

## MICROBIOLOGY

### We Get By with a Little Help from Our (Little) Friends

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To understand diseases of the heart, kidney, lung, and other organs, medical students first learn how these systems function under normal conditions. The principal exception to this health-before-disease approach is the study of the relationship between humans and microorganisms. Remarkably, we know far less about the thousands of species that make up our intrinsic microbiota than we know about the few dozen microbes that cause disease (1). Biologists are becoming increasingly aware that animals have co-evolved, and continue to coexist, with diverse assemblages of microorganisms that are required for normal health and development (2, 3). This awareness has opened our eyes to a new biological frontier, the subject of a recent workshop that explored the influence of beneficial bacteria on animal host biology (4).

#### We Are Not Alone

Genomic and evolutionary analyses show us that we are not the single “individuals” that we think we are. Instead, we and other complex organisms are composed of an interconnected ecosystem of eukaryotic and prokaryotic cells whose interactions can best be understood in the context of community ecology. Two workshop participants, Brendan Bohannon (Stanford Univ.) and Robert Seymour (University College, London), showed how mathematical modeling can predict the forces that shape and maintain microbial diversity in the host. In particular, activities of both the host and its microbiota create spatial and temporal niche heterogeneity, allowing complex and stable microbial consortia, such as those present in the intestinal tract, to coexist in stable assemblages. Ecological modeling may also predict how the microbial community reacts to natural processes, such as the normal developmental succession of microbiota that occurs during the maturation

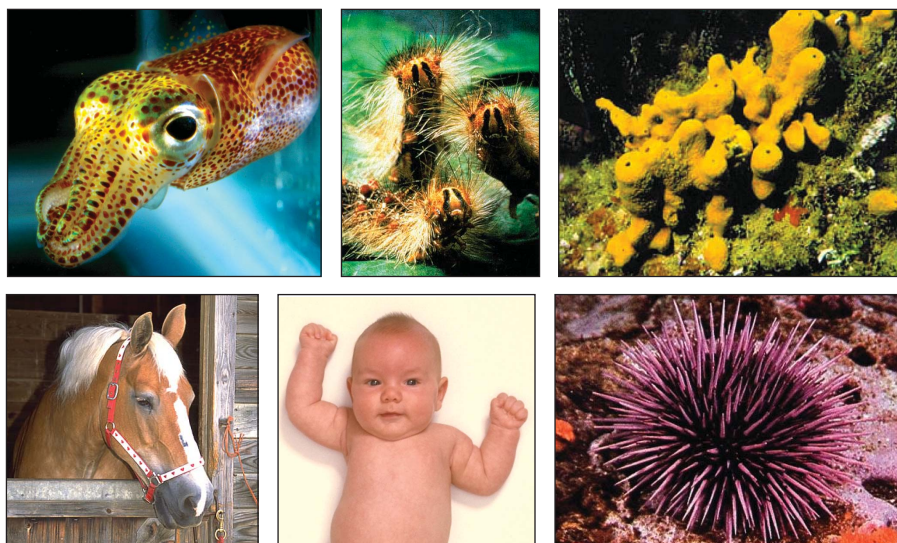
of an animal’s digestive tract. Jo Handelsman (Univ. Wisconsin, Madison) and others described how the microbial communities found in insects are typically less complex than those in vertebrates. Nonetheless, these invertebrate hosts have evolved highly integrated, species-specific associations with microbes. As such, invertebrate alliances with bacteria can serve as experimentally tractable models of vertebrate host-microbe communities.

