Spotlight

Neuronal mRNAs Caught in a TRAP

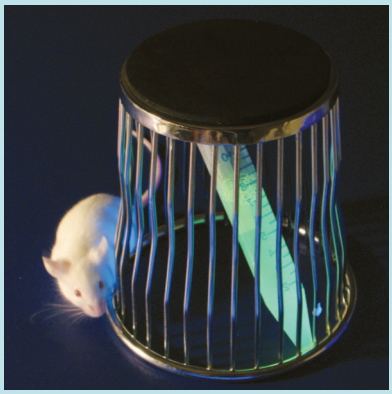

Image courtesy of Nathaniel Heintz.

The structural complexity of the mammalian brain has made gene expression profiling particularly difficult. While a skilled neuroanatomist can easily dissect the brain into the various regions such as cortex versus cerebellum, these brain structures contain hundreds of different cell types. To see whether a gene is expressed in a particular brain cell type, traditional methods have been limited to a gene-by-gene methodology using specific RNA probes or antibodies to the gene of interest and co-stains with marker genes. Now, a massive effort to address this problem has culminated in two publications that open up new doors into molecular neuroscience. These papers describe an optimized technical guide for profiling the mRNA population from various cell types in the brain and go on to take the reader on an unprecedented tour through the brain with global expression data on >24 cell types.

The first of the papers (Heiman *et al.*, *Cell* 2008, *135*, 738−748) unveils a technique to label and purify the ribosomes of living animals in a cell-type-specific manner. Ribosomal protein L10a was tagged with the green fluorescent protein (GFP) and placed under the control of a promoter known to be cell-type-specific. For example, the striatonigral neuronal subtype is known to produce the dopamine receptor Drd1a, so the promoter from this gene was used to drive the expression of the GFP-L10 fusion protein using transgenic mice; any cells that had the Drd1a promoter switched also produce the modified ribosomal protein. This GFP-tagged protein made its way into translating ribosomes within the living mouse brain to serve two purposes. First, the cells were lit up for histological purposes, but the second and more novel trick was to use GFP as an affinity tag. The authors developed what they term translating ribosome affinity purification (TRAP) to select the tagged ribosomes out of a homogenized mouse brain and then detect translating mRNA molecules by microarray. By this method, they could take a snapshot of the unique translating proteome extracted from one genetically defined cell type of the brain.

After the proof of principle, the Heiman *et al.* study proceeded to compare two extremely similar types of neurons that respond to dopamine, the Drd1a striatonigral and the Drd2 striatopallidal types, and found many previously unknown gene expression differences. The authors also treated the mice with cocaine, a drug that elevates dopamine levels in the synapse, and then compared the TRAP profiles with the placebo group. The drug caused hundreds of gene changes in each cell type, including striatonigral-specific changes for signaling pathways of another neurotransmitter, GABA. This type of observation was not possible using previous methodologies, and this simple drug example with just two neural subtypes shows the power of the TRAP platform in comparing stimuli-induced expression changes.

The second study (Doyle *et al.*, *Cell* 2008, *135*, 749−762) used this technique on a grand scale and placed the tagged ribosomes into 24 different cell populations of the mouse central nervous system. The result is a new type of brain atlas that gives detailed information about the gene expression programs driving the fate and function of cell types in the brain. The authors showed that a primary source of diversity between the cell types is actually those proteins on the outside, the channels and receptors. Neurons that respond to different neurotransmitters, glia, oligodendrocytes, and more are all archived in a database resource for basic and applied scientists to query. Of keen interest will be the cell subtypes that connect to human disease, such as GABA neurons and epilepsy or dopaminergic neurons and Parkinson's disease. These huge data sets will also enable computational methods to look for coregulated genes and pathways. With this impressive resource, let the mining begin.

Jason G. Underwood, Ph.D.

A Workhorse Organism Joins the Workforce

Dwindling oil reserves in the ground are shifting the focus to above the earth and what fuels can be manufactured in an oil-independent fashion. The field of biofuels is rapidly growing, and new organisms and genetic variants are being tested for production of combustible hydrocarbon chains and alcohols. Now, an old friend of the biologist, *Escherichia coli*, is getting a mutational makeover to pro-

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duce a product that is not native to its metabolic repertoire. The approach by Zhang *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2008; DOI: 10.1073/pnas.0807157106) combined overexpression of some metabolic enzymes, addition of some new ones, and a dash of rational active site design to turn regular bacteria into tiny factories for unnatural alcohols.

