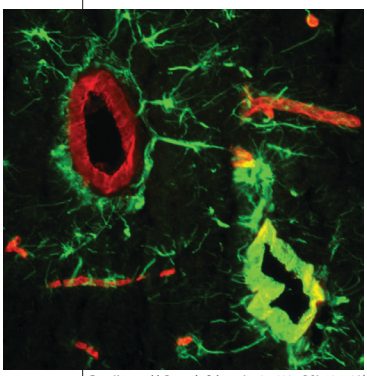
Spotlight

How Cells Get Their Vitamins



From Kawaguchi, R., *et al.*, *Science*, Jan 25, 2007, DOI: 10.1126/science.1136244. Reprinted with permission from AAAS.

We all know it is important to get our vitamins. But once we eat that carrot or swallow that pill, how do those vitamins actually make their way into our cells to carry out their duties? Kawaguchi *et al.* (*Science* 2007, *315*, 820–825) now report the identification of a cell membrane receptor that helps transport vitamin A into cells.

Well known for its importance in vision, vitamin A (also known as retinol) is a molecule critical for cell growth and differentiation. Vitamin A is stored in the liver and hitches a ride with retinol binding protein (RBP) for transport through the bloodstream for delivery to other organs. Clever construction of a histidine-tagged RBP conjugated to a photoreactive group enabled cross-linking of RBP to its cell surface receptor. Nickel resin purification of RBP complexed with its putative binding partner followed by mass spectrometry analysis led to the identification of stimulated by retinoic acid gene 6 (STRA6), an 11-transmembrane domain-containing protein with no previously known function. The authors demonstrated that cells containing STRA6 on their surface bound RBP and exhibited highly increased vitamin A uptake. Investigations into the mechanism of STRA6-mediated vitamin A uptake argued against endocytosis or simple diffusion, even though vitamin A is capable of diffusing through cell membranes. Given that too much vitamin A can be toxic and too little vitamin A can lead to blindness and other diseases, the existence of an efficient, specific, and controlled vitamin A delivery system could be important for regulating its levels throughout the body. Future investigations into the function of STRA6 will help define the mechanisms that control vitamin A uptake and enable proper execution of its many important functions. **Eva J. Gordon, Ph.D.**

The Medicine Map

If personalized medicine, or health care that is customized to the precise molecular and genetic properties of the individual, were a car, then the kids would be in the back seat shouting, "Are we there yet, are we there yet?" Unfortunately, we are not there yet, but advances in genotyping coupled with investigations into the molecular basis for disease and drug side effects promise to substantially improve medical care as well as provide numerous economic and societal advantages. However, implementation of an effective personalized medicine program requires a detailed plan that addresses the scientific, economic, and legal hurdles that we currently face. Fortunately, M. J. Ratain (*Clin. Pharmacol. Ther.* 2007, *81*, 321–322) has proposed the creation of a genomic prescribing system (GPS) to help us navigate the twists and turns to get there.

A GPS would link genotyping, pharmacogenetics tests, and secure data management methods to enable personalized health care. Certainly, more research in pharmacogenomics is needed, but these elements are within our grasp. However, because pharmacogenetics tests need be performed only once in an individual's lifetime, the market for a GPS contains significant unknowns, and a distinctly different business model is required to ensure the availability of adequate funding. Ratain reasons that because

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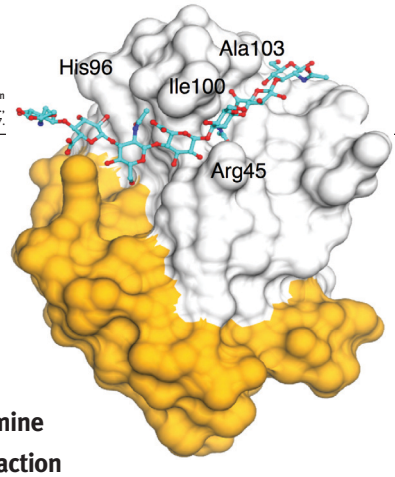

CD44 Surprise

CD44 is a cell surface receptor that binds hyaluronan (HA), a high-molecular-weight copolymer of *N*-acetylglucosamine and glucuronic acid. This interaction mediates cell migration in both normal and pathological processes, including inflammation and tumor metastasis. Low- and high-affinity conformations of the receptor appear to regulate CD44–HA interactions, depending on the activation status of the cell, but the structural and molecular basis of this regulation had not been determined. Banerji *et al.* (*Nat. Struct. Mol. Biol.*, *14*, 234–239) now present the crystal structure of CD44 in complex with HA, uncovering surprising details that provide clues into the mechanism of its regulation.

