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Animals in a bacterial world: a new imperative for the life sciences

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In the past two decades, the widespread application of genetic and genomic approaches has revealed a bacterial world astonishing in its ubiquity and diversity. This review examines how a growing knowledge of the vast range of animal-bacterial interactions, whether in shared ecosystems or intimate symbioses, is fundamentally altering our understanding of animal biology. Specifically, we highlight recent technological and intellectual advances that have changed our thinking about five questions: how have bacteria facilitated the origin and evolution of animals; how do animals and bacteria affect each other's genomes; how does normal animal development depend on bacterial partners; how is homeostasis maintained between animals and their symbionts; and how can ecological approaches deepen our understanding of the multiple levels of animal-bacterial interaction? As answers to these fundamental questions emerge, all biologists will be challenged to broaden their appreciation of these interactions and to include investigations of the relationships between and among bacteria and their animal partners as we seek a better understanding of the natural world.

inflammation | B cell | T cell | type 2 diabetes | obesity

Biologists have long appreciated the roles that microbes play in the two distinct disciplines of pathogenesis and ecosystem cycling. However, it wasn't until the late 1970s that Carl Woese and George Fox opened a new research frontier by producing sequence-based measures of phylogenetic relationships, revealing the deep evolutionary history shared by all living organisms (1). This game-changing advance catalyzed a rapid development and application of molecular sequencing technologies, which allowed biologists for the first time to recognize the true diversity, ubiquity, and functional capacity of microorganisms (2). This recognition, in turn, has led to a new understanding of the biology of plants and animals, one that reflects strong interdependencies that exist between these complex multicellular organisms and their associated microbes (3).

While the biosphere comprises many diverse taxonomic groups, our focus here is principally on the interactions between one group of microorganisms, the domain Bacteria, and one group of complex multicellular organisms, the animals. Although we chose to focus on animal-bacterial interactions, we expect the application of new technology to reveal similar trends among

and between Archaea, fungi, plants, and animals. We begin by describing what we know about the evolution of animals and their interactions with bacteria, and about the influence that these relationships have had on the present-day genomic makeup of the partners. We review the wealth of new data on the roles of bacteria in animal development and physiology, and conclude with a discussion of the nesting of animal-bacterial relationships within their larger ecological frameworks. We argue that interactions between animals and microbes are not specialized occurrences, but rather are fundamentally important aspects of animal biology, from development to systems ecology.

In addition to the references of the main text of this article, we include a list of useful citations to provide the reader a broad opening to the subtopics covered in this contribution (SI References).

Bacteria and the Origin of Animals

Understanding how associations among bacteria and animals first evolved may reveal the foundations of ecological rules that govern such interactions today. Animals diverged from their protistan ancestors 700-800 million years ago, some three billion years after bacterial life originated and as much as a billion years after the first appearance of eukaryotic cells (4) (Fig. 1). Thus, the current-day relationships of protists with bacteria, from predation to obligate and beneficial symbiosis (5, 6), were likely already operating when animals first appeared. Attention to this ancient repertoire of eukaryote-bacterial interactions can provide important insights into larger questions in metazoan evolution,

