ACS chemical biology

Spotlight

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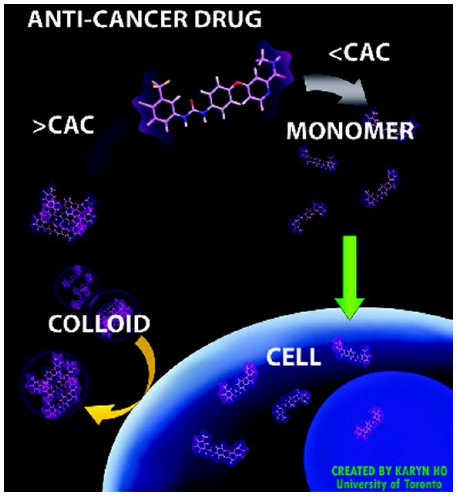
BEST OF CHEMICAL BIOLOGY 2012

2012 was another wonderful year for chemical biology, and we've used our Spotlight and In This Issue sections to highlight new developments every month. Now, looking back, the Editors have compiled a list of articles representing some of the most exciting and relevant research from 2012. Please note that it is simply not possible to recognize all of the amazing work being published, and this list is not meant to be comprehensive. However, we hope that this feature will at least provide a sense of the richness and quality of research being published in chemical biology as we move forward into a new decade. Happy New Year!

COLLOID CHARACTERIZATION

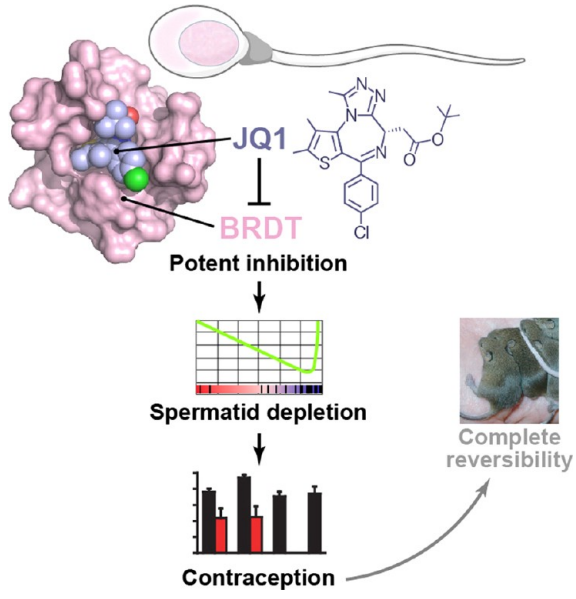


In aqueous solution, many small organic molecules can form colloids, or microscopic aggregates that are evenly dispersed throughout the solution. When in solution with proteins or other biomolecules, colloids can alter protein activity and cause nonspecific inhibition, suggesting that this phenomenon could skew the interpretation of small molecule activity in various high-throughput screens. Owen *et al.* (*ACS Chem. Biol.* 2012, *7*, 1429−1435) now explore the tendency of 7 anticancer drugs and 1 diagnostic agent to form colloids and assess the biological consequences of their colloid formation.

All 8 of the molecules studied were found to form colloids in biochemical buffer and in cell culture medium. Moreover, in cell-based assays the activity of the colloidal form of 3 of the drugs was found to be dramatically reduced compared to the monomeric form. In addition, investigation of the colloidal properties of the diagnostic compound revealed surprising new details regarding how this compound functions as an agent to measure vascular permeability. This study offers compelling insights into how colloidal formation affects biological activity and could lead to new strategies to manipulate colloid formation and behavior.

Eva J. Gordon, Ph.D.

"THE PILL" FOR MEN!



Reprinted from *Cell*, *150*, Matzuk, M. M. *et al.*, Small-Molecule Inhibition of BRDT for Male Contraception, 673−684. Copyright 2012, with permission from Elsevier.

Proteins involved in epigenetic regulation, such as the bromodomain and extraterminal (BET) subfamily of epigenetic reader proteins, have emerged as intriguing drug targets for various conditions such as cancer, inflammation, and metabolic disorders. The protein BRDT, a BET family member that recognizes acetyl-lysine moieties on histones, is expressed in testis and is essential for sperm production. The tissue-selective expression, coupled with the association of mutations in the *BRDT* gene with infertility in both animals and humans, points to BRDT as a potential target for the design of male contraceptives. To this end, Matzuk *et al.* (*Cell* 2012, *150*, 673−684) report that the small molecule JQ1, a known inhibitor of another BET family protein called BRD4, is a potent inhibitor of sperm production.