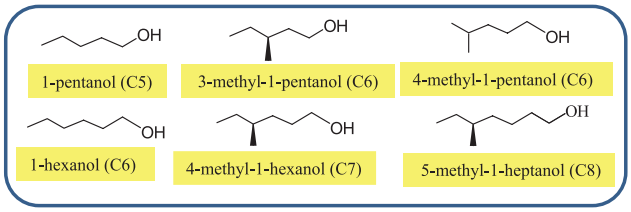
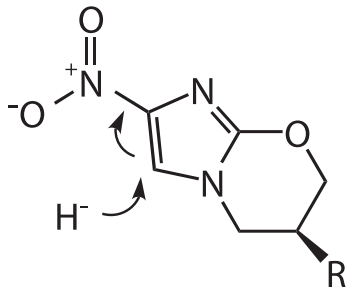


Image courtesy of Kechun Zhang and James Liao.

Using existing metabolic maps for *E. coli*, the authors reasoned that a metabolite on its way to being the amino acid L-isoleucine, 2-keto-3-methylvalerate, might take another path to grow the carbon chain. Naturally, bacteria use acetyl-CoA as a carbon elongation unit and the Leu operon, encoding 4 enzymes that convert a similar precursor, 2-keto-isovalerate, into the amino acid L-leucine. Upon overexpression of this operon, the methylvalerate derivative was indeed grown to a methylhexanoate. To push this on into an alcohol, a decarboxylase and an alcohol dehydrogenase from other organisms were also overexpressed with the operon. The result was a bacterial strain that could now produce 3-methyl-1-pentanol, a C6 alcohol that was not detectable in the wild-type *E. coli*. With this as a starting strain, computer modeling was used to zoom in on the active site of the LeuA enzyme, which elongates the carbon chain and the decarboxylase, which turns the acid into an aldehyde. Switching sidechains in the active sites of these two enzymes to accommodate the more bulky substrate resulted in a >100-fold increase in the C6 alcohol yield. The many mutants and alcohol products explored in this study just scratch the surface of what is to come in this realm of microorganism tinkering for the purpose of fuel production. **Jason G. Underwood, Ph.D.**

Saying NO to Tuberculosis

Tuberculosis affects one-third of the world's population, and the 9 million new infections and 2 million deaths each year have made it one of the world's deadliest diseases. In searching for treatments for this rampant and evasive killer, it is particularly important to target nonreplicating cells, which contribute to the difficulties in effectively treating the disease and are thought to be responsible for latent tuberculosis. The bicyclic nitroimidazole PA-824 is active against both replicating and nonreplicating bacteria, making it a promising drug candidate for tuberculosis treatment. Now, Singh *et al.* (*Science* 2008, *322*, 1392−1395) present important insight into the complex mechanism by which PA-824 kills nonreplicating *Mycobacterium tuberculosis*.



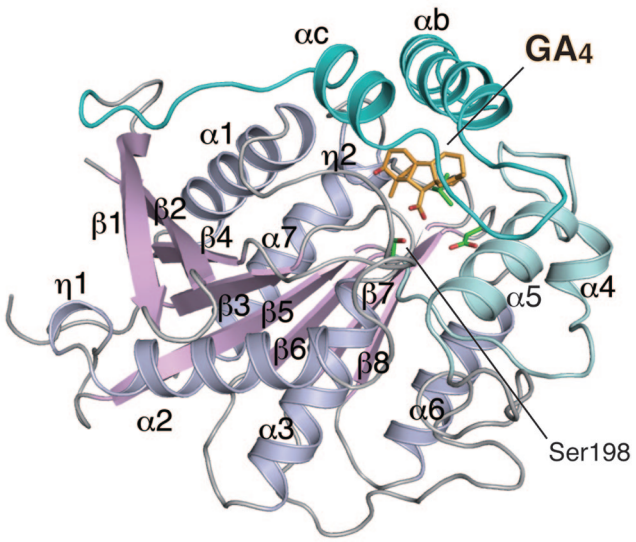
From Singh, R., *et al.*, *Science*, November 28, 2008, DOI: 10.1126/science.1164571. Reprinted with permission from AAAS.

