ACS chemical biology

Spotlight

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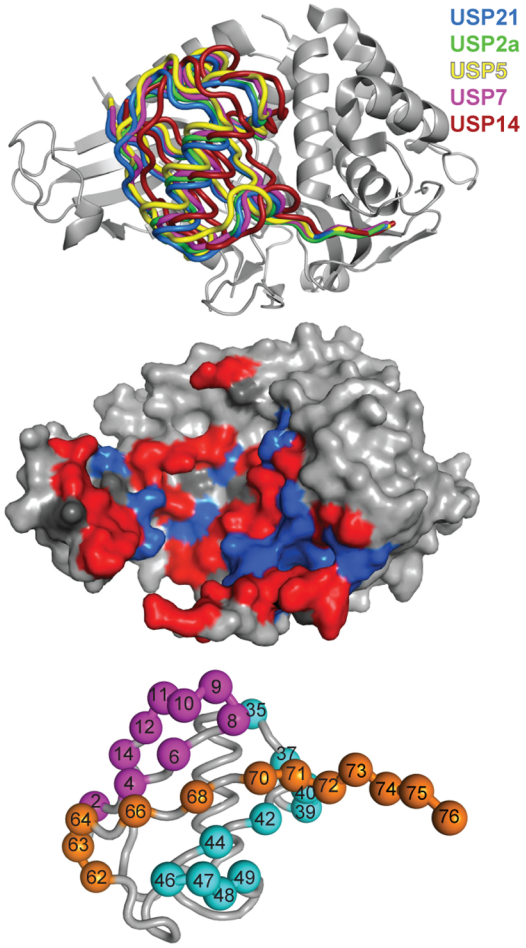
UBIQUITOUS TOOLS FOR EXPLORATION OF UBIQUITINATION



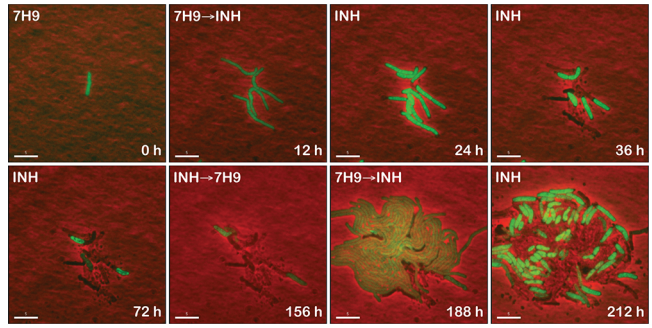
From Ernst, A. *et al.*, *Science*, January 3, 2013, DOI: 10.1126/science1230161. Reprinted with permission from AAAS.

Protein ubiquitination, the mastermind of protein degradation in cells, is also emerging as a regulator of many other processes including the cell cycle, DNA repair, and immune function and has been implicated as a therapeutic target for a variety of diseases. Ubiquitination is a dynamic process; proteins are ubiquitinated and deubiquitinated through a series of enzymatic reactions that collectively help maintain protein homeostasis in the cell. The human genome encodes for hundreds of enzymes involved in this process, making their characterization a daunting task. Toward developing molecular tools for exploring ubiquitination, Ernst *et al.* (*Science*, published online January 3, 2013; DOI: 10.1126/science1230161) now present a strategy for generating potent and specific ubiquitin-derived modulators of enzymes involved in the ubiquitination process.

The strategy was based on the notion that because ubiquitin binds to its partners via a large surface area, it should be possible to create variants of the protein with higher specificity and affinity for its targets. Such variants were constructed using combinatorial phage-displayed libraries, which were used for selection against the various ubiquitin-binding enzyme families. Variants specific for four deubiquitinases, enzymes that remove ubiquitin from target proteins, as well as for other ubiquitin-binding proteins including ubiquitin conjugating enzymes and ubiquitin ligases, were identified. Notably, some of the variants were potent inhibitors of their target enzymes, while other enhanced their activity. Examination of the complex between the ubiquitin variants and the deubiquitinases using X-ray crystallography offered insight into the structural basis for their activity, and confocal microscopy and mass spectrometry verified and helped characterize the interactions in live cells. This clever approach for exploring ubiquitination will continue to deepen our understanding of ubiquitin dynamics and could contribute to the design of therapeutic agents targeting the ubiquitin pathway.

Eva J. Gordon, Ph.D.

PERSISTENCE PAYS OFF FOR BACTERIA



From Wakamoto, Y. *et al.*, *Science*, January 4, 2013, DOI: 10.1126/science.1229858. Reprinted with permission from AAAS.

In a given bacterial population that is exposed to an antibiotic, a small number of cells often persist. This phenomenon is typically a phenotypic distinction rather than a genotypic one and likely supports the survival of a population in stressful environments. With antibiotic exposure, these "persisters" were thought to be those cells that were slowly dividing or not dividing at all, since that characteristic would make them inherently resistant to drugs that predominantly target dividing cells. However, the mechanisms that direct the behavior of persisters are not well understood. Now, Wakamoto *et al.* (*Science* 2013, *339*, 91−95) use single cell methods to explore this phenomenon, and their findings challenge the current model of persistence.

