Spotlight

Big Signaling from the Smallest Molecule

Many small molecules function as signaling molecules. Perhaps not to be underestimated is the signaling that occurs through the smallest "molecule" of all, the proton. Recent evidence has implicated proton signaling in central nervous system function, but the mechanisms that govern the signal transmitting properties of protons are unclear. Now, Beg *et al.* (*Cell* 2008, *132*, 149–160) demonstrate that, through specific proteins that regulate proton release, protons can mediate muscle contraction in the nematode *Caenorhabditis elegans*.

The defecation cycle in *C. elegans* is characterized by three independent muscle contractions, the first of which is contraction of the posterior body muscles. Although muscle contraction is normally mediated by neurotransmitters, posterior body contraction in *C. elegans* appears to be regulated through a non-neuronal mechanism. In the search for proteins required for this motor step, two genes, *pbo-4* and *pbo-5*, were identified. Sequence analysis revealed that the PBO-4 protein is homologous to Na^+/H^+ exchangers, which mediate the exchange of a Na^+ ion into the cell for a H^+ ion out of the cell, whereas *pbo-5* and its homolog *pbo-6* encode subunits of the cys-loop ligand-gated ion channel superfamily, which are typically activated by classical neurotransmitters. However, phylogenetic analysis and biochemical evidence suggested that PBO-5 is not likely activated by known classical neurotransmitters. The authors further demonstrated that PBO-4 is required for the transient acidification of the extracellular space that precedes posterior body contractions and that PBO-5 and PBO-6 can function as a proton-gated ion channel. Furthermore, localization studies revealed that

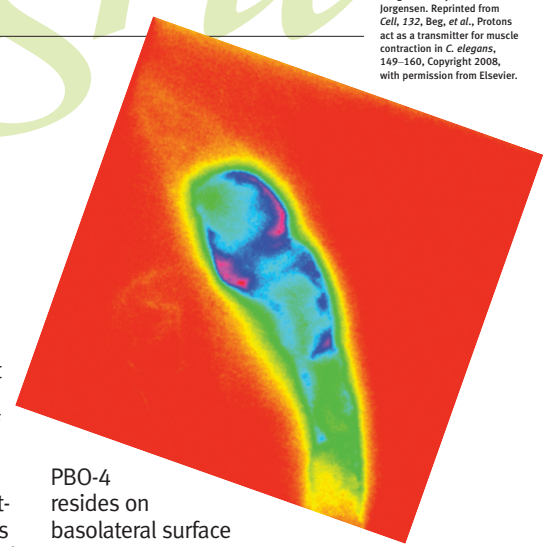

Image courtesy of Erik Jorgensen. Reprinted from *Cell*, *132*, Beg, *et al.*, Protons act as a transmitter for muscle contraction in *C. elegans*, 149–160, Copyright 2008, with permission from Elsevier.

PBO-4 resides on basolateral surface of the intestine, whereas PBO-5 and PBO-6 were expressed in the posterior body wall muscles. The authors propose a model in which PBO-4 mediates the release of protons to the extracellular space immediately prior to posterior body contractions, while PBO-5 and PBO-6 form the proton-gated cation channel that facilitates depolarization of the cell and muscle contraction. **Eva J. Gordon, Ph.D.**

Antisense Teams Up with Targeted Therapy

One of the tricky things about treating cancer is finding a way to kill cancer cells without destroying healthy ones. Targeted therapy is a treatment strategy in which a toxic agent is attached to a molecule that specifically binds to tumor cells, such that normal cells are left unharmed. Another challenge in cancer research is finding a suitable, effective target. The transcription factor Id1 is an attractive cancer target because it is implicated in tumor invasiveness, metastasis, and angiogenesis, albeit an elusive one, as it is located in the nucleus of cells and is difficult to inhibit with small molecules. Henke *et al.* (*Nat. Biotechnol.* 2008, *26*, 91–100) tackle both these challenges in the development of a targeted therapy approach against Id1 by combining an antisense oligonucleotide with a peptide-based targeting agent.

