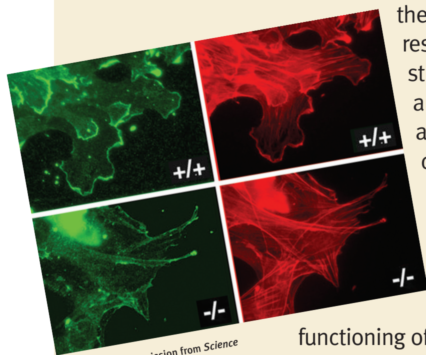
Spotlight

Actin Arginylated

Protein arginylation is a post-translational modification in which an arginine residue is transferred to the N-terminus of a protein by the enzyme arginine-transfer RNA protein transferase (Ate1). Ate1 knockout mice are embryonic lethal, having severe defects in cardiovascular development and angiogenesis, but the molecular basis of and the proteins affected by post-translational arginylation have remained ambiguous for >40 years. Toward understanding the biological role of N-terminal arginylation, Karakozova *et al*. (*Science* 2006, *313*, 192–196) decipher the biochemical and cellular effects of β-actin arginylation.

A combination of 2D gel electrophoresis and mass spectrometry on samples derived from embryonic fibroblasts was used to confirm that β-actin is arginylated *in vivo*. To determine how the physical properties and biological function of actin are affected by arginylation, the authors compared embryonic fibroblasts from wild-type mice and mice deficient in Ate1 (Ate1$^{-/-}$). *In vitro* biochemical examination revealed that β-actin derived from Ate1$^{-/-}$ cells was as stable and interacted with the same profile of proteins as β-actin from wild-type cells. However, in contrast with the single β-actin filaments that are formed in normal cells, actin from Ate1$^{-/-}$ cells formed filamentous aggregates, hindering the ability of Ate1$^{-/-}$ cells to move as proficiently as wild-type cells. In addition, Ate1$^{-/-}$ cells had defects in spreading, lamella formation, and intracellular localization of β-actin. On the basis of these results and the crystal structure of actin, the authors propose that arginylation of actin coats the filaments with a positive charge that prevents aggregation, contributing to proper functioning of the protein. The importance of Ate1 in embryonic development highlights the need to understand the biological role of Ate1 function and the consequences of perturbing this protein. These results not only shed light on the role of N-terminal arginylation in actin function but also pave the way toward understanding the global role of N-terminal arginylation. **EG**



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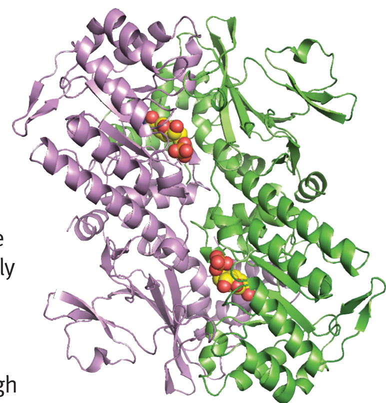
A Light in the NAmPRTase Tunnel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Nicotinamide phosphoribosyltransferase (NAmPRTase) is an important enzyme in the biosynthesis of nicotinamide adenine dinucleotide (NAD$^+$), a molecule intimately involved in biochemical redox reactions during vital processes such as glycolysis and the citric acid cycle. Interestingly, depletion of NAD$^+$ levels in tumors through inhibition of NAmPRTase activity has demonstrated encouraging anticancer effects. Because NAmPRTase catalyzes the conversion of free nicotinamide to nicotinamide mononucleotide (NMN), which is a key step in the salvage pathway of NAD$^+$, inhibitors of the enzyme may have potential as cancer drugs. Khan *et al*. (*Nat. Struct. Mol. Biol*. 2006, *13*, 582–588) and Wang *et al*. (*Nat. Struct. Mol. Biol*. 2006, *13*, 661–662) now report the crystal structures of free NAmPRTase, NAmPRTase bound to NMN, and NAmPRTase bound to the inhibitor FK866. The structures provide insights into the substrate specificity and the mechanism of the enzyme and jumpstart the rational design of novel NAmPRTase inhibitors.

