RESEARCH ADVANCES

Genes not Genomes Called Key Driver of Bacterial Diversity

Marcia Stone

Mutation-encoding genes can sweep though bacterial populations, driving differentiation, according to B. Jesse Shapiro and his colleagues at the Massachusetts Institute of Technology (MIT) in Cambridge. Analysis of genomic sequences from two recently diverged bacterial populations from different habitats suggests that genomic fragments rather than whole genomes drive differentiation. “Despite differences in how adaptive alleles are acquired, our results suggest that they spread in a more uniform manner within both bacterial and eukaryotic populations than previously imagined,” these researchers say. Details appeared in the April 6, 2012 Science (336:48–51).

Shapiro and his collaborators focused on closely related, ecologically distinct strains of Vibrio cyclitrophicus living on particles of different size and composition in the Atlantic Ocean. The bacterial cells had identical 16S genes and more than 99% average amino acid identity, making them ideal for studying early ecological differentiation, according to Shapiro. The main genetic differences between these two populations were restricted to a few small patches within the core genomes. Tight genotypic clusters emerge as a result of preferential recombination within, rather than between, cells in each of the habitats without genome-wide selective sweeps. “We are very excited about this finding because it explains the organization of microbial communities into ecologically differentiated genotypic clusters,” says Martin F. Polz, one of two principal investigators (PI) with whom Shapiro works.

Quickly adaptable genomes appear to be shaped far more by horizontal gene transfer (HGT) than by clonal descent, says the other PI, Eric J. Alma. A few genome regions appear to have swept through subpopulations in a habitat-specific manner accompanied by gradual separation of gene pools, he adds. This work confirms their earlier research showing that genes, not genomes, serve as major units of bacterial evolution and that ecology governs HGT.

Because of the high rate of HGT in bacterial populations, a significant percentage of genes from closely related bacterial strains can have different evolutionary histories. However, the Vibrio strains that they studied shared a common ancestor until relatively recently and, since then, about 99%, of the genome recombined, Shapiro notes. “This is very much Darwinian because it’s driven by selection of ecologically important genes.”

“There is no question that genes move around between all kinds of organisms, even us,” says Norman R. Pace at the University of Colorado in Boulder. “But it’s also clear that many genes, those most critical to the nucleic acids-based processing and information systems, for example, are phylogenetically stable in the sense that their phylogeny tracks with the rRNA tree. The bacterial cell has a core of genes . . . which are pretty stable. The rest are more or less volatile because of sweeps as is so nicely described by Shapiro and colleagues.”

“This is very exciting work because Shapiro and colleagues show that bacteria may be asexual but they’re not simply clonal,” says R. Thane Papke of the University of Connecticut in Storrs. “Therefore, theories of diversity based
on clonality are inaccurate and bacterial speciation is far more like that of eukaryotes than previously thought. It’s a breath of fresh air in a debate that sorely needs these observations.”

Marcia Stone is a science writer based in New York City.

RESEARCH ADVANCES

Shifts in Microbiota Influence Mating Choices among Fruit Flies

Shannon Weiman

Although commensal microbes are very much in the scientific and medical spotlight, their influences may far exceed our wildest expectations, judging from research presented at the 2012 ASM General Meeting, held in San Francisco, Calif., last June. Commensal microbes can influence behavior, sexual selection, and other evolutionary processes, says Eugene Rosenberg of Tel Aviv University in Tel Aviv, Israel, who spoke during the plenary session, “Who’s in Charge? How Microbes Affect Animal Behavior.”

Some flies choose their mates based in part on diet-dependent, gut microbial signatures, Rosenberg says. *Drosophila melanogaster* fruit flies that feed on starch-containing growth medium exhibit positive sexual selection, choosing to mate with each other while rejecting flies that are reared on a different growth medium, called basic CMY, and vice versa. Rosenberg revisited these experiments, adding antibiotics to the growth media to probe the underlying mechanism. Antibiotic treatment abolished mating preference—starch-fed flies mated at random with CMY-fed flies despite having lived for generations on different diets.

To Rosenberg, these results pointed to gut microbiota as the likely mediator of the changes in fly mating behavior. Indeed, *Lactobacillus plantarum*, a starch-metabolizing bacterium that typically accounts for only 3% of gut microbiota in CMY-fed flies, had expanded up to 26% of gut microbiota in starch-fed flies. When *L. plantarum* was added to antibiotic-treated, randomly mating flies, this single bacterial species restored positive sexual selection, Rosenberg says.