The anticancer agent, termed Id1-PCAO, was created by covalently coupling the Id1 antisense oligonucleotide to the F3-peptide, a fragment of the high mobility group protein N2 known to home in on tumor cells and to localize to the nuclei of endothelial cells. After the authors demonstrated that Id1-PCAO internalized in endothelial cells, down-regulated Id1 protein expression, and localized to the nucleus, they tested it in several *in vivo* mouse tumor models. Intravenous injection of Id1-PCAO in a breast cancer model resulted in complete loss of Id1 expression in tumor vessels. In addition, Id1-PCAO treatment led to enhanced hemorrhage and hypoxia, as well as substantial suppression of tumor growth and metastasis. When delivered in combination with the heat shock protein 90 inhibitor 17-AAG, complete growth suppression of aggressive breast tumors was accomplished. Furthermore, subcutaneous administration of Id1-PCAO resulted in significantly reduced tumor growth and increased hemorrhage in a Lewis lung carcinoma model. This strategy represents a promising new approach for treating cancer, especially for targets difficult to tackle by traditional methods. **Eva J. Gordon, Ph.D.**

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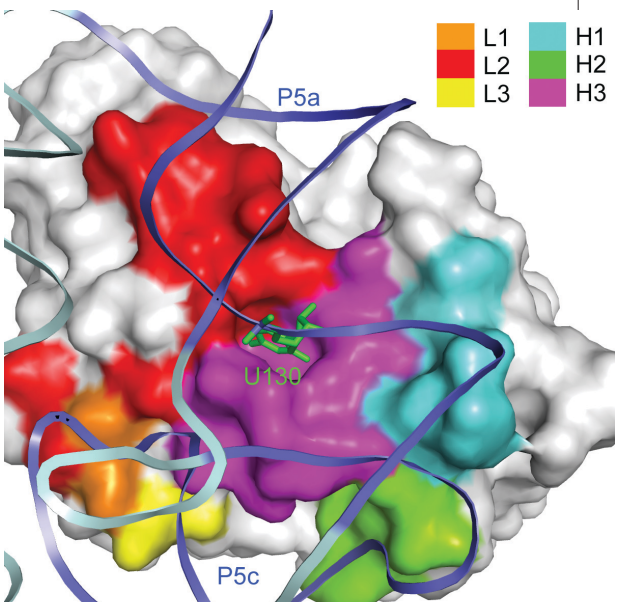
RNA Function No. 160: Antigen?

Structural biologists sometimes throw molecular handcuffs on a particularly flexible protein in the interest of X-ray crystallography. For instance, co-crystallizing a protein with an antibody that recognizes the protein as an antigen can be advantageous, because such an antibody interaction can hold the polypeptide chain in a more rigid state and promote crystal contacts. This often results in a complex that is more amenable to crystal formation and better diffraction data for solving the structure. But, what if the molecule of interest is the flexible biopolymer RNA? Injecting this molecule into an animal to produce an immune response would probably just result in immediate degradation of the RNA. To get around this caveat and in pursuit of antibodies for RNA co-crystallization, Ye *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2008, *105*, 82–87; Epub Dec 27, 2007) use a phage display methodology to select antigen-binding fragments (Fabs) that specifically bind to a structured RNA molecule.



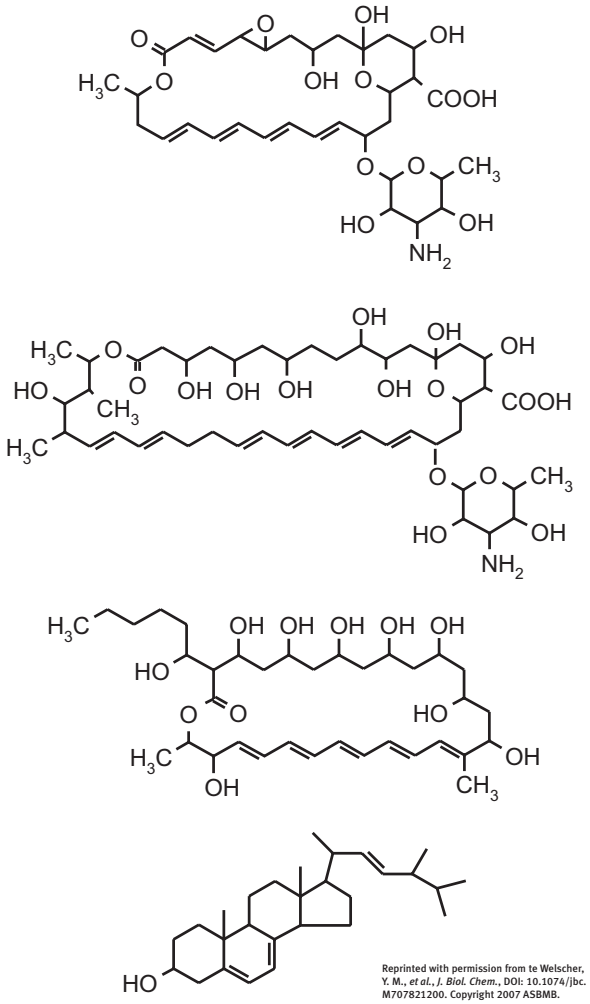
Ye, J. D., *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, *105*, 82–87. Copyright 2008 National Academy of Sciences, U.S.A.