The structure of NAmPRTase revealed that it belongs to the dimeric class of type II phosphoribosyltransferases, which include nicotinic acid phosphoribosyltransferase (NAPRTase) and quinolinic acid phosphoribosyltransferase (QAPRTase). The proteins can each be organized into three domains composed of a mixture of β-strands and α-helices, and all three proteins possess an extensive dimer interface. However, it is a few key structural differences among these enzymes that ultimately expose the basis for their substrate specificity. NAmPRTase is quite a bit larger (~100 amino acids) than either NAPRTase or QAPRTase, and distribution of these additional residues over the structure, along with differences in domain orientations, has a dramatic impact on the active-site environment of NAmPRTase.

Structures of NAmPRTase bound to NMN and FK866 revealed that the active site of the enzyme is located at the dimer interface. In fact, the nicotinamide ring of NMN participates in π-stacking interactions with a phenylalanine from one monomer unit and a tyrosine from the other monomer unit. The basis for the substrate specificity centers at an aspartate residue, which is not present in NAPRTase or QAPRTase and which takes part in a direct hydrogen bond with the amide group of NMN. The importance of the aspartic acid residue in defining the substrate specificity of NAmPRTase was also confirmed by mutagenesis and kinetic studies. The significance of the dimerization of NAmPRTase is reinforced upon examination of the binding of the inhibitor FK866. At the dimer interface, FK866 binds in a tunnel, with some resemblance to the binding of NMN. Notably, structural and kinetic data indicate that FK866 is a tight-binding competitive inhibitor of NAmPRTase, in contrast to previous reports that FK866 inhibits NAmPRTase *via* a noncompetitive mechanism. The unique presence of the tunnel confers specificity of FK866 for NAmPRTase over NAPRTase and QAPRTase, because FK866 is exquisitely shaped to partake in favorable interactions upon slithering into place. The information gained from these structures will contribute significantly to furthering our understanding of the enzyme's mechanism and role in biology. **EG**

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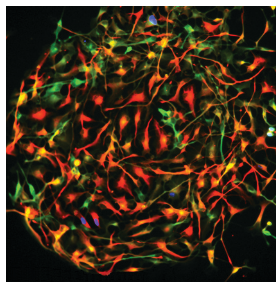
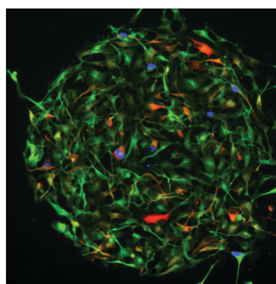
Understanding Fate

The process of cell differentiation is driven by complex spatial and temporal signaling mechanisms. Systematic exploration of the molecular factors that contribute to the fate of a cell will help scientists navigate the murky waters of cellular differentiation. To this end, Soen *et al.* (*Mol. Syst. Biol.*, published online July 4, 2006, doi:10.1038/msb4100076) present a microarray-based method for investigating the phenotypic effects of exposing neural precursor cells to different combinations of extracellular signaling molecules.

A microarray was generated of defined combinations of 13 recombinant signaling molecules, many of which have been implicated in neuronal cell differentiation. Bipotent neural cells capable of differentiating into neurons or glial cells were allowed to attach to the microarray surface and were incubated under conditions favorable for differentiation. Analysis of neural and glial cell differentiation markers on each cell enabled the effects of the molecular microenvironments to be assessed. The researchers determined that different combinations of signaling molecules resulted in four distinguishable outcomes relating to the differentiation state of the cells. Whereas certain mixtures of signaling factors promoted differentiation toward glial cells, others nudged cells toward becoming neurons. Interestingly, some combinations appeared to decrease both differentiation markers, in essence retracting the cells into an "undifferentiated-like" state that coincided with an increased proliferative phenotype. Still other mixtures increased both differentiation markers, with the cells classified as being in an indeterminate state of differentiation. Analysis of the relationships within mixtures of signaling factors pointed to additional subtleties, including sometimes unexpected dose–response and kinetic profiles and the ability of certain molecules to have dominant effects over others. This powerful method can be adapted to the investigation of additional molecular factors with a variety of cell types, enhancing our understanding of the molecular environment involved in cell differentiation and progressing the exciting prospect of manipulating the fate of a cell. EG





