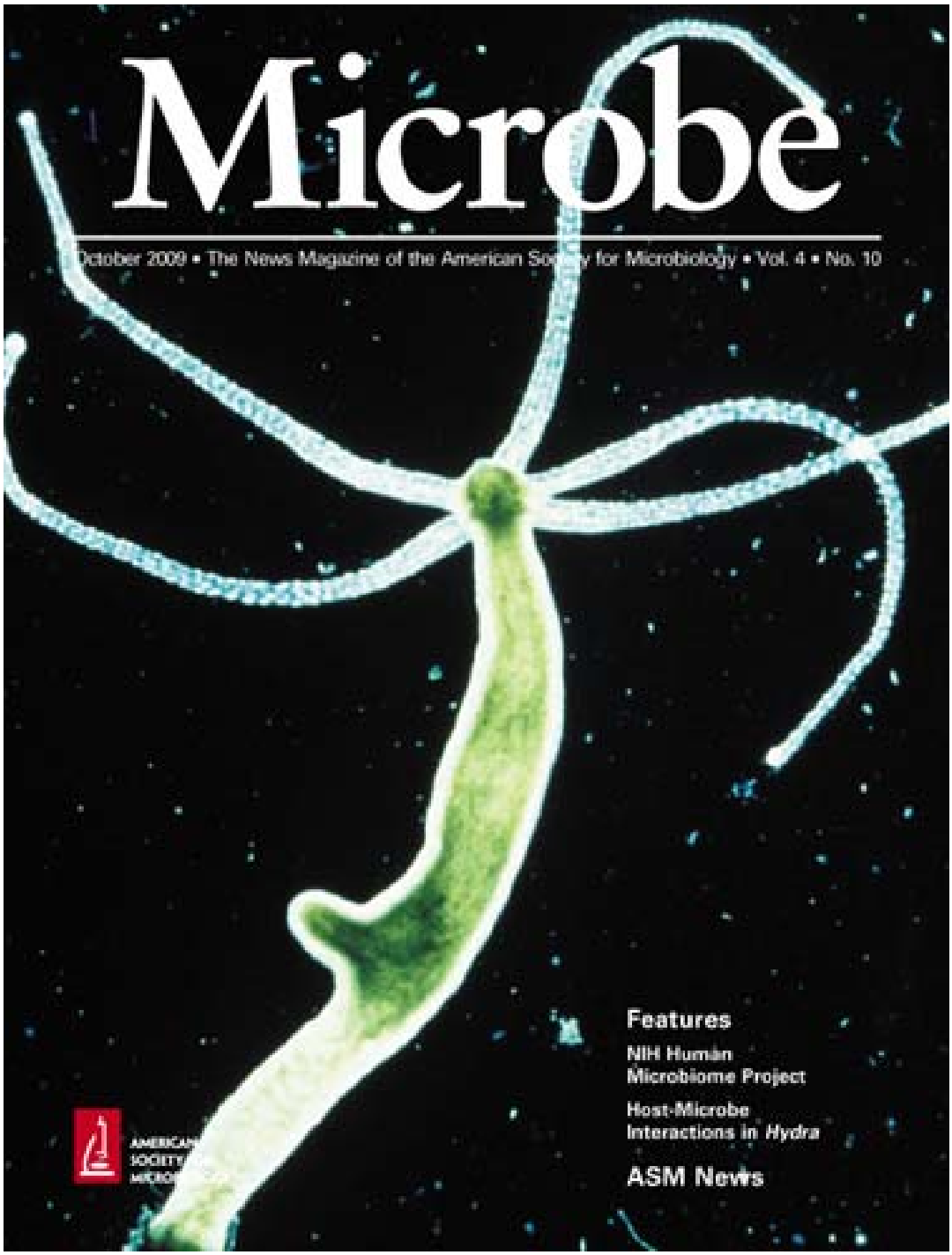
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Host-Microbe Interactions in Hydra
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Exploring Host-Microbe Interactions in *Hydra*

Hydra prove a suitable organism for addressing how hosts interact with epithelial-bound microorganisms

Sebastian Fraune, René Augustin, and Thomas C. G. Bosch

The freshwater polyp *Hydra,* which belongs to an ancient animal phylum, still must defend itself against pathogens, much like other more complex organisms. Further, microorganisms colonize the epithelial layers of *Hydra*, much as they colonize other organisms. How do hosts distinguish benign epithelial colonizers from threatening pathogens, and what role do those colonizers play for *Hydra* in its freshwater environment, which is teeming with microbes of all sorts, including potential pathogens?