The ribo-antigen of choice was a well-characterized subdomain of the *Tetrahymena* Group 1 ribozyme termed P4-P6, an RNA with a distinct fold and a previously solved crystal structure. The phage library encoded Fab fragments spiced with partially randomized amino acids at the solvent-accessible positions. After several rounds of selection and enrichment, seven Fabs emerged and were further characterized by binding assays and, as a proof of principle, one was solved in complex with the P4-P6 RNA *via* X-ray crystallography. The RNA was in the native conformation, and the diffraction data were even better than the previous RNA-alone structure. The Fabs displayed binding K_d values in the nanomolar range, and the structure displayed a whopping 1300 Å^2 of buried surface between the RNA antigen and the Fab. That is nearly twice the typical surface area of a Fab interaction with a protein. The recognition is specific to P4-P6 RNA and seems to be mediated largely by hydrogen bonds, with some nonpolar or stacking interactions making contributions as well. Notably, the types of interaction in the Fab–RNA structure are very similar to those seen in structures of natural RNA-binding proteins. This study paves the way for other Fab selections toward those challenging structured RNA molecules that flip and flop to avoid the crystallographer's watchful eye. It also opens up the door to using antibodies toward RNAs in other cell biological and biochemical assays normally set aside for protein antigens. **Jason G. Underwood, Ph.D.**

Not All Polyenes Poke Holes

Exploring how antibiotics kill their targets is becoming increasingly important, because it provides insight into the development of better drugs and can help combat the development of drug resistance. Natamycin is an antibiotic that is part of the polyene group of antifungal agents. Other polyenes are known to kill yeast by interacting with ergosterol, a component of the yeast cell membrane, causing membrane permeabilization and cell death. te Welscher *et al*. (*J. Biol. Chem.*, published online Dec 27, 2007, DOI: 10.1074/jbc.M707821200) investigate whether natamycin works in a similar fashion and unveil an unexpected revelation about its mechanism of action.

Various *in vitro* and *in vivo* approaches were used to investigate natamycin's mechanism of action. Phosphatidylcholine model membranes and isothermal titration calorimetry were used to determine that natamycin does indeed interact with ergosterol in a membrane environment and with an affinity comparable to that of other polyenes. Yeast mutants containing sterols other than ergosterol were then employed to explore the relationship between sterol structure and natamycin activity. Growth inhibition and binding studies with these mutants revealed that double bonds in the B ring of the sterols, which are present in ergosterol but not in the sterols made in several of the other strains tested, are important for natamycin's activity. Finally, leakage assays were used to investigate the ability of natamycin to permeabilize the yeast cell membrane. In contrast to other polyene antibiotics, natamycin did not promote leakage of the small-molecule carboxyfluorescein or even protons from ergosterol-containing membrane vesicles or of protons from yeast cells. The authors propose that, rather than permeabilizing the membrane, natamycin activity may be linked to the natamycin–ergosterol complex, preventing ergosterol from performing some of its critical functions, such as participating in endocytosis, exocytosis, or vacuolar fusion. **Eva J. Gordon, Ph.D.**



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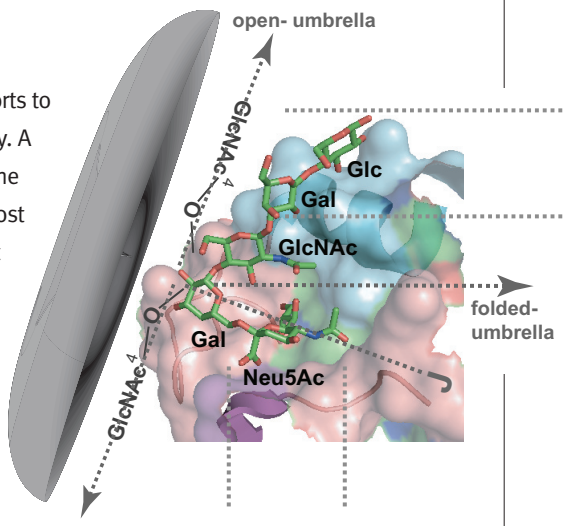
Microbial Fuel Factories

