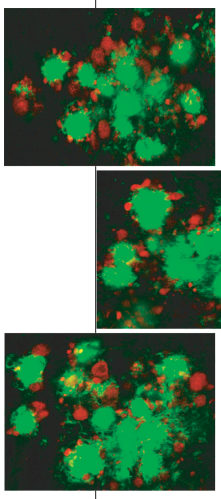
Spotlight

Targeting Tau



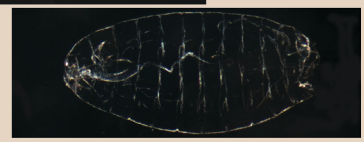
From Roberson, E. D., *et al.*, *Science*, May 4, 2007, DOI: 10.1126/science.1141736. Reprinted with permission from AAAS.

Alzheimer's disease (AD) is characterized by deposits of aggregated amyloid-β (Aβ) peptide, a proteolytic product of the amyloid precursor protein (APP), and the somewhat less notorious culprit tau, a microtubule-associated protein. Ample evidence has been uncovered linking Aβ aggregates to cognitive impairments in AD, leading to intense efforts to combat the disease by targeting Aβ formation. However, the potential therapeutic benefits of targeting tau are not as well defined and have thus remained somewhat under the radar. Now, Roberson *et al.* (*Science* 2007, *316*, 750–754) report that reducing endogenous tau levels ameliorates the behavioral deficits in mouse models of AD.

To address the role of tau in AD, the authors generated transgenic mice that express human APP and two, one, or no tau alleles. Remarkably, mice with reduced levels of tau did not exhibit the abnormalities in learning, memory, and exploratory activity observed in mouse models of AD. Investigation into the mechanism behind this finding revealed that mice expressing human APP with normal tau levels were hypersensitive to excitotoxins such as the glutamate receptor agonist kainate and the γ-aminobutyric acid type A receptor antagonist pentylenetetrazole. In contrast, mice with reduced tau levels were resistant to these excitotoxic agents. This unexpected discovery suggests a previously unknown role for tau in modulating neuron sensitivity to excitotoxins, an especially intriguing finding given that excitotoxicity has been implicated in the pathogenesis of AD. The authors propose that although additional studies are necessary, tau's involvement in regulating neuronal activity could be exploited as a new therapeutic strategy for treating AD and other excitotoxicity-related disorders. **Eva J. Gordon, Ph.D.**

Noncoding? Not So Fast

The complete genomes of 12 *Drosophila* species unlocked a veritable gold mine for computational biologists who compare genomes. Though all flies may seem very similar, the most divergent species of the 12 probably shared a common ancestor ~40 million years ago. This data deluge is further illuminated by complementary DNA cloning projects and microarrays that look at the transcribed RNAs in the organisms. Combined, these efforts uncover RNAs of unknown function, and many have little or no coding potential. Often these are labeled as noncoding RNAs because they have just short open reading frames that are unlikely to code for a functional protein. Now, a new study from Kondo *et al.* (*Nat. Cell Biol.* 2007, *9*, 660–665) clearly demonstrates that one fly RNA that flew below the reading frame radar is not noncoding after all.



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The authors studied one candidate noncoding RNA with an interesting pattern of expression during development. The RNA was switched on in epithelial cells that make the cuticle, a layer that forms the insect's exoskeleton. A function

(continued on page 363)

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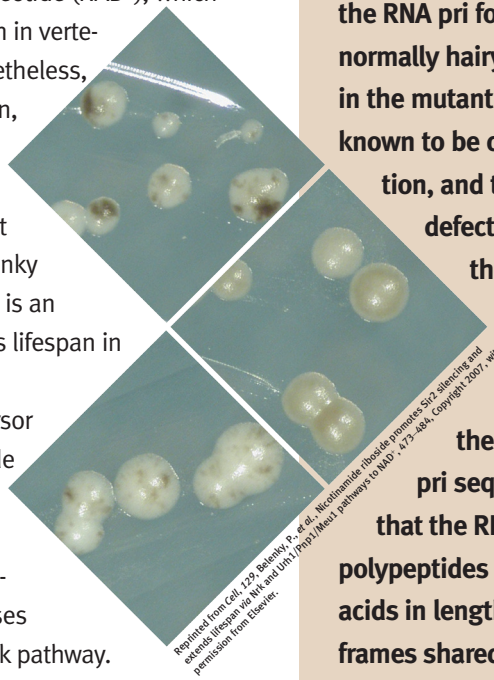
Nicotinamide Riboside: Two Routes to Longevity