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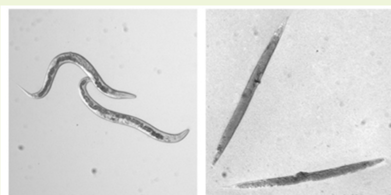
Worming Our Way into New Antibiotics

The growing number of infections caused by bacteria resistant to known antibiotics is a worldwide health concern. However, the critical need for new antibiotics has been plagued by the limitations of traditional screens. Typical screens are unable to recognize toxic molecules, compounds with poor pharmacokinetic properties, or molecules that cannot penetrate the multidrug-resistance barrier of Gram-negative bacteria. In addition, most *in vitro* screens barely resemble the biological systems they are attempting to replicate, and this calls into question their relevance. Moy *et al*. (*PNAS* 2006, *103*, 10414–10419) now report an innovative, high-throughput, live-animal antibiotic screen using the nematode *Caenorhabditis elegans* and the human pathogenic bacteria *Enterococcus faecalis*.

E. faecalis is a human opportunistic bacterium that, like many human bacterial pathogens, also infects the nematode intestinal tract. When *C. elegans* are infected with *E. faecalis*, half the worms die within 5 days, but antibiotic treatment upon infection can rescue the worms from death. On the basis of this system, a screen was developed to identify novel antimicrobials that could cure nematodes infected with *E. faecalis*. Infected worms were transferred to a liquid medium in 96-well plates, and 6000 synthetic molecules and 1136 natural product extracts were tested for their ability to cure the infection. Visual inspection with a dissecting microscope easily distinguished live worms, which adopt a sinusoidal posture, from dead worms, which become straight and rigid because of bloating from the *E. faecalis* cells. Eighteen of the small molecules and nine of the extracts were found to promote survival of infected worms. This screen has a number of advantages over traditional antibiotic discovery screens, including the ability to identify prodrugs, compounds that target virulence factors, and molecules that enhance the host's defense system. In addition, the assay selects for nontoxic compounds that are effective *in vivo*. This live-animal screen presents an intriguing new method for antibiotic discovery. **EG**



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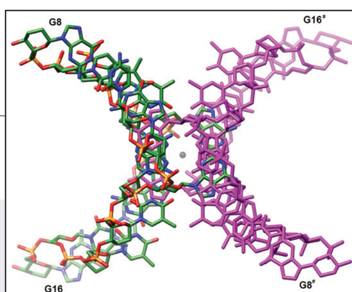
ACS chemical biology

Spotlight

Rationalizing the Ribose



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Many polymers in biology assemble from monomers that display unifying chemical properties. Proteins are assembled from amino acids that all share L-chirality, whereas DNA and RNA are sugar-coated strictly with pentose in the backbone. Such stringent choices have remained a ponderous point for biologists and chemists who envision founder macromolecules emerging from a prebiotic chemical soup. A new study by Egli *et al.* (*J. Am. Chem. Soc.*, ASAP Article 10.1021/ja062548x S0002-7863(06)02548-0) explored this theme by asking DNA to trade in its standard ribose for a hexose sugar. The group synthesized a hexose-based nucleic acid, termed homo-DNA. Although at first glance the functional groups and geometry looked rather similar to DNA, a high-resolution view of homo-DNA demonstrated radical differences. The X-ray crystal structure of a double-stranded octamer revealed base-pairing and helical properties that are quite foreign to the textbook rules for DNA. The duplex resembles a slowly twisting ribbon rather than the tight coil of DNA. The steps between each base pair varied considerably, and the intrastrand base stacking found in nature's double helix was completely lacking. Some likenesses were observed, such as cross-strand base stacking and the antiparallel architecture, but the elegant uniformity that DNA uses to store genetic information was largely absent. The researchers postulate that stable base-pairing systems are highly unlikely with hexose-based nucleic acids, and this might explain why nature chose pentose over hexose. This study is also particularly interesting because of the techniques used to solve the structure of the duplex. An old friend to protein crystallographers, selenium, was used in the form of a phosphoroselenoate in the homo-DNA backbone. These compounds are usually too reactive for the time scale of crystal growth, but in this case, the researchers miraculously timed their synthesis, crystallization, and data collection to make this unique structure possible. **JU**

Derepressing Antidepressants

Histone methylation is one of several modifications of chromatin structure that play a key role in the regulation of gene expression. BHC110, an enzyme found in a number of multiprotein complexes involved in nucleosome modification, is capable of demethylating histone H3 lysine 4 (H3K4) and consequently causes repression of gene expression. BHC110 shares sequence homology with monoamine oxidase (MAO) enzymes, which are targets of several antidepressant drugs. Lee *et al.* (*Chem. Biol.* 2006, *13*, 563–567) now report that certain MAO inhibitors are also potent inhibitors of BHC110 and that cells exposed to these inhibitors exhibit transcriptional derepression of BHC110 target genes.

