Current Topics

Microbial Energy on the Dark Side Juxtaposed to Nanowire Crosstalk

In terms of energy exchange, intercellular crosstalk, and biochemical inventiveness, the microbial world continues to furnish surprises. Eventually, some of that microbial mystique might provide us sluggish humans with novel approaches for harnessing energy. Meanwhile, probing provides insights as to how microorganisms function under unusual—and, in some cases, extreme—environmental circumstances, according to several researchers who spoke during separate symposiums, including “Melanized Fungi: the Dark Side of Medical Mycology” and “Electromicrobiology and Extracellular Electron Transfer,” convened during the 108th ASM General meeting, held last June in Boston, Mass.

**Geobacter sulfurreducens** bacteria generate electrons while metabolizing uranium in the cytoplasm, and then transmit those electrons to cellular outer membranes, according to Gemma Reguera of Michigan State University in East Lansing, Mich., who spoke during the electromicrobiology symposium in Boston. Those electrons then can be transmitted between cells via pili that extend outward from the outer membranes. Those pili, which contain the same or similar proteins as other nontransmitting pili, thus are a natural form of “nanowire,” she says.

When such cells grow at low densities, their nanowires form “networks” and “confer a growth advantage,” Reguera continues. This network of nanowires and cells is in some ways the “opposite” of the quorum-sensing system that operates among many types of bacterial cells while they grow at high densities, she points out. In quorum sensing, cells typically communicate by means of low-molecular-weight, readily diffusible chemicals such as homoserine lactone derivatives. In the case of **G. sulfurreducens**, however, that communication appears to be electronic via the nanowire network—perhaps the microbial equivalent of the Internet or, at least, the telephone system.

The nanowires form helical bundles that are “very conductive,” Reguera says. Although they are “like semiconductors,” the nanowires appear to be “always conducting,” which seems to set them apart from typical synthetic semiconductor materials, she points out.

Although nanowires are readily sheared from **G. sulfurreducens** cells in culture, in more natural settings they can form “massive structures” that are “sticky,” gather into “clumps,” and are “more like biofilms than planktonic,” Reguera says. In such settings, they can become overlaid with uranium, perhaps trapping that material outside cells, shielding them from the toxic effects of uranium or similar materials, she adds.

“If cells don’t express pili, they die soon after exposure to uranium.”

In somewhat similar fashion, melanin-forming fungi, including the pathogen **Cryptococcus neoformans**, its relative **C. spheospermum** that was found in a nuclear reactor, and **Wangiella dermatitidis**, another pathogen, trap potentially damaging ionizing radiation within that pigment—protecting themselves and, in some cases, also converting that radiation into a metabolically useful form of chemical energy, according to Ekaterina Dadachova of Albert Einstein College of Medicine in Bronx, N.Y., who spoke during the “Melanized Fungi” symposium in Boston. Such fungi are extraordinarily hardy, withstanding ionizing radiation in nuclear reactors, including components of the reactor that was damaged in 1986 at Chernobyl, Ukraine, then part of the Soviet Union. “Melanized fungi inhabit some of the most extreme environments on the planet,” she says.

Living fungal cells and intact (but not “crushed”) ghost cells can withstand very high doses of radiation, with melanin playing a key role, according Dadachova. In terms of an objective measure, intact cells or ghosts of fungal cells are about 50% as effective in protecting light-sensitive photographic film as is metallic lead when exposed to ionizing radiation, she notes.

Even more remarkably, both **W. dermatitidis** and **C. neoformans** cells “can harness ionizing radiation for metabolic energy—not unlike photosynthesis,” in which chlorophyll molecules harness light energy, Dadachova says. However, in the case of these fungi, she adds, “We don’t know the structures” that are responsible for converting that radiation energy into a form that cells use.

Not so much is known about melanin itself, according to Helene Eisenman, another speaker at the symposium, who also is from Albert Einstein College of Medicine. The crosslinked polymer, which is considered a “virulence factor” when found in some species of pathogenic fungi such as **W. dermatitidis** and **C. neoformans**, derives from L-Dopa or other similar
Toxin from *H. pylori* Rides Sphingomyelin Rafts into Target Cells

*Helicobacter pylori*, an acid-tolerant bacterium that can cause stomach ulcers, secretes a protein called vacuolating toxin A (VacA), that enters and damages human gastric cells by attaching to sphingomyelin in lipid rafts within host cell membranes, according to microbiologist Steve Blanke at the University of Illinois (UI), Urbana, and his collaborators. “This is the first example of a bacterial virulence factor that uses sphingomyelin as a receptor,” he says.