Ponce de León never found the fountain of youth, but chemical biologists continue to look for biological factors and pathways that contribute to vitality. In yeast, lifespan can be extended by calorie restriction (CR) *via* a pathway dependent on the protein lysine deacetylase Sir2 and the coenzyme nicotinamide adenine dinucleotide (NAD^+), which is also a Sir2 substrate. CR also extends lifespan in vertebrates and increases brain and liver NAD^+. Nonetheless, lifespan had never been extended with a vitamin, even in yeast cells. Nicotinamide riboside (NR) is a newly discovered NAD^+ precursor vitamin whose contribution to NAD^+ metabolism has not been fully characterized. A new report from Belenky *et al.* (*Cell* 2007, *129*, 473–484) reveals that NR is an NAD^+-boosting vitamin that significantly extends lifespan in yeast cells grown in non-CR conditions.



Reprinted from *Cell*, *129*, Belenky, P., *et al.*, Nicotinamide riboside promotes Sir2 silencing and extends lifespan via Nrk and Urh1/Pnp1/Meu1 pathways to NAD+, 473–484, Copyright 2007, with permission from Elsevier.

NR was initially discovered as an NAD^+ precursor whose activity depends on nicotinamide riboside kinase (Nrk), which is conserved between yeast and humans. Using yeast mutants, the authors observed that adding NR promotes Sir2-dependent repression of recombination, increases gene silencing, and extends longevity *via* the Nrk pathway. In addition, they determined that a second, Nrk-independent pathway exists that converts NR to NAD^+. Further studies of the two NR salvage pathways suggested that NR is a normal NAD^+ metabolite, even in the absence of NR supplementation, and established that NR extends the lifespan of wild-type yeast cells on high glucose through both pathways. Genetic evidence in mice suggests that NR may be valuable in the treatment of neurodegenerative diseases and in the prevention of chemotherapy-induced peripheral neuropathy. Further evidence suggests that NR may protect against *Candida glabrata* infection and that NR might provide the high-density lipoprotein-elevating effects of nicotinic acid without causing painful flushing. Accordingly, clarification of the basic chemical biology of endogenous NR metabolism and identification of the gene expression consequences of varied NAD^+ levels may lead to new medicines and new insights into drug mechanisms.

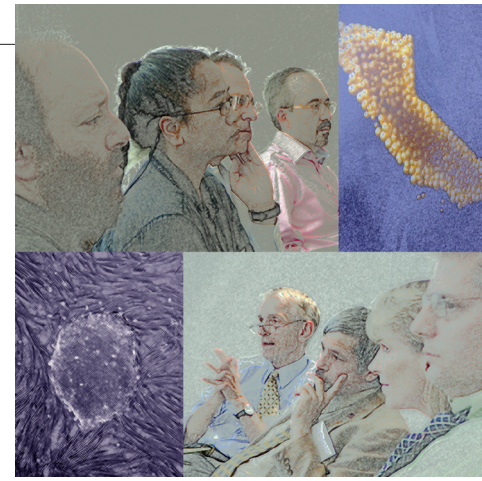
Eva J. Gordon, Ph.D.

Noncoding? Not So Fast,

continued from page 362

for the RNA was implicated when the researchers found exoskeleton defects in a fly mutant lacking the RNA. They named the RNA pri for polished rice, because the normally hairy cuticle fibers were smooth in the mutant. Filamentous actins are known to be critical for the cuticle formation, and the pri mutant showed a defect in the normal assembly of these proteins during development. But how might pri RNA regulate actins? On further examination of the *Drosophila melanogaster* pri sequence, the authors found that the RNA could encode five short polypeptides that are 11 or 32 amino acids in length. Four of the reading frames shared a common septamer peptide motif. Comparing genomes with the other 11 flies helped solve this mystery, because other species often displayed different pri nucleotide sequences but the same encoded peptide. The four related peptides appear functionally redundant, as a re-expression of just one peptide in the mutant pri line provided a full rescue. These results are striking and give researchers a good reason to take another look at the tiny open reading frames that might be hiding in the genomes. Jason G. Underwood, Ph.D.

The Best "Best Practices"



Reprinted from *PLoS Med.*, Lomax, G. P., *et al.*, Responsible oversight of human stem cell research: the California Institute for Regenerative Medicine's medical and ethical standards, DOI: 10.1371/journal.pmed.0040114; e114.