Reserved for Publication Footnotes

Fig. 1

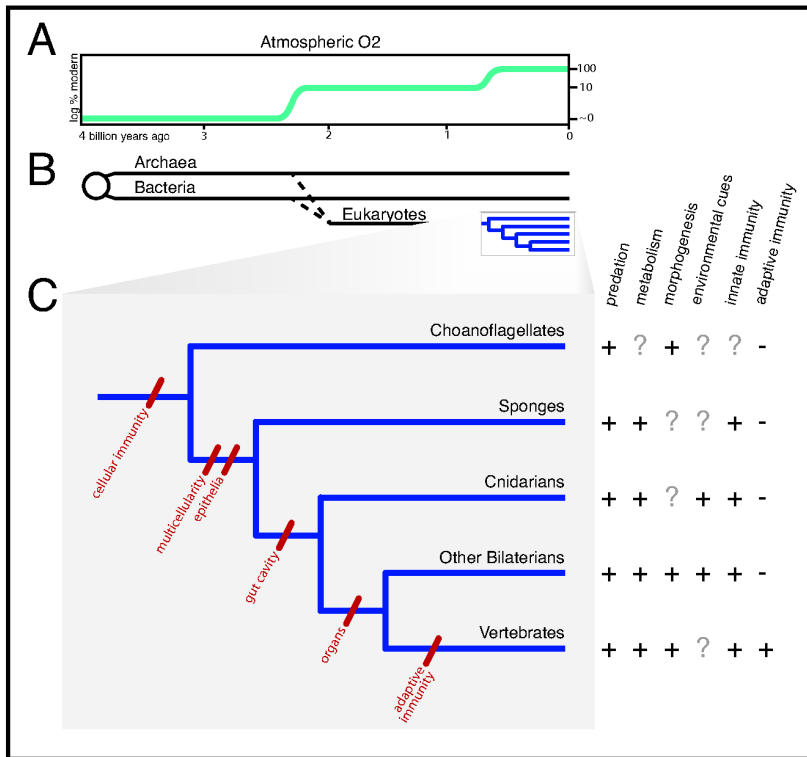


Fig. 1. Animals through time. **A.** Upper, atmospheric oxygen concentration, as a percent of current levels, plotted against geological time. **B.** The phylogenetic history of life on Earth, scaled to match the oxygen timeline. Note that the origin of the eukaryotes and the subsequent diversification of animals both correspond to periods of increasing atmospheric oxygen. **C.** Left, a phylogeny of choanoflagellates and selected animals, annotated to indicate the evolution of characters particularly relevant to interactions with bacteria. Right, interactions between bacteria and eukaryotes, corresponding to the phylogeny. Bacteria are prey, sources of metabolites, inducers of development in symbiosis (morphogenesis) and in larval settlement (environmental cues), and activators of immune systems.

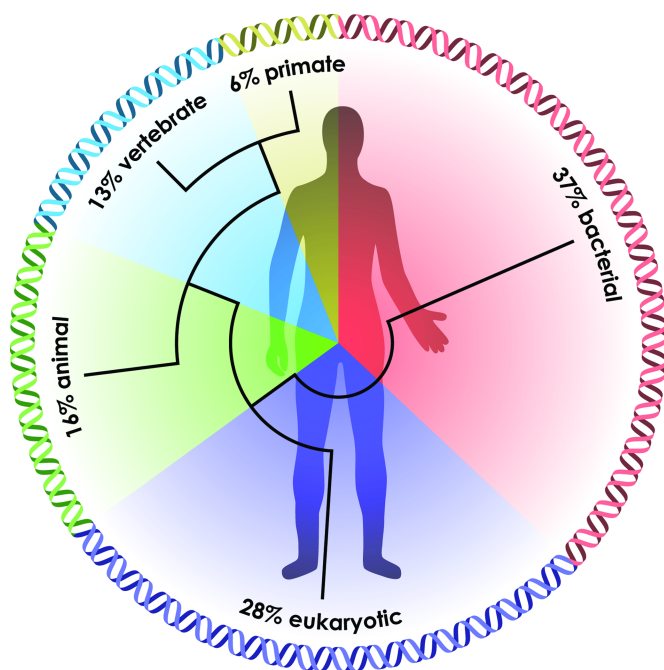


Fig. 2. The ancestry of humans reflected in the genomic signature. A phylogenetic analysis of the human genes reveals the relative percentage of the genome that arose at a series of stages in biological evolution.

from the origins of complex multicellularity to the drivers of morphological complexity itself.

Based on molecular and cellular data, animals and choanoflagellate protists are now considered sister groups,

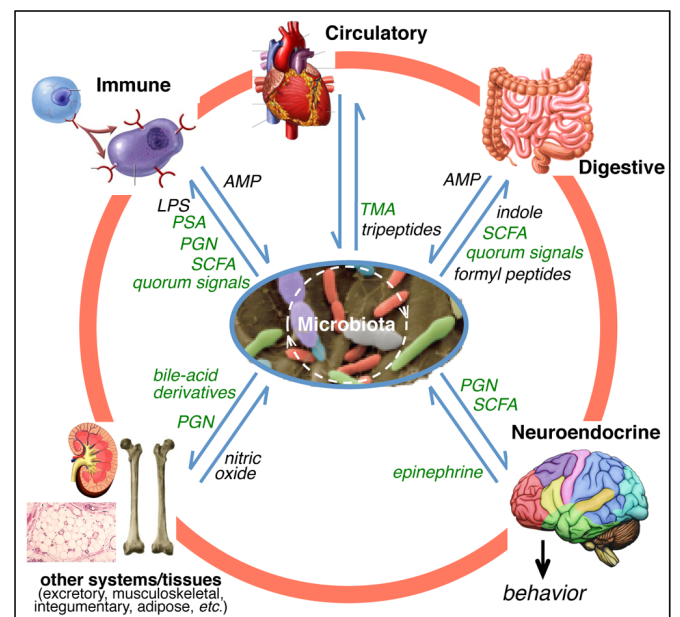


Fig. 3. Signaling within and between the animal and its microbiota. Members of the microbiota, such as those in and on the gut, oral cavity, and skin, communicate amongst themselves, and exchange signals with the animal's organ systems, participating in the body's homeostasis. Some of the signals promoting this balance are mentioned in the text (green), while other representatives are not (black; Table S1). The microbiota also influence animal behavior, creating a direct interface with other organisms. AMP, antimicrobial peptides; LPS, lipopolysaccharide; PGN, peptidoglycan; PSA, polysaccharide A; SCFA, short-chain fatty acids; TMA, trimethylamine oxide.