Various biochemical and structural characterization methods, including a homogeneous luminescence proximity assay, isothermal titration calorimetry, and X-ray crystallography, demonstrated that JQ1 is a potent and competitive inhibitor of BRDT. Pharmacokinetic studies in male mice showed that JQ1 can effectively permeate the blood−testis barrier. In addition, it was shown that JQ1 treatment significantly reduces testis volume, sperm number and motility, and production of new sperm, and genome-wide expression analysis demonstrated a reduction in expression of a number of spermatogenic genes. Notably, mice treated with JQ1 were phenotypically similar to mice deficient in BRDT, supporting the notion that JQ1 acts through inhibition of BRDT. The contraceptive effect of JQ1 was shown to be dose-dependent and reversible within a few months, and the compound did not appear to affect testosterone levels, mating behaviors, or developmental processes in offspring. These compelling results

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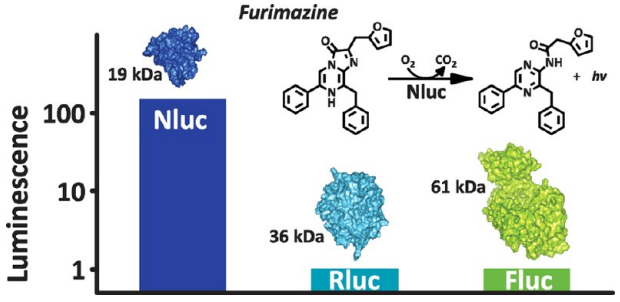
designate JQ1 as a promising lead compound for development of an oral contraceptive drug for men.

Eva J. Gordon, Ph.D.

A BRIGHT FUTURE FOR ENGINEERED SHRIMP LUCIFERASE

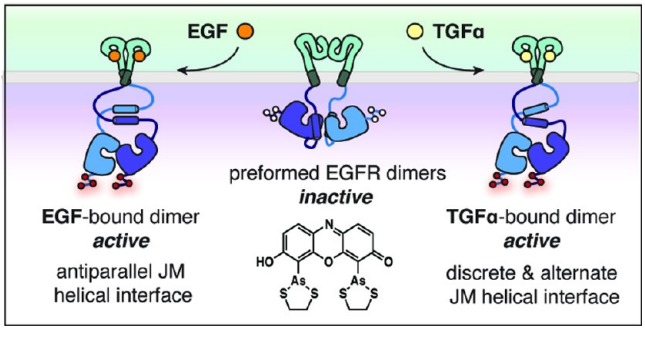


A variety of microbes, insects, and marine organisms produce light for variety of purposes including to attract prey, to repel predators, as a means of communication, or as a light source. They use enzymes called luciferases to oxidase a small molecule substrate called luciferin to generate such bioluminescence, and these enzyme−substrate pairs have become invaluable molecular tools for exploring biological processes. Now, Hall *et al.* (*ACS Chem. Biol.* 2012, *7*, 1848−1857) report the engineering of an engineered luciferase-luciferin-derived system with superior light-emitting and physicochemical properties.

As a starting point, the researchers looked to the luciferase from the deep sea shrimp *Oplophorus gracilirostris*, which emits bright clouds of blue light to defend itself from predators. They manipulated the structure of the small subunit of this heteromeric enzyme to increase its stability and simultaneously tweaked the structure of its substrate to create a molecule called furimazine. This novel enzyme−substrate pair generates much brighter luminescence than the traditional pair and is highly stable in culture medium and cells, suggesting it as an improved bioluminescence system for biological discovery and analysis.

Eva J. Gordon, Ph.D.

