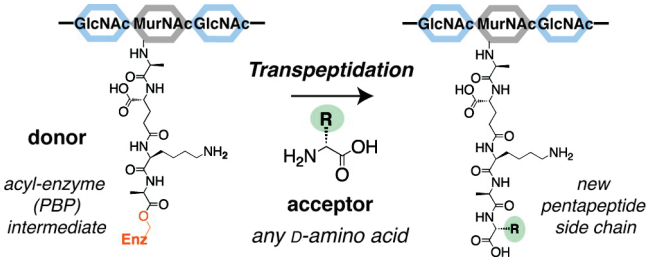
Garkavtsev, I., *et al., Proc. Natl. Acad. Sci. U.S.A., 108,* 11596-601. Copyright 2011 National Academy of Sciences, U.S.A.

Adhesion of endothelial cells, which line the blood vessel walls, is an appealing process to target because of its critical role in blood vessel function. To find compounds that perturb endothelial cell adhesion, 50,000 small molecules were screened for their ability to disrupt cell attachment to the well of a 384-well plate. Eighty-six compounds were selected for additional screening for their ability to affect the remodeling of actin filaments, which is a consequence of the cell adhesion process. Of the 12 remaining compounds, the plant natural product dehydro-α-lapachone (DAL) was chosen for further study because of its favorable toxicity profile. In a zebrafish model, DAL interfered with blood vessel formation during development and wound healing, and in mouse breast cancer models, DAL treatment reduced vascular density in tumors and inhibited tumor growth. Investigation into its mechanism of action demonstrated that DAL inhibits actin-dependent processes such as cell spreading and motility by promoting the proteosomal degradation of the Rho GTPase Rac1. This study points to new target pathway for antivascular drug development and illuminates an exciting jumping off point for development of a novel antivascular agent. **Eva J. Gordon, Ph.D.**

Making Peptidoglycan

The bacterial cell wall is made up of a polymer called peptidoglycan, which consists primarily of linear glycan chains cross-linked by peptide bonds. The enzymes responsible for peptide bond formation, called transpeptidases, are the targets of the β-lactam antibiotics. Though the therapeutic importance of these drugs has fueled extensive characterization of the interactions between transpeptidases and β-lactams, much less is known about the interactions between transpeptidases and their natural substrates. Transpeptidases catalyze a two-step reaction that involves enzyme-mediated activation of a donor substrate followed by reaction of an acceptor substrate with an amine moiety. By exploring each step individually, Lupoli *et al.* (*J. Am. Chem. Soc.* 2011, *133,* 10748−10751) reveal key aspects of the structural requirements underlying this important biosynthetic reaction.



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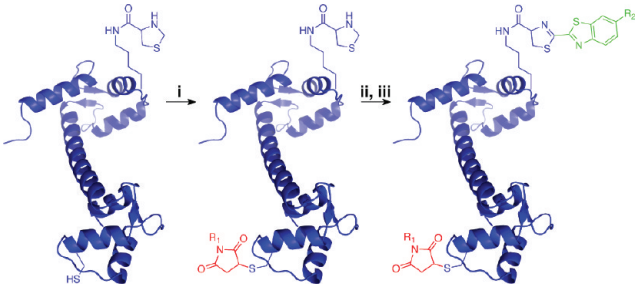
Investigation of the reaction steps indicated that the donor substrate must be a polymeric fragment of peptidoglycan, while the acceptor substrate can be any of a variety of structurally diverse peptides and amino acids provided there is an amine nucleophile in a D-stereocenter in the molecule, as in the native acceptor substrate. Appropriate donor substrates were generated by polymerization of a synthetic, radiolabeled glycopeptide-derived lipid using a peptidoglycan glycosyltransferase. Polymerization of the glycolipid is necessary; monomeric derivatives do not react with the enzyme. Unexpectedly, all classes of D-amino acids functioned as acceptors in the reaction. Notably, studies have shown that naturally occurring incorporation of noncanonical D-amino acids into the cell wall of diverse bacteria influences various structural and functional properties of bacteria, including cell shape, biofilm formation, and sensitivity to β-lactams. This study describes new tools for investigation of this underappreciated but remarkably versatile ability of transpeptidases to build, retool, and even influence cell signaling through the bacterial cell wall. **Eva J. Gordon, Ph.D.**