Hydra, each about 0.5 cm in length, prove a particularly suitable organism for addressing questions of how hosts interact with epithelial-bound microorganisms. *Hydra* are cnidarians, which includes corals, jelly fishes, and sea anemones—an early branch on the animal side of the tree of life, whose closest sister group is the Bilateria (Fig. 1A). The last common ancestor of the Cnidaria and Bilateria, likely the first animal with a nervous system, branched off about 700 million years ago.

Hydra has a relatively simple three-dimensional tissue structure (Fig. 1B-D), and its malleable body consists of two cell layers, the ectoderm and endoderm. separated by a thin matrix called the mesoglea. The polar body has a head and tentacles at one end, and a foot at the other end of a hollow column (Fig. 2B). Epitheliomuscular cells cover the outside and line the gastric cavity, while interstitial cells between those two layers differentiate into nerve cells, cnidocytes, gland cells, and gametes. Although *Hydra* lacks migratory phagocytic cells, hemolymph, and permeability barriers, members of our lab determined that its epithelial cells activate in response to invading pathogens, increasing expression of genes encoding antimicrobial peptides and thus mediating this organism's innate immune responses.

Summary

- *Hydra* epithelial cells respond to invading pathogens by increasing expression of genes encoding antimicrobial peptides and other agents to mediate its innate immune responses.
- *Hydra* produce a variety of antimicrobial agents, including peptides with broad-spectrum activity as well as a serine protease inhibitor that inhibits several gram-positive pathogens.
- The *Hydra* epithelium apparently imposes selective pressures that shape its microbiota, which, if depleted, can damage host health and well-being.
- Changes in cell composition of *Hydra* mutants can lead to significant changes in the *Hydra*-associated microbial community.

Hydra, a Potential Source of Antimicrobial Drugs

Once induced, *Hydra* tissues produce highly selective antimicrobial peptides. For example, the endodermal epithelium produces the cationic 60-amino acid hydramacin-1, which contains eight cysteines, after being induced by microbial products. Once isolated from such tissues, hydramacin-1 can kill effectively several types of gram-negative pathogens that infect humans, including strains of *Escherichia coli*, *Klebsiella oxytoca*, and *Klebsiella pneumoniae* that produce extended spectrum beta-lactama-

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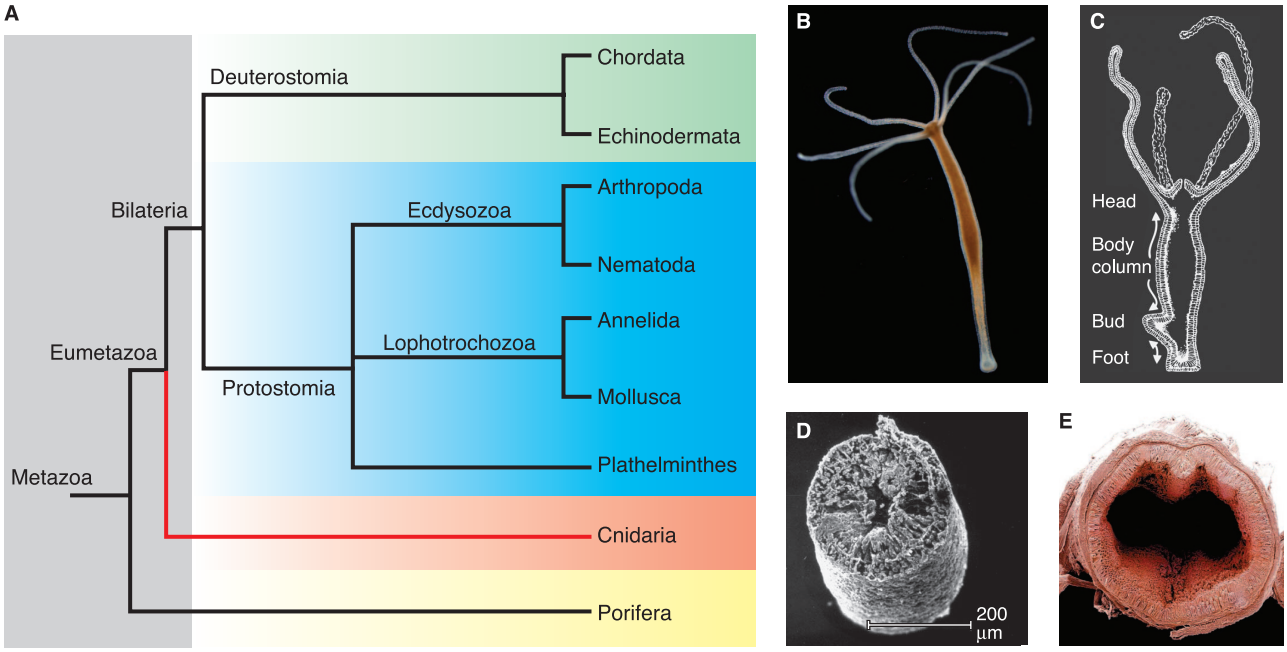
FIGURE 1