PA-824 is a prodrug and thus must be activated in the cell for activity. Discovery of a rare class of bacterial mutants suggested that a protein of unknown function, named Rv3547, might be involved in the activation of PA-824. Indeed, in the presence of a reduced form of the deazaflavin cofactor F_{420} known to be required for PA-824 activity, Rv3547 was capable of reducing PA-824, resulting in the formation of three products. Structural characterization revealed the main product as that of PA-824 minus the nitro group. Mechanistic studies suggested that the reaction resulted in the release of reaction nitrogen species, including nitric oxide. Moreover, using a series of PA-824 derivatives, the authors demonstrated that the nitric oxide release correlated directly with anaerobic killing activity, which is important for targeting nonreplicating bacteria. Thus, PA-824 and similar bicyclic nitroimidazoles may be a highly specific class of nitric oxides donors that require a unique deazaflavin (F_{420})-dependent nitroreductase like Rv3547 to promote their activity under anaerobic conditions. The insights gained into this intriguing mechanism could lead to the design of compounds with improved activity against latent tuberculosis. **Eva J. Gordon, Ph.D.**

Removing the Gibberish from Gibberellin Recognition

Though discovered in 1926, the gibberellin (GA) class of plant hormones gained significant fame in the 1950s, when it was found that they could positively affect the growth of seedless grapes. GAs actually comprise a large family of tetracyclic diterpenoids that have diverse biological roles in plant growth. To increase our understanding of the origin and function of GAs, Shimada *et al.* (*Nature* 2008, *456*, 520−523) investigate the structural basis of GA recognition by the nuclear receptor Gibberellin insensitive dwarf1 (GID1).

GID1 has sequence similarity to hormone-sensitive lipases (HSLs), which are enzymes involved in lipid metabolism. Indeed, the crystal structure of GA_4 with GID1 from the rice species *Oryza sativa* revealed an α/β-hydrolase fold resembling that of HSLs. One



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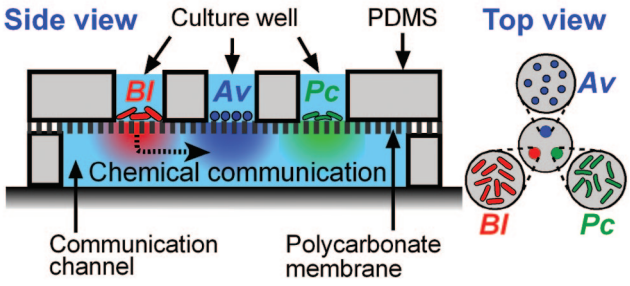
notable exception appears in the function of an amino-terminal lid. In HSLs, the lid covers the substrate-binding site and opens upon substrate binding. In contrast, in GID1, the lid is open in the absence of substrate and closes upon GA_4 binding. GA_4 is held in place by a network of hydrogen bonds, as well as a host of nonpolar interactions that are thought to facilitate the closing of the lid over the binding pocket. Mutants in which residues thought to be involved in GA-binding were changed to alanine showed little to no activity, confirming their critical role in GA recognition. The observation that most of the residues important for GA-binding are conserved within plant GID1s but not in HSLs suggests that GID1 likely evolved from HSL, recruiting residues that resulted in high affinity and selectivity for specific GAs that induced a desired biological response. Interestingly, some lower plant GID1s contain nonconserved residues and as a result have lower affinity and specificity for certain GAs. The authors propose that GID1s in higher plants have evolved highly specific recognition elements that enable them to capitalize on the growth-stimulatory properties of specific GAs. **Eva J. Gordon, Ph.D.**

Microbes Need Their Space

Communities of microbes coexist in nature, and the organisms within the community rely on each other to create a thriving environment. For example, different soil microorganisms perform certain tasks, such as nitrogen processing, decomposition of organic matter, and remediation of environmental contamination, that are essential for the stability and function of their community. Attempts to replicate such communities in the laboratory have been largely unsuccessful, as one species often dominates and, in a quite unneighborly fashion, effectively kills off its neighbors. Building on the observation that, in nature, such communities coexist within matrices with defined spatial structure, Kim *et al.* (*Proc. Natl. Acad. Sci.* 2008, *105*, 18,188−18,193) create a community of three different species of soil bacteria in which the spatial structure is controlled using microfluidics.