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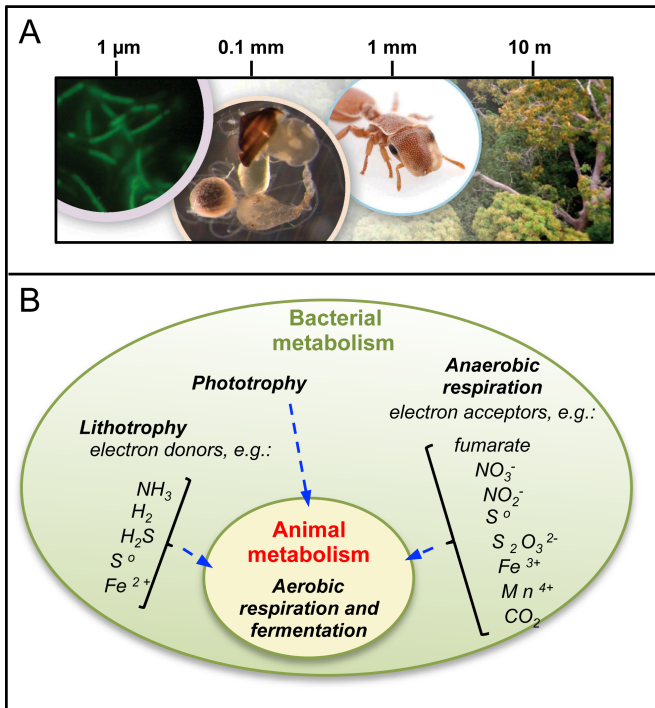


Fig. 4. Nested ecological interactions of animals and bacteria and their underlying metabolic bases. **A.** A forest canopy insect illustrates the cascading effects of animal-bacterial interactions across multiple spatial scales. Bacterial symbionts (left), residing in the gut (middle, left), are essential to nutritional success of insect species (middle, right) in tropical forest canopies (right), where they often make up a majority of animal biomass. **B.** Diversity of energy metabolism in bacteria and animals. Animals can ferment and aerobically respire, but are unable to perform the vast diversity of other, ecologically vital, energy-harvesting processes. Beyond phototrophy, which they share with plants, bacteria can also contribute to primary production by using inorganic energy sources (lithotrophy) to fix CO_2 . Animals are directly or indirectly dependent on bacteria for extracting energy and cycling biomolecules, while animals actively contribute to bacterial productivity through bioturbation, nutrient provisioning, and as habitats for colonization and shelter.

descended from a common choanoflagellate-like ancestor (Fig. 1) (7). The major underpinnings of animal-bacterial interactions – nutrition, recognition, cell adhesion, and signaling – guide two types of choanoflagellate behavior that may have been key to the origin of animals: predation (8) and colony formation (9). Extant choanoflagellates have homologs of animal signaling and adhesion proteins (e.g., cadherins and C-type lectins) that may have arisen as critical facilitators of bactivory (8). Diverse animals respond to bacterial signals as triggers for morphogenesis or behavior (e.g., larval settlement). Thus, the discovery that at least one choanoflagellate, *Salpingoeca rosetta*, responds to signals from specific bacteria to initiate colony formation through cell division hints at an ancient involvement of bacteria in the initiation of multicellularity (9). It will be important to learn whether intercellular cohesion in sponges, which are known to harbor hundreds of bacterial species (10–12), similarly depends on the presence of bacteria. The origin of multicellularity has been a topic of intense debate in biology, and many hypotheses have been developed about how this evolutionary milestone was achieved (13). A microbial role in animal origins does not obviate other perspectives on the evolution of -complex multicellularity, but adds a necessary functional and ecological dimension to these considerations.

As early animals diversified, animal-bacterial interactions continued to shape evolution in new ways (Fig. 1C). Bacteria took

on a new role in animal nutrition, serving not only as prey, but also as producers of digestible molecules in the animal gut. This role may have become more diverse with the evolution of a tubular gut, with one-way passage of food from mouth to anus. Bacterial influence on gut evolution certainly intensified with the subsequent origin of the coelom, a body cavity in which the organs are suspended. The advent of the coelom made gut elongation and regional specialization possible, facilitating both massive ingestion and storage for later digestion. Although the degree to which microbes have driven gut evolution is unknown, the radiation of several animal groups (e.g., ruminants) was undoubtedly enabled by alliances with their gut-associated microbiota. The evolution of form and function in other organ systems (e.g., respiratory, urogenital) may have also been influenced by interactions with bacterial partners (14). Furthermore, it is likely that the evolution of these organ-system niches drove radiation of particular clades of animal-associated bacteria (15), such as the genus *Helicobacter* in vertebrate guts (16).

Evolution with animals, whether in symbiosis or via shared habitats, has also influenced the distribution and diversification of bacteria. For example, 90% of the bacterial species in termite guts are not found elsewhere (17). Such specialization, while increasing efficiency, comes with a cost: for every animal species that goes extinct, an unknown number of unique bacterial lineages that have evolved to depend on this animal niche disappear as well (18). On a broader scale, the evolution of animals provided novel physical environments for bacterial colonization, such as aerated deep sediments resulting from animal burrowing. Finally, human activities, which make a range of molecules not previously found in nature, such as halogenated hydrocarbons, have driven selection on bacterial catabolic pathways (19), leaving a signature of our presence in microbial metabolism.

