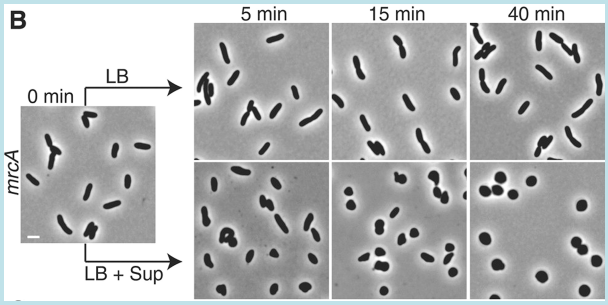
Spotlight

Deciphering the D-Amino Acid

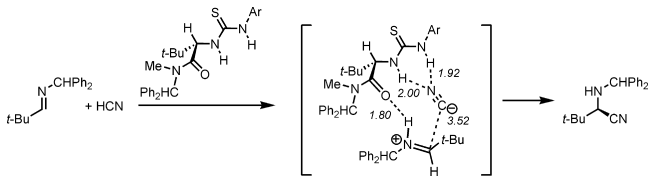


From Lam, H., *et al.*, *Science*, 2009, *325*, 1552. Reprinted with permission from AAAS.

Conventional wisdom says that organisms from all kingdoms of life predominantly use L-amino acids; one well-known exception is the presence of D-alanine and D-glutamate in the main component of the bacterial cell wall, peptidoglycan. One of the many roles of peptidoglycan is to help control bacterial cell shape, but the mechanisms that regulate the composition and structure of this dynamic biopolymer are not well-defined. Discovery of a bacterial mutant that was round instead of rod-shaped led Lam *et al.* (*Science* 2009, *325*, 1552−1555) to find that bacteria produce additional D-amino acids and that these compounds have a structural and regulatory role in peptidoglycan function.

A mutant strain of the bacteria *Vibrio cholerae*, while rod-shaped during its growth phase, was found to adopt a spherical morphology during its stationary phase. A search for the sphere-inducing factors resulted in the identification of four amino acids, methionine, leucine, valine, and isoleucine, in the stationary phase supernatant, and it was the D-forms of these amino acids that triggered the rod to sphere transformation. Enzymes called racemases convert L-amino acids to D-amino acids, and indeed a racemase was identified in the periplasm of *V. cholerae* that produced D-isomers of the four amino acids. Investigation into how the D-amino acids affected peptidoglycan structure and function demonstrated that D-amino acids were incorporated into the polymer structure, which likely affects peptidoglycan strength and flexibility. In addition, D-amino acids were found to negatively regulate the amount of peptidoglycan produced during stationary phase. Taken together, the data suggest that D-amino acids may signal for and facilitate the alteration of peptidoglycan composition and structure in preparation for entering into stationary phase. These findings may point to a broader model in which modulating amino acid chirality is a tool used by bacteria to sense and adapt to changing environments. Eva J. Gordon, Ph.D.

Accessing α-Amino Acids



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α-Amino acids are important biomolecules on several fronts. They are the building blocks of proteins; they are components of various drugs; they are ingredients in a variety of molecular tools created to probe biological processes; they are elements of chiral catalysts used in organic synthesis. Methods to synthesize α-amino acid derivatives over a range of scales, purities, and enantioselectivities are needed for these diverse applications. The Strecker synthesis, which involves addition of a cyanide to an imine, followed by hydrolysis, can be an effective method for generating enantiomerically enriched, unnatural α-amino acids, but the complexity of the catalyst and the hazardous nature of the cyanide reactant have prevented use of this method on a large scale. Zuend *et al.* (*Nature* 2009, *461*, 968−970) tackle these issues in the development of a catalytic asymmetric Strecker reaction for an economical and ecologically friendly synthesis of unnatural α-amino acids.

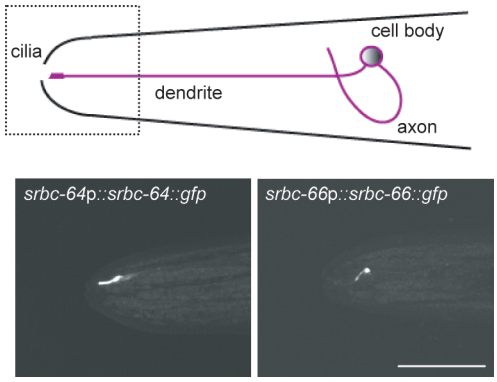
The key to the success of this Strecker reaction variation was the discovery that easily prepared amido-thiourea derivatives were effective catalysts for the reaction, solving the catalyst issue. However, the hazardous nature of the cyanide reagent remained. Potassium cyanide is an attractive alternate source of cyanide but was not appropriate with previous generation catalysts because of its insolubility in organic solvent and the catalyst's incompatibility with aqueous solutions. Exploiting the fact that the amido-thiourea catalyst does not possess sensitive functional groups, the reaction was successfully carried out under biphasic conditions using potassium cyanide. The wide range of imines suitable as the starting materials enabled incorporation of diverse substituents at the α-position, and no chromatography was required to isolate the products from this high-yielding, enantioselective transformation. **Eva J. Gordon, Ph.D.**