JUSTIFYING THE JUXTAMEMBRANE DOMAIN

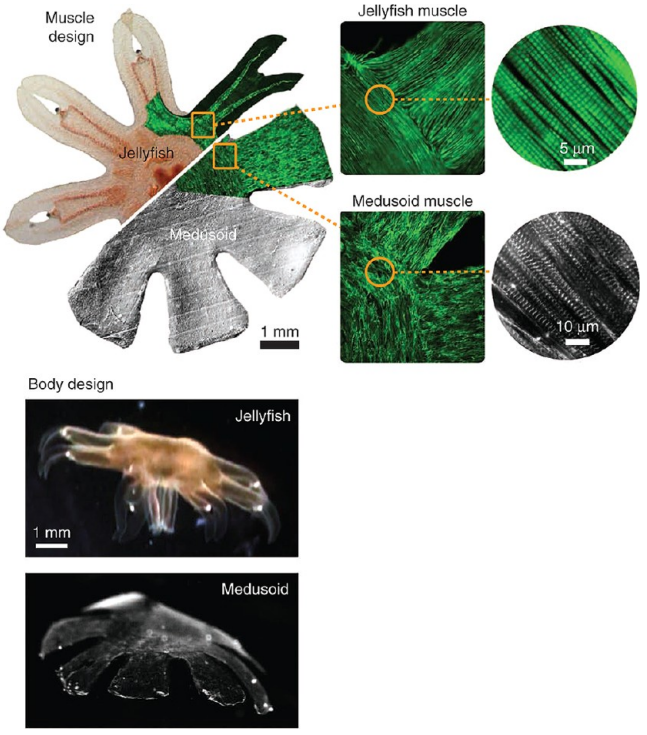


Receptor tyrosine kinases such as the epidermal growth factor receptor (EGFR) are important liaisons between the outside and the inside of a cell, transmitting information from extracellular ligand binding events to the intracellular environment. While the functional consequences of these binding events have been intensively studied, the structural changes within the receptor that accompany them are less well understood. Scheck *et al.* (*ACS Chem. Biol.* 2012, *7*, 1367−1376) now report the use of a clever chemical tool called bipartite tetracysteine display to characterize conformational changes in EGFR that take place in response to ligand binding.

In bipartite tetracysteine display, a fluorescent signal is produced when a structure of interest is folded and assembled properly. Using this approach, the structure of a key intracellular region of EGFR called the juxtamembrane domain was examined. Intriguingly, when the EGFR binds one of its ligands, EGF, the juxtamembrane domain adopts one structure, but when it binds a different ligand, TGFα, it forms a different structure. These findings offer insight into how structural changes within receptor tyrosine kinases might influence the functional ramifications of distinct ligand binding interactions.

Eva J. Gordon, Ph.D.

JELLYFISH BY DESIGN



Reprinted by permission from Macmillan Publishers Ltd: *Nat. Biotechnol.*, Nawroth, J. C., *30*, 792−797, copyright 2012.

Tissue engineering is an exciting field with various diagnostic and therapeutic applications. The relatively simplistic, umbrella-like motion used by adult jellyfish, also called medusae, to move through the water presents an attractive opportunity for designing a synthetic muscular pump. Jellyfish, made up of a bell-shaped, transparent body surrounded by a symmetric display of tentacles, require just a few specific cell types, including motor neurons and striated muscle cells, and efficient interactions with fluid to support their movement. Nawroth *et al.* (*Nat. Biotechnol.* 2012, *30*, 792−797) now combine computational design methods, rat tissue, and versatile biomaterials to reverse engineer a "synthetic jellyfish", referred to as a medusoid, capable of moving like a real jellyfish.

To simulate the rhythmic movement of the jellyfish body, the authors use a sheet of cultured rat cardiac muscle cells synchronized by an electrical field. To mimic the jellyfish contraction and expansion strokes, they construct a bilayer composed of the muscle cells, which provide the force to contract the bell, and jellyfish-shaped

