Spotlight

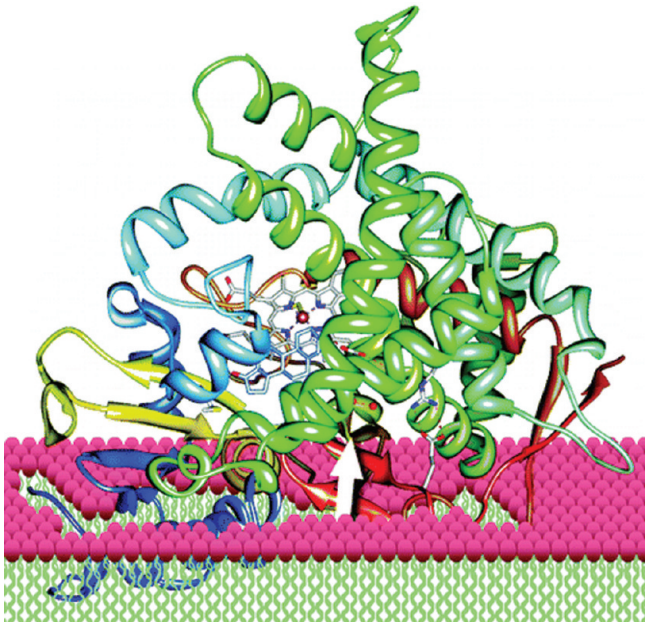
Best of Chemical Biology in 2009

2009 was an exciting year for chemical biology and we've used our Spotlight section to highlight new developments every month. Now, looking back, the Editors have compiled a list of articles representing some of the most interesting and relevant research from all of 2009. As with our monthly Spotlights, it is simply not possible to recognize all of the amazing work being published, and this list is not meant to be comprehensive. However, we hope that this feature will at least provide a sense of the richness and quality of research being published in chemical biology as we move forward into a new decade. Happy New Year!

The Difference between Male and Female?

The differences between men and women may be too many to count, but at the molecular level, a single enzyme is responsible for converting androgens, the characteristic male sex hormones, to their female counterparts, estrogens. Aromatase, which resides as a membrane-bound protein in the endoplasmic reticulum (ER), is a cytochrome P450 that is responsible for hydroxylating and aromatizing androgens such as testosterone to estrogens like 17β-estradiol. Given the pivotal, detrimental role that estrogen plays in certain breast cancers, development of aromatase inhibitors is a promising anticancer strategy. However, despite many years of study, the structure of the enzyme and the mechanism of the aromatization step have remained elusive. Ghosh *et al.* (*Nature* 2009, *457*, 219–224) now report the crystal structure of aromatase in complex with one of its natural substrates, androstenedione.

The structure revealed that aromatase retains the typical cytochrome P450 fold but also exhibits several androgen-specific characteristics. A cleft precisely complementary in shape to the steroid backbone, supported by a slew of hydrophobic, van der Waals, and hydrogen-bond interactions, gives rise to the high selectivity of aromatase for its androgen substrates. In addition to pointing to residues critical for the hydroxylation steps, the structure also offers alluring clues regarding the mechanism of aromatization. Based on the locations of three key residues, a threonine, an alanine, and an aspartate, along with the help of a water molecule and a heme group, a mechanism involving hydrogen abstraction and enolization en route to aromatization was proposed. The locations of certain hydrophobic helices and key residues indicative of lipid integration suggested the manner in which aromatase is integrated into the ER membrane, providing a rationale for how its lipophilic substrates gain access to its active site. These exquisite details of aromatase structure and function will no doubt facilitate design of more sophisticated aromatase inhibitors in the future. **Eva J. Gordon, Ph.D.**

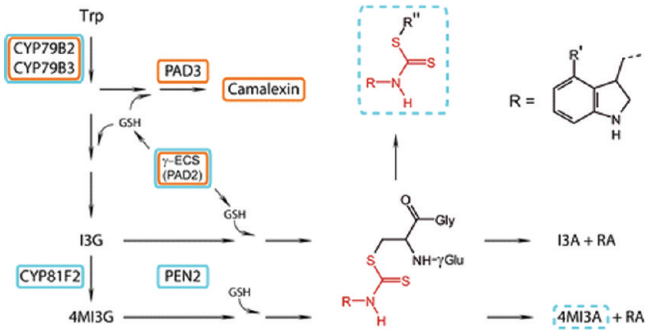


A Bitter Pill for Plant Pathogens

In addition to causing the bitter flavor in foods such as mustard, horseradish and Brussels sprouts, glucosinolates can also be a bitter pill to swallow for certain plant predators. Glucosinolates, which comprise a thioglucoside linked via a carbon atom to a sulfated nitrogen moiety, are plant secondary metabolites that play an important role in deterring insects. Recent evidence also suggests that glucosinolate metabolism is induced upon plant exposure to certain microbial pathogens. However, the pathways involved in this response have remained elusive. Now, two reports (Clay *et al.* (*Science* 2009, *323*, 95–101) and Bednarek *et al.* (*Science* 2009, *323*, 101–106)) uncover details of a novel glucosinolate metabolic pathway in the small flowering plant *Arabidopsis* and elucidate key aspects of its role in the plant innate immune response.