New Approach to Protein Labeling

The site-specific labeling of proteins with novel synthetic molecules provides new ways for studying proteins and their function in a cell. Recently, several approaches for labeling proteins with fluorescent dyes, chemical cross-linkers, and other functional moieties have been described. However, these approaches are typically quite slow and require consumptive amounts of reagents or alter protein structure through the use of protein fusions or supplementary amino acid residues. Nguyen *et al.* (*J. Am. Chem. Soc.* 2011, Epub ahead of print July 7, 2011, DOI: 10.1021/ja203111c) report the development of an efficient new way to site-specifically label proteins that circumvent these drawbacks.



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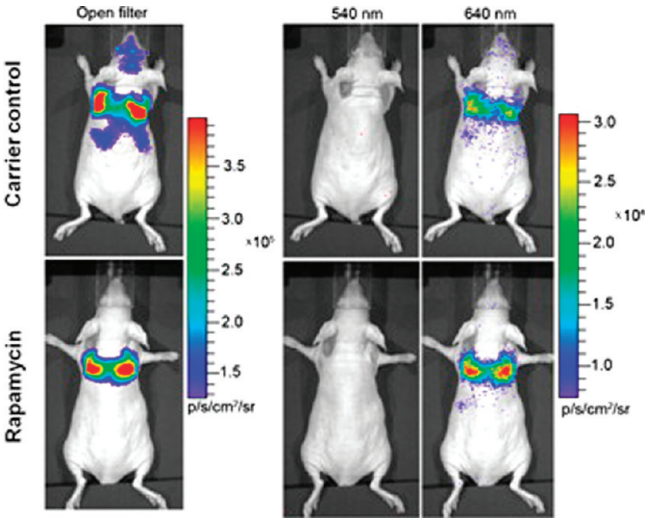
For this novel approach, the authors utilized the pyrrolysyl-tRNA/tRNA synthetase pair (*Mb*PylRS/$tRNA_{CUA}$) from *Methanosarcina barkeri*, whose cognate substrate is typically the 22nd genetically encoded amino acid, L-pyrrolysine. Using the *Mb*PylRS/$tRNA_{CUA}$ pair, N^{ε}-L-thiaprolyl-L-lysine, N^{ε}-D-cysteinyl-L-lysine, and N^{ε}-L-cysteinyl-L-lysine were site-specifically introduced into recombinant proteins *via* amber codon suppression. These unnatural amino acids possessing 1,2-aminothiols were incorporated into myoglobin and green fluorescent protein. However, the efficiency of incorporation of these novel residues was relatively poor. The authors thus altered the *Mb*PylRS residues involved in the recognition of the pyrroline ring of pyrrolysine. A screen identified tRNA synthetases more efficient at incorporating the novel residues into proteins. These new tRNA/tRNA synthetase pairs increased production of derivatized recombinant protein by up to 16-fold. Proteins altered with N^{ε}-L-thiaprolyl-L-lysine possessing the 1,2-aminothiol chemical handle were rapidly labeled using a cyanobenzothiazole condensation reaction carrying a fluorescent probe. Importantly, 1,2-aminothiols are not present in naturally occurring proteins and therefore the cyanobenzothiazole condensation reaction could be performed without perturbing the derivatized protein. When paired with cysteine labeling, a protein could be simultaneously labeled with two distinct, site-specific probes. Thus, this novel

approach furthers the quest for quick and efficient site-specific labeling of proteins with biophysical probes. **Jitesh A. Soares, Ph.D.**

Deep Tissue Messages from BRET

Many proteins have critical partners and even minor mutations at a binding surface which can perturb protein−protein interaction, sometimes with profound consequences. Despite the wealth of genetic and biochemical techniques for identification of protein−protein interactions, understanding the finer mechanistic details usually means leaving the cell and entering the controlled environment of the test tube. Now, a recently developed technique (Dragulescu-Andrasi *et al. Proc. Natl. Acad. Sci. U.S.A.* 2011, *108*, 12060−12065) called bioluminescence resonance energy transfer, or BRET, brings the study of such interactions back to the organism with detection inside of a living mouse.