Published online November 20, 2009 • 10.1021/cb900274u CCC: $40.75

Spotlight

Finding a Pheromone Receptor

Diverse organisms use small signaling molecules called pheromones to elicit a specific behavior or trigger a certain response, either internally or in other members of the species. For example, the nematode *Caenorhabditis elegans* employs pheromones when it arrives at a fork in its developmental road and must decide whether to continue toward the reproductive cycle or enter into a developmentally arrested state called the dauer stage. Recent studies have revealed that the dauer pheromone, a mixture of chemicals including derivatives of the dideoxysugar ascarylose, guides this decision. However, the receptors through which dauer pheromone signals are not well-defined. Now, Kim *et al.* (*Science* published online October 1, 2009; DOI: 10.1126/science.1176331) describe the role of two heterotrimeric GTP-binding protein-coupled receptors (GPCRs) in mediating dauer formation.

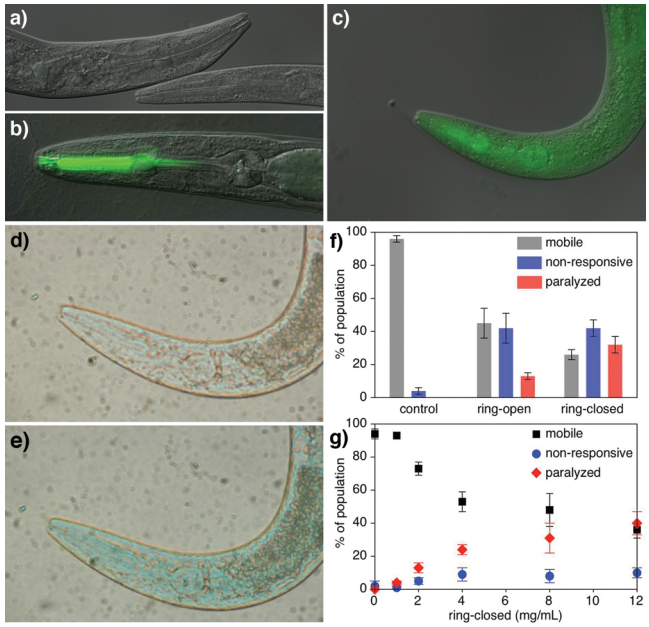


From Kim, K., *et al.*, *Science*, October 1, 2009; DOI: 10.1126/science.1176331. Reprinted with permission from AAAS.

Dauer pheromone promotes dauer formation and also downregulates expression of certain genes. It was found that dauer formation in response to certain ascarylose derivatives, termed C6, C9, C7, and C3, was strongly limited in animals mutant for the GPCR genes *srbc-64* and *srbc-66*. In addition, the repression of gene expression normally induced by these ascarosides was diminished in the mutants as well. The GPCR receptors were localized to the sensory cilia of ASK chemosensory neurons, and these cells were found to be critical to dauer formation. Building on previous findings, it was suggested that addition of pheromone inhibits a guanylyl cyclase, thereby decreasing intracellular cyclic nucleotide levels in the neurons, to promote dauer formation. Notably, expression of the receptors in a human kidney cell line was sufficient to observe pheromone-dependent responses. Taken together, the data support a model in which SRBC-64 and SRBC-66 function in ASK neurons as receptors for a subset of dauer pheromone components. This model helps delineate how pheromones orchestrate such profound developmental and behavioral responses, in *C. elegans* as well as more complex species. **Eva J. Gordon, Ph.D.**

Biochemical Freeze Tag

Reversible photochemical switches integrate functional responses into the structures of new synthetic materials, changing those materials' properties such as color or refractive index. They also offer an opportunity to trigger particular biological functions within organisms, by shining a light on the molecules to activate them. But designing switchable molecules that regulate biological activity is not easy: the switch molecule cannot immediately break down in the living host, and each isomer must have unique functional activity within the organism.



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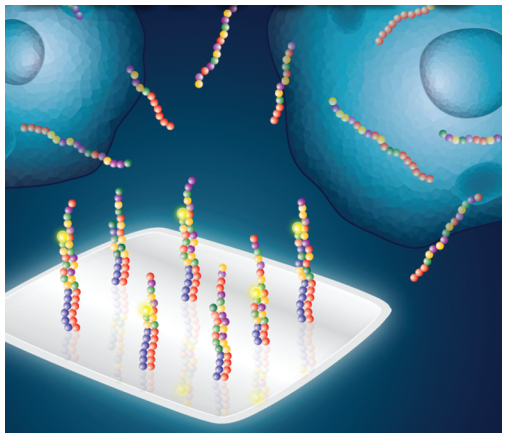
However, researchers have now demonstrated that reversible photoswitches can control biological functions in a living organism. Taking on these challenges, Al-Atar *et al.* (*J. Am. Chem. Soc.* 2009, *131*, 15966−15967) used a diarylethene photoswitch that reversibly paralyzes nematodes (*Caenorhabditis elegans*). In this case, the switch molecule was a charged bis(pyridinium) diarylethene that undergoes a reversible ring-closing reaction between a colorless ring-opened form and a blue ring-closed form. When they incubated the nematodes with the ring-opened isomer, the worms continued to move. After exposure to UV light (365 nm), the same nematodes immediately turned blue, indicating conversion to the ring-closed isomer, which stunned the worms over the next 15 min. Shining visible light (490 nm) transformed the photoswitch back to the ring-opened isomer, freeing the nematodes to move again. Although both molecules are eventually toxic, researchers could switch the paralysis on and off at least 3 times. Although the exact paralysis mechanism is not yet known, the researchers are studying whether the ring-