#### Trifling with Mother Nature

The tremendous impact of humans on global ecosystems such as rainforests and coral reefs is well documented, but little is known about how our activities are influencing the internal ecosystems of animals. Mounting evidence suggests that social practices and medical intervention in industrialized nations have profound and lasting repercussions. For example, Lora Hooper (Univ. Texas Southwestern Medical Center) explained the importance of indigenous microbiota in the normal development of the mammalian digestive and immune systems, and how disruption of this microbial com-

munity may result in inflammatory bowel disease (IBD). IBD has the highest incidence in industrialized nations. Environmental influences, such as the misuse of antibiotics, may create an imbalance in the normal bacterial consortia that contributes to the induction of disease. Furthermore, in the dense, gene-exchanging microbial communities of the gut, antibiotic resistance develops efficiently but in poorly understood ways. An individual whose normal microbiota has evolved antibiotic resistance may become a vessel for the transfer of resistance genes into potential microbial pathogens. Julian Davies (Univ. British Columbia) emphasized the need to better understand the dynamics of these processes in the normal microbial consortia of animals if we are to design and apply antibiotics more effectively and safely.

Similarly, the distancing of industrialized societies from the natural world has reduced the carriage of certain microbes, such as *Helicobacter pylori* (Rino Rappuoli, Chiron Corp.), an event whose full consequences to human health remain unknown (5). Other researchers have suggested that the presence of this bacterium may lower the occurrence of esophageal disease (6). Further, widespread vaccination efforts have purposely eliminated other members of the normal microbiota without an understanding of their significance. For example, about 10% of the human population harbor the opportunistic pathogen *Neisseria meningitidis*, but only one in



**Dynamic partnerships.** Diverse model systems are used to study the interactions between beneficial bacteria and their animal hosts. These include (clockwise, from top left): the bobtail squid, the gypsy moth caterpillar, a marine sponge, the purple sea urchin, a human infant, and a horse. These animals all develop specific associations with single bacterial species or microbial consortia.

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## PERSPECTIVES

100,000 individuals develops meningitis. How does carrying this microbe influence the host immune system? What is the significance of a population that carries low levels of a potential pathogen? Catharina Svanborg (Lund Univ.) explained that the propensity for carrying nonpathogenic strains of *Escherichia coli* is a heritable trait that can confer increased resistance to urinary tract infection. Such a phenomenon may accompany the carriage of many other “commensal” bacteria.

### Evolving Partnerships

All organisms are products of their evolutionary history, and Carole Hickman (Univ. California, Berkeley) considered how microbes may have influenced the origin and diversification of animal taxa. Under the harsh conditions of early Earth, alliances between microbes and multicellular eukaryotes apparently increased the evolutionary potential of both species. For instance, symbiotic partnerships invaded ancient hypersaline or sulfide-rich habitats that would have remained inaccessible to either partner species on its own. Extant invertebrate systems are providing insight into how such associations can drive evolutionary processes. For example, Eugene Rosenberg (Tel Aviv Univ.) reported that reactive oxygen-producing symbiotic microalgae protect corals from bacterial pathogens that cause the bleaching and death of coral reefs. Greg Hurst (University College, London) described how interactions between insects and the intracellular bacterium *Wolbachia*, which influences insect reproduction by inducing parthenogenesis or feminization, may have accelerated speciation or caused extinction within host populations. Conversely, Aziz Heddi (Institut Nationale Scientifique Applique, Lyon) discussed host-driven bacterial evolution resulting in, for example, a reduction in the genome size and complexity of insect-associated symbionts.

We are unlikely to adequately understand immune systems without recognizing the influence of nonpathogenic bacteria on their evolution. Courtney Smith (George Washington Univ.) used comparative expressed sequence tag (EST) analysis to show that although sea urchins lack the tremendous complexity of the vertebrate combinatorial (or acquired) immune system, they have a surprisingly diverse array of molecules dedicated to immune function. Have beneficial bacteria aided the evolution of such immune molecules in this and other invertebrate hosts? Margaret McFall-Ngai (Univ. Hawaii) discussed how invertebrates, such as the Hawaiian bobtail squid (see the figure), often have associated microbiota comprising far fewer species than the micro-

biota of vertebrates. This feature correlates with the absence of a combinatorial immune system, the evolution of which in vertebrates may have been permissive to, or may have been driven by, the host's relationship to its beneficial microbial partners. Mathematical models developed by Seymour, which predict that imposition of a complex immune system will increase the diversity of microbial assemblages, support this thesis. Similarly, Hooper described the development of the relationship between the mammalian host and its enteric microbiota as a complex succession of species assemblages. She also commented on the microbiota-induced maturation of the combinatorial immune system in the mouse.

