Spotlight

Mice Thrown Off the Scent

The mouse olfactory system is an excellent model for studying how the scent of odorants is processed in other mammals such as humans. In mice, odorants are recognized in the brain through information that is passed through any combination of ~1000 kinds of olfactory sensory neurons (OSNs) located in the olfactory epithelium of the nasal cavity. The olfactory epithelium is divided into discrete dorsal and ventral zones, and all OSNs found in one zone project to a similar region in the olfactory bulb. To figure out how the organization of spatially separate OSNs affects odorant recognition in the brain, Kobayakawa and colleagues create and characterize mutant mice strains in which OSNs in a specific region of the olfactory epithelium are lacking (*Nature* 2007, *450*, 503–508; DOI: 10.1038/nature06281).

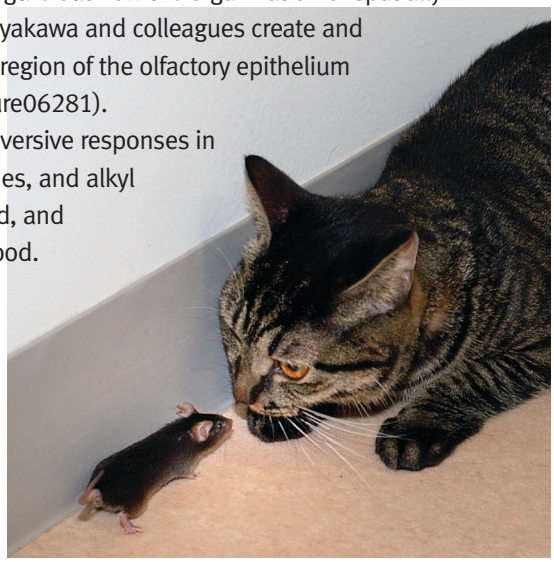


Image courtesy of Ko and Reiko Kobayakawa.

The authors used chemical compounds known to induce aversive responses in mice. Normal mice avoided aliphatic acids, aliphatic aldehydes, and alkyl amines, all of which are odorants associated with rotting food, and were instead attracted to chemical odorants reminiscent of food. Mutant mice lacking dorsal OSNs, on the other hand, were able to detect both groups of odorants but displayed no aversion to either. Quite remarkably, mutant mice could also detect odorants with similar chemical structures such as pentanal and hexanal. They could also detect odorants such as the normally fear-inducing trimethyl-thiazoline (secreted from fox glands) and the pleasant-smelling eugenol, just like wild-type mice. Mutant mice could also be trained to avert these odorants but lacked the innate avoidance and fear responses found in normal mice. From this study, the authors concluded that dorsal OSNs are responsible for inducing innate fear and aversive behaviors.

Anirban Mahapatra, Ph.D.

RNAi, Just Add Light

The discovery of RNA interference (RNAi) revolutionized both the realm of large-scale biological screening and the more common "your favorite gene" knockdown experiments. Researchers can now synthesize small interfering RNAs (siRNAs) composed of two 21-nucleotide RNA strands, anneal them together, and introduce these into mammalian cells by formulation with cationic lipids. These siRNAs program endogenous silencing complexes, and the result is cleavage and rapid destabilization of a target mRNA. As with any gene manipulation technique, the ability to induce silencing at a specific time is critical to some experimental designs. Inducible vector systems, which transcribe the silencing RNA in a drug-dependent manner, have been one answer to this request. However, the extraordinary potency of RNAi makes any leakiness of these systems a technical barrier. Now, a new method takes on the inducible RNAi challenge with a special ray of light.