Like several other bacterial toxins, VacA interacts with plasma membranes of mammalian cells, but in this case only with sphingomyelin, a membrane lipid that not only plays a structural role but also has signaling properties. To prove that sphingomyelin is crucial for VacA intoxication, Blanke and his collaborators stripped mammalian HeLa cells of this lipid by treating them with sphingomyelinase. Although VacA induces untreated cells to form large vacuoles, few to no vacuoles form in cells treated with sphingomyelinase. However, adding extra sphingomyelin to sphingomyelin-depleted HeLa cells restores their capacity to form vacuoles, according to the UI researchers. They report similar results when they tested two human gastric epithelial cell lines (AGS and AZ-521).

“Sphingomyelin is absolutely necessary for the toxin to be able to interact with and subsequently intoxicate cells,” Blanke says. The amount of sphingomyelin within plasma membranes is critical for determining how much VacA binds to cells and the extent of its damage to those cells, he adds. Details are reported in the May 23, 2008 *PLoS Pathogens* (4:e1000073).

Sphingomyelin molecules can cluster, forming “islands,” or “rafts,” within membranes along cell surfaces. VacA preferentially binds and enters cells through such rafts, according to Blanke. Moreover, VacA intoxicates cells only when it enters them via sphingomyelin in rafts, he adds. Whether VacA binds preferentially to such rafts or whether its binding to sphingomyelin leads such rafts to form is not known.

Sphingomyelin clustered in lipid rafts appears to be “a bona fide receptor for VacA,” says Patrice Boquet, a cell biologist at INSERM in Nice, France, about the new findings. However, the direct detection of VacA on sphingomyelin-containing liposomes by electron microscopy would strengthen the claim, he notes.

Individuals with *H. pylori* infections typically are treated with several antibiotics. Identifying agents that target VacA to use along with conventional antibiotics could prove beneficial, according to Blanke. “It’s important to understand the biology of bacterial toxins to develop treatments that complement antibiotics,” he says.

Besides inducing gastric and duodenal ulcers, long-term *H. pylori* infections are a significant risk factor for stomach cancer, which is the second leading cause of cancer deaths worldwide. Identifying the VacA receptor raises the possibility of finding new ways to block the action of the toxin on cells and perhaps to reduce the risk of stomach cancer. “This is a potentially ideal target because sphingomyelin is extracellular, so you don’t have to worry about getting a drug inside cells,” Blanke points out.

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Early-career researchers are having a harder time than their predecessors, according to a scientific panel convened by the American Academy of Arts & Sciences (AAA&S) and led by Thomas Cech, president of the Howard Hughes Medical Institute. It takes them longer to train, and finding grant support is far tougher than a generation ago. Further, the challenges now faced by young researchers are distracting them from doing high-risk innovative research, according to the recent report from that AAA&S panel, ARISE: Advancing Research In Science and Engineering.

Research resources for young investigators are notably scarce. In 1980, for example, 86% of new investigators received a grant on their first try. However, by 2000, that figure dropped to 59% and, in 2007, to only 28%. “Time spent submitting repeated grant applications is a distraction from the research endeavor itself and poorly utilizes the potential of this highly creative resource,” the panel members note in their report. To make matters worse, current early-career principal investigators lag their older colleagues by about five years from the outset—the average age for a first grant jumping from 37 to 42 years. These challenges, in turn, make it likely that frustrated early-career faculty will directly or indirectly dissuade some of their students from pursuing careers in science, panel members point out. “This is a very bad strategy for building the nation’s future research enterprise.”

Tight research budgets are not only damaging morale; the constant hunt for support is fostering conservative thinking, causing both researchers and funding agencies to shy away from conducting high-risk, high-reward research. People who are afraid to take chances are unlikely to come up with breakthrough results that transform a field. “Riskier research should be nurtured,” panel members advise. “Science benefits greatly from work that has the potential to disrupt complacency and conventional thinking.”

The AAA&S report not only documents obstacles facing young scientists and the impact that they are having on research, it also calls for changes in the way science is funded. For starters, the report recommends that universities pay a greater proportion of research faculty salaries and lab costs. Meanwhile, federal agencies that support research are advised to provide ample, long-term seed funds to early-career faculty, enabling them to explore new ideas. Because high-risk proposals tend to suffer and the stressed peer-review system is not equipped to appreciate them, the grant application and review processes need to be strengthened, according to the report. It also urges institutions to provide “trustworthy, convenient, and affordable child care” to young faculty who become parents as they try to establish their research careers and also to permit them tenure “timeouts,” if needed.

The AAA&S report is “right on,” says microbiologist Derrick Brazill, a recent recruit to Hunter College, City University of New York in New York, N.Y. “Junior researchers spend most of their first few years writing grants. Add teaching responsibilities to that and there isn’t much time or energy left over for research.” Brazill notes that graduate students and postdocs are increasingly discouraged by what they see and often come to him for advice. “Having special pools of funding specifically for beginning investigators to help ease the burden of those first few [research] years” is a practice that would help to overcome some of those difficulties, he says.