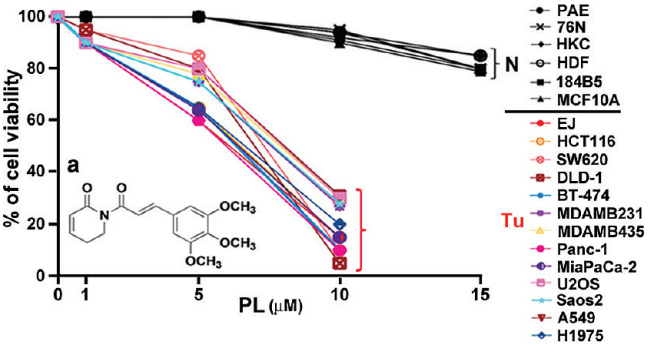


Dragulescu-Andrasi , A., *et al., Proc. Natl. Acad. Sci. U.S.A., 108*, 12060−12065. Copyright 2011 National Academy of Sciences, U.S.A.

The concept is similar to fluorescence resonance energy transfer, or FRET, but BRET instead uses light from bioluminescence. In nature, the sea pansy luciferase produces the donor light and a green fluorescent protein accepts that light and emits in a new wavelength. The researchers employed a modified luciferase paired with red-shifted fluorescent protein, resulting in a final emission light over 600 nm in wavelength. This was critical since the signal of shorter wavelength light is efficiently attenuated by tissue or blood. Several flavors of new and improved BRET pairs were first tested in a tumor cell line by directly expressing the two functional proteins as one contiguous polypeptide. Then, candidate tumor cell lines were injected into the mouse tail vein causing accumulation of the cells in the lungs. Both the direct luminescence of the luciferase and the BRET generated signal could then be measured using a sensitive digital camera while the mouse lay unconscious. The new red-shifted BRET pair showed impressive detection of the injected cells in deep tissue. The luciferase and RFP variants were then tethered to separate proteins, FRP and FKBP12, two proteins that are known to interact in the presence of the drug rapamycin. In this experiment, rapamycin treatment resulted in BRET activation as visualized by high signal in the lungs of the injected animals. This method should prove useful to others seeking a novel way to validate and visualize protein−protein interactions within the context of a whole organism. **Jason G. Underwood, Ph.D.**

Targeting cancer cells based on their stress response

One of the greatest challenges in cancer chemotherapy is the discovery of molecules that are powerful enough to kill cancer cells but operate with a mechanism selective enough to avoid harming normal cells. Among existing therapies, few molecules meet those criteria. But now Raj *et al.* (*Nature* 2011, *475*, 231−234) report that a small molecule, piperlongumine, selectively kills cancer cells in culture and in mouse models by targeting those cells' revved up stress response to reactive oxygen species (ROS).



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Piperlongumine is a plant-derived natural product with demonstrated cytotoxicity. When Raj *et al.* treated cultured cancer cells with this compound, p53 was activated in the cancer cells, inducing apoptosis. Normal and immortalized noncancerous cells remained viable even with treatment at high concentrations. When the researchers transformed normal cells into cancer cells, piperlongumine killed these newly oncogenic cells also, suggesting that the processes that confer malignancy also make cells sensitive to this compound. Further studies of cancer-related genes showed that the transcription levels of 55 different genes were affected in response to the compound: pro-apoptotic factor transcription levels were enhanced while pro-survival levels decreased. Piperlongumine also shrank tumors in mice.

Raj *et al.* then did proteomics studies to identify the proteins that piperlongumine targets, most of which were involved in the cellular response to ROS such as glutathione-*S*-transferase pi 1 (GSTP1) and carbonyl reductase 1 (CBR1). The compound binds to GSTP1 and blocks its activity. Studies with whole cells treated with piperlongumine showed an increase in ROS in cancer cells and in normal cells engineered with cancer genes. In normal cells treated with the compound ROS levels remained constant. Treatment with antioxidants or overexpression of GSTP1 and CBR1 in cancer cells can reverse the effect of piperlongumine.

In general, normal cells and even stem cells have low levels of ROS while cancer cells have higher levels, but further study will be needed to determine which cancers might respond to this treatment. Overall, however, this ability to target diseased cells based on their elevated stress response to ROS presents an intriguing new strategy for the development of new cancer therapies. **Sarah A. Webb, Ph.D.**