Published online January 15, 2010 • 10.1021/cb1000026 CCC: $40.75

Bednarek *et al.* begin by investigating the molecular basis for antifungal defense by *Arabidopsis*. Wild-type strains and strains defective in PEN2 (a protein thought to function as a glycosyl hydrolase and known to be involved in plant defense pathways) were exposed to the powdery mildew fungus *Blumeria graminis*. Metabolic profiling experiments performed on leaf extracts, followed by structural analysis, indicated that several indole glucosinolate-related metabolites were involved in the plant response to the fungus. It was also determined that the cytochrome P450 CYP81F2 monooxygenase is involved in production of the indole glucosinolate 4-methoxy-I3G, which then appears to serve as a substrate for PEN2 activity as a myrosinase, an enzyme that hydrolyzes glucosinolates. This finding was unexpected on the basis of the predicted catalytic residues of PEN2. Notably, the metabolism of glucosinolates initiated by PEN2 produces compounds different from those triggered by damage from chewing insects, indicating that separate glucosinolate metabolic pathways exist depending on the specific immune response induced in the plant.



From Bednarek, P., *et al.*, *Science*, 2009, *323*, 101. Reprinted with permission from AAAS.

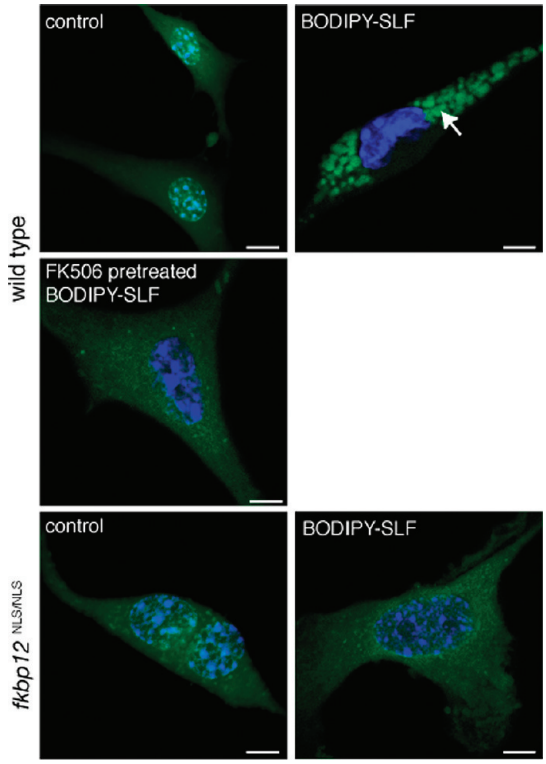
In a related study, Clay *et al.* employed a phenotypic assay to search for genes involved in a classic innate immune response by plants to bacteria, that of the deposition of callose, a glucan polymer, upon exposure to Flg22, a synthetic peptide derived from bacterial flagellin. Several genes involved in defense signaling by the hormone ethylene and in the biosynthesis and breakdown of glucosinolates were identified as necessary for the Flg22-induced callose response. Notably, evidence suggested that a hydrolytic product of the glucosinolate 4-methoxy-I3G, but not the glucosinolate itself, acts as a signaling molecule for callose deposition. Further transcriptional and metabolic profiling studies of *Arabidopsis* mutants revealed that the myrosinase PEN2, along with the genes encoding the phytochelatin synthase PCS1 and the ABC transporter PEN3, are responsible for hydrolysis and other breakdown reactions of the glucosinolates produced upon Flg22 exposure. Moreover, the glucosinolate biosynthetic pathway was found to be regulated by feedback inhibition upon accumulation of both the glucosinolates and their hydrolysis products. Taken together, the results demonstrated that, in addition to the previously defined role of glucosinolates in discouraging hungry insects, these compounds play an important role in plant defense against bacterial pathogens.

These two studies offer enlightening insight into the intricate pathways of plant defense. Glucosinolates, long studied for their properties as insect deterrents, are now exposed as key compounds in the innate immune response of plants against a wide range of microbial pathogens. With the help of transcriptional and metabolic profiling methods, the signaling pathways that govern this response, as well as the key genes and proteins involved, are beginning to be uncovered. Further exploration of this intriguing pathway will help unravel the many complex pathways involved in plant defense. **Eva J. Gordon, Ph.D.**

Pharmacologically Stabilized

Despite extraordinary progress over the past two decades, drugs developed to treat AIDS are often plagued by poor pharmacological properties. As a result, treatment with these therapeutics requires high doses and/or co-administration with metabolic enzyme inhibitors. This not only increases treatment costs but can lead to adverse side effects and poor patient compliance. While the use of prodrugs is a common strategy for overcoming some of the pharmacological issues surrounding anti-HIV therapeutics, such compounds require enzymatic processing for activity. Marinec *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2009, *106*, 1336–1341) stray from this conventional approach, presenting an innovative strategy for creating a "pharmacologically stabilized" derivative of the HIV protease inhibitor amprenavir.