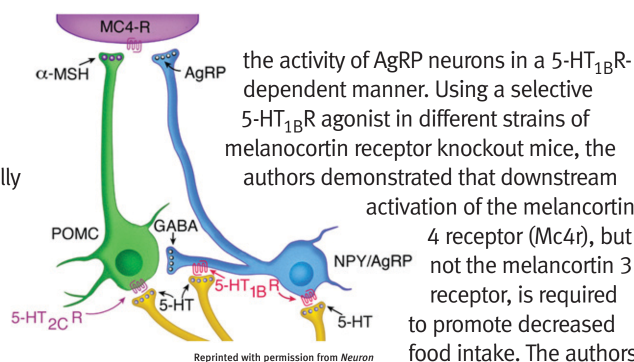
Three selective and three nonselective MAO inhibitors were tested for their ability to inhibit histone and nucleosome demethylation *in vitro* by recombinant BHC110, and two of the nonselective inhibitors showed dose-dependent activity against the enzyme. The most active compound, tranylcypromine (brand name Parnate), had an IC_{50} of <2 μM, which notably is 10-fold less than the IC_{50} of the drug against MAO enzymes. Tranylcypromine was next tested for its ability to inhibit histone demethylation in live cells. The transcriptional activity of two BHC110 target genes, *Oct4* and *Egr1*, was analyzed in response to tranylcypromine exposure. Quantitative reverse-transcriptase PCR and chromatin immunoprecipitation experiments in embryonic carcinoma cells revealed that tranylcypromine treatment results both in derepression of *Oct4* and *Egr1* gene expression and in enhanced global histone methylation levels. This discovery offers new insights into the mechanisms of some antidepressant medications and provides additional tools for exploring the role of histone demethylation in important molecular and cellular processes, such as gene expression, cellular differentiation, and oncogenesis. **EG**

Prying into Prion Mechanisms

Proteinaceous infectious particles, or prions, are unique protein pathogens thought to be responsible for several fatal diseases, including scrapie, Creutzfeldt–Jakob disease, and bovine spongiform encephalopathy ("mad cow" disease). One mysterious and remarkable characteristic of prion pathogenesis is that different conformations of the same misfolded protein produce different disease phenotypes. Tanaka *et al.* (*Nature* 2006, *442*, 585–589) provide insight into this phenomenon by demonstrating that the brittleness of prion aggregates can affect the rate of prion division, ultimately leading to distinct physiological consequences.

The researchers used synthetic prion forms of the yeast protein Sup35 as a model to investigate the physiological impact of different prion conformations. The color phenotype of the [*PSI*$^+$] prion state, which results from Sup35 aggregation, varies depending on the physical properties of the Sup35 aggregate in the [*PSI*$^+$] strain, and this provides an easily monitored system. The intrinsic fiber growth rate and the frangibility, or propensity to fragment, of three infectious amyloid conformations of the prion-forming domain of Sup35 (Sc4, Sc37, and SCS) were characterized by atomic force microscopy. Unexpectedly, it was found that the strain with the strongest phenotype, Sc4, has the slowest intrinsic growth and is the most likely to fragment. Further investigation using a variety of assays revealed that Sc4 also possesses the fastest rate of division in cells, easily compensating for the slower growth rate. In addition, the Sc4 prion particles are noticeably smaller than those found in the other strains, but the number of fibers per cell is considerably higher. Taken together, the data indicate that the strength of a prion strain phenotype is directly related to the frangibility of the infectious prion aggregate. This revelation not only demystifies a piece of the mechanism behind prion pathogenesis but also points to new strategies for restraining the infectious competence of prion aggregates. **EG**

