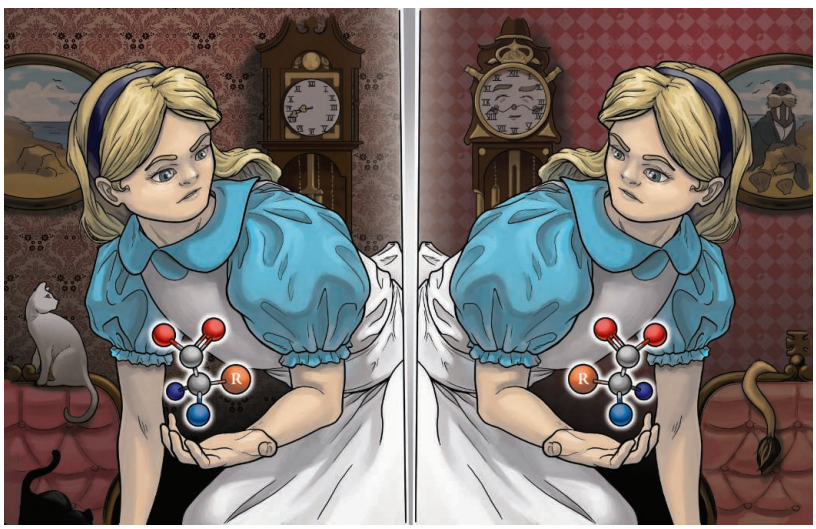
CELL BIOLOGY

Expanding Functionality Within the Looking-Glass Universe

Although once thought to be largely irrelevant in the biological world, new and unexpected functions for D-amino acids continue to emerge.

Steven R. Blanke

After she stepped through a looking glass into a mirror image of her own world, Lewis Carroll's adventuress Alice soon recognized that the two worlds were perhaps not so similar after all. She intuits that something about the very make-up of "looking-glass milk" was fundamentally different from that of the wholesome beverage she typically enjoyed (*1*). Indeed, nature has stocked our universe with biological substances that exist as two mirror image forms of one another. Nineteen of the 20 naturally occurring amino acids that make up proteins are "chiral," meaning that each can be arranged in two orientations around a central carbon atom. The result is a mixture of "mirror-image compounds" called L- and D-amino acids, which cannot be superimposed (see the figure), much in the way a person's left and right hands are not superimposable. One of the great mysteries of life has been the emergence of a strictly "left-handed" protein world where great attention has been paid to the L-amino acid building blocks (*2*–*4*), while D-amino acids are largely regarded as red-headed cousins who are easy to ignore. On page 1552 of this issue, Lam *et al*. challenge this perception, describing a role for D-amino acids in controlling bacterial responses to environmental cues (*5*).



Which universe? Additional functions for D-amino acids are being discovered in both bacterial and eukaryotic systems. This challenges the notion that D-amino acids are relatively unimportant in a universe dominated by left-handed (L-amino acid) proteins.

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Alice had good reason to be concerned, because while pairs of "mirror-image molecules" are identical in many respects (e.g., molecular mass, bond angles, and bond lengths), they often exhibit highly divergent biological properties, with potentially far-reaching ramifications (including exactly how Alice would manage to digest and metabolize milk proteins composed exclusively of D-amino acids). The apparent predominance of L-amino acids raises fundamental questions about the extent to which D-amino acids have major roles in biological systems. However, new functions for D-amino acids are now emerging, fueling new discussions. Adding to the debate, Lam *et al*. have determined that D-amino acids regulate cell wall remodeling in bacteria.

The cell wall provides the strength and rigidity needed for bacteria to withstand environmental stresses. It is constructed primarily of peptidoglycan, an elastic polymer consisting of sugars and amino acids arranged in a mesh-like lattice encasing the plasma membrane. One of the first biological roles identified for D-amino acids was the cross-linking of long peptidoglycan sugar chains by short peptides containing D-alanine and D-glutamate, which are thought to confer resistance to degradative enzymes that selectively hydrolyze linkages between L-amino acids.

For bacteria to thrive and survive, peptidoglycan must demonstrate a degree of plasticity. Bacterial cells that are dividing rapidly in nutrient-rich medium must allocate peptidoglycan to new daughter cells. In times of stress, peptidoglycan must be remodeled to function as the stress-bearing component of the bacterial cell wall. However, the factors controlling cell wall remodeling as bacterial cells pass from a nutrient-rich to a nutrient-poor environment had been largely unidentified.

While studying a mutant form of *Vibrio cholerae*, the etiologic agent of the pandemic diarrheal disease cholera, Lam *et al*. noted that changes in bacterial morphology consistent with cell wall remodeling were associated with an accumulation of several amino acids in the spent bacterial growth medium.

Surprisingly, the D- rather than L-form of two particular amino acids, methionine and leucine, were the most active agents in causing a decrease in peptidoglycan synthesis and alterations in peptide cross-linking. A direct role for D-methionine and D-leucine in the regulation of cell-wall remodeling was supported by discovery of a novel *V. cholerae* enzyme necessary for synthesis of these two D-amino acids. Interestingly, the D-forms of two different amino acids were most active in inducing peptidoglycan alterations in the Gram-positive *Bacillus subtilis*.