The strategy draws upon on the unique properties of the natural product FK506. FK506 is a macrolide that binds with high affinity to FKBP, a protein that resides in the cytoplasm of blood cells. The authors hypothesized that by creating a bifunctional molecule composed of an FK506-like component covalently linked to an amprenavir-like component, the HIV protease activity associated with amprenavir could be recruited to the cytoplasm of blood cells and away from key metabolic enzymes that can only access free drug in the plasma. To this end, a bifunctional inhibitor termed SLFavir was synthesized, and *in vitro* experiments confirmed that the compound retained antiprotease activity. To explore the metabolic stability of SLFavir, its localization in mouse whole blood samples and in live mice was determined. Indeed, whereas amprenavir divides its time relatively equally between blood cells and plasma, SLFavir clearly prefers to reside in the cells. Moreover, it was demonstrated that SLFavir retains a dramatically increased half-life in both blood samples and in mice when compared with amprenavir. Hinting at the promising therapeutic potential of this strategy, an SLFavir derivative exhibited activity significantly higher than that of amprenavir when tested for activity against live HIV in cultured cells. Notably, this novel approach for improving the pharmacological stability of HIV inhibitors might be extended to other drug classes as well. **Eva J. Gordon, Ph.D.**

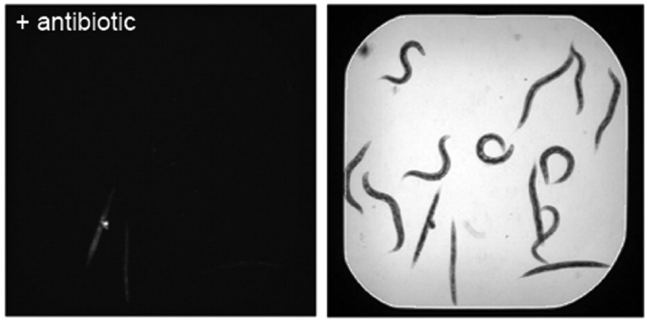


Marinec, P. S., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, *106*, 1336–1341. Copyright 2009 National Academy of Sciences, U.S.A.

Worming Our Way to New Antibiotics

The hunt for effective new antibiotics faces many challenges; the increasing emergence of drug-resistant pathogens and the lack of new drug targets are at the top of the list. Typically, new antimicrobial agents are discovered by searching for compounds that kill a pathogenic organism. However, these compounds often do not translate to effective drugs, in part because the environment in which they are discovered (a test tube or cell culture) differs dramatically from the environment in which they need to work as drugs (inside a human being). New large-scale discovery methods that better approximate this environment might yield more effective drugs. To this end, Moy *et al.* (*ACS Chem. Biol.* 2009, *4*, 527–533) describe the development of a high-throughput screen for new antibiotics that is conducted in live animals.

The high-throughput screen was adapted from a previously developed, small-scale screen designed to identify compounds that enhance the survival of the nematode *Caenorhabditis elegans* that has been infected with *Enterococcus faecalis*, a pathogen that resides in the human gastrointestinal tract. The new method enabled screening of over 37,000 small molecules and natural product extracts in live, infected worms. Several automated processes, including a large particle sorter for dispensing the nematodes, an automated microscope for acquiring images, and a modified cell image



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analysis program for quantifying worm survival, were instrumental to the success of the screen, which identified 132 compounds and 4 natural product extracts that enhanced worm survival. Of these, 80 were known antibiotics or had structural similarities to known antibiotics, validating that the screen can identify compounds with antibiotic activity. Most notable, however, was the identification of 28 novel compounds and extracts not previously reported to have antimicrobial activity. In addition, 6 distinct structural classes were found that cured infected animals but did not affect the growth of the pathogen *in vitro*. These intriguing compounds likely function through new mechanisms of action, an exciting step forward on the path toward new antibiotics. **Eva J. Gordon, Ph.D.**

