A Beeline for a Chemical Signal

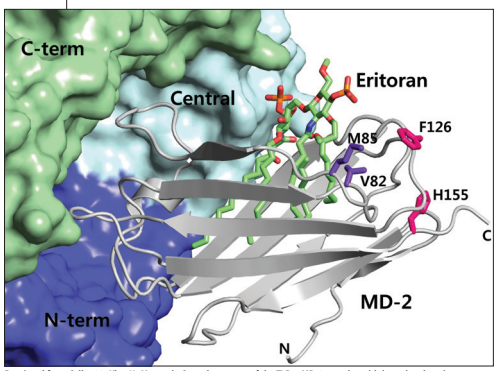
Wanner, K. W., *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, *104*, 14,383–14,388. Copyright 2007 National Academy of Sciences, U.S.A.

Honey bees are social insects with complex behavior. Colonies of honey bees contain female bees in either of two forms. A solitary female queen honey bee is involved in reproduction, while thousands of sterile worker bees perform most of the other duties. Often, an unsuspecting mammal is painfully reminded of one such duty: the defense of the hive. In comparison, male drone bees live much simpler lives. Attracted to a component of the queen retinue pheromone, known as 9-oxo-2-decenoic acid (9-ODA), males mate with the queen and die shortly thereafter. It has been known since the early 20th century that olfaction plays a role in mating. The chemical signal involved in mating, 9-ODA, was discovered almost half a century ago. Now, Wanner *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2007, *104*, 14,383–14,388) identify an odorant receptor for 9-ODA by analyzing the differential expression of receptor genes in male drone and female worker honey bee antennae.

To test the specificity of this odorant receptor, AmOr11, in an *in vivo* milieu, Wanner *et al.* examine *Xenopus* oocytes that are injected with honey bee odorant receptor complementary RNA. It is known that insect odorant receptor activation in the presence of a coexpressed stabilization partner results in an electrophysiological response using a two-electrode voltage-clamp, although how exactly this occurs is unknown. In this experiment, AmOr11, in the presence of a coexpressed partner, responds specifically to 9-ODA, but not to any other chemical compound tested. In addition to AmOr11, Wanner *et al.* also identify three candidate odorant receptors for which there are no known pheromone ligands. It is known that the queen retinue pheromone is composed of a number of chemical components apart from 9-ODA. A possible area of future research is the characterization of ligand-specific responses with other candidate odorant receptors. Ultimately, details on the mechanisms underlying the responses to these chemical signals will aid in understanding the morphological and behavioral development of social insects. **Anirban Mahapatra**

Looking at LPS

Lipopolysaccharide (LPS) is a component of Gram-negative bacteria that triggers a strong innate immune response, which ultimately protects the host from any harm the invading pathogen might be planning. However, exposure to LPS can also induce a potentially fatal septic syndrome. Understanding the molecular details behind LPS activity could help get an immune system gone awry back under control. To this end, Kim *et al.* (*Cell* 2007, *130*, 906–917) report the structural characterization of the LPS receptor bound to the LPS analogue Eritoran.



Reprinted from *Cell*, *130*, Kim, H. M., *et al.*, Crystal structure of the TLR4–MD-2 complex with bound endotoxin antagonist Eritoran, 906–917, Copyright 2007, with permission from Elsevier.

LPS binds to a complex of Toll-like receptor 4 (TLR4), a transmembrane protein critical for the innate immune response, and the protein MD-2, which binds to the extracellular domain of TLR4. Recombinant, full-length ectodomains of the proteins were used to determine the structure of the TLR4–MD-2 complex. However, in order to obtain crystals of the heterodimer in complex with Eritoran, the authors developed a novel method, termed the Hybrid LRR Technique, in which truncated fragments of TLR4 were fused with components from other proteins that contain leucine-rich repeats (LRRs), like those found in the extracellular segments of TLRs. The crystal structure of the complex containing the hybrid TLR4, MD-2, and Eritoran demonstrated that Eritoran binds exclusively to the hydrophobic pocket of MD-2 in the TLR4–MD-2 complex. Further structural characterization using gel filtration chromatography, native gel electrophoresis, and cross-linking experiments showed that binding to Eritoran does not significantly affect the apparent size of the complex, whereas interaction with LPS induced formation of a TLR4–MD-2 heterotetramer. Furthermore, mutagenesis studies led to the identification of the specific residues that participate in receptor dimerization. These structural insights enabled the researchers to propose a model in which binding of LPS induces a structural change that facilitates dimerization of the receptor, leading to initiation of a signaling cascade that activates innate immunity. Notably, the model provides a compelling rationale for why binding of LPS triggers an immune response but binding of Eritoran does not. **Eva J. Gordon, Ph.D.**