Intertwining Genomes

The long history of shared ancestry and alliances between animals and microbes is reflected in their genomes. Analysis of the large number of full genome sequences presently available reveals that most life forms share approximately one third of their genes, including those encoding central metabolic pathways (20). Not surprisingly, many animal genes are homologs of bacterial genes, mostly derived by descent, but occasionally by gene transfer from bacteria (21). For example, 37% of the ~23,000 human genes have homologs in the Bacteria and Archaea, and another 28% originated in unicellular eukaryotes (20) (Fig. 2). Among these homologous genes are some whose products provide the foundation for signaling between extant animals and bacteria (22).

The intertwining of animal and bacterial genomes is not just historical: by co-opting the vastly more diverse genetic repertoire present in its bacterial partners (23), a host can rapidly expand its metabolic potential, thereby extending both its ecological versatility and responsiveness to environmental change. For instance, many invertebrates have intracellular bacterial symbionts whose genes encode metabolic capabilities lacking in animals, such as the synthesis of essential amino acids (24), photosynthesis (25), or chemosynthesis (26). Certain marine invertebrates that feed on algae maintain algal plastids as photosynthetically active ‘symbionts,’ a behavior that allows the host to use photosynthate as a food source for extended periods (27). These metabolic ‘add-ons’ allow the animal to thrive by adapting to otherwise non-competitive lifestyles (e.g., feeding on nutrient-poor diets such as plant sap) (28) or environments (e.g., oligotrophic habitats) (26). Further, such phenomena fit the definition of epigenetic features. Recent studies have revealed that bacterial pathogens (29) and other environmental factors (30) can alter the activities of epigenetic machinery. It is to be anticipated that such influences will extend to all types of animal-bacterial interactions, including those described above.

409 Microbial communities in the vertebrate gut respond to the
410 host diet over both daily and evolutionary time scales, endowing
411 animals with the flexibility to digest a wide variety of biomolecules
412 and cope with and even flourish under conditions of diet change
413 (15, 31). For example, the gut microbiome of most people in
414 the United States is adapted to digest a high fat, high protein
415 diet, while populations in rural Malawi and the Amazonas of
416 Venezuela have distinct microbial consortia and functional gene
417 repertoires optimized for breaking down complex carbohydrates
418 (32). The gut microbiome adapts to changing diets and conditions
419 not only by shifting community membership, but also by changing
420 gene content via horizontal gene transfer. For instance, the gut
421 bacterium *Bacteroides plebeius*, found in some Japanese people,
422 bears a gene transferred horizontally from the marine bacterium
423 *Zobellia galactanivorans*, giving the gut symbiont the capacity to
424 degrade seaweed polysaccharides (33). More generally, human-
425 associated bacteria have a 25-fold higher rate of gene transfer
426 than do bacteria in other environments, highlighting the impor-
427 tant role of gene transfer in host-associated bacterial commu-
428 nities (34).

429 Bioinformatic analyses have revealed that interactions with
430 animals also influence the size and content of the genomes of their
431 bacterial partners. Although not all genome-size reduction occurs
432 in symbiosis, a long history of intimate association with insects
433 has resulted in highly reduced genomes in their intracellular
434 symbionts; for example, the endosymbiont *Candidatus* Hodgkinia
435 cicadicola of the Arizona cicada has a genome size <144 kilobase
436 pairs, smaller than that of some organelles (35). Recent studies
437 have shown that genome reduction also occurs in segmented
438 filamentous bacteria (*Candidatus* Savagella), members of the
439 mammalian microbiota that are critical for the maturation of the
440 immune system (36). Conversely, in *Bacteroides thetaiotaomicron*,
441 another member of the mammalian intestinal microbiota, adapta-
442 tion to a gut habitat rich in complex carbohydrates has driven the
443 expansion of at least two gene families: glycan-utilization genes,
444 which constitute 18% of this species' genome (37); and diverse
445 sulfatases that allow *B. thetaiotaomicron* to digest host mucin
446 (38). The genomic basis for other microbial adaptations among
447 gut microbes is less clear. One possible selection pressure is host
448 temperature. In aquatic environments such as the deep sea, host
449 fishes and invertebrates conform to the temperature of the en-
450 vironment, so temperature-driven coevolution would be unlikely
451 in these habitats. In contrast, terrestrial environments often have
452 broad, short-term (daily) and long-term (seasonal) fluctuations in
453 temperatures. It is in these habitats that endothermy (maintaining
454 a constant body temperature by metabolic means) evolved as a
455 shared character in birds and mammals. Most enteric bacteria of
456 birds and mammals have growth optima at ~40 °C, suggesting
457 the unexplored possibility that this trait resulted from coevolution
458 of these bacteria with their endothermic hosts. The reciprocal
459 may also be true, i.e., an animal's microbial partners may have
460 played a role in selecting for the trait of endothermy. Constant
461 high temperature speeds up bacterial fermentation, providing
462 rapid and sustained energy input for the host. These benefits are
463 apparent when comparing conventional to germ-free mammals,
464 which require 1/3 more food to maintain the same body mass
465 (39). Keeping their microbes working at optimum efficiency likely
466 offered a strongly positive selection pressure for the evolution
467 of genes associated with the trait of endothermy in birds and
468 mammals.