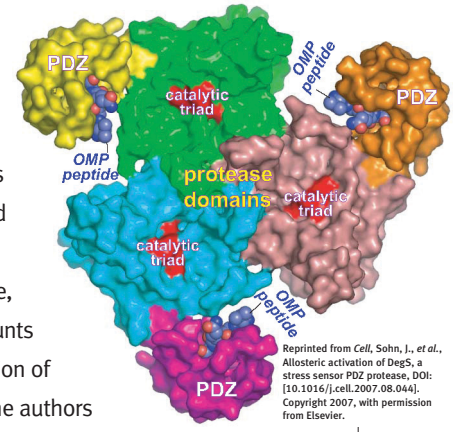
In a new paper, Mikat and Heckel (*RNA* 2007, *13*, 2341–2347) use caged nucleotide monomers during the synthesis of their siRNAs. The cage chemistry, an

Published online December 21, 2007 • 10.1021/cb700242h CCC: $37.00

Periplasm, Peptide, and Proteolysis

When a signal needs to cross between cellular compartments, ligand-activated proteolysis can act as a signaling mechanism. In *Escherichia coli*, the DegS protease is activated by outer-membrane proteins (OMPs) within the periplasmic space. The OMPs present their carboxy termini when they are misfolded, and these bind to the trimeric DegS enzyme and somehow switch on the proteolytic activity. Reminiscent of other signaling cascades and the game dominoes, this protease then goes on to cleave a substrate protein, RseA, which in turn activates another protease on the opposite side of the membrane. With bacteria, the end result of the DegS pathway involves up-regulating many of the genes involved in periplasmic maintenance. But, how does DegS control its potent serine protease and only turn on the cutting power when a misfolded protein is encountered? A recent study by Sohn *et al.* (*Cell* 2007, *131*, 572–583; DOI: 10.1016/j.cell.2007.08.044) demonstrates that this heterotrimeric enzyme shows hallmarks of the classic allosteric regulation found in biochemistry textbooks.

The authors used an improved proteolysis assay and rigorous enzymology to show that DegS is allosterically controlled by peptides derived from the OMP carboxy-termini. A key player in the DegS regulation is the protein's PDZ domain, which binds to OMP peptides and turns on the protease. Deletion of this domain led to a constitutively active form of DegS. To further support the activation hypothesis, the structure of the protein missing the PDZ domain was solved by X-ray crystallography and compared with previously published DegS structures with and without peptide ligand. It appears that the peptide binding allows a more relaxed conformation for the enzyme, and this accounts for the activation of proteolysis. The authors also demonstrated positive cooperativity for this enzyme and did a battery of mutagenesis trials to delve more deeply into the structural predictions. Many of the important residues for the activation appear to be conserved in other PDZ-containing proteases. This impressively complete study demonstrates that classic enzymology is far from dead and may just need a little peptide poke sometimes. **Jason G. Underwood, Ph.D.**



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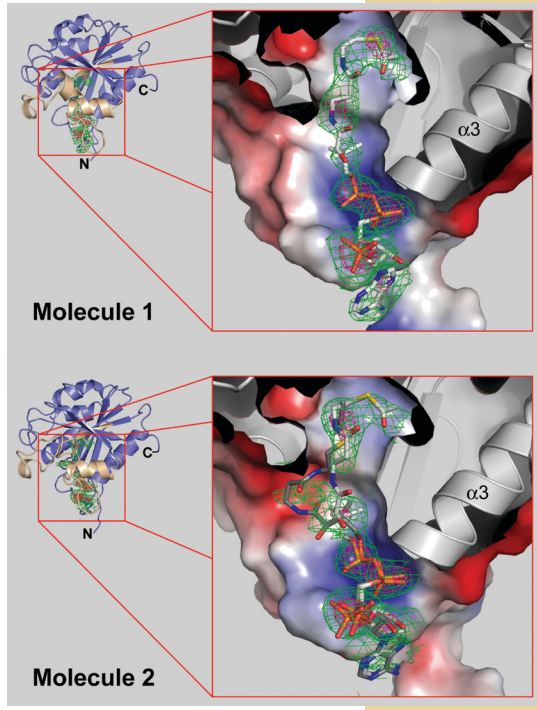
NPP, 2-(2-nitrophenyl)propyl moiety, enables uncaging of a nucleotide by exposure to UV light. The authors synthesized siRNAs targeting the GFP with caged nucleotides in a variety of positions. They then tested the efficacy of these siRNAs in HeLa cells *via* a co-transfection assay with vectors producing GFP and a red fluorescent protein as a normalization control. The modified siRNAs showed little silencing in cells, but a post-transfection UV light treatment fully restored the activity of the siRNA. Only caging groups at the very heart of the siRNA, where the antisense strand guides the sequence-selective messenger RNA degradation, were highly effective. This is the region in the siRNA that is most mismatch-sensitive, and the caged nucleosides can also be seen as light-curable pairing mismatches. The method is definitely in its first stages of development; given enough time, some of the caged siRNAs lost their modifications *in vivo* and began silencing independent of UV light. Even with early caveats, the idea of transfecting an siRNA and then activating it at a specific time makes this study a step toward an attractive new tool. Jason G. Underwood, Ph.D.