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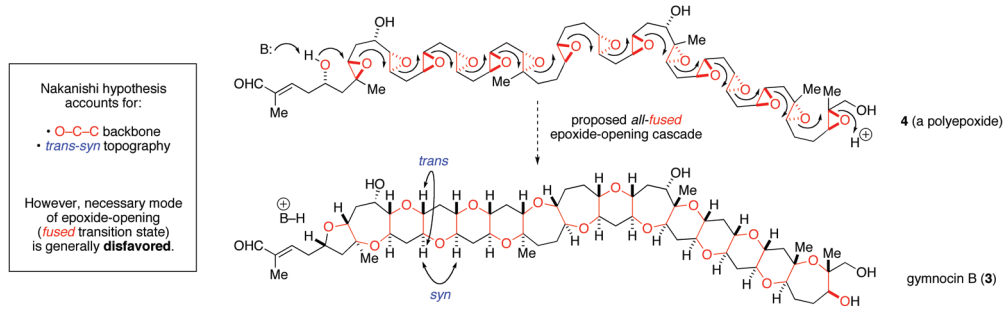
A Water-Propelled Step up the Ladder Polyethers

The "ladder" polyether natural products, such as brevetoxin B, are responsible for the toxic effects observed in so-called red tides, which are caused by the rapid accumulation of ladder-polyether-producing algae near the surface of the water. The intriguing structures and biological properties of these compounds have prompted great interest in the mechanism of their biosynthesis and in methods for their chemical synthesis. Though a hypothesis for the mechanism of their formation, a cascade of selective epoxide-opening reactions, has existed for >20 years, little evidence has emerged to support it. On the contrary, the proposed ring-opening process is generally thought to be disfavored. Now, Vilotijevic and Jamison (*Science* 2007, *317*, 1189–1192) provide the first compelling evidence supporting the cascade hypothesis.

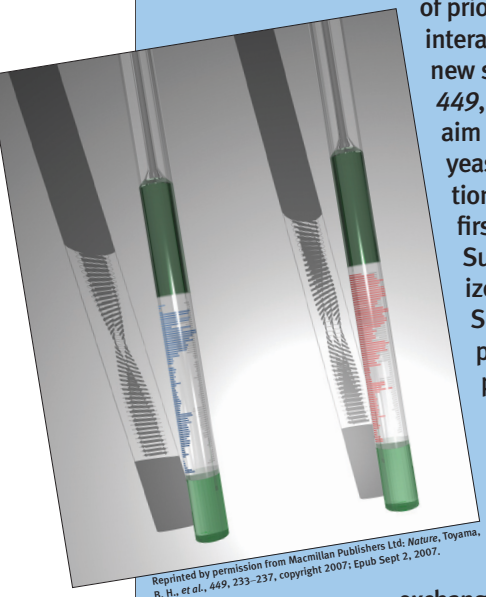
Epoxide-opening reactions typically proceed to generate five-membered tetrahydrofuran rings, not the six-membered tetrahydropyran (THP) rings found in the majority of ladder polyethers. After analyzing the factors that might govern the regioselectivity of the epoxide opening, the authors reasoned that providing a template that already had one THP group in place could be just the trick to reverse the entropic *versus* enthalpic issues that control the outcome. Indeed, the reaction of an appropriate THP-containing epoxy-alcohol did provide some of the desired di-THP product. Examination of the pH dependence of the reaction led to the remarkable finding that the desired selectivity increases substantially as the pH of the reaction approaches neutral. It was further observed that plain old water appeared to increase the rate and selectivity of the reaction. When the reaction conditions were attempted on a substrate for a cascade of epoxide-opening reactions, the THP-selective product was observed in impressive yields. The authors propose that the presence of the THP template and the water may be replicating the environment of conformational constraints and hydrogen-bond activation found in an enzyme active site. While chemists continue to contemplate the mechanisms that promote ladder polyether generation, this superior method for their synthesis will also allow biologists to better explore their mechanism of action. **Eva J. Gordon, Ph.D.**