The first unexpected finding came upon examination of the general nature of the binding interaction. The contacts between HA and CD44 were governed mainly by hydrogen bonds and van der Waals forces, not ionic and CH-π stacking interactions as might be expected from a charged polysaccharide like HA. The next revelation emerged upon comparison of three different crystal structures, one of CD44 alone and two with HA bound, where two distinct conformations of CD44 in complex with its ligand were observed. One conformation was similar to the structure of CD44 in the absence of HA, whereas the other contained an altered orientation of a key arginine residue such that several subtle but significant structural differences throughout the receptor resulted. Conformational changes associated with HA binding apparent in NMR studies were similar to the differences between the two HA-bound CD44 conformations observed in the crystal structures. The researchers propose that this conformational switch in CD44 could be induced either upon HA binding or by changes in sialylation of regulatory glycan chains, which would provide allosteric mechanisms for adopting the high-affinity conformation necessary for its activity. Eva J. Gordon, Ph.D.

The Medicine Map, *continued*

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the public and payers stand to gain considerably from a GPS through improved medical care and lower medical costs, they should consider footing the bill. One option, put forth by Senator Barack Obama, a 2008 U.S. presidential candidate, last August in the "Genomics and Personalized Medicine Act of 2006", provides increased funding for genomics research, offers a tax credit for the development of pharmacodiagnostic tests that can improve drug safety, and addresses the need to protect genetic privacy. Another approach would be the creation of a GPS foundation, financed through a combination of philanthropic funds, contributions from foundation members such as insurance companies, and fees for services such as genotyping, data management, and education.

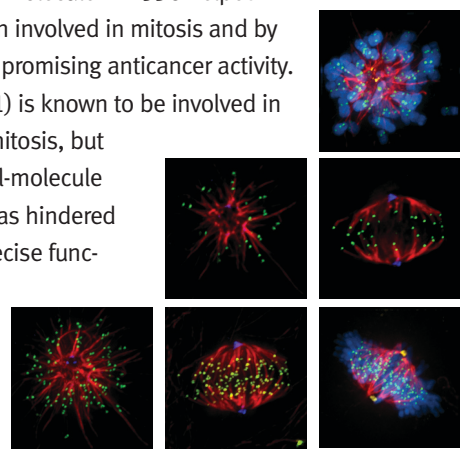
Unlike the direction that a GPS (global positioning system) provides you in your car, the personalized medicine GPS route is not yet mapped out. Discussions among scientists, physicians, economists, payers, philanthropists, and the government (and perhaps a cartographer or two) are necessary to ensure that the promising findings in pharmacogenomics research will actually be transported into truly better health care.

Eva J. Gordon, Ph.D.

The Power of a Small Molecule

Chemical biologists often discuss the value of small molecules as molecular tools for biological discovery or as potential therapeutic agents for a specific disease. Now, two recent reports by Lénárt *et al.* (*Curr. Biol.* 2007, *17*, 304–315) and Steegmaier *et al.* (*Curr. Biol.* 2007, *17*, 316–322) highlight chemical biology at its best by describing how the small molecule BI 2536 helped clarify the role of a protein involved in mitosis and by demonstrating BI 2536's promising anticancer activity.

Polo-like kinase 1 (Plk1) is known to be involved in many steps throughout mitosis, but the lack of selective small-molecule inhibitors of the kinase has hindered efforts to elucidate its precise functions. Screening of a small-molecule library in search of inhibitors of Plk1 activity led to the identification of BI 2536, a dihydropteridinone. BI 2536 was shown to disrupt several Plk1-dependent processes, and cells treated with the compound exhibited the same phenotype as those subjected to Plk1 RNA interference; this provided compelling evidence that BI 2536 specifically inhibits Plk1 activity. Thus, by treating various cell lines with BI 2536, the authors were able to more clearly define Plk1's many predicted activities. For example, they determined that Plk1 is not essential for entry into prophase, the first phase of mitosis, but is required for timely entry into the second phase of mitosis, prometaphase. They also found that Plk1 is necessary for degradation of Emi1, an inhibitor of the anaphase-promoting complex/cyclosome that targets proteins for degradation during exit from mitosis. Furthermore, in addition to the known functions of Plk1 in the release of the protein cohesin from chromosome arms and the maturation of the centrosomes (where microtubules are organized), they showed that Plk1 is required for the establishment of stable attachment of microtubules to kinetochores, the structures that link chromosomes to microtubules. The potent and specific activity of BI 2536 has addressed many controversial hypotheses regarding Plk1 function and provides an important tool for future exploration of mitosis.