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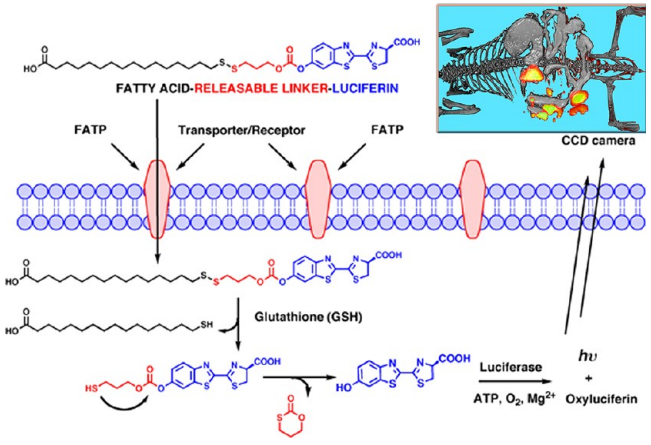
polydimethylsiloxane elastomer, which facilitates restoration of the original shape. Finally, the medusoid was designed to contain wedge-shaped lobes separated by gaps, a geometry that optimizes the efficiency of fluid transport in the construct. Remarkably, medusoids with these parameters achieved similar propulsion and contraction movements, velocities, and fluid currents as real jellyfish. The medusoids presented here highlight the incredible potential of tissue engineering for developing designed biomaterials with various functionalities, including those simulating entire organisms.

Eva J. Gordon, Ph.D.

ILLUMINATING FATTY ACID UPTAKE

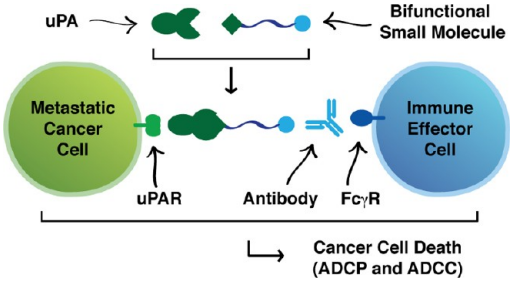


Fatty acids are important sources of energy for most cell types and play key roles in diverse normal and pathological processes. Cellular uptake of these long hydrocarbon chains topped with carboxylic acids is affected and altered by numerous factors in the biological milieu, but monitoring fatty acid location has proved technologically challenging. Now, Henkin *et al.* (*ACS Chem. Biol.* 2012, *7*, 1884−1891) use bioluminescence imaging to enable real-time, quantitative visualization of fatty acid uptake in cells and animal models.

Key to their strategy was the synthesis of a bioactivatable fatty acid derivative conjugated *via* disulfide bond to the bioluminescent compound luciferin, which emits light upon oxidation by the enzyme luciferase. The conjugate is stable outside cells, but upon disulfide cleavage in the reducing environment of the cytoplasm, luciferin is released. This innovative probe enabled visualization of fatty acid uptake in two cell types, adipocytes and fibroblasts, and also in several organs in mice genetically engineered to express luciferase.

Eva J. Gordon, Ph.D.

ARMING THE IMMUNE SYSTEM TO FIGHT CANCER



Over 500,000 people in the United States died from cancer in 2010, and especially deadly are cancers that have metastasized, or spread to other parts of the body. While the immune system may not be capable of killing metastatic cancer cells on its own, one promising approach to fighting cancer is to devise a method to help the immune system get rid of unwanted toxic elements. Jakobsche *et al.* (*ACS Chem. Biol.* 2012, *7*, 316−321) now describe a chemical strategy for luring cytotoxic agents of the immune system directly to metastatic cancer cells.

The strategy relies on the fact that many metastatic cancer cells contain higher levels of a protein called urokinase-type plasminogen activator receptor, or uPAR, on their surface than do healthy cells. uPAR binds to an enzyme called urokinase-type plasminogen activator (uPA). The authors created a bifunctional small molecule, called ARM-U, that binds to and inhibits the activity of uPA and contains an antibody-recruiting antigen. When an ARM-U−uPA complex binds uPAR-containing cancer cells, the antigen acts as bait, luring disease-fighting antibodies to the cancer cells.

Eva J. Gordon, Ph.D.

CODING, NON-CODING, ENCODEING

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Five years ago, a large consortium announced the initial results of a bold project dubbed ENCODE, or Encyclopedia of DNA Elements. At the time, this impressive collection of data peered into 1% of the human genome to identify coding and noncoding RNAs, functional DNA elements and methylation states, transcription factor binding sites, and histone marks. This giant data set became publicly available, and the nearly boundless opportunity for cross-comparison and correlation spawned