From Vilotijevic, I., and Jamison, T. F., *Science*, Aug 31, 2007, DOI: 10.1126/science.1146421. Reprinted with permission from AAAS.

Conformational Switch-Hitter

Infectious proteins, termed prions, are the root cause of Creutzfeldt–Jakob disease in humans and the infamous "mad cow" disease, bovine spongiform encephalopathy. The detrimental effects of these proteins stem from their propensity to fold into multiple conformations. Some of these conformations can form large insoluble oligomers in the cell. The flip-flopping shape and aggregation properties have made atomic-level characterization of prion proteins and their deleterious interactions a particular challenge. Now, a new study by Toyama *et al.* (*Nature* 2007, *449*, 233–237; Epub Sept 2, 2007) takes aim at these challenges by using the yeast prion protein, Sup35, and solution NMR. A polypeptide comprising the first 253 amino acids of Sup35 protein, SupNM, can adopt two well-characterized polymer conformations, Sc4 and Sc37, with a strong or weak *in vivo* phenotype, respectively. These fiber populations were independently purified to homogeneity and subjected to solvent isotope exchange over a time course extending from 1 min to 1 week. Fibers were then dissolved in DMSO, and NMR was performed to monitor the extent of exchange. Amides hidden from the solvent exchange far more slowly and are presumably involved in the tight interface between monomers. One class of protons exchanged in the first minute, whereas another class remained protected from exchange after an entire week. Both fiber types, Sc4 and Sc37, showed protection within the first 40 amino acids of the protein, but the latter displayed a protected portion that extended an extra 32 amino acids. To further address the differences in these conformations, the authors introduced proline mutations into SupNM protein and used techniques such as atomic force microscopy to monitor the ability of these mutants to join a wild-type Sc4 or Sc37 fiber. Prolines break the β-sheet-forming ability of a polypeptide, and because β-sheets are critical for prion oligomerization, the mutations can track whether a region is involved in the homotypic interactions. The mutation data agreed quite well with the NMR data, showing that mutations in the first portion of the protein affected both fibers, whereas mutations in the subsequent sequence affected Sc37 only. This study takes on this difficult biophysical problem with sophisticated techniques, and it paves the way for future studies of other prion or amyloid proteins that form polymers. **Jason G. Underwood, Ph.D.**