Renewable sources of energy such as biofuels have recently generated a lot of interest as alternatives to fossil fuels. Ethanol, produced primarily by the microbial fermentation of sugars, is currently the most widely used gasoline substitute. However, because of its relatively low energy density and high volatility, ethanol is a poor alternative. Far better than ethanol are higher alcohols such as 1-butanol that are not hygroscopic, have higher energy densities, and are comparatively less volatile. Better yet are branched-chain higher alcohols such as isobutanol that have higher octane numbers than straight-chain equivalents. Unfortunately, no known microorganism produces large amounts of isobutanol from the simple sugar glucose. In a recent study, Atsumi *et al.* (*Nature* 2008, *451*, 86–89) perform the remarkable feat of engineering a nonfermentative metabolic pathway for the production of isobutanol in *Escherichia coli*.

acs chemical biology

Strictly for the Birds

In the hopes of avoiding a pandemic in humans by the deadly bird flu virus, intense efforts to determine the molecular features responsible for species-specific infection are underway. A key step in the process by which influenza A viruses infect their hosts is the binding of the viral hemagglutinin (HA) protein to α2-3 and α2-6 sialylated glycans on the surface of host epithelial cells. Now, Chandrasekaran *et al.* (*Nat. Biotechnol.* 2008, *26*, 107–113) report the discovery that it is not just the sequence but also the shape of sialylated glycans that helps govern the species specificity of different viral subtypes.



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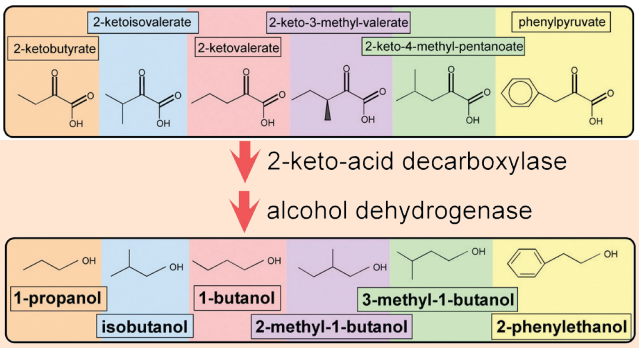
Several complementary approaches were used to investigate HA binding α2-3 and α2-6 sialylated glycans. First, the distribution of α2-6 sialylation patterns in human epithelial cells was evaluated by staining human tracheal tissue sections with various lectins and by mass spectrometry profiling of N-linked sialylated glycans in an upper-respiratory epithelial cell line. The presence a diverse set of sialylated glycan structures, including α2-6 with long glycan chains, prompted structural analysis of various HA-glycan crystal structures, data mining analysis of HA binding to glycan microarrays, and examination of human tissue binding to recombinant HAs. These investigations revealed that, whereas avian-adapted HAs favored binding to α2-3 and α2-6 with short glycan chains, which adopt a conelike topology, human-adapted HAs can accommodate α2-6 glycans with longer chains, which adopt an umbrella-like topology. The data suggest that human adaptation of influenza viruses is correlated with the ability of the viral HA to bind to the umbrella-like topology of the long-chain α2-6 glycans found in human epithelium. These findings provide compelling insight toward the development of new drugs and vaccines against influenza viruses. In addition, they may help point to methods for keeping highly pathogenic influenza strains such as the avian subtype H5N1, shall we say, strictly for the birds. **Eva J. Gordon, Ph.D.**



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The authors started off by examining intermediates in known metabolic pathways in *E. coli* that might serve as precursors in alcohol synthesis. They identified 2-keto acids that are intermediates in amino acid biosynthesis routes as potential starting points. Using a scheme in which the 2-keto acids are converted into aldehydes by 2-keto-acid carboxylases, and subsequently into alcohols by alcohol dehydrogenases, the authors were able to shunt these intermediates into a new alcohol synthesis pathway. To increase the yield of isobutanol, the authors overexpressed genes encoding enzymes catalyzing the synthesis of the precursor 2-keto acid, 2-ketoisovalerate. They then systematically deleted genes contributing to byproduct formation to increase yield. In one case, they substituted native genes with a non-native gene with altered affinity. Combining all these efforts, the authors were able to obtain an astonishing yield of 86% of the theoretical maximum possible from glucose.

This study has a number of broader implications. The ability to engineer *E. coli* to produce alcohols nonfermentatively by rationally creating an alternative pathway is noteworthy. The authors also demonstrated general applicability by engineering the high production of 1-butanol using a similar strategy. This strategy can be modified for other organisms such as *Saccharomyces cerevisiae*. Finally, the authors demonstrated that organisms such as *E. coli* that have no natural tolerance to high concentrations of alcohol can be adapted to improve tolerance to these alcohols, setting the stage for the use of this model organism as a powerful microbial fuel factory. **Anirban Mahapatra, Ph.D.**