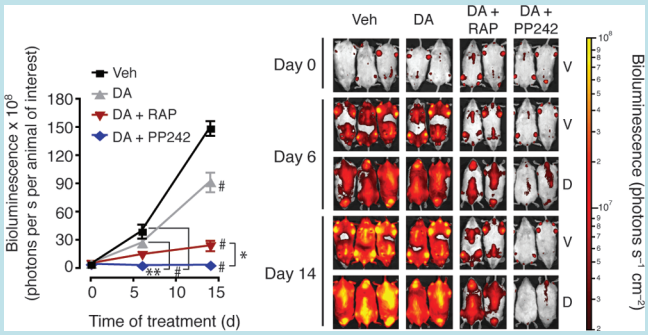
Spotlight

Getting the B-ALL Rolling Against Cancer



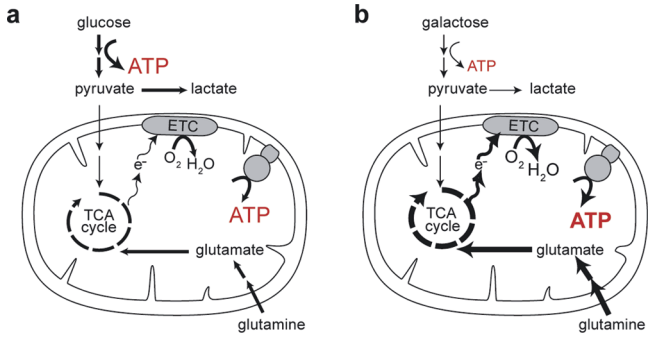
The mammalian target of rapamycin (mTOR) is a pivotal cog in the machinery that drives the growth, proliferation and survival of normal cells; it is also exploited by cancer cells for their own survival. The natural product rapamycin functions as an allosteric inhibitor of mTOR kinase activity, but as it turns out its prowess as an immunosuppressant far outshines its somewhat feeble ability to kill cancer cells. Janes *et al.* (*Nat. Med.* 2010, *16*, 205−214) now demonstrate the promising anticancer activity of an active site-competitive inhibitor of mTOR, PP242, in three mouse models of leukemia.

PP242 is a pyrazolopyrimidine that selectively targets the ATP-binding site of mTOR. *In vitro*, PP242 led to cell cycle arrest and death of a B-cell line linked to a type of leukemia termed Ph^+ B precursor acute lymphoblastic leukemia (B-ALL), inhibited growth of numerous solid tumor cell lines, and worked synergistically with known anticancer agents (tyrosine kinase inhibitors imatinib and dasatinib) to kill leukemia cells. In three separate mouse models of leukemia, PP242 consistently exhibited promising anticancer activity, such as reducing the prevalence of leukemia cells in the spleen and bone marrow, delaying the onset of leukemia, and again working synergistically with dasatinib to minimize progression of the disease in affected mice. Importantly and in stark contrast to rapamycin, PP242 did not exhibit immunosuppressive activity; growth and function of normal lymphocytes were relatively unaffected in the mice treated with the ATP-competitive mTOR inhibitor. Notably, other active-site mTOR inhibitors that also target a kinase that functions upstream of mTOR have impressive anticancer activity but exhibit immunosuppressive activity as well, calling into question their ability to be tolerated. The results presented in this study illuminate selective, active-site directed mTOR inhibitors as promising anticancer therapeutics. **Eva J. Gordon, Ph.D.**

Metabolic Manipulation

Glycolysis and mitochondrial respiration are the two major pathways used by cells to metabolize sources of energy, such as glucose. Growing evidence suggests that shifts in metabolism toward one or the other of these pathways may have intriguing therapeutic potential. For example, pharmacological agents that tip the metabolic scales toward mitochondrial respiration can retard the growth of tumors, whereas compounds that nudge metabolic processes toward glycolysis can attenuate oxidative tissue damage that can occur after heart attack or stroke. However, few molecular tools exist for manipulating the relative activities of these two metabolic pathways. Now, Gohil *et al.* (*Nat. Biotechnol.* advance online publication February 14, 2010; DOI: 10.1038/nbt.1606) describe a high-throughput screen that can identify small molecules capable of shifting energy metabolism toward glycolysis from mitochondrial respiration.