469 Partners in Animal Development

470 Animal development has traditionally been viewed as an
471 autonomous process directed by the genome. Because it both
472 originated and evolved in a microbe-rich environment, animal
473 development deserves a re-examination, at least in part, as an or-
474 chestration of animal-encoded ontogeny and inter-domain com-
475 munication (40, 41). Although relatively few studies have been

477 reported until recently, these early data lead us to anticipate that
478 microbes play a role in providing signals for multiple developmen-
479 tal steps.

480 From their earliest stages of development, animals employ
481 sophisticated mechanisms to manage their microbial environ-
482 ment. Physical barriers, such as capsules, chorions, and mucus
483 protect eggs by excluding microbes, and chemical barriers, in-
484 cluding antimicrobial peptides (AMPs), shape the composition
485 of the associated microbiota (42). Conversely, several animals
486 recruit specific bacteria to their embryonic surfaces to provide
487 protection against potential pathogens (43). For example, the
488 shrimp *Palaemon macrodactylus* is protected from the fungus
489 *Lagenidium callinectes* by 2,3-indolinedione that is produced by
490 an *Alteromonas* sp. on the embryo's surface (44). Although many
491 animals, including a wide variety of insects, have transovarial (i.e.,
492 via the egg to the embryo) transmission of bacterial partners (28,
493 45), we have no persuasive evidence to date that these microbes
494 or their metabolites influence embryogenesis. While develop-
495 mentally important symbioses have been documented throughout
496 the postembryonic (larval and juvenile) stages of vertebrate and
497 arthropod life cycles, the roles of symbiotic microbes during
498 normal embryonic development are just beginning to be stud-
499 ied. Unlike vertebrates whose embryos develop inside enclosures
500 that physically block bacterial associations, many invertebrates
501 acquire their symbionts through the female germ line. Here, we
502 may expect to find regulatory signals being generated by microbes
503 and interactions between host and symbiont development (46). It
504 is apparent that evolution has selected for anatomical, cellular,
505 and molecular determinants that act during this period to prepare
506 newborn animals for interactions with the microbial world.

507 Ample evidence shows that microbes act directly as agents of
508 post-embryonic development. For example, fucosyltransferases
509 decorate the surface of the embryonic mammalian intestine with
510 fucose residues that provide a nutrient source for gut microbes,
511 including *B. thetaiotaomicron*, as they colonize the newborn (47).
512 In the squid-vibrio system, a complex organ forms during embryo-
513 genesis that facilitates subsequent colonization by the symbiotic
514 bacterium *Vibrio fischeri* (48). The products of horizontally ac-
515 quired microbes can be essential for a range of developmental
516 functions, including influences on larval growth rate and body size
517 in invertebrates (49), postembryonic maturation and renewal of
518 epithelia in invertebrates and vertebrates (50-53), development
519 and specification of the gut-associated lymphoid tissues in ver-
520 tebrates (54), activation of the immune system in tsetse flies (55)
521 and normal brain development in mammals (56, 57). Intriguingly,
522 the host regulatory pathways that control immune responses to
523 microbes appear also to have central roles in animal development,
524 underscoring the intimate relationships between development
525 and host-microbe interactions (58, 59).

526 Perhaps the most pervasive example of microbial signaling in
527 animal development is the induction of settlement and metamor-
528 phosis of many marine invertebrate larvae (60). This transition
529 is an absolute requirement for completion of the animal's life
530 cycle and is contingent upon induction by exogenous morpho-
531 genetic cues, many of which are produced by bacteria associated
532 with a particular environmental surface (60). Marine invertebrate
533 metamorphoses offer valuable models for exploring the basis of
534 bacterial signaling in animal development in a setting where the
535 very persistence of marine ecosystems depends upon it.

536 Coming full circle, the influence of microbes on animal repro-
537 duction can be observed with particular clarity in invertebrates
538 (61). Most insect orders carry vertically transmitted parasites
539 that can affect the processes of sexual determination, matura-
540 tion, and reproductive success. For example, various *Wolbachia*
541 strains feminize crustacean genetic males, kill males, or induce
542 clonal production of females in some insects (62). However, in
543 one case, the association with a *Wolbachia* strain has become
544

545 essential for reproduction; the wasp *Asobara tabida* requires
546 this microbe for egg formation (63). Recent studies have shown
547 that, in both invertebrates and vertebrates, the microbiota can
548 even influence reproductive behavior (64). Changes in cuticular-
549 hydrocarbon profiles linked to specific bacterial symbionts in
550 the gut of *Drosophila melanogaster* correlate with mate choice
551 (65), and several lines of evidence suggest that olfactory cues
552 associated with mate choice in vertebrates are produced by their
553 resident microbiota (66).

554 Inter-Domain Communication

555 Although animals and bacteria have different forms and
556 lifestyles, they recognize one another and communicate in part
557 because, as described above, their genomic 'dictionaries' share a
558 common and deep evolutionary ancestry. One modality of inter-
559 domain communication, that occurring during bacterial patho-
560 genesis, has been extensively explored for over a century. But how
561 might bacterial signaling structure the biology of the healthy host?