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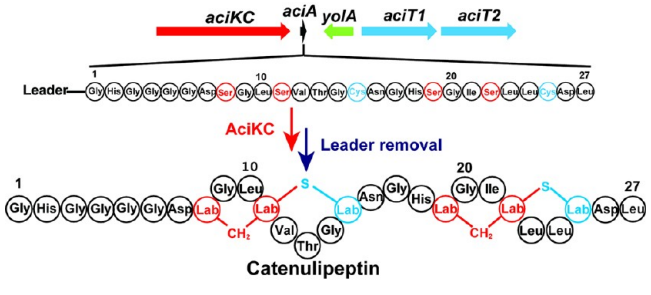
hundreds of research projects. The years that followed the pilot study saw rapidly decreasing sequencing costs and many new discoveries that proved that the human genome was still ripe with secrets beyond the nucleotide code. Recently, the ENCODE team announced the completion of the project with this rich data set of the entire human genome now available on the web and published as a collection in a special issue (*Nature* 2012, *489*, 57−74).

Though the notion that the human genome is full of "junk DNA" was debunked years earlier, the complete look offers finer classification of the vast regions that do not encode proteins. Drawing from parallel data sets on 147 normal and tumor cell types, the study estimates that 80% of the genome can be assigned a biochemical function in at least one cell type. A biochemical function was defined as an RNA or chromatin-associated event in that portion of the genome. In addition, much of the remaining DNA lies close to a regulatory event with 99% of the genome being within 1,700 nucleotides of a biochemical event characterized and measured by ENCODE. Interestingly, most of the SNPs, or single nucleotide polymorphisms, that were previously associated with a disease lie in or near ENCODE-defined functional noncoding elements and not in protein coding sequence. The cross-comparison between chromatin states, transcription factor binding and RNA production shows a strong correlation in most cases. This new encyclopedia stands as a gold standard data resource that will both assist basic scientists to better assign how the cell's rules are written and medical scientists to better understand what goes wrong in a cancer cell. As generating these type of data becomes even less costly, new cell types or cancer lines will be mined for their secrets and, just as with the first complete human genome sequence, the ENCODE data will be the critical resource against which all others are compared.

Jason G. Underwood, Ph.D.

CHARACTERIZING CATENULIPEPTIN

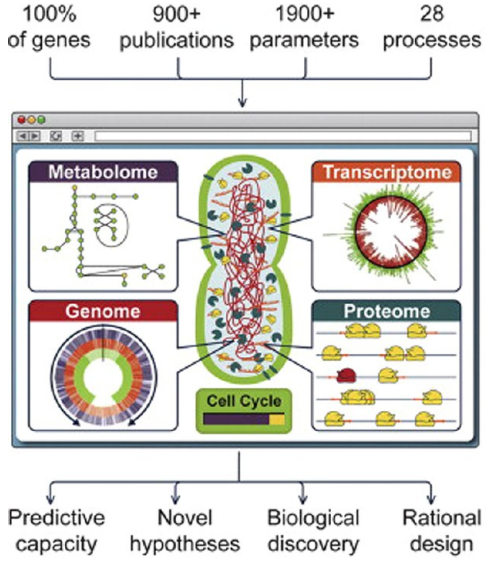


Lantipeptides are a family of polycyclic peptides characterized by the presence of thioether cross-links. They are produced by various bacteria and have diverse biological activities ranging from promoting the formation of branching filamentous structures called hyphae in bacteria to functioning as potent antibiotics. A new class of lantipeptides has been discovered recently that contains unusual cyclic structures called labionins, in which two serines and a cysteine are linked through the activity of multifunctional enzymes containing both kinase and cyclase activities, called LabKCs. Now, Wang and van der Donk (*ACS Chem. Biol.* 2012, *7*, 1529−1535) explore a previously uncharacterized LabKC in the bacteria *Catenulispora acidiphila*, referred to as AciKC.

They discover that AciKC is responsible for several key steps in the biosynthesis of novel labionin-containing lantipeptide called catenulipeptin, including dehydration and cyclization of the peptide substrate as well as installation of the labionin moieties. Examination of the biological activity of catenulipeptin demonstrated its ability to restore hyphae growth in the bacteria *Streptomyces coelicolor*.

Eva J. Gordon, Ph.D.

ENTER THE AGE OF "VIRTUAL CELL BIOLOGY"



Reprinted from *Cell*, *150*, Karr, J. R. *et al.*, A Whole-Cell Computational Model Predicts Phenotype from Genotype, 389−401. Copyright 2012, with permission from Elsevier.