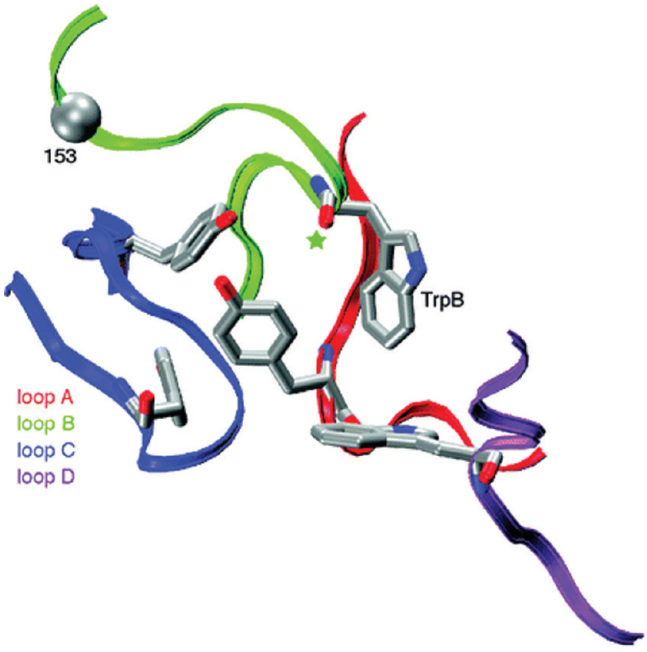
The Cannabinoid Conundrum

Recently, the endogenous receptors for marijuana's active agent, tetrahydrocannabinol, have gained attention in both the neuroscience and medical communities. Two endogenous ligands for these receptors, anandamide (AEA) and arachidonoylglycerol (2-AG), are present in humans and regulate a number of physiological responses including appetite, pain, and memory formation. Teasing apart the effects of these two different ligands has proven difficult because of a lack of effective tools for manipulating their levels. To add to the complexity, the carefully controlled degradation of each ligand in the brain relies on two different enzymes. Since degradation of AEA by fatty acid amide hydrolase (FAAH) was the better understood of the two, a new screen looked for ways to manipulate 2-AG levels to help sharpen the overall cannabinoid picture. Long *et al.* (*Nat. Chem. Biol.* 2009, *5*, 37–44) screened a chemical library for inhibitors of the candidate 2-AG degrading enzyme, monoacylglycerol lipase (MAGL) using a elegantly simple gel based assay. After refining a lead compound with further chemical decorations, the authors settled on compound JZL184 because of its high selectivity and potent inhibition for MAGL. This agent was active for MAGL inhibition in live mice and showed minimal effects on other serine hydrolase family members as judged by activity-based proteomics. The treated mice displayed higher levels of extracellular 2-AG but no effect on the other endogenous cannabinoid, anandamide. With

this specific monkey wrench in hand, the researchers were poised to ask what effects the increased 2-AG in the brain might have on behavior. They were encouraged to find that treated mice showed behavioral effects that are common to cannabinoid receptor agonists, including analgesia, hypomotility, and hypothermia. Looking forward, the availability of a specific inhibitor for both FAAH to increase anandamide levels and MAGL to increase 2-AG levels will be highly useful for neuroscientists interested in the cannabinoid pathway. In addition, JZL184 or related inhibitors may be of interest in the clinic since nonopiate pain relief still remains a pharmacological challenge due to addiction issues. **Jason G. Underwood, Ph.D.**

"Outside-the-Box" Nicotine Addiction

A clear picture of the molecular basis for nicotine addiction would be a valuable step toward preventing the more than four million smoking-related deaths that occur annually across the globe. Nicotine binds to the acetylcholine (ACh) family of receptors, which are located both in muscle and in the brain. In the brain, the $\alpha4\beta2$ ACh receptors have been linked to nicotine addition, but the distinct interactions responsible for such physiological consequences have remained elusive. Xiu *et al.* (*Nature* 2009, *458*, 534–537) now demonstrate the remarkable role played by a cation-π interaction between nicotine and $\alpha4\beta2$ receptors.



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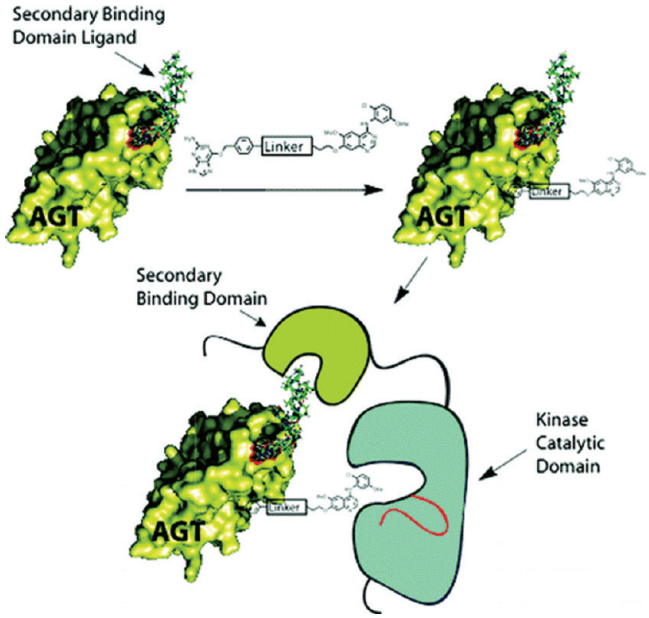
A common method for evaluating cation-π interactions is through the incorporation of fluorinated amino acid analogues into the receptor. Indeed, a decrease in cation-π energy for both acetylcholine and nicotine was observed when the tryptophan residue capable of the cation-π interaction in $\alpha4\beta2$ was replaced with fluorinated analogues. Notably, a similar decrease was not observed in the analogous experiment using the muscle ACh receptor. Furthermore, a hydrogen bond between nicotine and the same tryptophan residue was found to be enhanced in $\alpha4\beta2$ relative to the muscle receptor. Five residues comprise an aromatic box in which the cation-π and hydrogen bond interactions occur, and these residues are identical in both the brain and muscle receptors. However, additional mutagenesis experiments indicated that a single amino acid difference—lysine in the brain receptor *versus* glycine in the muscle receptor—positioned "outside the box" but near the tryptophan alters the shape of the binding site such that nicotine interacts much more strongly with the brain receptor. These insights into the molecular basis of nicotine interactions in the brain will facilitate drug design efforts for nicotine addiction, as well as numerous other diseases for which ACh receptors are established targets. **Eva J. Gordon, Ph.D.**