562 Biologists now know that bacteria have social behaviors,
563 communicating with each other through chemical signaling, such
564 as quorum sensing (67, 68); more recently, inter-domain quorum
565 signaling between bacteria and their eukaryotic partners
566 has become evident (22, 69-71). In addition to quorum signals,
567 bacteria use cell surface-derived molecules to communicate with
568 their hosts, affecting host processes both at the cellular level [e.g.,
569 apoptosis, toll-like receptor (TLR) signaling (52, 72)], as well
570 as at the organ-system level (Fig. 3). Conversely, host-derived
571 signal molecules like nitric oxide (NO) can be sensed directly by
572 microbes (73). It is intriguing to consider that these kinds of com-
573 munication evolved to maintain an association's balance with its
574 hundreds of beneficial species, and that pathogens have 'hijacked'
575 these conversations to enhance their fitness through disease.
576 For example, *Salmonella typhimurium* has adapted the quorum-
577 sensing regulator QseC to act as a receptor for the host hormone
578 norepinephrine and, thereby, tie the regulation of virulence genes
579 to the hormone's presence in the tissue (74). Some hosts, such
580 as the marine macroalga *Delisea pulchra*, respond to quorum-
581 signaling pathogens by producing halogenated furanones that act
582 as signal mimics, blocking the microbes' communication (75).

583 The gut is likely the site of the most dynamic and conse-
584 quential bacteria signaling that benefits animal hosts, because
585 of the sheer numbers and diversity of its microbes and the in-
586 herent permeability and sensitivity of the gut epithelium. For
587 example, acetate, a short-chain fatty acid (SCFA) produced by
588 the gut bacterium *Acetobacter*, stimulates insulin signaling in
589 *Drosophila melanogaster*, thereby promoting host growth rates
590 and reducing sugar and lipid levels (49). In mammals, SCFAs
591 affect fat deposition, appetite-related hormone titers, and food
592 consumption, which in turn can modulate the composition of the
593 microbiota, and have major consequences for health and behavior
594 (76, 77). Not surprisingly, the composition of the gut microbiota,
595 and its SCFA production, are influenced by diet. The resultant
596 interplay among diet, the microbiota and their metabolites is, in
597 turn, implicated in the development of major metabolic disorders
598 including obesity and diabetes (78). As much as a third of an
599 animal's metabolome – e.g., the diversity of molecules carried in
600 its blood – has a microbial origin; thus, the circulatory system
601 extends the chemical impact of the microbiota throughout the
602 human body (79), transporting metabolites that influence the
603 physiology and metabolism of distant organs and, perhaps, other
604 bacterial communities (80, 81). Some dietary constituents can
605 be modified by gut microbiota into deleterious compounds; for
606 example, the conversion of dietary phosphatidylcholine into the
607 pro-atherosclerotic metabolite, trimethylamine, can jeopardize
608 cardiovascular health (82). Furthermore, recent studies link the
609 gut microbiota to brain physiology and animal behavior (83).
610 For instance, germ-free mice have defects in brain regions that
611 control anxiety (57), and feeding probiotic bacteria to normal

612 mice reduces depression-like behaviors (84, 85). The finding that
613 toll-like receptors, which transduce bacterial signals to host cells,
614 are present on enteric neurons reveals one mechanism by which
615 microbiota can communicate with the central nervous system
616 through the brain-gut axis (72). Thus, maintaining homeostasis
617 with the normal microbiota is essential to a healthy nervous
618 system.

619 As the guardian of an animal's internal environment, its
620 immune system coordinates cellular and biochemical responses to
621 alterations in the molecular landscape (86, 87), creating a robust
622 equilibrium between the healthy host and its normal microbiota.
623 The complexity of components that comprise this system re-
624 flects the great chemical diversity present in the microbial world.
625 Pattern-recognition receptors (PRRs) of the innate immune sys-
626 tem can have enormous repertoires, particularly in the inverte-
627 brates. PRRs recognize microbe-associated molecular patterns
628 (MAMPs), such as bacteria-specific cell surface molecules (88).
629 For example, peptidoglycan (PGN), a cell-wall constituent of
630 bacteria, interacts with PRRs to induce developmental processes
631 in vertebrates and invertebrates (52, 54). The gut-associated
632 lymphoid tissues of mammals mature with the presentation of
633 peptidoglycan monomer by the gut microbiota during their early
634 establishment, and the same molecule induces the regression of
635 a juvenile-specific epithelium that facilitates colonization by the
636 symbiont in the squid-vibrio system. Similarly, a polysaccharide
637 produced and exported by *Bacteroides fragilis*, a constituent of the
638 normal microbiota, signals the PRRs of immune cells to suppress
639 gut inflammation (89). Disturbance of equilibria maintained by
640 MAMP-PRR interactions can lead to a wide variety of pathologic
641 states, including inflammatory bowel disease and diabetes (90,
642 91). Further, SCFAs produced by gut bacteria help the host de-
643 fend against enteric infections (92), revealing molecular symbiosis
644 between the microbiota and the immune system. Finally, immu-
645 nologists are beginning to examine the possibility that, in addition
646 to a role in pathogenesis, a principal selection pressure acting on
647 the form and function of the adaptive immune system is the need
648 to maintain balance among the complex, coevolved consortia that
649 form persistent symbioses with the mucosal surfaces of several
650 organ systems in the vertebrate host (86, 93-95).