The screen is based on the fact that cells grown with galactose as the only source of sugar are forced to rely on mitochondrial oxidative processes for energy metabolism. Approximately 3700 compounds, many of which are FDA-approved drugs, were screened for their ability to differentially affect the proliferation and viability of cells grown in galactose-containing media *versus* glucose-containing media. Numerous compounds, many of which are used clinically for various indications, shifted the metabolic tendency. The investigators focused on the over-the-counter anti-nausea drug



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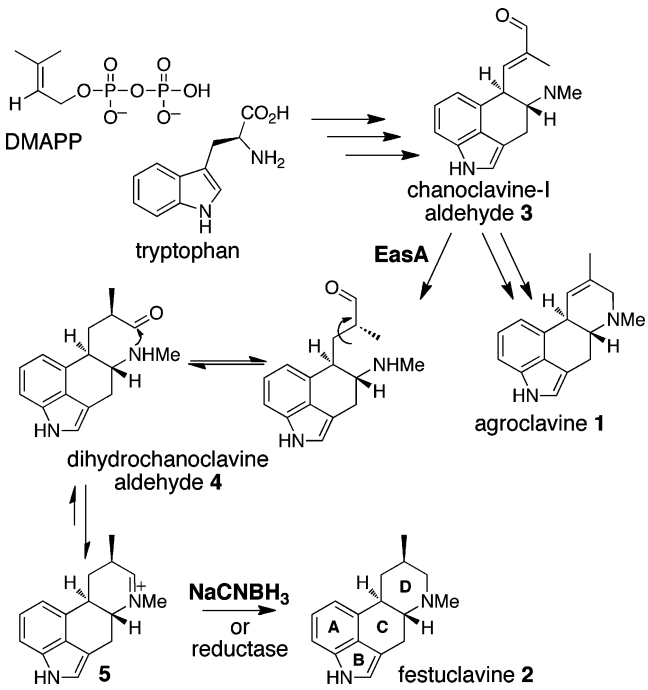
meclizine, which shifted cell metabolism toward glycolysis. After probing its mechanism of action, it was determined that meclizine blocks mitochondrial respiration indirectly and in a manner that is distinct from that which gives it its anti-nausea activity. Furthermore, in mouse models of stroke and heart attack, treatment with meclizine significantly attenuated oxidative damage to brain and cardiac tissue. This clever screening strategy can be extended both in scale and toward use of other nutrients for identification of additional small molecule tools that manipulate energy homeostatsis. **Eva J. Gordon, Ph.D.**

A New Role for Old Yellow

Ergot alkaloids are tetracyclic fungal secondary metabolites that exhibit diverse biological activities, as is showcased by their notorious, semisynthetic member lysergic acid diethylamide (LSD). Exploration into the biosynthesis of ergot alkaloids points to a tryptophan-derived, α,β-unsaturated aldehyde-containing derivative of the tricyclic alkaloid chanoclavine as a potential candidate for a precursor common to all ergot alkaloids. Cheng *et al.* (*J. Am. Chem. Soc.* 2010, *132*, 1776−1777) now demonstrate that EasA, a protein encoded in the ergot gene cluster of the fungus *Aspergillus fumigatus* and a homologue of Old Yellow Enzyme, catalyzes the reduction of the alkene functionality of this chanoclavine derivative, leading to formation of the D ring of the ergot alkaloids.



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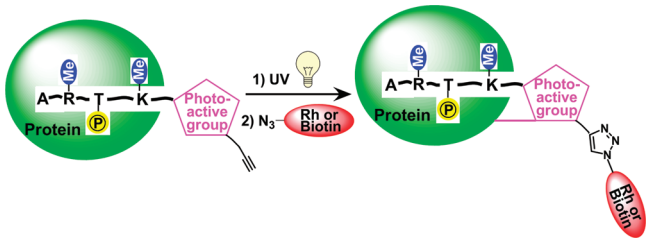
EasA was recombinantly expressed in *Escherichia coli* and tested for reactivity with the α,β-unsaturated chanoclavine derivative in the presence of NADPH, which is a cofactor for Old Yellow Enzyme. Liquid chromatography−mass spectroscopy, high resolution mass spectrometry, and nuclear magnetic resonance analysis each provided evidence that the enzyme reduced the α,β-unsaturated compound, which then quickly cyclized to generate a tetracyclic imine precursor to the festuclavine class of ergot alkaloids. This reactivity is consistent with the phenotype of mutant strains of *A. fumigatus* lacking the *easA* gene, which exhibit a build-up of chanoclavine compounds and do not produce downstream ergot alkaloids. Using mechanistic studies of Old Yellow Enzyme as a guide, a mechanism for EasA was proposed in which numerous key residues facilitate the transfer of a hydride ion from the cofactor to the substrate. EasA has been discovered to be a crucial enzyme in formation of the D ring, a necessary step in assembly of the tetracyclic core of all ergot alkaloids. **Eva J. Gordon, Ph.D.**

To Catch a Histone Reader

To crack open the puzzle of epigenetics, researchers will need to unravel the complex web of interactions between histones with specific post-translational modifications and the proteins that recognize these sequences. That process has been tricky: such interactions are relatively weak and transient and can happen in timing with other interactions. As a result, researchers have had difficulty matching these modifications, which have specific cellular functions, with proteins that "read" for these sequences within the cell. However, Li and Kapoor (*J. Am. Chem. Soc.* 2010, *132*, 2504−2505) now describe a versatile labeling method to identify and isolate these proteins.