Hydra is member of an ancient animal phylum. (A) Schematic representation of the metazoan phylogeny. The Cnidaria are a sister group to all Bilateria. (B) Live image of a *Hydra* polyp. (C) Schematic representation of a *Hydra* polyp. (D) Scanning electron microscopy of a section through a *Hydra* polyp. (E) Section of the human small intestine. (Panels D and E modified from T. C. G. Bosch, Biologie In Unserer Zeit 2/2009 **39:**114–122, 2009.)

ses (ESBL). Additionally, this peptide is highly active against gram-positive strains such as *Bacillus megaterium* ATCC14581.

Such findings suggest that the structure of hydramacin-1 is a good starting point for designing antibiotics to combat infections in humans caused by pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), the vancomycin-resistant strains of *Enterococcus faecium* and *Enterococcus faecalis*, and ESBL strains of *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

We screened *Hydra* tissues for other agents with antimicrobial activity. For example, in addition to peptides, a serine protease inhibitor that we designated kazal-2 is expressed in endodermal gland cells. It inhibits growth of *S. aureus* by blocking a specific serine protease of this gram-positive bacterium. Thus, kazal-2 and similar protease inhibitors might constitute a new class of antibiotics that, once optimized, could become highly effective against staphylococcal pathogens.

Finding this antimicrobial serine protease inhibitor in *Hydra* sheds new light on host defense mechanisms that developed early during metazoan evolution. Meanwhile, other findings from our lab suggest not only that antimicrobial peptides from *Hydra* can kill particular bacteria, but that they can help to shape the composition of the microbiota. For instance, overexpressing an endogenous antimicrobial peptide reduces overall bacterial levels while also drastically changing the resident microbial biodiversity. Thus, antimicrobial peptides apparently took part in the molecular conversation between bacteria and this host species during its evolution.

Hydra Epithelial Cells Select and Modulate Microbiota

The human intestine (Fig. 1E) is colonized by a complex and dynamic community of microorganisms that support a variety of functions. The stepwise microbial colonization of the intestine begins at birth and continues during the early phases of life, forming a microbiota that differs from one individual to another.

Bosch: from the Classics to Classical Music, amid a Steadfast Focus on *Hydra*

Thomas Bosch spent his early years in Augsburg, Germany, immersed in the classics under the tutelage of Benedictine monks at a boarding school within a monastery, the same school his father had attended—and with some of the same teachers. Although science played only a minor role in the curriculum, "somehow I always felt attracted to biology, to animal behavior, and to nature," he recalls. "The monks supported that and allowed me to spend most of my spare time in the monastery's wonderful garden, breeding guinea pigs—which I sold to the pathology department in the city to earn some pocket money—bees, and roses. I enjoyed the spirit of the school greatly."

Bosch, 54, now is professor of zoology and director of the zoological institute of the Christian-Albrechts University of Kiel, where he studies molecular mechanisms controlling the development and differentiation of the cnidarian *Hydra*. "Our data suggest that ancient organisms, such as *Hydra*, hold promise for detecting novel antimicrobial molecules and treating infections caused by multi-[drug] resistant bacteria," he says.

Bosch, many of whose ancestors were dentists—his father was one—or physicians, did not begin his formal studies of biology until he went to college. As an undergraduate at the University of Munich, he won a one-year scholarship to the University College of Swansea in Wales. There he learned English, which proved a valuable addition to his earlier devotion to Latin, Greek, and philosophy. He also grew increasingly fascinated with biological systems, after being assigned to conduct research in a lab. This exposure to experimental science was unlike his experiences in Germany, where undergraduates "sit in large classes and work on ready-made school experiments," he says.