The authors characterized the killing of the bacteria *Mycobacterium smegmatis* by the tuberculosis prodrug isoniazid, which is activated by a catalase-peroxidase enzyme within bacteria called KatG. Using a microfluidic device to culture the bacteria and time-lapse microscopy to track them, they find that the bacteria periodically produce KatG, and these bursts of enzyme are positively correlated with cell death. Interestingly, the KatG pulses are positively correlated between siblings, suggesting that they may be under epigenetic control. In addition, they find that the persistent population was

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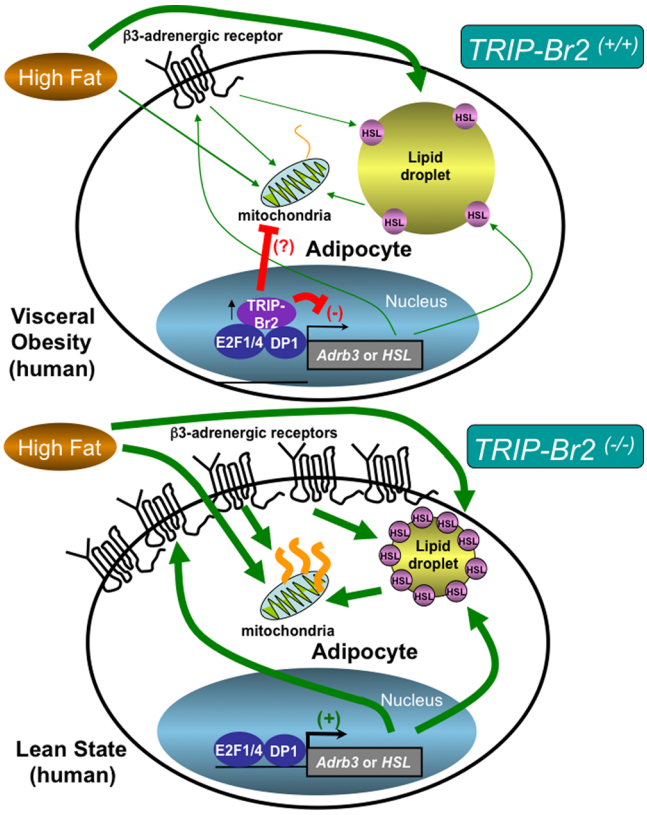
characterized by a balanced number of dividing and dying cells, contradicting the theory that persistent cells are solely comprised of nonreplicating cells. That persistent cells may grow and divide in the presence of an antibiotic suggests that the phenotypic behavior of persistence may enhance the genotypic consequence of resistant mutants. The authors propose that isoniazid-mediated selection could result in gradual adaptation in a survival of the fittest-type process, or the antibiotics could themselves trigger adaptive responses.

Eva J. Gordon, Ph.D.

TRIPPING OUT OBESITY



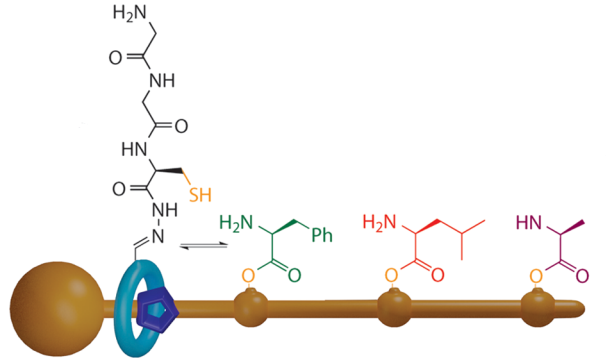
Adapted from and reprinted by permission from Macmillan Publishers Ltd.: *Nat. Med.*, advance online publication, 6 January 2013, DOI: 10.1038/nm.3056.

A staggering 500 million people worldwide are considered obese, a disease whose association with diabetes, cancer, and heart disease contributes to its position as the fifth leading risk factor for death across the globe. Fat cells, or adipocytes, store energy as fat, and a better understanding of energy metabolism in these cells could lead to new strategies for the prevention and treatment of obesity. To this end, Liew *et al.* (*Nat. Med.* published online January 6, 2013; DOI: 10.1038/nm.3056) report that a transcription factor called TRIP-Br2, previously known for its role in cell-cycle progression, is also a master regulator of energy metabolism in adipocytes.

The authors determined that TRIP-Br2 expression is elevated in fat tissue from obese mice and human. Mice lacking the TRIP-Br2 gene do not become obese, even when they consume similar amounts of high fat chow as control mice. These mice were also protected from developing the adverse effects of obesity, such as insulin resistance, high cholesterol, and fatty liver disease. Mice lacking TRIP-Br2 had generally smaller adipocytes and increased lipid breakdown (lipolysis) and expended more energy as evidenced by increased mitochondrial oxidative metabolism and heat generation (thermogenesis) than control mice. Clues to the mechanism by which TRIP-Br2 modulates these effects was found in two genes, HSL and Adrb3, whose transcription is regulated by TRIP-Br2. The authors propose a model in which TRIP-Br2 is permissive for the development of obesity by reducing energy expenditure and fat metabolism. The identification of the key role of TRIP-Br2 in energy metabolism implicates this transcriptional coregulator as a promising new target for obesity.