Targeting Kinases, Two Sites at a Time

The thought of deciphering the functions of the hundreds of protein kinases that comprise the human kinome is mind-boggling to say the least. However, kinase function is intricately involved in cell



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signaling mechanisms, and understanding signaling pathways has critical implications in the search for drug targets for many diseases. The use of kinase inhibitors is integral toward decoding kinase function, but since most inhibitors target the highly conserved ATP-binding site, designing compounds that are selective for specific kinases is notoriously difficult. Hill *et al.* (*J. Am. Chem.*

Soc. 2009, *131*, 6686–6688) now present a chemical genetic approach for creating selective, bivalent kinase inhibitors.

The approach relies on the increased affinity and selectivity that can be gained by targeting two binding sites on a given kinase instead of just one. To this end, bivalent inhibitors were designed to contain a small molecule that targets the ATP site and a peptide containing a polyproline motif that targets the SH3 domain of either Src or Abl, two closely related tyrosine kinases. The small molecule and peptide components of the inhibitors were displayed on a protein scaffold by exploiting the reactivity of O^6-alkylguanine-DNA alkyltransferase (AGT), an enzyme that can transfer small molecules to its active site cysteine provided they are linked to its natural substrate O^6-benzylguanine (BG). Thus, fusion proteins containing AGT linked to specific polyproline motifs were expressed and reacted with BG-conjugated derivatives of the ATP-competitive inhibitor 4-anilinoquinazoline. The resulting protein-small molecule conjugates were tested against Src, Abl, and a series of other protein kinases and found to be potent inhibitors that were selective for their intended target. As most kinases are bisubstrate enzymes, this clever strategy can be extended to the design of selective, bivalent inhibitors for a large portion of the kinome. **Eva J. Gordon, Ph.D.**

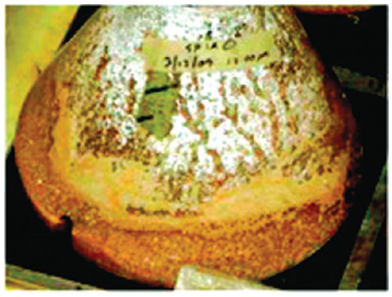
The Acetylation Nation Revealed

The role of reversible post-translational modifications (PTMs) in connecting chromatin biology to gene regulation continues to be a swirling brew of hot topics. Areas of recent interest are the acetylation of proteins by histone acetyltransferases (HATs) and the removal of this mark by histone deacetylases (HDACs). Other PTMs like phosphorylation and methylation have far reaching roles in nearly all cellular processes, but the role of acetylation has remained poorly understood due to technical limitations. Now, a new methodology (Choudhary *et al. Science* 2009, *325*, 834–840) emerges which enriches for acetylated proteins and then uses quantitative mass spectrometry to identify and measure what the authors term the "acetylome", a peptide inventory of acetylation sites in several human cell lines.

The proof-of-principal came from the histones and the changes induced by HDAC inhibitors, while the proteins unrelated to chromatin demonstrate that acetylation probably tweaks nearly every complex process in the cell. In the cytoplasm, dynamic macromolecular machines like the ribosome and actin cytoskeleton complexes were found to contain dozens of target sites for acetylation. Bridging the gap with the nucleus were many of the nuclear pore complex proteins and their cargo. Finally, back in the nucleus, hundreds of proteins other than the known chromatin targets were uncovered, and these spanned nearly every functional role. Proteins involved in DNA repair, RNA splicing and control of the cell cycle all receive lysine acetylation marks. So, could reversible acetylation play regulatory roles in events like alternative splicing or trafficking of proteins and RNAs within the cell? Phylogenetic conservation scores and positioning for the target lysines argue that the answer is probably yes. This modification is ancient and the modified amino acids appear more often in conserved regions with predicted protein secondary structure. This study not only displays the power of an elegant new technique, but also defines a new area for exploration into how acetylation tinkers with cellular metabolism. **Jason G. Underwood, Ph.D.**

A Proteomic OASIS

Polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs) are mutlicomponent biosynthetic machines that catalyze the synthesis of two classes of secondary metabolite natural products, polyketides and nonribosomal peptides. The wide range of antimicrobial, anticancer, and immunosuppressant activities exhibited by these metabolites has fueled intense investigation into their biosynthesis by PKSs and NRPSs, but our understanding of the regulation and activity of these enzymes lags behind their genetic characterization. Meier *et al.* (*ACS Chem. Biol.* 2009, *4*, 948–957) present a method, termed orthogonal active site identification system (OASIS), for the global identification and characterization of PKSs and NRPSs at the proteomic level.