Exactly how D-amino acid–dependent cell wall remodeling occurs remains to be determined. However, the finding that D-amino acids other than the canonical peptidoglycan components D-alanine and D-glutamate can be incorporated into cross-linking peptides suggests a direct mechanism by which altering the composition of peptidoglycan modulates the strength and flexibility of this polymer within the cell wall. However, it is possible that D-amino acids may also regulate the function of periplasmic enzymes that synthesize and modify the peptidoglycan polymer.

The repertoire of biological roles for D-amino acids continues to expand, with notable examples of potential regulatory functions. Several *Bacillus* species regulate the development of spores into vegetative

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cells by altering the relative concentrations of available D- and L-alanine, which, respectively, inhibits or induces germination initiation by modulating the function of germination receptors on the surface of spores (*6*). The outermost capsule layer of some *Bacillus* species, including *B. anthracis*, the etiologic agent of anthrax, comprises extensive polymers of D-glutamate, presumably providing resistance to degradative proteases (*7*). D-Amino acids are increasingly being identified in naturally occurring antibiotics, immunosuppressive drugs, and antitumor agents (*8*). Although once thought to be exclusively the domain of bacteria, roles for D-amino acids in eukaryotic physiology have emerged. Endogenous D-serine functions as a neurotransmitter in the mammalian brain (*9*). D-Aspartic acid is found at high concentrations in some mammalian cells and organs, and modulates hormonal secretion in neuroendocrine tissues (*10*).

In retrospect, Alice's musings were probably no accident, as her creator, Lewis Carroll, also led an alternate (although not quite mirror-image) life as the Oxford mathematician Charles Dodgson. Dodgson may have been familiar with and influenced by the work of a contemporary, the great French chemist and microbiologist Louis Pasteur. Pasteur had already published his findings on the chiral properties of tartaric acid, a common component of several palette-pleasing delectables found on this side of the looking glass, including wines, and some fruits and plants. The continuing discovery of additional functions for D-amino acids will continue to challenge the notion that D-amino acids have been largely irrelevant during the rise of the left-handed protein universe.

References and Notes

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ECOLOGY

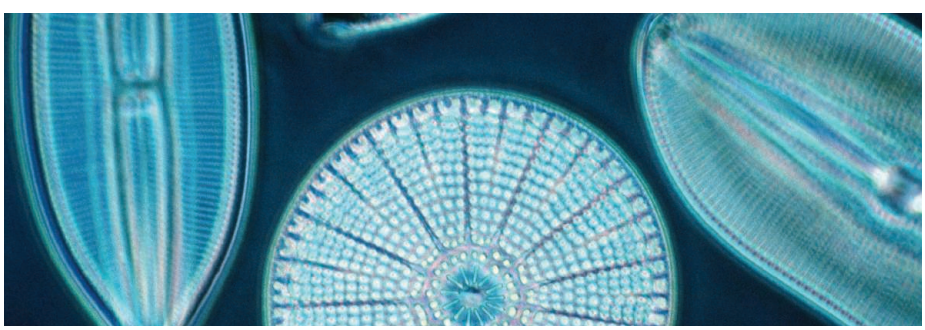
Seeing the Big Picture on Microbe Distribution

The distribution patterns of marine microbes are shaped by dispersal on a global scale.

David J. Patterson

Statements about the distribution of organisms can be made at all levels of phylogenetic scope and taxonomic resolution. The distributions of organisms on Earth reflect the interplay of dispersive trends with opportunity, obstruction, and extinction over time. If dispersal dominates, taxa tend to be widely distributed, and we rationalize presences and absences with greater reference to ecology. Where dispersal is constrained, taxa are more endemic, and we seek explanations by reference to history. For microbes, the idea that "everything is everywhere, but the environment selects," first mooted by Sprengel almost 200 years ago, still maintains support (*1*–*5*), but a recent review of molecular data suggested that it is simplistic or wrong (*6*). Two reports in this issue (*7*, *8*) help to evaluate which concept best describes the distributions of marine microbes. In doing so, they raise broader issues about the nature of biological knowledge.

On page 1539, Cermeño and Falkowski (*7*) report distribution patterns of marine diatoms (see the figure) in space and time, based on analysis of the Deep Sea and Ocean drilling projects' Neptune database. They show that diatom communities from polar locations resemble each other in a way that vertebrate communities do not. The same is true for more temperate communities that are geographically remote. The authors did not find biogeographical traces of historical climate change. Their conclusions are consistent with the dispersal-dominated model.



Traveling far and wide. Studies of marine diatoms (such as those shown here) (*7*) and thermophilic bacteria (*8*) show that dispersal plays a powerful role in shaping microbial distributions.

On page 1541, Hubert *et al*. (*8*) add interesting metrics to the picture. They find that microbial communities in the cold Arctic seabed included thermophilic bacteria that could not be metabolically active. The stocks of such bacteria were constantly being replenished, with 10,000 spores arriving from elsewhere on every square centimeter of the sediments every year. These results establish that dispersal is extremely powerful and can have a global impact.

The results bear on another recent insight into microbial diversity and abundance: the "rare biosphere" (*9*). Using high-throughput sequencing technologies, Sogin *et al*. discovered that there were massively more species of microbes out there than have previously been documented. The formal catalog of prokaryote species contains only about 6000 taxa, yet the team found evidence of orders of magnitude more taxa in individual samples. Most of these taxa are represented by only a few individuals. Two models can explain this distribution. One is that each habitat includes a massive number of microbial niches, and that samples draw on very complex, diverse, and active communities. Alternatively, only a small proportion of the species are metabolically active, and the remainder consists of

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