Top-notch scientists across the globe are racing to realize the potential of human embryonic stem cells. In the U.S., however, limits on federal funding have placed state governments at the forefront of ensuring that this incredibly important research area continues to forge ahead. In 2004, California emerged as a pioneer in this field. State funding of $3 billion was approved for stem cell research, to be administered through the California Institute of Regenerative Medicine (CIRM). Lomax *et al.* (*PloS Med.* 2007, *4*; e114) discuss the regulations developed by CIRM to ensure the highest ethical standards in human stem cell research.

The CIRM regulations were based on several overall objectives. First, to ensure best practices for ethical conduct of human stem cell research, a Stem Cell Research Oversight Committee will be created at each institution funded by CIRM. Considerable flexibility is built into the guidelines of the committee to accommodate the rapidly advancing field and empower those on the committee to develop best practices. Second, careful consideration was given to limit required documentation and procedures to those truly necessary to protect against ethical lapses. Third, given the strong public opinions on human stem cell research, input from the public on ethical issues was encouraged during formulation of the regulations and incorporated into the final document. Fourth, existing laws, regulations, and ethical guidelines were taken into account to facilitate a productive coexistence with other funding sources and regulatory bodies. Finally, acknowledging the tremendous strengths in scientific collaboration, the CIRM defined core requirements for use by CIRM-funded researchers while building in some inherent flexibility in recognition of differences in international standards.

Certain particularly innovative features of the CIRM regulations are worth noting, especially as other states consider their own regulations for stem cell research. For example, to ensure an appropriate balance between donors' wishes and scientific progress, prospective donors are required to be informed in detail of the possible uses of their donations, and restrictions in uses by the donors must be respected. In addition, much thought was put into protecting oocyte donors from the medical and financial risks involved and ensuring that they are not only informed but actually comprehend the implications and potential consequences of their donation.

The regulations for best practices devised by the CIRM were designed with the best intentions: to promote scientific progress while respecting ethical issues. The attention to public interest as well as research standards makes these regulations an inspiring model for continuing progress in stem cell research.

Eva J. Gordon, Ph.D.

Toward Life or Death?

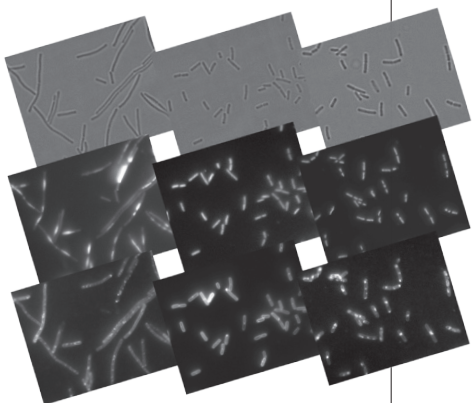
Retinoic acid (RA), a metabolite of vitamin A, has well-characterized antiproliferative activities that have led to its use as an anticancer agent. However, in a few specific cell types such as skin cells and neurons, RA appears to promote cell survival instead. Indeed, RA is also clinically used as a topical skin repair agent. So how do the cells decide how to respond to RA? Schug *et al.* (*Cell* 2007, *129*, 723–733) now report that two different receptors for RA help determine the fate of the cell.

Inhibition of cell growth by RA is mediated through its interaction with RA receptors (RARs), which form heterodimers with the retinoid X receptor (RXR) to modulate gene transcription. But RA also binds to the nuclear receptor peroxisome proliferator-activated receptor (PPAR)β/δ, which also influences gene transcription by forming a heterodimer with RXR. The authors demonstrate that two intracellular lipid-binding proteins, CRABP-II and FABP5, control whether RA is delivered to RAR or PPARβ/δ, respectively. Interaction with CRABP-II and subsequent activation through RAR result in the induction of antiproliferative genes such as those that promote cell-cycle arrest or apoptosis. In contrast, binding to FABP5 and consequent activation through PPARβ/δ has the opposite effect, inducing prosurvival activity instead. Because RA interacts more strongly with CRABP-II/RAR, only those cells that have high levels of FABP5, such as skin cells, experience the antiapoptotic effects of RA. This remarkable dichotomy of RA signaling clarifies the apparent opposing effects of RA, and these studies will help guide RA-based therapeutic strategies for cancer and other disorders. **Eva J. Gordon, Ph.D.**