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The key to OASIS is the use of strategically designed molecular probes that target the active sites of PKS and NRPS enzymes. Specifically, a coenzyme derivative and a fluorophosphonate are used to target the carrier protein and thioesterase domains, respectively, of PKS and NRPS enzymes in the model organism *Bacillus subtilis*. The probes enable enrichment of the enzymes of interest, and because they rely on enzyme activity, provide information regarding catalyst function as well. Enzymes targeted by the probes are subsequently identified using a mass spectrometry-based system referred to as MudPIT (multidimensional protein identification technology). OASIS was used to compare the PKS/NRPS pathways in two strains of *B. subtilis*, revealing distinctions in the levels and activities of enzymes involved in the synthesis of various natural products, including the antibiotic surfactin. These initial studies indicate several far-reaching

applications of OASIS technology for the proteomic investigation of PKS/NRPS pathways. For example, OASIS is a powerful complement to traditional genetic and biochemical methods for enzyme detection, identification, and characterization. In addition, it enables facile comparison of PKS/NRPS enzymes between different bacterial strains and could facilitate the discovery of new PKS/NRPS gene clusters. **Eva J. Gordon, Ph.D.**

Entrained to the Membrane

The purification and characterization of specific cell types has historically involved antibody labeling and cell sorting. Cells of the immune system in particular are often classified by what proteins reside on the outside of the cell. More recently, the surface proteome is emerging as a key obstacle for stem cell biologists wishing to define the various lineages that arise during cell differentiation. Now, a new proteomics method takes a crack at the surface topology problem without the aid of antibodies. Wollscheid *et al.* (*Nat. Biotechnol.* 2009, *27*, 378–386) took advantage of the reactivity of a post-translational modification commonly found on membrane proteins, N-linked glycosylation.

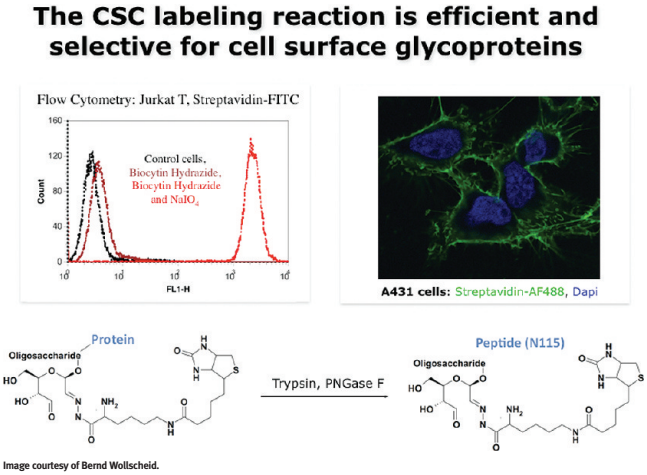
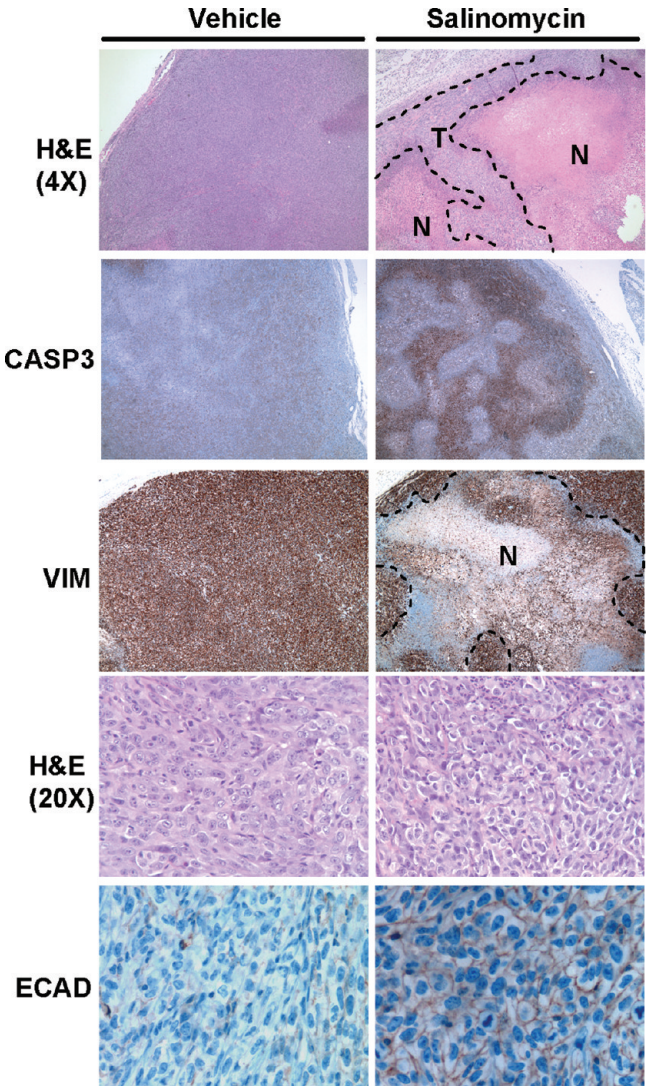


Image courtesy of Bernd Wollscheid.