How can a fly possibly respond to the microbial content of its mate’s intestinal tract? “The odor of many animals results from microbial modifications of compounds secreted by the host, or released by microorganisms themselves,” note Rosenberg and his collaborators. In the case of fruit flies on different diets, the researchers find significant differences in five cuticular hydrocarbon pheromones from the flies that play a major role in their mating behavior. Treating flies with antibiotics reduces overall levels of pheromones and reduces differences in cuticular hydrocarbon profiles between flies fed CMY and those fed starch. These studies point to gut microbiota as the likely source of these compounds that dictate sexual behavior. The scent glands of spotted hyenas in Kenya typically contain commensal anaerobic bacteria that produce volatile odorants, such as alcohols, ketones, and short-chain fatty acids that are common components of scent markings, according to another speaker in the session, Kevin Theis of Michigan State University in East Lansing. While all spotted hyenas harbor the same commensal species, the proportions of these bacteria vary according to traits of the individual host, including age, sex, pregnancy, and clan association. These unique microbial profiles generate signature mixtures of volatile compounds that can be “read” by a passerby.

Odor influences sexual behavior from insects to large mammals. These studies provide evidence that microbes can influence odor-related mate selection behavior. Moreover, microbe-mediated sexual selection can lead to restricted genetic exchanges between populations, allowing for genetic divergence and, potentially, speciation, Rosenberg says. Thus, microbes may be driving evolution by influencing sexual selective pressure in host populations.

Shannon Weiman is a freelance writer in San Francisco, Calif.
MINITOPIC
Silk-Based Material Stabilizes Vaccines, Antibiotics

A silk protein-based material stabilizes several types of antibiotics and vaccines, enabling some of them to withstand storage at 140°F (60°C), for more than 6 months, according to David Kaplan of Tufts University in Medford, Mass., and his collaborators. The silk films wrap around the antibiotics and vaccines, protecting them while extending their shelf-lives. The chemistry and structure of the silk protein provide a stabilizing environment for such molecules, the researchers say. Additionally, the silk films protect one of the antibiotics tested against the detrimental effects of light. Details appear in the July 9, 2012 Proceedings of the National Academy of Sciences doi: 10.1073/pnas.1206210109.

RESEARCH ADVANCES
... Changes to Micro- and Mycobiome Affect Some Cancers, Too

Marcia Stone

Endogenous microbes may help in driving or suppressing cancer development and may also affect therapy outcomes, according to Giorgio Trinchieri from the National Cancer Institute in Frederick, Md., part of the National Institutes of Health. He and other experts spoke last June during a President’s Research Seminar at the Rockefeller Research Laboratories in New York, N.Y.

Several examples stand out. For instance, commensal gut bacteria are directly linked with colitis-associated cancers, while several pro- and anti-inflammatory bacteria modulate other gastrointestinal malignancies. In addition, Helicobacter pylori is the well-known instigator for gastric cancer, part of a relationship that Trinchieri calls the “canonical example” of a malignancy associated with a single species. These bacteria are thought to upset intestinal microbiota homeostasis, causing a state of “dysbiosis,” according to Gerardo Nardone from Federico II University in Naples, Italy.

Gut commensals typically are restricted to the intestinal lumen, its epithelial surface, or underlying lymphoid tissues. Even though well contained, however, “the sheer number of microbes in the intestinal tract makes an occasional breach inevitable,” says Lora V. Hooper from the University of Texas Southwestern Medical Center in Dallas. Escaping cells are typically quickly eliminated via phagocytosis. However, some translocated microorganisms are carried to mesenteric lymph nodes by dendritic cells (DCs), where they stimulate immune responses that damage or destroy microbes roaming the body. Thus, the gut microbiome “affects all phases of cancer, from initiation at the single-cell level to early growth, progression, and dissemination,” says Trinchieri. Details appear in the 8 June 2012 Science (336:1268–1273) and the 2012 Annual Review of Immunology (30:677–706).