Bosch disliked the research project itself because it involved "isolating parasites from seagull feces kept in cages, and collecting ectoparasites from fish while being seasick on a research vessel," he says. Nevertheless, "I started to realize that organisms live together often in very complex and not-yet-understood relations." For example, he continues, "parasites have to take care that they do not kill the host too early—and the host has to develop defenses against the parasites. In some cases, parasites are not really harmful, and hosts seem even to like and attract them. Why? How did associations of different organisms of different origin and complexity co-evolve? Why are certain microbes associated with certain hosts? How do the organisms coordinate their interactions at the molecular level? That is, what is the molecular language in this 'inter-kingdom' communication? These are among the central questions of my research today."

When Bosch returned to the University of Munich, many classes that interested him no longer were available, with one exception. While he was abroad, the university had hired a professor for zoology and developmental biology, Charles David, from the Albert Einstein College of Medicine in New York. While conducting postdoctoral research at the Max Planck Institute in Tübingen, Germany, David "had been introduced to a simple animal model for understanding pattern formation and development: the freshwater polyp *Hydra*," Bosch says. "When I returned from the U.K. to Munich, Professor David's class was the only one not overbooked—simply because nobody knew him at that time. So I started working with him and spent the most influential years of my academic education in his lab."

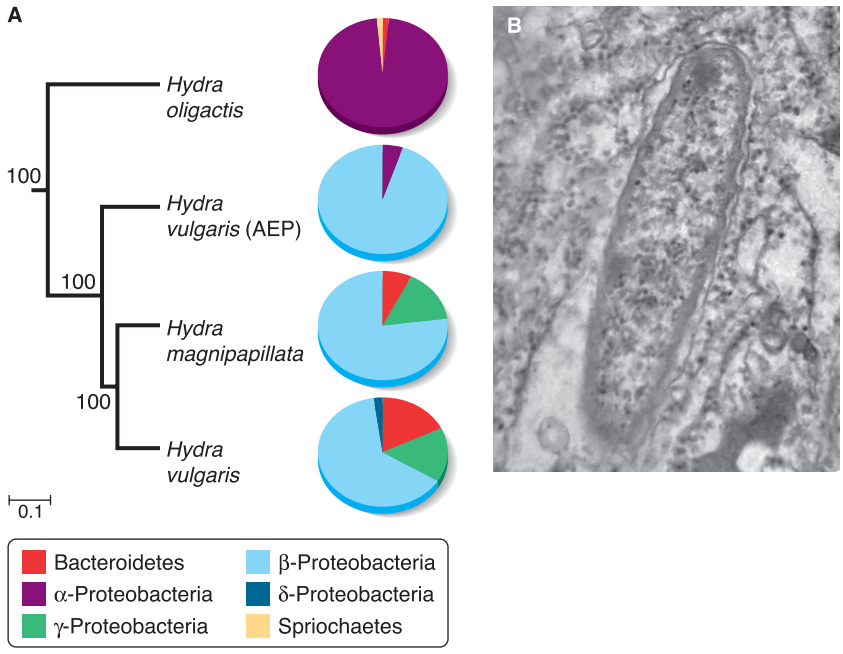
Bosch finished his undergraduate work in 1983 and earned his Ph.D., also from the University of Munich, in 1986. He spent 1986–88 as a postdoctoral fellow at the University of California, Irvine doing research at its developmental biology center. "The [center] was housed in barracks and containers outside the main campus," he recalls. "The entrance hall, with shaky doors, served also as a seminar room where, at lunch time, world-famous scientists were giving talks on a regular basis. Space was very limited. It was a good example of how to establish a truly interactive environment. The structure of my lab in Kiel recalls that experience at least to some extent."

Bosch says that his current life leaves little room for non-work activities. He does enjoy traveling to deliver talks and papers, teach, and to develop scientific collaborations. For example, he has traveled many times to Russia during the last 20 years, visits he describes as "something special," particularly in watching "the transition from the former Soviet state into the Russia of today."

He and his wife, a veterinarian, have been married 23 years, and have an 18-year-old daughter. "When time allows—and my wife has organized the tickets—an evening in a classical concert is wonderful," he says. "Very rarely, I play the piano by myself at home. And since we live in a countryside house, nature is always near when I am at home."