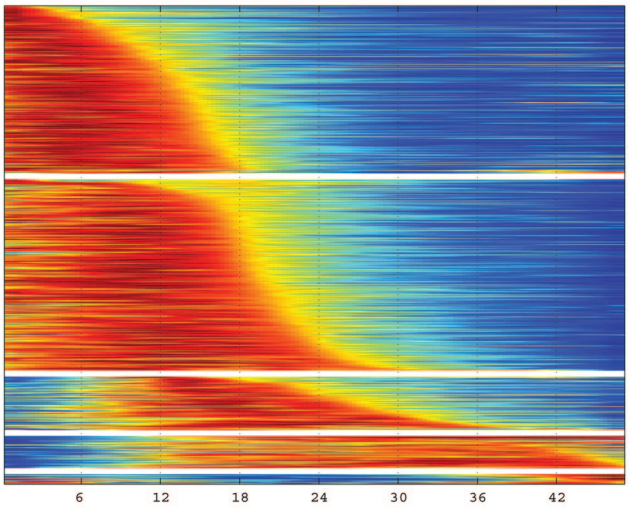
Kim, H. J., *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, *105*, 18188−18193. Copyright 2008 National Academy of Sciences, U.S.A.

The three bacterial species were selected such that each can perform a function necessary for the survival of the community as a whole. *Azotobacter vinelandii* (Av) can employ its nitrogenase to supply nitrogen sources, *Bacillus licheniformis* (Bl) can make use of its β-lactamases to reduce antibiotic pressure from penicillin supplied in the culture medium, and *Paenibacillus curdlanolyticus* (*Pc*) can utilize its cellulases to provide a carbon energy source. When the three species were simply cocultured in a test tube, one species would invariably overtake the population. However, when a microfluidic device was employed to spatially segregate each species but still enable the flow of chemical communication among them, the community was stable. Notably, if only one or two members of the community were present, its population size decreased or remained at initial levels, signifying the need for all three species to support the community. A mathematical model was additionally devised to describe how spatial structure might facilitate stabilization of the community. Given the importance of microbial communities in the environment and in human health, these studies offer an innovative approach toward understanding and exploiting these communities for environmental and medical purposes. **Eva J. Gordon, Ph.D.**

The Power of the Individual

Drugs, especially those that combat challenging diseases such as cancer, can have very powerful effects on cells. Little is known, however, about how and why individual, seemingly identical, cells can respond differently to the same drug. To address this puzzling behavior, Cohen *et al.* (*Science* 2008; DOI:10.1126/science.1160165) investigate proteome dynamics in individual human cancer cells that have been treated with the anticancer drug camptothecin.

In tackling this daunting task, the authors used a retrovirus-based approach called "CD tagging" to fluorescently label ~1000

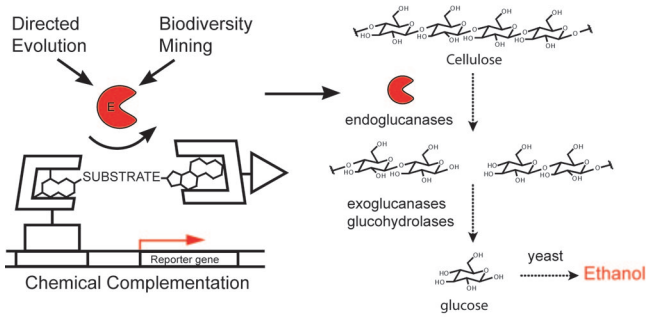


From Cohen, A. A., *et al.*, *Science*, November 20, 2008, DOI: 10.1126/science.1160165. Reprinted with permission from AAAS.

different proteins, designed such that any given cell expresses a single fluorescent protein. Time-lapse fluorescence microscopy was used to take movies of the cells over several days, and the levels and locations of the tagged proteins were tracked. The proteins directly affected by drug activity rapidly underwent changes in localization; this was followed by slower, more wide-ranging changes in protein levels due to degradation or accumulation over time. While most proteins displayed only moderate variability among individual cells, a small subset of proteins exhibited distinct and quite intriguing behavior, such as having increased levels in some cells but decreased levels in others. For example, levels of the RNA-helicase DDX5 increased dramatically in cells that survived drug treatment but decreased markedly in cells that appeared headed toward death. Moreover, this response was specific to treatment with camptothecin, because DDX5 did not exhibit this behavior in cells treated with other anticancer drugs. Thus, DDX5 may play a functional role in helping some cells circumvent cell death upon exposure to camptothecin. This approach not only provides a unique view into how proteins in individual cells respond to drug actions but also offers a method for identifying those proteins that may help cells escape their fate after drug treatment. **Eva J. Gordon, Ph.D.**