Serotonin Weighs In



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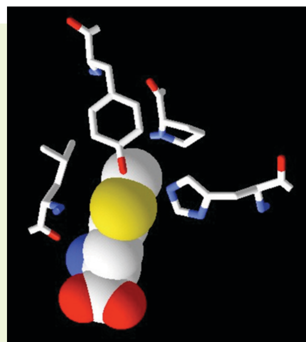
Levels of the neurotransmitter serotonin (5-HT) inversely correlate with food consumption, making some 5-HT analogues excellent weight-loss agents. However, 5-HT is involved in a host of metabolic and neurological activities that can also affect eating behavior, and this complicates the identification of the precise mechanism by which 5-HT regulates food intake. Now Heisler *et al.* (*Neuron* 2006 *51*, 239–249) further define the role that 5-HT plays in appetite suppression by demonstrating that the melanocortin system, a group of pituitary peptide hormones and their receptors known to be involved in a variety of biological activities, including feeding, is a critical component of the regulation of food intake by 5-HT.

5-HT is synthesized from the essential amino acid tryptophan in the brain, where it interacts with several types of 5-HT receptors, including 5-HT$_{1B}$R. Using transgenic mice, the authors observed that 5-HT$_{1B}$R receptors are anatomically positioned to regulate neurons containing the melanocortin agonist α-melanocyte-stimulating hormone (α-MSH) and the melanocortin antagonist agouti-related protein (AgRP). Both of these molecules are potent regulators of food intake. In addition, light and electron microscopy experiments revealed that 5-HT terminals are located such that both neuronal activity and release of products from the axon are likely affected by 5-HT. Electrophysiology experiments further indicated that 5-HT both increases the activity of α-MSH neurons and reduces the activity of AgRP neurons in a 5-HT$_{1B}$R-dependent manner. Using a selective 5-HT$_{1B}$R agonist in different strains of melanocortin receptor knockout mice, the authors demonstrated that downstream activation of the melancortin 4 receptor (Mc4r), but not the melancortin 3 receptor, is required to promote decreased food intake. The authors propose a model in which reciprocal regulation of melanocortin agonist and antagonist-containing neurons, in concert with downstream activation of Mc4r, is the key pathway through which 5-HT exerts its appetite-suppressing activity. Additional molecular insights into this pathway will enhance our understanding of food regulation and may facilitate the development of more-effective weight-loss agents. **EG**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Cell Surface Mutants

The incorporation of noncanonical amino acids into recombinant proteins enables the generation of innovative tools with which to manipulate molecular and cellular function. Aminoacyl-transfer RNA synthetases (aaRS), the enzymes that ligate specific amino acids to their cognate tRNAs, can be mutated to permit incorporation of noncanonical amino acids into proteins without affecting wild-type aaRS activity. The use of mutant aaRS offers unique control over recombinant protein production, but the generation of effective mutants can be a tedious process. Link *et al.* (*PNAS* 2006, *103*, 10180–10185) now describe a high-throughput, flow-cytometry-based method for identifying mutant aaRS that efficiently incorporates noncanonical amino acids into target proteins.

On the basis of previous studies, the authors chose a variant of the *Escherichia coli* outer membrane protein C (OmpC) for incorporation of the unnatural amino acid azidonorleucine (ANL) by the *E. coli* aaRS methionyl-tRNA synthetase (MetRS). The authors selected four sites for mutagenesis within MetRS by examining the crystal structure and identifying the residues most critical for methionine binding, and they used a modified PCR gene assembly process to generate a saturation mutagenesis MetRS library. After transformation of the library into bacteria, cells expressing recombinant OmpC containing ANL were covalently labeled *via* reaction of the azide of ANL with a molecule containing a biotinylated cyclooctyne functionality. Staining with fluorescent avidin followed by flow cytometry and cell sorting analysis led to the identification of three mutant MetRS proteins. Remarkably, all three contained the same leucine to glycine (L13G) mutation, and a protein containing this single mutation was subsequently generated and found to be the most efficient of the MetRS mutants evaluated for ANL incorporation. These results demonstrate the power of rapid identification of mutated aaRS for the expression of cell surface proteins possessing unique reactivity, and this methodology can easily be expanded to the generation of additional mutant aaRS with other noncanonical amino acids. **EG**

Spotlights written by Eva Gordon and Jason Underwood