Marlene Cimons
Marlene Cimons is a freelance writer in Bethesda, Md.

FIGURE 2



Hydra polyps are colonized by species specific microbiota. (A) Bacterial communities identified from different *Hydra* species. (B) Transmission electron microscopy of bacterial endosymbiont in the cytoplasm of an ectodermal epithelial cell of *Hydra oligactis*. (Panel B modified from S. Fraune and T. C. G. Bosch Proc. Natl. Acad. Sci. USA **104:**13146–13151, 2007.)

Similarly, a complex and dynamic community of microbes colonizes the *Hydra* epithelium and, here also, individuals from different *Hydra* species differ greatly in their microfauna (Fig. 2). Moreover, the microbiota of individual *Hydra* that were kept for years under controlled conditions are similar to the microbiota of individual polyps that are more recently isolated from lakes. This microbial continuity suggests that the colonizing bacteria become resident species and are not mere "tourists" passing through individual *Hydra* along with food, water, and other environmental components. The microbiota differences among *Hydra* species and their maintenance over long periods suggest that the *Hydra* epithelium imposes selective pressures on microorganisms, thus shaping this microbial community.

Meanwhile, environmental conditions can modulate bacterial communities that associate with particular types of *Hydra*. For example, when we cultured polyps of *H. oligactis* from the wild, that shift to laboratory conditions had drastic effects on the composition of its bacterial community. In particular, α-Proteobacteria remain the dominant species following long-term culture. However, other bacteria gradually disappear from the tissue collected in the wild following the shift to growth in the lab. Thus, *Hydra* not only is associated with specific symbiotic bacteria but that microbiota responds to changes in environment. Those environmentally responsive differences within microbiota fall within a narrow range, and, even under long-term culture, the bacterial communities from different *Hydra* species remain very different from one another. Thus, each *Hydra* species appears to select its own specific microbial community.

Associated Microbiota Influence and Benefit *Hydra* Hosts

Depleting microbiota damages the health and well-being of *Hydra* as well as mammals. Humans or other mammals lacking in intestinal microbiota, for example, tend to develop severe immune-response defects. Moreover, host genetic defects in immune responses along the epithelial layer can predispose individuals to inflammatory diseases. Although detailed explanations for these phenomena are lacking, there is little doubt that symbiotic bacteria help to maintain the balance between health and disease for their hosts.

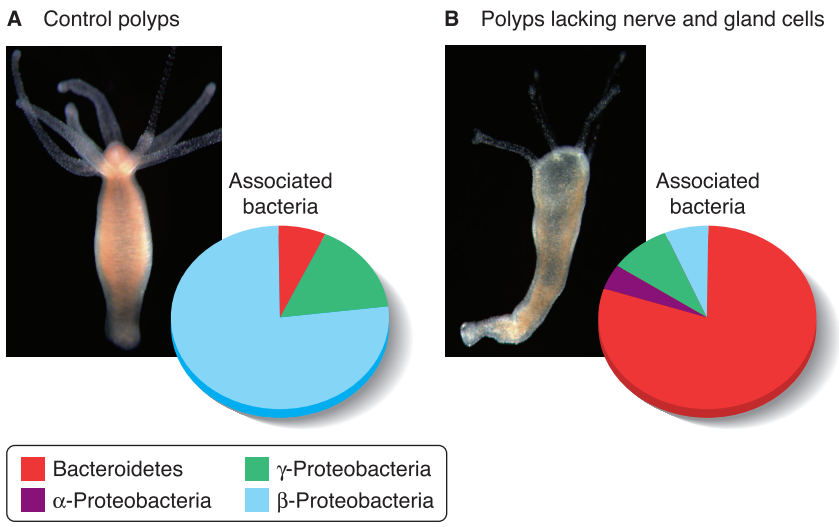
Bacteria exert other profound effects on *Hydra*. For instance, microbiota-free *Hydra* cannot proliferate asexually by budding, according to findings reported in 1982 by Menachem Rahat and Chanan Dimentman at the University of Jerusalem in Israel. However, reinoculating such *Hydra* with medium containing bacteria from standard cultures of this freshwater animal restores its capacity to bud. Thus, resident microbiota strongly affect development in *Hydra*, albeit through mechanisms that we do not yet understand.