Sweet but Powerful

For such a sweet small molecule, glucose is also an enormously powerful energy source, both for living things and for industrial fuels. In the production of biofuels, a rich source of glucose is cellulose, the β-1,4-glucose polymer that comprises approximately one-third of all plant matter. However, the degradation of cellulose is a major bottleneck in the development of cost-effective methods to convert biomass to biofuels such as ethanol. More efficient cellulases, the enzymes that break down cellulose into glucose, could make this process cost-competitive, but to date only medium-throughput screens are available to search for cellulases with improved activity. Now Peralta-Yahya *et al.* (*J. Am. Chem. Soc.* 2008; DOI: 10.1021/ja ja8055744) present a chemical complementation strategy that enables high-throughput screening for high-activity cellulases.



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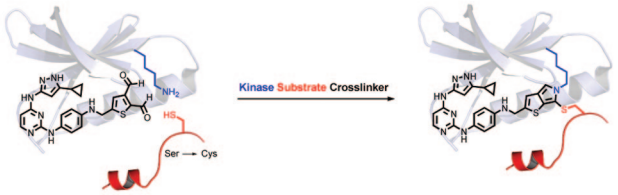
The screen is based on a yeast two-hybrid design that cleverly links cellulase activity with cell survival. The key component is a trifunctional molecule with the following properties: a methotrexate moiety that binds to a DNA-binding domain fusion protein, a tetrasaccharide cellulase substrate, and a dexamethasone group that binds to an activation domain fusion protein. When the cellulase substrate is intact, expression of a toxic gene is activated and cell death results. However, when the tetrasaccharide is cleaved by a cellulase, toxic gene expression is halted and the cell survives. A DNA library of 100 million unique cellulase variants was screened, and two active variants picked at random were found to exhibit a substantial increase in activity over native enzymes. This approach can be applied for both directed evolution methods designed to improve the activity of specific cellulases as well as biodiversity mining strategies aimed at discovering new cellulases. In addition, the chemical complementation strategy developed here can be expanded to the study of other glycosylhydrolases for a variety of applications. **Eva J. Gordon, Ph.D.**

Kinase Entrapment

For the interested reader, here are some notable phosphorylation facts: the human genome encodes >500 protein kinases, and thousands of phosphorylation sites exist in the proteome. This impressive network of kinases and their substrates is a major constituent of the marvelously complex information transfer processes that are so essential for cells to function properly. Attempts to decipher this network have resulted in the identification of numerous phosphorylation substrates, but finding many of the responsible kinases has proven significantly more challenging. Statsuk *et al.* (*J. Am. Chem.*

Soc. 2008; DOI: 10.1021/ja807066f) now present the use of an innovative chemical cross-linker that enables kinases that phosphorylate a known substrate to be covalently trapped.



The idea behind the covalent trapping of kinase−substrate complexes was to create a bifunctional compound capable of targeting a protein kinase while employing two reactive sites, one poised to react with a lysine on the kinase and the other positioned to react with a cysteine on the substrate (in place of the serine/threonine that would be phosphorylated), to covalently cross-link the kinase to its substrate. Though a similar cross-linker has been reported previously, its poor selectivity and high reactivity made it unsuitable for use in cell lysates. Design of a more selective, less reactive cross-linker encompassed making some key changes to the kinase-targeting and reactivity properties of the compound. First, the adenosine part of the cross-linker, which targets kinase active-site, was replaced with the higher-affinity kinase inhibitor scaffold. Second, the structure of the reactive, dialdehyde-containing fragment of the cross-linker was altered to attenuate its reactivity and to reduce the undesired side reactions. Indeed, the modified cross-linker could selectively cross-link recombinant Akt kinase with a cysteine-containing peptide substrate in the presence of cell lysates. This clever approach offers a viable strategy for identifying upstream protein kinases of known substrates, advancing our capacity to decipher the complex web of protein phosphorylation network in the cell. **Eva J. Gordon, Ph.D.**