Computer models are powerful tools for understanding and predicting biological phenotypes. An elusive goal has been to understand individual interactions on a "whole-cell" scale that lead to complex phenotypes. With the coming of age of genomics and high-throughput techniques, however, characterization has begun to reach a level of accuracy where comprehensive phenotypic prediction is within reach. In a recent article, Karr *et al.* (*Cell*, 2012, *150*, 389−401) provide a revolutionary study that thrusts us into a new era of "virtual cell biology".

The authors selected *Mycoplasma genitalium*, a human urogenital parasite with a small genome size (525 genes), for "whole-cell" computational modeling. The functionality of cells was subdivided into 28 independent modules (*e.g.*, metabolism, protein degradation, *etc.*) with each module modeled by an appropriate mathematical representation. These independent modules were subsequently integrated to interact and exchange variables in other modules at 1 s intervals. This integrated simulation was subsequently repeated several thousand times until termination when cell division is simulated to cease upon the septum diameter variable reaching zero. This "whole-cell" computational model accurately predicted known experimental data across multiple biological functions. Interestingly, the model also provided new insight into previously unobserved cellular behavior, such as predicting factors that regulate cell-cycle duration. On the basis of these predictive models it is easy to envision integrative "whole-cell" models revolutionizing experimental design and stimulating biological discovery.

Jitesh A. Soares, Ph.D.

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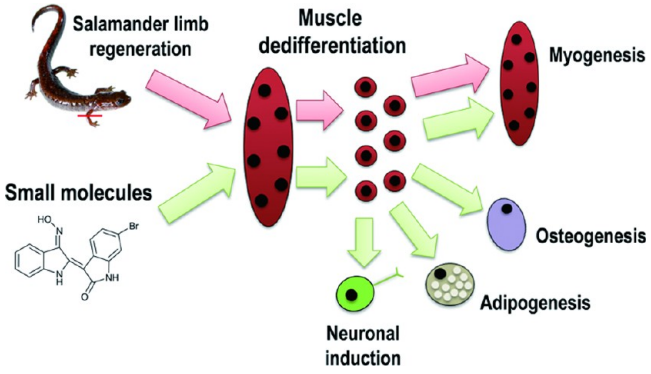
A NEWT METHOD FOR TISSUE REGENERATION

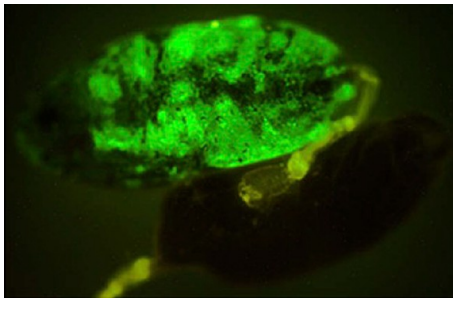


Urodele amphibians, such as salamanders and newts, have the extraordinary ability to regenerate their limbs, and understanding this process better could have profound implications for tissue repair and organ regeneration in humans. A key initial event in limb regeneration in amphibians is the conversion of the muscle cells near the site of injury to a special state from which they can develop into the different cell types necessary to generate new tissue. Small molecules that could induce similar changes in mammalian muscle cells have much therapeutic promise in regenerative medicine. Kim *et al.* (*ACS Chem. Biol.* 2012, *7*, 732−743) now report the identification of four small molecules that promote the growth of new cell types from mammalian muscle cells.

The four molecules, called lysophosphatidic acid, SQ22536, SB203580, and BIO, are known modulators of various cellular signaling pathways. Specially prepared mouse muscle cells treated with these compounds became capable of turning into new muscle cell types as well as other cell types such as precursors to fat, bone, and nervous system cells.

Eva J. Gordon, Ph.D.