651 Nested Ecosystems

652 Since the dawn of metazoan evolution, the ecology of animals
653 has depended on bacterial communities. The fossil record pro-
654 vides evidence that some animal forms in the Ediacaran grazed
655 on dense assemblages of bacteria on hard substrates (96) and that
656 burrowing animals originated in association with microbial mats
657 (97). Biologists increasingly recognize that, in extant animals,
658 developmental and physiological signaling are processes whose
659 understanding benefits from an ecological perspective (98).

660 Viewing animals as host-microbe ecosystems has given us new
661 insights into the maintenance of human health. The application of
662 ecological approaches, including successional assembly and diver-
663 sity analysis, has proven valuable in understanding how animal-
664 microbial alliances function (99-101). For example, human in-
665 fants born vaginally have a very different succession during the
666 early phases of gut colonization and, possibly, long-term com-
667 position of their microbiota than those delivered by Caesarean
668 section (102). The effects of this difference in infant delivery on
669 adult health remain to be discovered. We know that imbalances
670 in the mature human microbiome have been correlated with a
671 spectrum of diseases, including obesity and diabetes (77). A re-
672 cent metacommunity analysis of the gut microbiota of obese and
673 lean twins revealed that obesity is associated with a significantly
674 less stable and more variable microbial community (103). While
675 most research on consortia is currently focused on humans and
676 vertebrate model systems, such as mice and zebrafish, similarly
677 complex interactions occur in all animal species. Viewing bacte-
678 rial colonization of animals as an ecological phenomenon adds
679

681 clarity to an understanding of the mechanisms and routes by
682 which phylogenetically rich and functionally diverse microbial
683 communities become established and evolve on and within animal
684 hosts.

685 An ecological perspective influences not only our understand-
686 ing of animal-microbiome interactions, but also their greater role
687 in biology. The ecosystem that is an individual animal and its
688 many microbial communities [*i.e.*, the 'holobiont', (104)] does not
689 occur in isolation, but is nested within communities of other or-
690 ganisms that, in turn, co-exist in and influence successively larger
691 neighborhoods comprising ever more complex assemblages of
692 microbes, fungi, plants and animals (Fig. 4). Hydrothermal vent
693 communities illustrate the role of animal-microbe associations
694 in such nested ecosystems. At vents and other reducing habi-
695 tats, chemoautotrophic symbionts provide organic nutrients for
696 animal hosts in at least seven different phyla. The activities of
697 these individual symbioses contribute to larger communities that
698 include non-symbiotic animal and microbial species that are able
699 to exist through the symbiotic primary production that is not
700 driven by solar energy but rather by sulfide, hydrogen, methane
701 and other reduced energy sources (26, 105). Similarly, nested
702 within broader terrestrial ecosystems, bacterial communities in
703 floral nectar can influence the way animals such as pollinators
704 interact with plants. In these instances, the bacteria change the
705 chemical properties of the nectar making it more or less attractive
706 to the pollinator, which changes the pollinator-plant dynamic
707 (106).

708 Bacteria are critical determinants of animal population and
709 community structures, even in ecosystems where intimate sym-
710 bioses are not the driving force. Recent studies demonstrate
711 that the larvae of many benthic marine invertebrates require
712 specific microbial cues for their recruitment from the plankton,
713 and these larval responses to bacteria influence the structuring of
714 many marine benthic communities (60, 107). For example, cer-
715 tain strains of the biofilm-forming bacterium *Pseudoalteromonas*
716 *luteoviolacea* produce chemical cues that stimulate settlement
717 and metamorphosis by *Hydroides elegans*, a polychaete worm that
718 fouls docks and the hulls of ships worldwide (60, 108), as well
719 as a sea urchin (109) and a coral (107). Surface biofilms on
720 many marine animals serve important functions in determining
721 the very nature of the animals' ecological interactions with other
722 organisms (110). Similarly, the acquisition of an appropriate
723 microbiome at critical life-history stages of many animals affects
724 their subsequent behavioral patterns and thus the stability of their
725 ecological roles in their communities (64). Bacteria feeding on
726 dead animals in the sea, and likely on land, repel animal scav-
727 engers by producing noxious metabolites; these products allow
728 the bacteria to effectively out-compete organisms 10,000 times
729 their size (111).

730 Conversely, invasive animals can alter the activities of indige-
731 nous bacteria, with significant effects on their shared habitat. For
732 example, rats introduced onto small Pacific islands decimated
733 seabird populations, resulting in decreased sea-to-land transport
734 of nutrients (guano) and altered decomposition and nutrient
735 cycling by soil microbes (112). In another study, European earth-
736 worm species introduced to North American hardwood forests
737 led to significant changes in soil microbial biomass and the
738 metabolic quotient of the soil ecosystem (113). In each of these
739 situations, an introduction led to a substantial reduction in ecosys-
740 tem productivity. Applying metacommunity and network analyses
741 (114) to such animal-bacterial interactions will be essential for
742 the design of effective strategies for managing ecosystems in the face
743 of the environmental perturbations, such as pollution, invasive
744 species, and global climate change, that challenge the biosphere.