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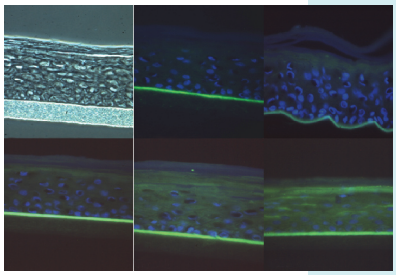
As if the utility of BI 2536 in probing mitosis were not exciting enough, a related group investigated the potential of BI 2536 as an anticancer agent. The essential role of Plk1 in mitosis along with its enriched expression in cancer cells indeed makes it an attractive cancer target, and the anticancer activity of BI 2536 was investigated in various cell lines and in animal models. Immunofluorescence microscopy and flow cytometry analysis of several cancer and non-cancer cell lines revealed that the compound causes mitotic arrest and induces apoptosis of proliferating cells. Also, when BI 2536 activity was examined in mouse xenograft models of colon, pancreatic, and non-small-cell lung cancer, marked tumor-growth inhibition resulted. In addition, optical near-infrared imaging and magnetic resonance imaging methods were implemented to assess the extent of apoptosis and structural changes in the tumors *in vivo*, and this will facilitate evaluation of BI 2536 activity in humans as well. In fact, the promising activity of this compound has recently led to its progression into clinical trials in patients with locally advanced or metastatic cancers.

If knowledge is power, then these studies are indeed a powerful addition to our war against cancer. The fact that Plk1's activity appears to be limited to mitosis of proliferating cells may have significant advantages in cancer treatment. For example, the anticancer agents that target microtubules can lead to various adverse effects, in part because of the diverse functions of microtubules in the cell. The enhanced understanding of Plk1 activity imparted by BI 2536 will help guide the design and implementation of future experiments in the research, preclinical, and clinical settings, accelerating the development of BI 2536 as a potential cancer drug. **Eva J. Gordon, Ph.D.**

Vitamin D_3: A Soldier for Innate Immunity

Vitamin D_3 has many important functions in the body, including regulating calcium and phosphorus levels in the blood and promoting bone formation. Recent studies have also implicated the secosteroid in various aspects of immune function, but its precise role in innate immunity is not clear. Schauber *et al.* (*J. Clin. Inv.*, *117*, 803–811) now report that the compound possesses soldier-like properties within the innate immune system, enabling skin cells to recognize and take action against microbes.

The expression of the microbial pattern recognition receptors TLR2 and CD14 and the production of antimicrobial peptides such as cathelicidin are inherent in the innate immune response. The authors discovered that several genes expressed after injury in skin cells were the same as those under the control of vitamin D_3, including TLR2, which was not previously known to be influenced by vitamin D_3. This suggested that injury may cause a local increase in enzymes responsible for producing vitamin D_3, and indeed, the enzyme CYP27B1, which converts an inactive vitamin D_3 precursor to active vitamin D_3, is induced in wounds. In addition, only skin cells treated with vitamin D_3 were able to induce production of cathelicidin upon exposure to TLR2 ligands, and this substantiates the role of vitamin D_3 in innate immunity. Taken together, the data suggest a model in which injury or infection in the skin results in the production of biologically active vitamin D_3, which in turn induces a chain of signaling events that enhances expression of critical components of the innate immune response. These findings further define the machinery of our incredibly complex immune system and may lead to vitamin D_3-based strategies for wound repair. **Eva J. Gordon, Ph.D.**



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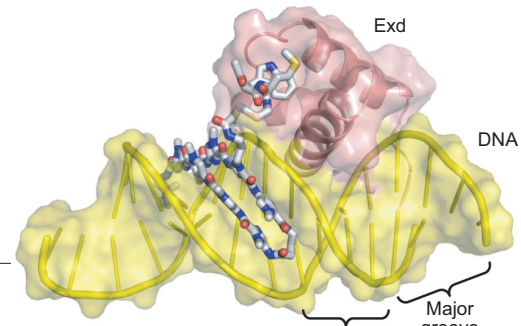
A Purpose for Slime