In a technique termed cell surface capture (CSD), a biotin linker was reacted with oxidized N-linked sugars before cell disruption and proteolysis. This clever trick added an affinity purification handle to extracellular peptides for subsequent capture and enrichment over the more abundant intracellular peptides. Then, using a sensitive mass spectrometry method, the peptides were mapped back to the genome to generate an extracellular inventory. By comparing coupling reactions performed with isotopically light or heavy reagents, they could even quantitatively compare two samples. In proof-of-principle experiments, the authors honed the method and showed its application to cultured cells, as well as cells from primary tissues and organs. They went on to ask several novel biological questions with this elegant new tool. What proteins are turned on during T and B cell activation, and further, what proteins change upon differentiation of pluripotent stem cells into neural precursor cells? The results not only confirmed several known changes in extracellular topology but also uncovered dozens of new proteins that change during these cellular transitions. This technique could be used in reverse for finding new targets for antibody-based enrichment strategies or could stand alone as a new tool to characterize and quantify those proteins poking out of the cell. **Jason G. Underwood, Ph.D.**

Targeting the Ugly Stepsister

The excitement around stem cells "stems" from their ability to differentiate into all cell types within a specific tissue or organism. Cancer stem cells (CSCs), which could perhaps be thought of as the ugly step-



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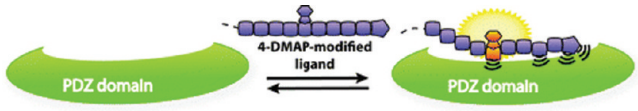
sisters of normal stem cells, can differentiate into all cell types within a tumor, intimating a unique accountability among tumor cells for their growth, metastasis, and recurrence. The broad resistance of CSCs to chemotherapeutic agents has hinted that they may be inherently impervious to drug treatment. Moreover, CSCs are often relatively rare within tumors and are not stable in cell culture, making searching for drugs that selectively target CSCs extraordinarily difficult. Now, Gupta *et al.* (*Cell* 2009, *138*, 645–659) report the development of a high throughput screen to identify small molecules capable of selectively inhibiting CSCs.

A key to the success of the screen was to gain access to sufficient quantities of CSC-like cells to screen against. This was achieved by inducing nontumorigenic immortalized mammary epithelial cells to pass through an epithelial-mesenchymal transition (EMT), which produces an enrichment of cells with stem-like properties and a drug resistance profile similar to that of CSCs. Of 16,000 compounds screened, 32 compounds were found to selectivity target cells that had undergone an EMT over cells that had not. When the compounds were tested against the corresponding tumorigenic cells, salinomycin, a potassium ionophore, emerged as a selective inhibitor. Strikingly, in *in vitro* experiments, salinomycin decreased the proportion of CSC cells by over 2 orders of magnitude relative to the common breast cancer drug paclitaxel. Importantly, salinomycin treatment also inhibits breast tumor growth in mice. Subsequent gene expression experiments indicated that salinomycin induces a loss of breast CSC gene expression, offering a molecular rationale for the observed effects. This proof-of-concept demonstration that CSCs can be selectively targeted outlines an exciting new approach for cancer drug discovery efforts. **Eva J. Gordon, Ph.D.**

Probing Dynamic Domains

PDZ domains are one of the most abundant protein interaction modules mediating cellular signaling in eukaryotic cells. These compact modules typically assemble macromolecular complexes by binding to the C-terminal regions of interacting proteins. PDZ domains bind transiently to multiple proteins with relatively weak (low micromolar) affinity and thus, are key elements in the plasticity of these protein assemblies. This promiscuity, together with structural features of the domains themselves, makes it difficult to develop binding probes based on a residue replacement strategy, where a conserved ligand residue is replaced by a reporter group with similar properties. However, Sainlos *et al.* (*J. Am. Chem. Soc.* 2009, *131*, 6680–6682) now report a clever method for developing fluorogenic peptides to probe the dynamics of PDZ-domain interactions.

The design of the probes was based on peptide sequences from natural ligands of PDZ domains, with the addition of a fluorophore from the dimethylaminophthalimide family that served as an environmental sensor. In an initial step, a peptide library based on the



C-terminal binding motif of the PDZ-binding protein Stargazin was used to determine the optimal location for the fluorophore and the ideal linker length between the peptide backbone and the fluorophore. Afterward, a new library of probes based on three different PDZ domain-binding sequences were then synthesized to afford probes with unique specificity for the partner PDZ domains. In each case, the increases in fluorescence (up to 265-fold) correlated with the binding affinity of the probes for their target PDZ domains. Fluorescence titrations and isothermal titration calorimetry studies indicated that the fluorescent peptides bound more tightly to their target PDZ domains than unlabeled peptides. Initial structural studies did not show an obvious origin for this enhanced binding and fluorescence emission, which underscored how this general approach could prove useful for designing probes for other similar domains when the rational replacement strategy falls short. **Sarah A. Webb, Ph.D.**