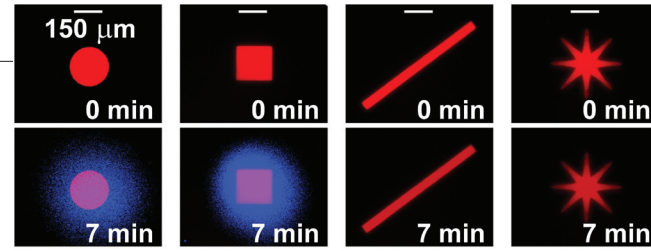
The Missing Link

To the average biochemist, DNA replication and glycolysis are two fundamental processes that belong several chapters apart in the basic biochemistry textbook. Indeed, on the surface, glycolysis, one of the major pathways of carbon metabolism, appears to have little in common with the replisome, the complex cellular machine responsible for copying DNA. However, several recent studies have hinted that these processes may be linked. Now, Jannière *et al.* (*PLoS ONE* 2007, *2*; e447) provide the first concrete evidence of the connection, uncovering specific proteins in the glycolysis pathway that affect the activity of key DNA replication enzymes.

The link between glycolysis and DNA synthesis emerged upon analysis of temperature-sensitive strains of *Bacillus subtilis*. In these strains, DNA replication ceases at the restrictive temperature because of mutations in the lagging strand polymerase DnaE, the primase DnaG, or the helicase DnaC. In the search for suppressors of this phenotype, mutations that restored full viability to the replication mutants were traced to five genes critical for the terminal reactions of glycolysis. The authors suggest that mutations that affect the activity of these metabolic enzymes may lead to a functional change in the replisome, perhaps from a conformational change in specific replisomal enzymes. This functional change results in thermoprotection, leading to growth restoration at restrictive temperatures. One hypothesis consistent with current data is that the metabolic enzymes could phosphorylate the DNA replication enzymes, resulting in the conformational changes necessary to suppress the replication defect. Although more research is necessary, these studies illuminate the path connecting DNA replication and cell metabolism. Further understanding of the molecular basis behind the link between these fundamental processes may indeed someday force us to rewrite the textbooks. **Eva J. Gordon, Ph.D.**



Reprinted from *PLoS ONE*, Jannière, L., *et al.*, Genetic evidence for a link between glycolysis and DNA replication, DOI: 10.1371/journal.pone.0000447; e447.



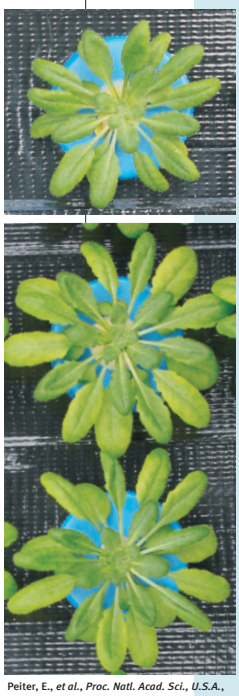
Reproduced with permission from *Angew. Chem., Int. Ed.* from Wiley-VCH, Kastrup, C. J., *et al.*, 2007, *46*, 3660.

The Shape of Things

At the molecular level, chemistry dictates biological processes, and the shapes of the molecules involved play a profound role in guiding these processes. Less is understood, however, about how shapes govern events at the cellular and organismal levels. For example, what fundamental processes guide insects to respond to the outline of a flower, or human white blood cells to take action against invading pathogens? Kastrup *et al.* (*Angew. Chem., Int. Ed.* 2007, *46*, 3660–3662) investigate this mysterious phenomenon by exploring the initiation of coagulation of human blood plasma after exposure to various shapes of a clotting stimulus.

Using photolithography, the authors patterned distinct shapes of the blood-clotting stimulus tissue factor in a microfluidic chamber. Plasma was exposed to the shapes, which were a circle, rectangles of varying dimensions, and a star. They determined the initiation of blood clotting by using bright field and fluorescence microscopy to monitor the formation of fibrin and thrombin, respectively. As expected, clotting initiation occurred on circular patches, provided they were above a threshold size. However, other shapes had striking effects on clotting initiation; wide but not narrow rectangles initiated clotting, and the star-shaped patch initiated clotting only half of the time. To examine the mechanism behind this phenomenon, the authors first used 3D numerical simulations of a simple reaction-diffusion system and then a simplified chemical model system of blood clotting. Their results indicated that the dynamics of the clotting network are regulated by a threshold response in which the initiation of clotting requires a certain threshold concentration of clotting activators, a threshold that was not attained, for example, with narrow rectangles. They further demonstrated that response to shape not only is dictated at the organismal level but also can emerge at the level of a biochemical network. Additional studies will address the generality of their findings and may help explain the fundamental processes that govern responses of complex biological systems. **Eva J. Gordon, Ph.D.**

Tolerating Manganese



Peiter, E., *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, *104*, 8532–8537. Copyright 2007 National Academy of Sciences, U.S.A.