Microbiologist Bonnie Bassler of the Department of Molecular Biology at Princeton University in Princeton, N.J., sympathizes with early-career researchers like Brazill, noting that the “ARISE report provides a frank assessment of the danger we face if . . . we lose our most promising scientists from the basic science arena.”

Microbial ecologist Tom P. Curtis at the University of Newcastle upon Tyne in England suggests that U.S. researchers “take a look over the pond where we have special earmarked schemes for new investigators and a wide range of fellowships for young researchers.” However, he and others note, the British government is rethinking their funding programs and considering putting less emphasis on innovative research in...
favors of programs that provide more immediate financial returns.

The ARISE panel report cautions generally against such short-term thinking. “Regardless of the size of the pie,” it notes, “strategic support for early-career investigators and potentially transformative research will be integral to the long-term competitiveness of our economy.” The ARISE report is available online from www.amacad.org/ARISE.

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Genes Underlying Bacterial “Persistor Cells” Identified

For microorganisms to persist in a dormant state that tolerates antibiotics that would otherwise inhibit or kill those cells, bacteria such as Escherichia coli depend on at least 10 newly identified and validated genes, according to Kim Lewis and his collaborators Marin Vulic and Sonja Hansen of Northeastern University (NU) in Boston, Mass. Analyzing those genes is expected to help in “determining the mechanism of persistor formation,” Lewis says, and thus “provides a rational approach for developing sterilizing antibiotics” to overcome bacteria that can enter the persistor state. Details appear in the August Antimicrobial Agents and Chemotherapy (52:2718–2726).

To probe the genetics underlying the persistor state, the NU researchers first tried screening a transposon insertion library of E. coli cells for knockout mutants that are not persisters. Earlier efforts to screen such libraries of E. coli or other bacterial species revealed genes involved in various survival functions associated with changes in status, including sporulation and biofilm formation. In this case, however, “It was not possible to identify a single E. coli knockout that made no persisters,” Lewis says, indicating that “redundant mechanisms are responsible for persistor formation, and knocking out a single one does not produce a strong phenotype.”

With this sobering view of the challenges they faced, the NU researchers obtained a comprehensive library of 3,985 E. coli knockout strains that was developed by Hirotada Mori and colleagues at Nagoya University in Nagoya, Japan. They used this collection to screen for mutants with altered antibiotic tolerance, and then searched for clones with a 5- to 10-fold decrease in persisters. From a set of 150 candidate genes, they identified two that appear to be involved specifically in persistor formation—ygfA, encoding a 5-formyltetrahydrofolate cyclo-ligase, and yigB, encoding flavin mononucleotide (FMN) phosphatase.

“YgfA can cause depletion of the folate pool, while YigB will deplete FMN,” Lewis says. Thus, when over-expressed, these proteins might deplete key metabolites, thereby leading cells to become dormant. Further, overexpressing each of these genes boosts the tolerance of cells to antibiotics. “None of the knockout strains had a complete lack of persisters; in most cases, the drop was about 10-fold,” the NU researchers note. “The preponderance of global regulators and chaperones among the genes affecting persistence strongly suggests that the function is encoded by redundant genes [and] mechanisms.”

Persistor cells typically make up only a small part of a bacterial population, accounting for about one in $10^6$ to $10^4$ cells within exponentially growing cultures, and about one in $10^2$ cells among those that are no longer growing rapidly because nutrients are depleted and they have reached stationary phase. Persistor cells are implicated in many bacterial infections, including those associated with implanted medical devices, such as catheters, and with dental disease, endocarditis, and cystic fibrosis.

“The mutants identified in the study provide new clues on the molecular mechanisms involved in formation and maintenance of persistor populations,”

WHO, Others Instituting Faster Tests for Diagnosing, Cheaper Drugs for Treating TB in Africa

A rapid molecular method to diagnose multidrug-resistant tuberculosis (MDR-TB) is being made available in Africa, according to officials at the World Health Organization (WHO), the Stop TB Partnership, UNITAID (an international consortium that purchases and provides drugs to treat HIV/AIDS), and the Foundation for Innovative New Diagnostics (FIND), who in June announced plans for several related projects to combat MDR-TB. As part of the first, rapid molecular tests, known as line probe assays, will be made available in 16 countries for use in determining whether individuals have MDR-TB; the tests produce answers within two days. Among the recipient countries, Lesotho is already equipped to start using these tests, Ethiopia soon will be, and another 14 countries will be phased in during 2009–2011, according to WHO. As part of a second project, UNITAID is to provide $33.7 million for drugs needed to treat MDR-TB in 54 countries, including those receiving the new diagnostic tests. This project is expected to achieve price reductions of up to 20% for second-line anti-TB drugs by 2010.
Experts Ask Whether Infectious Diseases Go Better with Koch

Federal officials and researchers began brainstorming early in 2006 over how to explore the human microbiome—that is, the full array of microorganisms directly associated with the human body. The early exploratory results of this undertaking already are being shared, even as researchers who are leading these efforts continue to wrestle with key questions, such as how many types of people need to be sampled to derive a true reading of the microbiome biodiversity.