THE BITE THAT KILLS BITES BACK



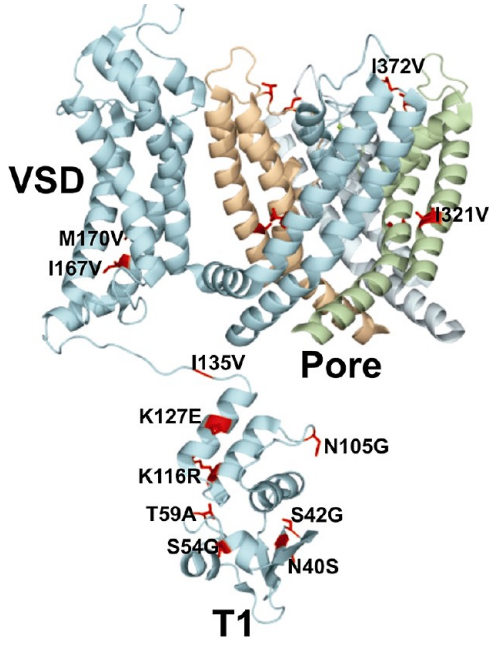
Wang, S. *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, *109*, 12734−12739. Copyright 2012 National Academy of Sciences, U.S.A.

Over one million people die each year from malaria, an infectious disease caused by the unicellular parasite *Plasmodium*. Unlike other killers like HIV, *Plasmodium* is not transmitted from person to person but instead depends on a sinister messenger to both mature and spread the organism. This messenger, the mosquito, ingests the gametocytes from one infected human's blood to begin a maturation process inside the insect. This cycle culminates in infectious sporozoites being deposited into mosquito saliva and, ultimately, into the next bite recipient. While insecticide tactics and antimalarial therapies have gained ground on *Plasmodium*, a new method takes the war against malaria inside of the insect host.

Wang *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2012, *109*, 12734−12739) genetically engineered strains of a symbiotic bacterium that normally lives in the mosquito midgut, a key incubator for the maturing malarial parasite. These strains of *Pantoea agglomerans* all harbored the *E. coli* hemolysin system, which facilitates secretion of proteins through a membrane pore. Each individual strain also expressed one or more known antimalarial proteins tagged for secretion. Taking advantage of the known life stage in the midgut, the various therapeutic proteins targeted the *Plasmodium* ookinete or the mosquito midgut players involved during invasion by the ookinete. After the researchers proved that the *Pantoea* strains displayed robust expression and secretion of the proteins, each were fed to mosquitos to colonize the midgut before administering a blood meal infected with *Plasmodium*. One week later, the mosquitos were assayed for the number of oocysts in the midgut, the next developmental stage after the ookinete that depends upon the insect host. The results showed that therapeutic strains of the *Pantoea* symbiote were successful in suppressing the transition to oocyst. Two of the strains displayed an impressive inhibition of over 97%. The same strains also showed therapeutic inhibition in a mouse model for malaria, *P. berghei*. Importantly, the authors showed that mosquito lifespan is not affected by having a genetically engineered symbiote instead of the wild type. They also discuss a hopeful future where this technique, known as paratransgenesis, could be implemented in the field so that mosquito soldiers can help fight the spread of this deadly parasite.

Jason G. Underwood, Ph.D.

THE OCTOPUS INKS A NEW SCRIPT



From Garrett, S. and Rosenthal, J. J. *Science*, 5 Jan 2012, DOI: 10.1126/science.1212795. Reprinted with permission from AAAS.

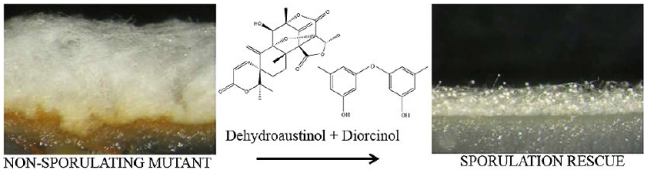
In many eukaryotes, RNA editing machinery expands mRNA diversity by catalyzing selected base changes in a post-transcriptional fashion. One type, adenosine to inosine, or A-to-I, editing occurs by a deamination event of the base. In turn, the translational machinery recognizes the inosine base as if it was a guanosine, often resulting in a change in the encoded amino

acid. The enzymes carrying out this recoding event in animals are known as ADARs, or Adenosine Deaminases Acting on RNAs. ADARs play an important role in the nervous system of worms, fruit flies and mammals, where editing events are critical for generating diversity in transcripts involved in synaptic transmission and neural plasticity. As such, ADAR mutants can have profound cognitive or behavioral phenotypes. Now, this fascinating phenomenon meets an unexpected new variable, environmental temperature, in a nervous system deep in the ocean. Garrett and Rosenthal (*Science*, 2012, 335, 848−851) demonstrate that A-to-I editing plays an important role in tuning the potassium (K^+) channel excitation properties of both an Antarctic and tropical octopus species.