### Friend or Foe

The historical emphasis on pathogenic bacteria and their diseases has led to an assumption that genes encoding virulence factors are specific to those relationships. However, several of the cellular and molecular mechanisms that underlie interactions between an animal and its beneficial microbiota are remarkably similar to those first found in pathogens. Svanborg described how molecules that enhance persistence at a site where a given microbe is a member of the normal microbiota can be the very factors that promote disease when these bacteria emigrate to other sites of the body. For instance, attachment structures (P-fimbriae) of intestinal *E. coli* may induce disease when these bacteria colonize the urinary tract. Jörg Hacker (Univ. Würzburg) explained how “pathogenicity islands”—large, laterally acquired, genetic loci bearing virulence-associated genes—were first discovered in harmful *E. coli*. Yet these same islands are common in beneficial or commensal strains of this and other bacterial species. Thus, the presence of these genes may indicate the potential for host interactions, which may be pathogenic or benign according to how these genes are regulated or the sensitivity of the tissue in which they are expressed. Similarities in origin and mechanism are also apparent in specific cell-cell signaling systems used by host-associated bacteria. For instance, Edward Ruby (Univ. Hawaii) described how acyl homoserine-lactone quorum sensing—a mechanism used by bacteria for sensing their own abundance in an environment—is required for normal tissue colonization by both beneficial and pathogenic bacterial species, even within the same genus. Similarly, the use of type III secretion systems, first described as a mechanism by which animal pathogens hijack their host's cell biology, have been implicated in mutualistic associations between nonpathogenic bacteria and their hosts (7).

How do genes arise that promote microbe-host associations? Paul Rainey (Univ. Auckland) explained how genetic modularity promotes the rearrangement of existing protein motifs, thereby creating new opportunities for the evolution of bacteria-host interactions. Similarly, Jeffrey F. Miller (Univ. California, Los Angeles) described a new reverse transcriptase-based genetic mechanism in which certain bacteriophage (viruses that infect bacteria) rapidly generate the capacity to target different bacterial cell-surface receptors. This discovery may provide insight into how microbes quickly switch host specificity.

Studies of pathogenic infections have revealed that host tissues respond to common bacteria-specific molecules, most notably two surface components: lipopolysaccharide (LPS) and peptidoglycan (PGN). Because their effects were first noted in infected tissues, such molecules have been referred to as pathogen-associated molecular patterns (PAMPs) (8). PAMPs trigger host responses such as activation of the Toll-like receptor/NF- $\kappa$ B pathway, elements of which are conserved among all animals and plants. McFall-Ngai showed that in the squid light-emitting organ, LPS and PGN produced by the luminescent symbiont *Vibrio fischeri* are required to signal the normal development of host tissues. Further, as discussed by Clare Bryant (Cambridge Univ.), the same LPS structure can act as an agonist (that is, stimulating a Toll-like receptor response) in one mammalian host and an antagonist (inhibiting the response) in another. This suggests that the host response does not result simply from recognition of a PAMP, but rather is a partner-specific feature. Andrew Neish (Emory Univ.) described a form of signaling by nonpathogenic bacteria that inhibits host responses to LPS and PGN. Such findings suggest that these bacteria-specific molecules are part of an ancient mechanism by which host-associated microbes “talk” with animal tissues, irrespective of the eventual outcome of the conversation.

### A Wide-Open Frontier

The workshop revealed that many scientific fields are accumulating evidence about the critical impact of intrinsic microbiota on all aspects of animal biology. As the depth of host-microbe interactions and the mechanisms underlying them continue to be unraveled, fundamental paradigms of pathogenic microbiology, developmental biology, and immunology will need to be reevaluated. For this reason, a specific recommendation arising from the workshop is that biology be taught in a new way, incorporating our growing knowledge about the