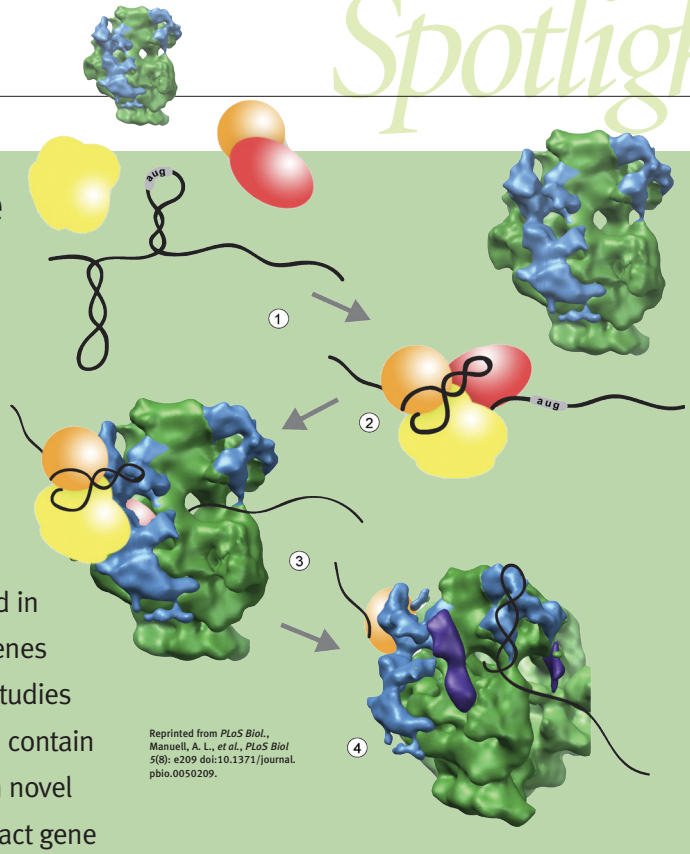


A Magnetic Approach

Global interest in the potential use of ethanol as a fuel has, well, fueled interested in improved methods for its production. This is particularly true in Brazil, a world leader in coercing the yeast *Saccharomyces cervisiae* to make ethanol from sugar cane molasses. From the State University of Campinas in São Paulo, Perez *et al.* (*Biotechnol. Prog.* 2007, *23*, 1091–1094) share their findings for improving ethanol production using a bioreactor coupled with two magnetic field generators.

On the basis of recent evidence that low-frequency magnetic fields can affect the growth and metabolism of microbial and mammalian cells, the researchers sought to determine the effects of an extremely low frequency electromagnetic field on ethanol production by *S. cervisiae*. The cellular suspension was externally recycled from the fermentor through a stainless steel tube inserted in two magnetic field generators, and the recycle velocity and intensity of the magnetic field were varied in a controlled manner. They found that when velocity and the magnetic field treatment were between 0.9 and 1.2 m s^{-1} and 20 mT plus solenoid, sugar consumption and ethanol productivity both increased by ~17%. In addition, maximal ethanol production occurred ~2 h earlier than in control experiments, in which the recycling loop was maintained during the fermentation process but no magnetic field was applied. Examination of the energetic character of the fermentation process enabled the authors to propose that the observed increase in ethanol production is due to the effects of the electromagnetic field on both membrane permeability and the redox system involved in the process. Because magnetic field treatment can be easily implemented on an industrial scale, these findings represent a compelling approach for increasing ethanol production. **Eva J. Gordon, Ph.D.**

Chloroplast Ribosome Structure Reveals Novel Regulatory Role



Reprinted from *PLoS Biol.*, Manuell, A. L., *et al.*, *PLoS Biol* 5(8): e209 doi:10.1371/journal.pbio.0050209.

Ribosomes are central components in translation, the conversion of an RNA message into proteins. Interest has been increasing in these molecular machines because they have also been shown to be involved in gene regulation, affecting the very genes that are being translated. Recently, studies have shown that chloroplasts, which contain their own ribosomes, also have both novel proteins and RNA elements that impact gene regulation. Because translation and ribosome structure in the chloroplast were traditionally considered bacterial-like, these novel components are of interest. Recent work indeed has shown some key differences. The ribosomes in chloroplast are extensively involved in coordinating gene expression between the plastid and nuclear genome by using a combination of regulatory messenger RNA (mRNA) elements and unique ribosomal proteins beyond what is typically seen in bacteria.

In a recent study, Manuell *et al.* (*PLoS Biol*, 2007, *5*(8): e209) use cryoelectron microscopy and single-particle reconstruction to show the structure of the chloroplast ribosome at a resolution of 15.5 Å. The structure revealed that previously identified novel ribosomal proteins are located on the ribosome in key positions that allow interaction with mRNA during translation initiation. Indeed, the proteins dominate not only the solvent exposed face of the small subunit (which interacts with mRNA and initiation factors) but also both the entrance and exit channels for the mRNA. This would allow previously identified mRNA elements to interact with the ribosome in ways not possible in the traditional bacterial-like model. Through the addition of novel proteins, the chloroplast ribosome plays a major role in the regulation of gene expression in the chloroplast. **Ross Larue**

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