After sequencing the channel-encoding genomic regions of the two octopi and comparing to cloned mRNA transcripts, they discovered that delayed rectifier K^+ channel mRNAs are extensively edited with some of the dozen edits displaying strong bias toward one thermal environment dweller or the other. One mutation, I321V, was highly edited in the cold water Antarctic octopus and electrophysiology experiments demonstrated that this mutation has a strong effect in increasing the rate of channel closure. This allows the channel to abbreviate the refractory period between channel firings and probably helps to compensate for the handicap of cold temperature. With this curious finding in hand, the authors went on to sample the K^+ channel editing of 6 more octopi living in water temperatures spanning from −2 to +37 °C. They uncovered a very strong correlation between the water temperature and the I321V editing event. As the water temperature dropped, the extent of RNA editing increased. Since ADARs usually act on structured RNA, one new direction will be to study how temperature affects the folding of the mRNAs near the editing site.

Jason G. Underwood, Ph.D.

SIGNALING FOR SPORES

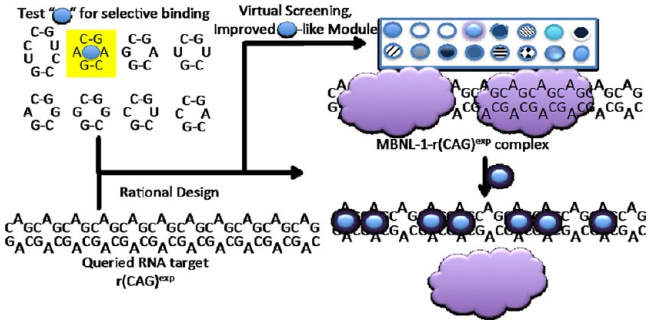


Filamentous fungi comprise a diverse group of microorganisms; they are the mold on your bread, produce life-saving medicines and environmentally friendly agricultural chemicals, and cause human disease. The thread-like structures that characterize these microbes are called hyphae, and when hyphae are exposed to the atmosphere, they produce spores that allow the fungi to reproduce. Studies using genetic mutants of the filamentous fungi *Aspergillus nidulans* that are unable to form spores have suggested that a factor secreted by wild-type *A. nidulans* is responsible for signaling for sporulation. Rodríguez-Urra *et al.* (*ACS Chem. Biol.* 2012, 7, 599−606) now report that this factor is actually two small molecules, the meroterpenoid dehydroaustinol and the orsellinic acid derivative diorcinol.

Using an assay measuring sporulation activity, dehydroaustinol and diorcinol were purified from extracts of wild-type *A. nidulans* cultures. It was found that the compounds form an adduct that prevents crystal formation on the surface of the hyphae and increases the lipophilicity of the signal, properties that may contribute to sporulation.

Eva J. Gordon, Ph.D.

ANOTHER WAY TO SPLICE IT



As our understanding of the many functions of RNA, such as gene transcription, catalysis, and regulation of gene expression, increases, the biomolecule is emerging as an attractive drug target for numerous diseases. For example, the 5′CAG/3′GAC RNA motif is present as expanded triplet repeats in various neurological disorders including Huntington's disease, where it is thought to sequester a protein involved in mRNA splicing. Kumar *et al.* (*ACS Chem. Biol.* 2012, 7, 496−505) now describe a new strategy for identifying compounds that bind to specific RNA motifs, cleverly using known DNA-binding agents as a jumping off point.

Initially, competition dialysis and fluorescence anisotropy were used to screen the DNA-binding agents for their ability to bind to the 5′CAG/3′GAC motif. The common DNA stain 4′,6-diamidino-2-phenyl-indole, known as DAPI, was identified and used as for a virtual screen to identify additional compounds with improved binding properties. The most potent compound discovered, 4-guanidinophenyl 4-guanidinobenzoate, improved splicing defects in cellular models of Huntington's disease as well as in cells derived directly from a patient with Huntington's disease.

Eva J. Gordon, Ph.D.

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