New Functions for an Old Enzyme

Polyketide synthases (PKSs) are multicomponent enzyme complexes that are involved in the synthesis of a number of secondary metabolites, many of which have known and potential therapeutic uses. PKSs catalyze the assembly of polyketide products from short-chain acyl-CoA through a complex process involving an initiation step and multiple rounds of elongation and processing.

Curacin A is a mixed-polyketide nonribosomal peptide that is obtained from the cyanobacterium *Lyngbya majuscula* and shows potential as a cancer drug lead. The detailed mechanism of synthesis of this compound is currently the subject of considerable investigation. A particularly mysterious feature of curacin A assembly involved a presumed chain initiation module of unknown function that contains a GCN5-related *N*-acetyltransferase (GNAT) domain. The GNAT superfamily contains >10,000 known representative enzymes that are found in all three domains of life and, as the name suggests, is representative of enzymes that catalyze acyl transfer to a primary amine. Now, Gu *et al.* (*Science* 2007, *318*, 970–974; DOI: 10.1126/science.1148790) demonstrate a unique bifunctional decarboxylase/S-acetyltransferase for the GNAT scaffold involved in curacin A chain initiation.



From Gu, L., *et al.*, *Science*, Nov 9, 2007, DOI: 10.1126/science.1148790. Reprinted with permission from AAAS.

First, the authors determined the involvement of the GNAT of the chain initiation module in loading an acyl group. Then, they showed that the GNAT catalyzed decarboxylation of malonyl-CoA followed by acyl transfer of substrate. Interestingly, the enzyme has lost most of the N-acetylation activity that is the hallmark of other GNATs. Crystal structures revealed the presence of two tunnels emerging on opposite sides of the protein, with one tunnel involved in CoA binding and the other the site of acetyl-group transfer *via* transthioesterification. Elucidation of novel roles for the loading GNAT module is significant because these domains are found in a large number of biosynthetic gene clusters for known natural products as well as in orphan metabolic systems from microbial genomes whose natural products await discovery. **Anirban Mahapatra, Ph.D.**

Toxins in the Path of Least Resistance

Toxins produced by the microorganism *Bacillus thuringiensis* (Bt) naturally kill insect pests such as insect larvae upon consumption. Because Bt toxins are specific for certain organisms and harmless to most others, transgenic crop plants modified to produce these toxins are used widely throughout the world. The most common Bt toxins are the crystal toxins that belong to the Cry1A family. Bt toxins belonging to this family bind to an extracellular domain of cadherin proteins spanning the midgut membrane of insect larvae. Unfortunately, a major obstacle in the long-term use of any pesticide is the development of resistance, and insects resistant to Cry1A toxins have evolved both in the laboratory and in the wild.

One hypothesis of how Cry1A toxins kill lepidopteran larvae suggests that after protease activates monomers of the toxin, these monomers bind to cadherin, allowing the cleavage of the N-terminus of the toxin, including a section designated helix α-1. Subsequently, the monomeric toxins oligomerize and cause the creation of pores in the midgut membrane. In an elegant test of this hypothesis, Soberón *et al.* (*ScienceExpress*, www.sciencemag.org/cgi/rapidpdf/1146453.pdf) analyze Cry1A toxins lacking helix α-1 and find that these modified toxins oligomerize in the absence of cadherin. Resistance to Cry1A native toxins is often linked to mutations in midgut cadherins; however, because the modified Cry1A toxins do not require cadherin to oligomerize, they are effective against tested larvae of resistant strains. From these observations, they deduced that in larvae cadherin promotes the toxicity of native Cry1A toxins by allowing these toxins to oligomerize through helix α-1 removal. Ultimately, what is most refreshing about this study is that the authors are successful in elucidating the mechanism of action of Cry1A toxins and in simultaneously providing a practical strategy for combating resistant strains. **Anirban Mahapatra, Ph.D.**