The cell surface of most bacteria is coated with an amalgamation of carbohydrate polymers, such as lipopolysaccharide (LPS) O-antigen, capsular polysaccharides (K-antigen), and colonic acid (CA or M-antigen), and this coating plays important roles in cell recognition, adhesion, and defense. Although some polysaccharides are covalently attached to the bacterial outer membrane, CA is only loosely associated, so it is characterized as an exopolysaccharide or a slime polysaccharide. It is thought that bacteria exploit the diverse mixture of available polysaccharide components to create suitable surfaces for given environments. However, the precise roles of the different polysaccharides and the circumstances surrounding their generation are not well defined. Now, Meredith *et al.* (*J. Biol. Chem.*, *282*, 7790–7798) describe a novel surface coating termed M_{LPS} that is generated in a specific mutant strain of *Escherichia coli* K-12, named KPM22, under certain environmental conditions.

KPM22 adopts a mucoid phenotype in hypotonic media, an indication of CA induction. However, characterization of the extracellular polysaccharides on this strain with gel electrophoresis, mass spectrometry, and NMR revealed that M_{LPS} is composed of repeating CA units that are covalently attached to LPS. In addition, the polysaccharides, although identical in composition and sequence to CA, differed at multiple positions in anomeric configuration, along with other minor differences. The authors also determined that the O-antigen ligase WaaL is responsible for attaching CA to LPS in M_{LPS}. The unique properties of M_{LPS} suggest that *E. coli* is empowered to change its polysaccharide coat in response to outer membrane perturbation. An M_{LPS} surface, which has a higher net negative charge and greater surface accessibility than O-antigen, may offer several structural and biochemical advantages for the bacteria. For example, it may help to stabilize the bacterial membrane under conditions of stress or provide enhanced accessibility of surface proteins that are important in biofilms, for which CA is an integral component. **Eva J. Gordon, Ph.D.**



Engineering an Interaction

For proper regulation of transcription in eukaryotes, DNA-binding proteins bind to cognate sequences within a promoter and stimulate the binding of other factors to activate or repress transcription. A prototypical transcription factor displays a multidomain structure where one domain binds DNA and a separate domain partners with other factors to modulate the promoter activity. Now, a new study by Stafford *et al.* (*J. Am. Chem. Soc.* 2007, *129*, 2660–2668) zooms in on a well-characterized interface between two transcription factors and asks whether such an interaction could be mimicked with an artificial dimerizer, a molecule that can both specifically bind DNA and another protein.

In the fruit fly, the homeobox transcription factor Ultrabithorax (Ubx) binds to DNA and facilitates the binding of the factor Extradenticle (Exd) to a neighboring DNA sequence. With a crystal structure of the ternary complex in hand, the authors engineered a bifunctional, artificial Ubx that would both bind to DNA and interact with Exd. A hairpin imidazole-pyrrole polyamide was used in place of the protein DNA-binding domain. These molecules can be programmed to bind in the minor groove of DNA with sequence specificity. Tethered to the DNA binder was the short peptide sequence from Ubx that fits into a binding pocket on Exd. Using DNA footprinting techniques, the authors demonstrated that the artificial transcription factor can bind to DNA and facilitate binding of Exd to the adjacent DNA. They then truncated the tethered peptide to find the minimum sequence required for dimerization of the artificial factor and Exd. Interestingly, the sequence could be cut down to just the tiny dipeptide of Ubx that is buried in Exd in the natural interface crystal structure. Variants of the peptide tryptophan-methionine were hooked to the polyamide hairpin and tested in gel mobility shift assays for the ability to recruit Exd in various conditions. The full-length peptide could perform the task on ice, at RT, or at 37 °C, whereas only one variant of the dipeptide could recruit Exd at all temperatures. Most unusual, it was the replacement of L-tryptophan with the unnatural amino acid D-tryptophan that caused the higher affinity and facilitated the ternary complex, even at 37 °C. The authors developed a model for the interface and speculate on how such an engineered transcription factor might be used to modulate transcription in living cells. **Jason G. Underwood, Ph.D.**