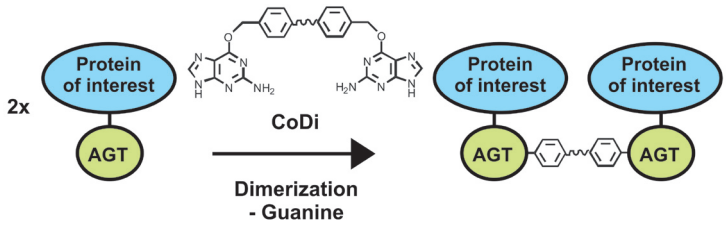
Manganese is an essential micronutrient for all living things. However, too much Mn can be toxic, and many plants and animals have developed mechanisms for tolerating high Mn concentrations. Though not extensively characterized, it is generally thought that Mn levels in plants are controlled mainly through export across the plasma membrane or compartmentalization in vacuoles, in contrast to animals, which also utilize secretory vesicles to eject Mn from cells. Peiter *et al.* (*Proc. Nat. Acad. Sci. U.S.A.* 2007, *104*, 8532–8537) now report the previously unobserved finding that plants also employ the secretory system to regulate Mn levels with the help of a cation diffusion facilitator called MTP11.

Scientists first discovered that MTP11 from *Arabidopsis* selectively restored Mn tolerance to wild-type levels in yeast mutants that were hypersensitive to Mn. To verify that MTP11 played a similar role in plants, the authors generated *Arabidopsis* mutants lacking functional MTP11, and these plants were indeed hypersensitive to Mn. In addition, plants overexpressing the protein were found to be hypertolerant of Mn. Notably, MTP11 from poplar rescued the Mn-hypersensitive *Arabidopsis* mutant, an indication of a general role for the protein in Mn homeostasis in plants. The authors investigated the mechanism by which MTP11 regulates Mn levels by expressing fluorescent MTP11 fusion proteins and examining their localization patterns. Surprisingly, MTP11 was found to localize to the Golgi apparatus and not the plasma membrane or vacuoles, where other transport systems involved in metal tolerance reside. Furthermore, compared with wild-type plants, mutant plants had a higher Mn content. This suggests that plants, like animals, also use the secretory pathway to manage Mn concentrations and prevent the toxic effects associated with elevated Mn levels. **Eva J. Gordon, Ph.D.**

Covalent Capture

Manipulating protein function inside cells is a powerful method to control and decipher complex biological processes. Various small molecules have been used to induce protein–protein interactions, which in turn trigger processes such as cell signaling events, protein degradation pathways, and transcriptional activation. However, many small-molecule protein "dimerizers" lead to protein dimerization products that are reversible, and this relinquishes a certain level of control over the system and precludes quantification of the extent of dimerization. Lemercier *et al.* (*Angew. Chem., Int. Ed.* 2007, *46*, 4281–4284) now report the synthesis and evaluation of covalent dimerization molecules (CoDi's), which generate irreversibly linked protein dimers in solution and in cells.

The CoDi system is based on the binding of the small molecule O^6-benzylguanine to O^6-alkylguanine-DNA alkyltransferase (AGT). Three synthetic dimers of O^6-benzylguanine were generated that differed only in the length and flexibility of the linker connecting the two binding elements. All three dimers were effective AGT cross-linking agents at nanomolar concentrations in solution and at micromolar concentrations in cells, with ~70% of the protein dimerized as determined by Western blot analysis. Exploiting the covalent nature of the resulting protein dimers, the authors also investigated the spatial proximity of



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protein pairs. AGT fusion proteins with different subcellular localizations were created, and only those targeted to the same locations were observed to form dimers. In addition, interactions between different AGT fusion proteins could be evaluated on the basis of the ratio of heterodimer to homodimer formation. The CoDi's described here expand the versatility of the tool kit of small molecules available for probing protein–protein interactions in cells. Eva J. Gordon, Ph.D.

UPCOMING CONFERENCES

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