closed isomer interrupts the metabolic electronic pathway in the worms.

Such molecules could provide new tools for designing targeted biomedical therapies. New noninvasive treatments could use photoswitches to target diseased tissue and then activate the bound molecules or selectively deliver drugs with light. **Sarah A. Webb, Ph.D.**

Reading RNA, One Molecule at a Time

The high-throughput sequencing era has arrived with new methods and machine upgrades arriving monthly while current technologies churn out the gigabytes of genomic data. Those interested in the transcriptome, or the RNA population expressed in cells, are jumping onboard as well. Most "RNA-seq" methods rely upon enzymatically converting RNA into single- or double-stranded complementary DNA (cDNA), amplifying these molecules and then using the DNA sequencers to read off the information. While this method proves functional for some applications, it does come with caveats. Adding more steps also adds more bias and errors, complicating the use of the sequencing data for quantitative purposes and discovery of novel RNA species. Enter a new technology that avoids the need for a middleman and directly sequences the RNA on a single molecule basis rather than a pool of amplified cDNAs.



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Ozsolak *et al.* (*Nature* 2009 *461,* 814−818) immobilized individual molecules of polyadenylated RNA on a solid surface by virtue of their annealing to an oligo dT strand. Eukaryotes, like the yeast used in their study, naturally possess polyA tailed messenger RNAs, but the researchers also enzymatically tailed RNAs lacking this polyA sequence. With individual RNA-DNA hybrids on the surface, a polymerase was added to begin the sequencing, but with special nucleoside triphosphates. These monomers, termed virtual terminators, contained a specific fluorescent dye added to each base and were designed in such a fashion that only one base could be added to the elongating nucleic acid chain. After each nucleotide addition, the machine monitored the color generated by each immobilized hybrid via an integrated fluorescence microscope at single molecule resolution. Once the four base terminators were cycled through 30 times, with images recorded at each step, the data is converted real time into sequence. About 50% of the sequences could be mapped back to the yeast genome, on par with the current DNA/cDNA-seq technologies. With an average read length of just 29 nucleotides, and a maximum of 50, the technology has room for improvement but these numbers are impressively similar to the very first high throughput DNA sequencing machines several years ago. If the trend follows, direct RNA sequencing will be headed for bigger numbers and a growing impact. **Jason G. Underwood, Ph.D.**

Sensing Your Soda

A bite of a fine meal washes over the tongue to begin the sensation of taste. The human brain registers that taste into culinary realms such as salty, sour, spicy or sweet, but what happens between the fork and the brain remains a key question for neurobiologists. Over the past decade, molecular triggers for many tastes have been uncovered and traced to specific cells of the tongue. In a recent study, Chandrashekar *et al.* (*Science* 2009, *206*, 443−445) pursued another popular sensation, the taste of carbonated soda water, and looked for the proteins involved in its reception.

Using a mouse tongue physiology assay, the researchers first showed that CO_2 gas or club soda triggered a dose-dependent spike in neural response. Then, using mice lacking specific types of taste receptor cells, they uncovered a fascinating functional overlap. Sour sensing cells expressing the ion channel PKD2L1 are also responsible for the taste of carbonation. A microarray experiment on sour cells indicated an attractive candidate gene to help explain the connection. PKD2L1 expression correlated with Car4, a gene encoding a carbonic anhydrase enzyme that anchors within the cell's extracellular membrane. Blocking the anhydrase activity with a cell-impermeable inhibitor resulted in a large decrease in neural firing during CO_2 stimulus, while a cell-permeable inhibitor led to nearly complete loss of response. Implicating Car4 specifically, animals that lacked a functional copy of the enzyme displayed very low physiological response to CO_2 but normal response to sour tastes. Further tests demonstrated that protons are the likely messengers since the other carbonic anhydrase reaction product, bicarbonate, did not elicit a response. This study points to how a complex sensory circuit can be traced to particular protein players and how different tastes can relay through both overlapping and distinct pathways. This elegant theme is reminiscent of the shared taste receptor proteins that make spicy items feel hot and minty items feel cold. **Jason G. Underwood, Ph.D.**