745 **The Challenges**

746 For much of her professional career, Lynn Margulis (1938-
747 2011), a controversial visionary in biology, predicted that we
748

749 would come to recognize the impact of the microbial world on
750 the form and function of the entire biosphere, from its molecular
751 structure to its ecosystems. The weight of evidence supporting
752 this view has finally reached a tipping point. The examples come
753 from animal-bacterial interactions, as described here, and also
754 from relationships between and among viruses, Archaea, protists,
755 plants, and fungi. These new data are demanding a reexamination
756 of the very concepts of what constitutes a genome, a population,
757 an environment, and an organism. Similarly, features once consid-
758 ered exceptional, such as symbiosis, are now recognized as likely
759 the 'rule', and novel models for research are emerging across
760 biology. As a consequence, the New Synthesis of the 1930s and
761 beyond must be reconsidered in terms of three areas in which
762 it has proven weakest: symbiosis, development and microbiology
763 (115). One of these areas, microbiology, presents particular chal-
764 lenges both to the species concept, as formulated by Ernst Mayr
765 in 1942, and to the concept that vertical transmission of genetic
766 information is the only motor of selectable evolutionary change.

767 It is imperative that human societies recognize the centrality
768 of the relationships between microbes and other organisms for
769 the health of both individuals and the environments in which they
770 live. The current focus on studies of humans and their microbiota
771 has provided compelling evidence that the composition and activ-
772 ity of resident microbes play crucial roles in shaping the metabolic
773 and regulatory networks that define good health, as well as a
774 spectrum of disease states. Nonetheless, the underlying ecological
775 mechanisms are still poorly defined, and the development of tools
776 to translate this understanding into novel therapies presents an
777 ongoing challenge.

778 In broader scale ecosystems, evidence is mounting that seem-
779 ingly minor environmental perturbations have major, long-term
780 impacts. A full understanding of the consequences will require us
781 to expand our investigations of the associated changes in micro-
782 bial communities in soil, freshwater and marine habitats. How are
783 such microbial assemblages affected by the introduction of non-
784 native species of plants and animals, the increases in temperature
785 due to global climate change, and the acidification of the oceans?
786 While a few studies (*e.g.*, (116, 117)) have revealed its impor-
787 tance, the impact of acidification has thus far focused largely on
788 eukaryotic calcification processes (118). This emphasis leaves us
789 still ignorant of how marine ecosystems may be changed if small
790 shifts in seawater pH or temperature alter the compositions of
791 bacterial communities that are crucial for recruitment of the next
792 generations of plants and animals into their native habitats. The
793 maintenance and restoration of ecosystems that support sustain-
794 able agriculture and carbon-neutral energy production depend on
795 recognition of the interactions between microorganisms and ani-
796 mals, plants and fungi, and the robustness of these relationships in
797 response to anthropogenic and other perturbations. Whether an
798 ecosystem is defined as a single animal or the planet's biosphere,
799 the goal must be to apply an understanding of the relationships
800 between microbes and other organisms to predict and manipulate
801 microbial community structure and activity so as to promote
802 ecosystem health.

803 These challenges present a vast and exciting frontier for the
804 field of biology, and call on life scientists to alter significantly
805 their view of the fundamental nature of the biosphere. Ambitious
806 large-scale, interdisciplinary research efforts, such as the Human
807 Microbiome Project and the Earth Microbiome Project, aim to
808 provide a basic understanding of microbial variation across a wide
809 range of body and environmental habitats in both the normal and
810 perturbed states. Effective project design and the resulting large
811 data sets are driving advances in quantitative methods, such as the
812 creation and refinement of techniques to improve approximation
813 algorithms, dimensionality reduction, and visualization of the
814 results (119). These efforts have highlighted the need for ge-
815 nomic standards, open-source integrated analysis pipelines, and
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increased low-cost computational power. A compelling goal for the future is to apply these technologies, the resultant data, and the emerging intellectual framework to a wide array of biological questions. Such a synthesis promises to generate a more accurate vision of life on earth.

Successful development of research on our microbial world will result only with the breakdown of existing intellectual barriers, not only between the subdisciplines of biology, but also across the natural sciences, mathematics, computer science and engineering. Such integration will be fostered by the active promotion of cross-disciplinary units at universities, collaboration among professional societies, and novel approaches by the funding agencies to support the development of this new frontier (120). The progress of change across the field will also require reformulation

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of educational goals, including development of ways of teaching biology that are as revolutionary as those that occurred in the 1950s in the wake of both the New Synthesis and the launch of Sputnik. Because of advances described here, we foresee a day when microbiology will be a centerpiece not only of biological research, but also of high school, undergraduate and graduate biology education.

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