In contrast, some commensal bacteria enhance the anti-inflammatory activity of adaptive immunity by directing T-regulatory (T_{REC}) cells and inducing expression of IL-10, says Hooper. When colonized with 46 strains of clostridia, for example, IL-10-secreting T_{REC} cells expand in germ-free mice. In another case, commensal flora protect transgenic mice against mammary carcinoma; moreover, treating them with antibiotics speeds tumor growth. Similarly, antibiotics also sometimes interfere with anticancer therapies, suggesting that commensals somehow regulate an individual’s response to treatment, according to Trinchieri, who is studying this phenomenon.

In addition to bacteria, the mammalian gut contains fungi—the mycobiome—that also interact with the host immune system, including through an innate immune receptor called Dectin-1. It can signal cells to produce inflammatory cytokines and induce T helper 17, according to David M. Underhill at the Cedars-Sinai Medical Center in Los Angeles. Mice lacking the Dectin-1 immune receptor gene show increased susceptibility to chemically induced colitis, which is a risk factor for colitis-associated cancers. Details appear in the June 8, 2012 Science (336:1314–1317).

RESEARCH ADVANCES
Genome for First RNA Virus of Archaea Tentatively Identified

John Ostrompke

Archaeae from acidic hot springs in Yellowstone National Park apparently harbor RNA viruses, according to Mark Young of Montana State University in Bozeman and his collaborators. “Scientists previously discovered DNA viruses of archaea, but before our work, no RNA viruses were discovered,” he says. “This was a big blank spot on the scientific map, but there’s no biochemical reason for them not to exist.” Young spoke about this first RNA virus, which was identified indirectly via metagenomic analysis, during the 2012 annual meeting of the Canadian Society of Microbiologists, held last June in Vancouver, British Columbia. Details appeared earlier this year in the February 29, 2012 Journal of Virology (doi: 10.1128/JVI.07196-11).

“There are about 5,000 viral parasites on this planet that are known, but... only 30 to 40 of those are from archaea,” Young says. The genes of viruses that infect archaea are unusual, suggesting that eukaryotes share an evolutionary relationship with archaea that is closer than that with bacteria, he says. However, because archaea or their viruses cause no diseases in humans, animals, or plants of agricultural interest, there is little interest in study-
ing these viruses except among a handful of scientists doing basic research.

Young and his collaborators relied on metagenomic analysis to assemble RNA segments from viral populations isolated from hot spring samples. One of the two putative RNA viruses in question has a genome containing approximately 5,600 nucleotides. It as well as another less fully analyzed partial genome in their samples includes a gene that appears to encode an RNA-dependent RNA polymerase (RdRp), which is considered a hallmark of positive-strand RNA viruses. The RdRp genes of archaeal viruses “form a unique group distinct from the RdRps of RNA viruses of Eukarya and Bacteria . . . and even more distant from known bacterial RNA viruses,” he and his collaborators note. “These positive-strand RNA viruses might be direct ancestors of RNA viruses of eukaryotes.”

Viruses of archaea were first identified in Japan, then later in Iceland, Kamchatka in Russia, and also in Lassen Volcanic National Park in northern California as well as in Yellowstone, according to virologist Kenneth Stedman of Portland State University in Oregon. “The results in Young’s work are very exciting,” he says, while also cautioning that the evidence for this RNA being the genome of an archaeal virus is indirect. “Right now all they have is sequences,” he says. “They found RNA in an ecosystem where there are quite a lot of archaea, but there are certainly not only archaea present there.” Whether their genomes consist of DNA or RNA, he adds, any viruses that infect archaea should not be called archaea “phage,” arguing that this term should remain reserved for the viruses that infect bacteria.

John Ottompke is a writer based in Chicago.

RESEARCH ADVANCES

Seeking Insights into Resistance to Two Antibiotics

Jeffrey L. Fox

A longstanding riddle underlying the resistance of particular bacterial pathogens to polymyxin B— itself a vintage antimicrobial peptide—yielded to analysis revealing a newly recognized self-modification process in gram-negative bacteria, according to Stephen Trent of the University of Texas (UT) at Austin and his collaborators. Meanwhile, a clinical trial evaluating a new boron-containing antibacterial agent, designated GSK2251052 (also called GSK052 or AN3365), was suspended early this year when patients with urinary tract infections being treated developed resistance to this experimental drug, according to ClinicalTrials.gov.