Although microbial imbalances underlie many human diseases, little is known about how epithelial homeostasis affects associated

microbial community structures. *Hydra* provides an opportunity for experimentally approaching some of these issues. For example, the mutant strain sf-1 of *Hydra magnipapillata* produces temperature-sensitive interstitial stem cells. When this mutant is held for several hours at the restrictive temperature, it loses its entire interstitial cell lineage. However, both the ectodermal and endodermal epithelial cells remain undisturbed, and the morphology of the mutant and the integrity of its epithelium remain intact (Fig. 3B).

Meanwhile, these changes in cell composition lead to significant changes in the *Hydra*-associated microbial community. In particular, two bacterial phylotypes change drastically following the temperature shift. The ordinarily dominant bacterial phylotype of β-Proteobacteria decreases in the temperature-shifted polyps that lack the usual interstitial cells and their derivatives. Following the shift, a bacterial phylotype belonging to Bacteroidetes increases (Fig. 3). Thus, the epithelia and microbiota apparently interact directly.

FIGURE 3



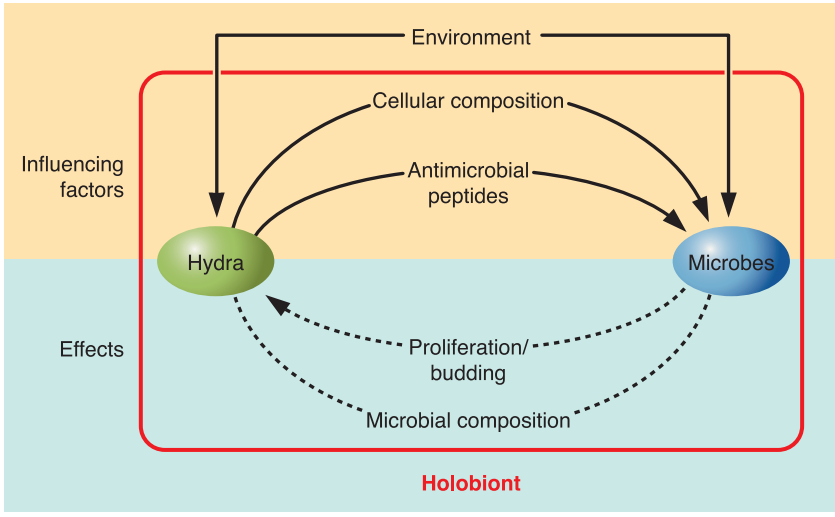
Disturbed tissue homeostasis affects the associated bacterial community. (A) Control polyp with its associated bacterial community. (B) Polyp lacking nerve and gland cells with its associated bacterial community.

What *Hydra* Offer to Our Understanding of Superorganisms

The numbers of symbiotic microorganisms and their aggregate genetic information far exceed the genetic information encoded by humans or other host animals. The human body thus belongs to its own diverse ecosystem, making it a superorganism. Because such communities formed long ago, microbes play important roles not only during the lives of individual hosts, but also while each of those host species evolved.

The holobiont, which consists of the host and its entire set of symbiotic microbiota, should be considered the entity that is subject to selective forces during evolution, according to Eugene Rosenberg at Tel Aviv University in Israel and his collaborators. Nonetheless, little is known about the part played by the microbiota and how microorganisms affect any species during evolution (Fig. 4). How do associated organisms coordinate their interactions at the molecular level? How do the genomes within a superorganism co-evolve? How do microorganisms affect their hosts and vice versa?

FIGURE 4



Schematic model of the *Hydra* holobiont.

Although probing these complex interactions remains a challenging task, *Hydra* with its epithelial defense mechanisms provides a particularly suitable model for approaching some of these questions. These organisms carry many of the same gene families found in bilaterians and retain many genes that were lost from *Drosophila* and *Caenorhabditis elegans*.

Because *Hydra*-associated microbial communities are so complex, fulfilling this analytic challenge will not be easy. Indeed, such communities are distinct from those in the surrounding water, are specific for each *Hydra* species, are spatially and temporally stable, and also likely play multiple roles in their interactions with the *Hydra* host. Disturbing the balance between *Hydra* and its colonizing microbiota appears to foster disease. Characterizing these host-microbe interactions therefore will provide both fundamental and applied insights into the common ancestor of *Hydra* and other animals as well as how symbiotic bacteria contribute to the balance between health and disease in their hosts.

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