Ubiquitous Ubiquitination Enzymes

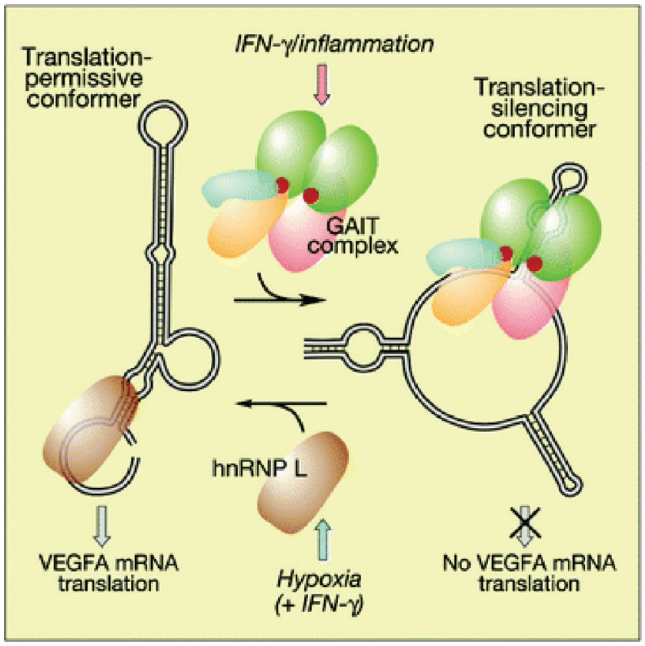
Modification of proteins with ubiquitin (Ub) is a critical component of protein regulation, providing instructions to the cell regarding activities such as gene transcription, protein trafficking or protein degradation. Misregulation of the ubiquitylation process has been implicated in several diseases, and thus targeting this pathway has intriguing therapeutic potential. An intricate yet somewhat undefined network of enzymes controls Ub attachment to and removal from target proteins, and while genomic profiling has implicated numerous proteins in the Ub pathway, this method offers no verification of their activity. Activity-based proteomic profiling, on the other hand, can offer insight into the structure and function of the proteins involved in this complex system and can also help in the design of small molecules capable of disrupting it. To this end, Love *et al.* (*ACS Chem. Biol.* 2009, *4*, 275–287) use activity-based profiling to search for additional enzymes involved in the ubiquitination system.

The profiling strategy is based on previous studies in which chemical ligation methods enabled installation of an electrophilic group at the C-terminus of an epitope-tagged Ub fusion protein. Redesign of the electrophilic group led to the creation of an expanded set of Ub-based chemical probes capable of tagging additional enzymes involved in ubiquitination. Once tagged, the enzymes can be isolated by immunoprecipitation and identified using tandem mass spectroscopy. Several enzymes involved in all classes of Ub modification, including the Ub conjugating and ligation machinery as well as deubiquitinating enzymes, were uncovered in this effort. More-

over, the probes provided a conduit into exploration of the structure, function, and mechanism of target enzymes. For example, examination of a ubiquitin ligase target by one of the probes revealed that three out of six cysteines residues present within the catalytic domain reacted with the probe, pointing to candidate residues for ubiquitin attachment. **Eva J. Gordon, Ph.D.**

Humans Flip a Riboswitch Too

Sensing the environment and adjusting gene expression to external cues are critical tasks for cells ranging from the tiniest bacterium to the largest mammal. In the past 10 years, a large body of work indicates that bacteria and fungi can sense their environments with the help of riboswitches. These RNA modules respond to a metabolite and change their conformational status such that a nearby gene involved in the synthesis of that metabolite is adjusted up or down in transcription or translation efficiency. But, could this type of mechanism be active in complex eukaryotes such as mammals? Now, a study by Ray *et al.* (*Nature* 2009, *457*, 915–919) answers this question with a resounding yes and a new mechanism for how RNA structure can modulate mammalian gene expression.



The study focused on the 3′ untranslated region (UTR) of an important human growth factor gene, VEGF. Under normal oxygen conditions, the protein displays weak translation, a property that was previously shown to be controlled by 3′ UTR sequences. Expression of VEGF is upregulated during hypoxic stress, such as when the cell is trapped within the confines of a tumor. By dissecting the RNA elements in the 3′ UTR, the authors found an interesting conformational switch. Instead of a small molecule ligand like the riboswitches, the VEGF RNA uses changes in protein concentration to switch between two states. Under hypoxic conditions, hnRNP L bound to an RNA element to promote a compact structural motif that favors translation of the VEGF RNA. Under normal oxygen conditions, hnRNP L was downregulated by protein degradation and the RNA formed a different conformation displaying low translational efficiency. Showing mutually exclusive binding with hnRNP L was the GAIT complex, an interferon-γ-regulated protein complex previously shown to downregulate VEGF translation. Thus, this 3′ UTR acts as a type of human riboswitch that integrates information from upstream signals, tweaks the level of key proteins, and unlocks RNA structures that turn gene expression on or off. Uncovering the first riboswitch in bacteria proved to be just scratching the surface, so mammalian cells may be the next frontier for this type of ribo-regulation. **Jason G. Underwood, Ph.D.**