Eva J. Gordon, Ph.D.

CHEMISTRY RIFFS ON THE RIBOSOME



Adapted from Lewandowski, B. *et al.*, *Science*, January 11, 2013, DOI: 10.1126/science.1229753. Reprinted with permission from AAAS.

Protein synthesis is orchestrated by a complex, ordered series of events on the ribosome. In addition to its role in RNA-catalyzed peptidyl transfer, this amazing macromolecular machine facilitates activities as diverse as binding, proofreading, unwinding, and translocation. Taking peptide synthesis out of the cell and into a pure chemical arena involves a series of reactions on a solid support. To avoid side reactions, monomers are traditionally protected with specific blocking groups that can be removed by a shift in pH. Synthesis proceeds via an iterative series of deprotection, washing, activation and coupling steps. Now, Lewandowski *et al.* (*Science* 2013, *339*, 189−193) have taken inspiration from both the biological and chemical methods of polypeptide synthesis to create a molecular machine that can catalyze the elongation of a peptide chain in an iterative fashion.

To achieve a stepwise peptidyl transfer reaction, the researchers synthesized a rigid molecular scaffold with each of the reactive amino acid monomers covalently attached in a specific order. Then, a ring-like molecule was threaded onto this scaffold at a specific end using click chemistry, forming a rotaxane. The product of this reaction both positioned a reactive moiety on the rotaxane for the first peptide bond formation and provided a steric block so the ring could only move along the scaffold in one direction. The reactive moiety of choice was a cysteine residue attached to the rotaxane. Once the machine's protecting groups were removed in an acid-catalyzed manner, the cysteine group was positioned for a transacylation reaction with the first reactive amino acid monomer along the scaffold. After this first peptide bond formation, the reactive thiolate group reacted with successive amino acids along the chain by a similar mechanism until the last amino acid was added. The researchers investigated the

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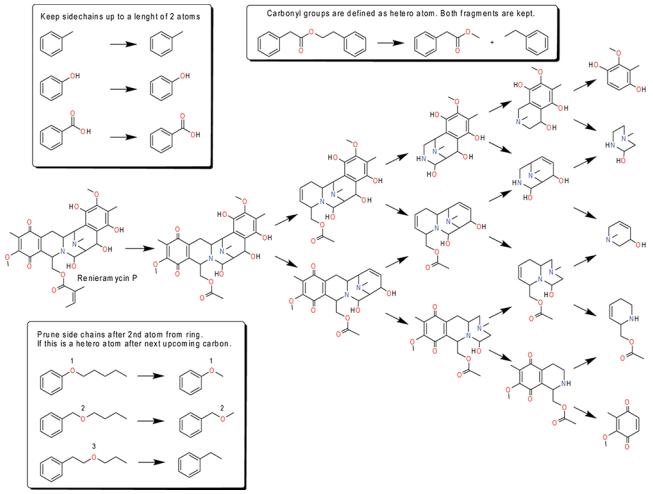
products of the reaction by both NMR and mass spectrometry to demonstrate that the intended hexapeptide was generated with the proper order of amino acids. While this unique method cannot compete with the ribosome's speed and efficiency, it demonstrates a new route to peptide synthesis and opens the door for new methods to produce peptides containing non-natural amino acids in milligram quantities.

Jason G. Underwood, Ph.D.

EXPANDING THE FRAGMENT LIBRARY



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In recent years, the drug discovery process has slowed as researchers struggle to access molecules with novel chemical features. Fragment-based drug discovery, a computational approach for breaking apart more complex structures to look for simpler components with desired chemistries, offers a way to speed the development of new drug candidates. But most compound libraries are currently derived from known drug molecules that are often flat (sp^2 hybridized) and well-characterized. Scientists have been looking for ways to increase the diversity of these libraries. Now Over *et al.* report a chemical informatics strategy for creating candidate fragments from natural products and demonstrate the value of this strategy by uncovering novel enzyme inhibitors (*Nat. Chem.* 2013, *5*, 21−28).

To develop the natural product fragments for study, the researchers used an algorithm to disassemble natural products into molecular pieces that retained sp^3 hybridization and critical functional groups while limiting the number of rings and bridging structures and retaining a sufficient number of hetero atoms. They clustered these fragments to filter out similar structures. Overall they whittled nearly 200,000 initial fragments down to a manageable number of 2,000.

The researchers used that data to purchase or synthesize molecules that were either members of these structural clusters or closely related compounds. For studies of p38α MAP inhibitors, the researchers purchased or synthesized 193 compounds and solved X-ray crystal structures for 9 fragments that bound to the enzyme and used assays to study their inhibitory activity. They also used this strategy to look for phosphatase inhibitors with novel chemical structures. They found novel inhibitors for both classes of enzymes.

The total synthesis of natural products is difficult and labor-intensive, and these obstacles have limited their use in drug discovery research. Although the synthesis of fragments will still require investment in synthesis methodologies, the fragment-based molecules should provide a faster and cheaper strategy for producing new drug candidate compounds.

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