A Fungus Takes on Cancer

Numerous natural products or chemical derivatives have made their way into the pharmacy, but a large number still don't have a molecular explanation for their benefits. The fungus *Aspergillus* produces a compound named for its original isolation from a green alga, (+)-avrainvillamide. This complex multiring alkaloid is decorated with a variety of methyl groups, but it's the antiproliferation effects of this avrainvillamide that have decorated its short history. This natural product and derivatives are capable of inhibiting cancer cell growth in the Petri dish. But how does this alkaloid put the brakes on cancer cells? In a recent study by Wulff *et al.* (*J. Am. Chem. Soc.* 2007, *129*, 14,444–14,451; DOI: 10.1021/ja075327f), a biotinylated derivative of avrainvillamide was used as bait in a molecular fishing expedition for the cellular partner of this interesting toxin.

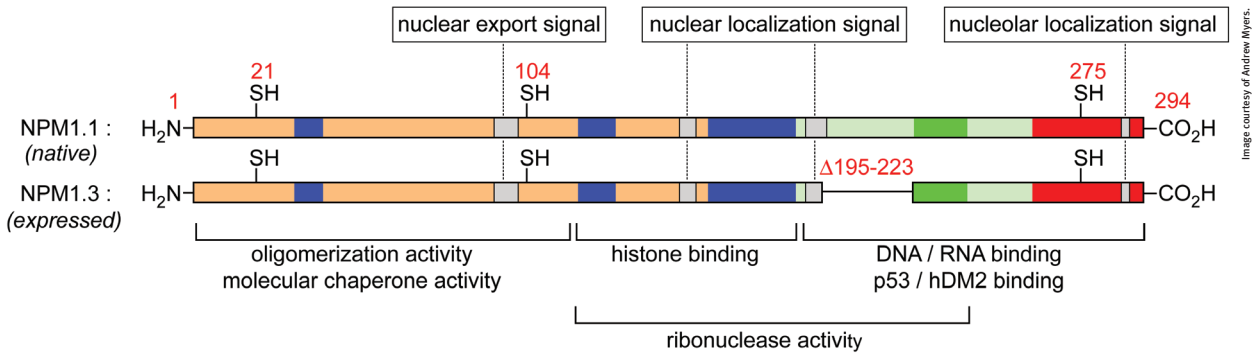


Image courtesy of Andrew Myers.

Breast cancer cells were treated with the drug, and then they were harvested, lysed, and bound to avidin beads to pull out the biotinylated compound. Mass spectrometry identified a binding partner, nucleophosmin, a nucleolar protein that is overexpressed or mutated in many cancers. A fluorescent derivative of avrainvillamide was used to treat cancer cells, and it displayed enriched nucleolar localization. Further, competitive binding assays were performed in which nucleophosmin's affinity for the biotinylated compound was challenged with unconjugated avrainvillamide or chemical derivatives. An impressive correlation was observed between the efficacy of the derivatives in antiproliferation assays and their affinity for nucleophosmin. By knocking down the levels of this protein in cancer cells and then treating with the compound, the authors show that the antiproliferative effects of avrainvillamide are, at least in part, through nucleophosmin. This protein has previously been tied to the intracellular levels of tumor suppressor p53, so this was an excellent downstream candidate. Indeed, the treatment of two different cancer cell lines with the drug increased p53 levels. This study brings together synthetic organic chemistry and cancer biology, only to leave the reader wondering: what other compounds are out there in the wild, and what might they target in the cell? **Jason G. Underwood, Ph.D.**