Some are calling the microbiome project the “second human genome project,” says symposium participant Claire Fraser-Liggett of the Institute of Genome Sciences and University of Maryland School of Medicine in Baltimore, Md. A major impetus for pursuing this project is that the human-associated complement of microorganisms is expected to be brimming with genes whose functions exceed those in the purely human repertoire, she says. Investigating those anticipated novel functions is appealing for its own sake and also because it offers the prospect for better understanding how those microbes and their genes might impinge on human health.

These early exploratory efforts are deepening the appreciation of the role played by microorganisms in human health, according to David Relman, another participant in these symposia, who is from Stanford University in Stanford, Calif. In some cases, human disease is more apt to reflect a “disturbed microbial community structure” instead of the singular mischief of a single pathogenic species, he says. “Think of communities as the pathogen.” With that notion comes a corollary that “enhancing the resiliency” among members of that microbial community might ward off disease in individuals. “It’s something we haven’t been thinking about as clinicians, and it’s beyond our reach at the present,” he cautions. Further, it remains a “challenge to build convincing arguments” that any particular disruption of a microbial community is the crucial underlying cause of a specific disease.

However, there is a growing sense that human health and disease states are deeply enmeshed with the microbiome, according to symposium participant Martin Blaser of New York University School of Medicine in New York, N.Y. This way of thinking puts some experts along the “frontier of a different understanding of disease causation,” he says, pointing to cancer as an example. The causes of cancer generally are consid-
ered “multifactorial,” he says. Perhaps it is just as apt to consider at least some infectious diseases as also being multifactorial, dependent on both a range of host factors as well as some portion of the host-associated microbial community. Thus some infectious diseases may result from a “community disturbance” instead of the more traditional “all-or-none” effects resulting from a specific pathogen—a way of thinking that is embodied in Koch’s postulates, he suggests.

When analyzing some as-yet-unexplained diseases in which microorganisms apparently are involved, investigators “should not assume there are bright lines in the sand,” Fraser-Liggett says. For example, for individuals with Crohn’s disease, in which episodic inflammatory responses painfully disturb bowel functions, it appears unlikely that there will be a particular signature microbiome that accounts universally for this “very complex disease,” she says. However, there is reason to think that microbial community census-taking could help in distinguishing one stage of Crohn’s disease from another, at least for some part of the population suffering from this disease. In the face of such complexity, partial clues from analyzing microbial profiles could prove useful.

The goal is “not to raise questions about classical pathogens,” Fraser-Liggett continues. Instead, the idea is to determine whether detectable shifts in commensal microorganisms “give a readout” or in other cases may prove “causal” for diseases and syndromes that are genuinely multifactorial and thus distinct from those infectious diseases in which one pathogen dominates and undeniably fulfills Koch’s postulates.

Jeffrey L. Fox

Several Developments on the Vaccine Front

Here are several noteworthy developments involving vaccines:

- Rotavirus activity among children during the 2007–2008 season appears to have started later and to be less severe than usual and thus likely reflects widening use of the RotaTeq vaccine that was introduced in 2006, according to officials from the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga.
- Poliovirus was attenuated, and thus might be suitable for vaccine use, by recoding the genome with codons that express correct amino acids but deviate from those codons found in natural isolates of this virus, according to Eckard Wimmer and his collaborators at Stony Brook University in Stony Brook, N.Y.; details appear in the 27 June 2008 Science.
- An experimental self-destructing, “biological containment” vaccine that is based on a genetically reprogrammed strain of Salmonella enterica serovar Typhimurium was injected into mice and, after 21 days, left no viable vaccine strain cells in host tissues, according to Roy Curtiss III at Arizona State University in Tempe and his collaborators; details appear in the July 8, 2008 Proceedings of the National Academy of Sciences.
- In July, officials at the National Institutes of Health (NIH) in Bethesda, Md., canceled plans to test an AIDS vaccine that uses adenovirus-5 as a vector because of safety and efficacy concerns; the move follows the collapse last year of several clinical trials involving another vaccine that may have left some recipients more susceptible than before the trial to HIV infection (Microbe, January 2008, p. 10).