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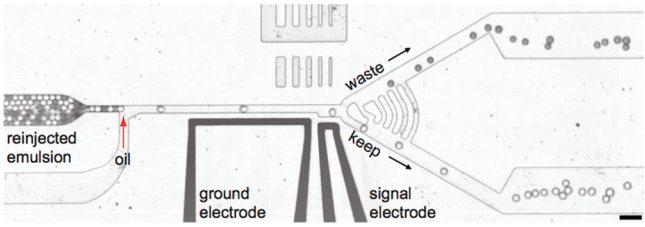
Guided by the structure of the histone modifications, the researchers designed activity probes to capture proteins that interact with specific histone modifications. In this initial study, Li and Kapoor mimicked a well-characterized post-translational modification, the trimethylation of lysine-4 on histone 3 (H3K4me3), which signals active chromatin and expression of the packaged DNA. On the basis of recently published structural data of H3K4me3 bound to a known binding domain, the plant homeodomain (PHD), the researchers designed a 16-amino-acid peptide probe based on the

H3K4me3 sequence. A benzophenone was substituted for the alanine side chain on the seventh residue to facilitate protein labeling with UV light. The final residue contains an alkyne side chain to react with reporter molecules *via* "click chemistry."

The probe labeled PHD domains in a concentration-dependent manner *in vitro* and competed with H3K4me3 for binding. The probe also captured tudor domains, another established H3K4me3 binding partner. In addition, when mixed with cell lysates, the probe selectively pulled out endogenous H3K4me3 binding proteins without labeling proteins known to bind to other histone modification sequences. This selectivity suggests that a similar strategy could be used to probe interactions with other modified histone sequences or a broader array of binding partners of other post-translationally modified proteins. **Sarah A. Webb, Ph.D.**

Cheaper, Faster, Better Screening and Sorting

With increasing understanding of biological systems, researchers are harnessing that knowledge to engineer biological molecules that are either more reactive than a natural enzyme or have entirely new chemistry. But taking proteins to the next level of reactivity is often hampered by the time, labor, and expense of screening thousands of possible mutants. However, researchers may have solved some of these issues with an innovative ultra-high-throughput microfluidic method that successfully screens and sorts millions of mutant enzymes at an infinitesimal fraction of the resources needed for existing techniques.



Agresti, J. J., *et al., Proc. Natl. Acad. Sci., U.S.A.*, DOI: 10.1073/pnas.0910781107. Copyright 2010 National Academy of Sciences, U.S.A.

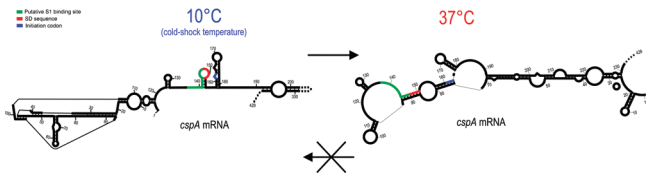
Agresti *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2010, published online February 8, 2010, DOI: 10.1073/pnas.0910781107) developed a two-part microfluidic device that suspends aqueous drops of just 6 pL within an inert oil and surfactant to create tiny reaction vessels that they can later sort on the basis of their fluorescence intensity. The researchers used horseradish peroxidase (HRP) enzyme to demonstrate how the system could speed the search for mutant enzymes with greater reactivity. To build a better HRP, the researchers expressed mutant HRPs on the surface of yeast cells. Each aqueous droplet in the microfluidic device encased an average of a single cell. Those yeast cell droplets were then incubated with substrate within a further chamber. A second device then sorted the drops on the basis of their fluorescence at a rate of 2000 per second.

Using this technique, the researchers initially screened a total of 10^8 mutants of the HRP enzyme, finding 50 unique sequences after 4 rounds with 2-fold enhanced activity. They then did a purifying selection, generating 10^7 mutants from the 18 most active first-generation enzymes, eventually finding 31 unique sequences with 5- to 12-fold enhanced activity.