MINITOPIC

Influenza Virus Carries Modulator Gene; Flu Dose Matters

The influenza virus encodes a gene, designated PA-X, that modulates an infected host’s immune responses, according to Paul Digard of the Roslin Institute at the University of Edinburgh, Scotland, and his collaborators at several institutions in the United Kingdom and the United States. “The flu virus has a very, very small genome—just 12 genes,” says one of those collaborators, Andrew Firth of the University of Cambridge. “Finding a new gene makes a pretty significant change to our understanding of this virus.” That gene, within segment 3 of the viral genome, is part of a second open reading frame that is accessed via ribosomal frameshifting. When active, it decreases pathogenicity in mice but, if unexpressed, leads to increases in host inflammatory responses as well as in apoptotic and T cell-signaling pathways. Details appear in the July 13, 2012 Science doi: 10.1126/science.1222213. Separately, the number of flu particles involved in causing an infection affects its course, according to Martin Richter of the Université de Sherbrooke and Centre de Recherche Clinique Etienne-Le Bel in Québec, Canada, and his collaborators. For instance, high viral concentrations stimulate a better host immune response and broader protection against other flu strains. Details appear in the July 2012 Journal of Leukocyte Biology (doi:10.1189/jlb.1011490).
which is maintained by the National Institutes of Health.

Soon after Anacor Pharmaceuticals of Palo Alto, Calif., developed AN3365, the company entered a partnership with GlaxoSmithKline (GSK), headquartered near London, United Kingdom, to bring the promising compound into clinical trials. The boron-containing molecule blocks protein synthesis by inhibiting aminoacyl tRNA synthesis in gram-negative bacteria—specifically, by binding to the editing domain of particular tRNA molecules and interfering with additions of the amino acid leucine (*Microbe*, November 2010, p. 466–468). Currently, GSK researchers are continuing to study GSK052 and intend to publish their results, according to John Tomayko, who is GSK Senior Director of Infectious Diseases Medicine Discovery and Development in Collegeville, Pa.

More than 50 years ago, researchers realized that *Vibrio cholerae* O1 El Tor, unlike close relatives of this gram-negative bacterium, is resistant to polymyxin, but the mechanism underlying this resistance remained a mystery, according to Trent and his collaborators. Recently they learned that the El Tor strains of *V. cholerae* modify components of their cell wall—specifically, adding the amino acid glycine or the dipeptide diglycine to the lipid A anchor of lipopolysaccharide. Such changes confer a neutral instead of negative charge to the cell surface, thereby rendering these bacteria resistant to the cationic polymyxin B peptide, the UT researchers report. Details appear in the May 29, 2012 *Proceedings of the National Academy of Sciences* (doi:10.1073/pnas.1201313109).

Resistance to polymyxin sets El Tor apart from classical O1 *V. cholerae* biotypes, and acquiring this resistance “could be a key for fitness,” helping to explain the worldwide emergence of El Tor, which is the pathogen responsible for the current—and seventh—cholera pandemic, Trent says. In 2010, 48 countries reported, in total, more than 300,000 cases of cholera, mainly in the Americas, particularly Haiti, and in Africa, according to the World Health Organization, which points out that these figures vastly underestimate cases, particularly from Asia.

A key difference between those two biotypes is that classical strains carry a mutation that apparently blocks them from transferring glycyl residues to the lipid A anchor of LPS, according to Trent and his collaborators. Another “cool” feature of this resistance mechanism is that it closely resembles what happens within many gram-positive bacteria, in which the amino acid D-alanine, when added to outer-surface teichoic acids, can confer resistance to peptide antibiotics, he says. Thus, both gram-positive and –negative bacteria appear to share a “charge-based [surface] remodeling strategy.”

The El Tor findings are “quite interesting, and I like the connection to the gram-positive modification of teichoic acid,” says Lynn Silver, a New Jersey-based consultant who focuses on antibiotics. “Resistance to polymyxin by a variety of gram negatives is becoming a hot topic.”

Jeffrey L. Fox is the *Microbe* Current Topics and Features Editor.