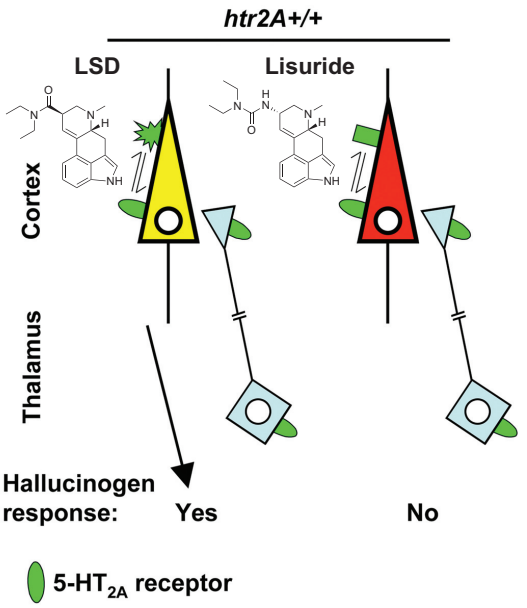
Protein Puzzles Get a New Solution

Post-translational modifications act as more than just decorations on eukaryotic proteins. Many are key to proper localization or in tuning the function of a particular protein. Some families of proteins, such as the histones that coat DNA, are decorated with an array of modifications at various sites. Acetylation, phosphorylation, and methylation of histone tails affect the packaging of DNA to regulate the activity of a particular genomic region. One question that has plagued the field of chromatin biology is how one class of modifications affects another and whether there is an order to these events. This is a technically difficult challenge because most purification methods and modification assays display the overarching status of a protein population but cannot reveal much about whether the modifications are on the same polypeptide. Taverna *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2007, *104*, 2086–2091) apply a newly developed method for mass spectrometry to one histone's tail as an impressive proof of principle.

The method, termed electron transfer dissociation (ETD), breaks the peptide almost independently of its sequence or length. This generally produces an extensive set of fragment ions. Then, a second ion–ion reaction, called proton transfer charge reduction (PTR), is employed to simplify the complex ETD spectrum. The resulting ETD/PTR spectrum can be analyzed to determine the amino acid sequence and the location of modifications. The ETD/PTR method enables the analysis of modifications on a single polypeptide. This provides a new look at the link between acetylation and methylation status on the tail of one histone. The authors chose histone H3 from the ciliate *Tetrahymena* and found several overarching themes. It appears that H3 is methylated at Lys4 and Lys27 in the transcriptionally active somatic macronucleus. Interestingly, only Lys4 trimethylation is found to coexist with hyperacetylated H3 N-termini, an indication of a link between Lys4 trimethylation and H3 hyperacetylation. This method represents a powerful step forward in post-translational modification analysis and is likely to provide new insights into the order and propensity for modification when many combinations are possible. Jason G. Underwood, Ph.D.

Hallucinogenic Signals

Hallucinogens have a colorful history of medical, religious, and recreational use, but only relatively recently have scientists begun exploring the molecular mechanisms of their, well . . . colorful effects. All known hallucinogens, including lysergic acid diethylamide (LSD), are serotonin receptor (specifically the 5-HT_{2A} receptor) agonists, but in a perplexing twist of biology, other closely related 5-HT_{2A} agonists such as (*R*)-lisuride do not elicit hallucinogenic behavior. Now, González-Maeso *et al.* (*Neuron* 2007, *53*, 439–452) report that interaction of LSD with 5-HT_{2A} triggers distinct signaling pathways in neurons that begin to explain the unique effects of hallucinogens.



Reprinted from *Neuron*, *53*, González-Maeso, J., *et al.*, Receptor-mediated signaling pathways to affect behavior, 439–452, Copyright 2007, with permission from Elsevier.

After development of a robust mouse model for hallucination in which treatment with LSD but not (*R*)-lisuride caused a "head twitch response" (the murine equivalent of "tripping out"), the researchers began looking at the genes affected by these compounds. Although both compounds induced expression of *c-fos*, a nuclear protein involved in growth-related transcriptional control, only LSD induced expression of two genes encoding members of the early growth response protein family, *egr-2* and *egr-1*. Delving further into the differences in signaling patterns elicited by the two compounds, the authors discovered that pertussis toxin, an inhibitor of $G_{i/o}$-mediated protein signaling, and PP2, an inhibitor of the protein tyrosine kinase Src, significantly attenuated the effects of LSD. In contrast, a phosphatidylinositol-3 kinase inhibitor did not affect the activity of either agonist, an indication that the unique effects of LSD are linked to signaling through $G_{i/o}$ proteins and Src. Notably, these signaling pathways were only affected in neurons in certain parts of the brain, such as cortical structures and the olfactory bulb, but not in the thalamus or cerebellum, a sign that specific neuronal populations are responsible for mediating hallucinogenic effects. By uncovering the signaling pathways activated by hallucinogens, this study clearly illuminates a path toward understanding the molecular basis of hallucination. **Eva J. Gordon, Ph.D.**