Within 7 h, the researchers screened libraries that would take a robot two years to complete, with millions-fold reduction in reagent volumes and overall costs. The method can be easily adapted to other optical detection strategies including absorbance and luminescence. With its affordability and speed, this method could make high-throughput screens a more practical strategy for many biological and chemical research questions. **Sarah A. Webb, Ph.D.**

RNA Takes On a Cold Case

For a human, adapting to temperature can be as simple as looking outside and deciding whether to put on a t-shirt or a sweater. For a unicellular organism, rapid adaptation to the ambient temperature must be innately programmed if the organism is to survive. For *E. coli*, mechanisms to cope with heat or cold shock are critical and act to regulate both transcription and translation. To deal with the cold, the bacterium turns down the volume on most genes but upregulates the *csp* genes, which encode a family of cold shock proteins. Among the best studied is CspA, an RNA binding protein whose level is controlled by post-transcriptional mechanisms at the RNA stability and translation levels. But how could a catalytic process like the translation of *cspA* mRNA be more efficient at cold temperatures?



Reprinted from *Mol. Cell*, 37, Giuliodori *et al.*, The cspA mRNA is a thermosensor that modulates translation of the cold-shock protein CspA, 21−33, Copyright 2010, with permission from Elsevier.

A new study on the *cspA* mRNA turns up the heat on this interesting topic by uncovering a temperature-dependent RNA structure to help explain the phenomenon. Giuliodori *et al.* (*Mol. Cell* 2010, *37*, 21−33) probed the structure of the mRNA *in vivo* using the chemical modification reagent dimethyl sulfate, at both normal growth temperature (37 °C) and after cold shock (10 °C). Though the RNA was easily detected at both temperatures, the reactivity of certain nucleotides indicated that it might be adopting a different structure at these two temperatures. After moving to an *in vitro* characterization system that recapitulated these temperature-dependent struc-

tural rearrangements, a series of deletions and mutations demonstrated that the *cspA* transcript acts as a temperature sensor.

During normal growth, the RNA folds to hide the Shine-Dalgarno and start codon sequences within a double helical region and thus reduces translation initiation. At cold temperatures, these same sequences are found within the loop region of an RNA structure and are more readily accessed by the translation machinery. The authors went on to develop several translation assays that were sensitive to the temperature at which the input *cspA* mRNA was refolded. The cold folded mRNA was more efficient at binding to 30S ribosomes and the initiator codon tRNA, plus it generated more CspA protein product during *in vitro* translation. Taken together, it appears that the *cspA* mRNA acts like a riboswitch, but in this case there's no ligand to change the conformation. Changing gene expression is as simple as a shift in the temperature. **Jason G. Underwood, Ph.D.**

Chromatin Talks to the Spliceosome

The tale of histone tails and their modifications continues to yield new secrets to how information is marked for silent storage or for active transcription along the chromosome. In parallel, emerging data continues to reveal that gene expression processes are coupled to one another. The transcription machinery seems to handoff the RNA to the splicing machinery, which in turn couples to the polyadenylation and transport machines. Now, recent results demonstrate that it is probably more complicated than a simple handoff from one machine to another, that instead there may be an interplay that reaches all the way from the packaging of chromosomes to the choice of exons during pre-mRNA splicing.

After several recent studies demonstrated differential histone deposition or modification along exons, a new study (Luco *et al. Science* 2010, *327*, 996−1000) looked at how histone marks might affect alternative splicing. Using the well-characterized FGFR2 locus, the group used quantitative chromatin-immunoprecipitation (ChIP) to map the chromatin modifications along the gene. The alternative splicing of FGFR2 is controlled by the splicing repressor protein, PTB. hMSC cells, which use PTB to repress the FGFR2 exon IIIb, have higher histone H3 K4 monomethylation and K36 trimethylation along the FGFR2 locus than cells that do not repress IIIb. After this pattern appeared in loci containing other PTB-regulated splicing events, the group went on to investigate the upstream connections.

Could tinkering with these methylation marks affect PTB-regulated splicing events? Overexpressing the enzyme that leaves the K36 mark, SET2, caused lower inclusion of PTB-regulated alternative exons, consistent with the ChIP observations. An RNAi experiment on the endogenous SET2 gave the opposite result, demonstrating that alternative splicing is quite sensitive to the H3 methylation status. Similar overexpression and RNAi knockdown experiments, plus co-immunoprecipitations on the H3 K36 trimethylation-binding protein, MRG15, indicated that this may be an important bridging factor between the chromatin and the splicing repressor protein, PTB. Biochemical experiments confirmed this notion. Finally, knockdowns of PTB, MRG15, and SET2 were assayed by transcriptome profiling to gauge alternative splicing genome-wide.

Hundreds of alternative splicing events changed in each case, and dozens showed correlations between PTB and MRG15. Now, with these interesting hints in hand, the stage is set for exploring how direct this connection might be *versus* how much is contributed by the indirect effects of altering chromatin and in turn changing expression levels of other regulators. As with all of these couplings, codes and connections, the story is bound to become more complex. **Jason G. Underwood, Ph.D.**