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**RESEARCH ADVANCES**

**Marine Cyanobacterium Produces Novel, Dual-Acting Drug Prospects**

Carol Potera

A cyanobacterium that resides in coral reefs produces chemical compounds with both anti-inflammatory and antimicrobial properties, according to William Gerwick of the Scripps Institution of Oceanography in San Diego,
MINITOPIC
Human Microbiota Data Bonanza

During June and July, researchers from a variety of universities and other institutions who are analyzing the human microbiome released their latest data in a giant wave. Highlights from reports delivered during the 2012 ASM General Meeting, held in San Francisco, Calif., last June or published in the June 8, 2012 *Science*, June 14, 2012 *Nature*, and several journals within the *Public Library of Science* include:

- More than 10,000 microbial species were found among 242 healthy U.S. volunteers—129 males and 113 females—in samples collected from 15 anatomic sites in men and 18 in women (including three vaginal sites), according to officials from the National Institutes of Health (NIH), who are overseeing the Human Microbiome Project.
- The human microbiome contributes some 8 million protein-coding genes—about 360 times more bacterial than human genes per individual host, whose own genome is endowed with about 22,000 protein-coding genes.
- The gut bacterium *Bifidobacterium dentium* proves capable of secreting large amounts of γ-amino butyric acid (GABA), a neurotransmitter of both the host enteric and central nervous systems, according to Karina Pokusaeva and James Versalovic of Baylor College of Medicine in Houston, Tex., and their collaborators. GABA modulates pain and may also inhibit inflammation, they say.
- The nasal microbial populations of patients with chronic sinus conditions are depleted of diversity and frequently overgrown with *Corynebacterium* spp., according to Nabeetha Nagalingam of the University of California, San Francisco, and collaborators.
- Mice colonized with bacteria ordinarily found in the human gut develop immune responses much like those of germ-free mice, suggesting that gut microbiomes are very much attuned to and may function properly only when matched with their appropriate host species, according to Dennis Kasper of Harvard Medical School in Boston and his collaborators. Their findings appear in the June 22, 2012 *Cell* doi:10.1016/j.cell.2012.04.037.

Potential therapeutics could be developed for either indication, but a product with both capabilities “would be a very powerful combination,” he says, citing an example an acne treatment that would block microbial colonization while reducing inflammation of the skin.

Mats of the cyanobacterium *Lep- tolyngbya crossbyana* were growing onto and overwhelming coral reefs in Honaunau Bay in Kona, Hawaii, a popular dive site, at least as early as 2008, says his Scripps colleague Jennifer Smith. Those bacterial blooms were smothering and bleaching corals on which they grew, while damaging nearby corals with which they had no direct contact. This damage at a distance suggested to Smith that *L. crossbyana* may be leaching toxic chemicals that can diffuse through sea water, prompting her to send samples to Gerwick for chemical analysis.

Postdoctoral researcher Hyukjae Choi isolated three natural products from *L. crossbyana*, which were named honaucin A, B, and C in honor of the bay where the cyanobacteria that produce them were collected. The honaucins consist of hydroxy-butyrolactone joined via ester linkage to chlorocrotonic acid.

All three of these honaucins block quorum sensing, which was a surrogate measure of their anti-infectiveness in a dose-dependent manner, according to Gerwick. The compounds were tested in *Vibrio harveyi* BB120 cultures that use bioluminescence as a marker for quorum sensing. When tested in mouse macrophage cells for anti-inflammatory activity, the honaucins reduce nitric oxide levels and the subsequent stimulation of proinflammatory cytokines, especially interleukin-1β, he says. Promising though these findings may be for human medicine, they offer no clues about how to protect coral reefs against damaging cyanobacteria, he adds, noting that environmental pollutants are the likely stimulus for growth of those cyanobacteria.

Several brominated and iodinated honaucins are being evaluated for changes in their anti-infective and anti-inflammatory activities, Gerwick says. For example, one such analog, 4’-bromohonaucin, has enhanced activity and is being further developed. Details appear in the May 25, 2012 *Chemistry & Biology* [doi:10.1016/j.chembiol.2012.03.014].

“Marine natural products offer the unique opportunity to discover novel mechanisms of action that can be translated into treating human disease,” says Keith Glaser, an adjunct professor of pharmacology at Midwestern University in Downers Grove, Ill. The honaucins offer a novel scaffold for blocking inflammatory mechanisms and pathogens that drive inflammatory responses, he says. Moreover, Gerwick and his collaborators took the trouble early on to address what has proved a major shortcoming for many others seeking to develop natural products—ensuring an adequate supply of raw materials—by isolating honaucins and developing derivatives for further development, he adds. “This maintains our natural marine resources for the future discovery of more novel compounds.”

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