Cnidarian-Microbe Interactions and the Origin of Innate Immunity in Metazoans

Thomas C.G. Bosch

Zoological Institute, University of Kiel, 24098 Kiel, Germany;
email: tbosch@zoologie.uni-kiel.de

Annu. Rev. Microbiol. 2013. 67:499–518

First published online as a Review in Advance on June 28, 2013

The *Annual Review of Microbiology* is online at micro.annualreviews.org

This article's doi:
10.1146/annurev-micro-092412-155626

Keywords

host-microbe interactions, evolution, innate immunity, *Hydra*, metaorganism, hologenome theory of evolution, antimicrobial peptides, transcription factor FoxO

Abstract

Most epithelia in animals are colonized by microbial communities. These resident microbes influence fitness and thus ecologically important traits of their hosts, ultimately forming a metaorganism consisting of a multicellular host and a community of associated microorganisms. Recent discoveries in the cnidarian *Hydra* show that components of the innate immune system as well as transcriptional regulators of stem cells are involved in maintaining homeostasis between animals and their resident microbiota. Here I argue that components of the innate immune system with its host-specific antimicrobial peptides and a rich repertoire of pattern recognition receptors evolved in early-branching metazoans because of the need to control the resident beneficial microbes, not because of invasive pathogens. I also propose a mutual intertwinement between the stem cell regulatory machinery of the host and the resident microbiota composition, such that disturbances in one trigger a restructuring and resetting of the other.

Contents

Symbiotic interaction: a close and usually obligatory association between two or more different organisms of different species that live together, often to their mutual benefit

Microbes: microbial life forms including bacteria, archaea, and viruses

INTRODUCTION

When did symbiotic interactions with microbes first appear in the animal kingdom? The answer to this question—one of the most fundamental in biology—depends on how one defines "symbiotic interactions" and "microbes." Early animals diverged from their protistan ancestors 700–800 mya, some three billion years after bacterial life originated and as much as one billion years after the first appearance of eukaryotic cells (49, 64). That is, microbes were around long before the animals appeared on the evolutionary scene. Therefore, interactions with microbes have likely been operating since animals first originated. In addition to prokaryotic microbes, there is also evidence for interactions between animals and symbiotic protists at an early date in evolution. Of particular interest in this context are corals because they are members of the ancient animal phylum Cnidaria (**Figure 1**), which is associated with symbiotic algae. Paleontological findings

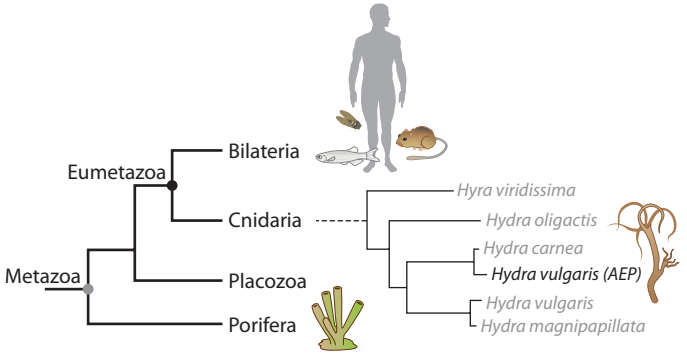

Figure 1

Schematic phylogenetic tree showing the main branches in metazoan evolution. The Porifera are a sister group to all eumetazoans, while the Cnidaria represent a sister group to all Bilateria. Cnidaria are divided into five classes, one of which is the Hydrozoa, which include the species *Hydra viridissima*, *Hydra oligactis*, *Hydra carnea*, *Hydra vulgaris*, and *Hydra magnipapillata*.

of coral fossils from basal Cambrian rocks in China containing exceptionally well-preserved remains of antipatharian corolla (4) might be signs of symbiotic interactions in the pre-Cambrian (542 mya). Predictions of the timing of coral phylogeny derived from molecular phylogenetic evidence (5, 96) support the view that Cnidaria were members of the pre-Ediacaran fauna between 1,200 and 600 Myr ago and diverged from the main line of metazoan evolution long before the pre-Cambrian radiation. Cnidarians not only are among the earliest known phyletic lineages to form natural symbiotic relationships with photosynthetic algae but also have retained many genes that have been lost in *Drosophila melanogaster* and *Caenorhabditis elegans* (41, 51, 67, 82, 99).

An important discovery during the past few years was that individuals from different species differ greatly in their microbiota and that individuals living in the wild are colonized by microbiota similar to that found in laboratory-grown individuals, pointing to the maintenance of specific microbial communities over long periods (9, 35, 75). Today there is an increasing appreciation that microbes are an essential part of the animal phenotype, influencing fitness and thus ecologically important traits of their hosts (36, 63, 73, 76). The widespread application of genetic and genomic approaches has revealed a vast range of animal-bacteria interactions in both invertebrates and vertebrates and has shown that bacteria facilitated the origin and evolution of animals (1, 72), that animals and bacteria affect each other's genomes (26, 43, 46), that normal animal development depends on bacterial partners (37, 81), and that complex mechanisms must exist so that homeostasis is maintained between animals and their symbionts (11, 64).

Because all epithelia in animals appear to be colonized by microbial communities, any multicellular organism must be considered a metaorganism composed of the macroscopic host and synergistic interdependence with bacteria, archaea, fungi, and numerous other microbial and eukaryotic species. The metaorganism concept (19) considers the dynamic communities of bacteria on epithelial surfaces as an integral part of the functionality of the respective organism itself. The discovery that individuals are not solitary, homogenous entities but consist of complex communities of many species that likely evolved during a billion years of coexistence led to the hologenome theory of evolution (87, 88, 108), which considers the holobiont with its hologenome as the unit of selection in evolution.

Disease onset is seen as a complex set of interactions among a variety of associated partners that affect the fitness of the collective metaorganism (89). Complex environmental diseases ranging from coral bleaching to inflammatory bowel disease and allergies in humans can only be understood if the relationships between the interacting infectious agents present at a given time in a given territory are recognized.

In this review I aim to discuss recent advances in the field of *Hydra*-microbe interactions, with particular emphasis on a survey of the components of the innate immune system involved and the novel concepts that have emerged from integrated genomic, genetic, and cell biology approaches. I challenge the prevailing view that immune systems evolved exclusively to control invading pathogens with mounting evidence that components of innate immunity play a profoundly generative role in establishing a host-specific microbiota. The evidence that major factors of innate immunity systems such as antimicrobial peptides shape the microbiota is by now incontrovertible. My thesis then is that immune systems evolved because of the need to control the resident beneficial microbes. Although the examples in this review are almost exclusively from *Hydra*, it is reasonable to assume that the inferences drawn apply to invertebrates and vertebrates in general. Strikingly, in contrast to other model systems such as *D. melanogaster* (21), *Hydra* is stably associated with only a few specific bacterial phylotypes. What, therefore, can *Hydra* tell us about the fundamental principles that underlie all host-microbe interactions?

Microbiota: microbial life forms within a given habitat or host

Metaorganism: an association composed of the macroscopic plant or animal host and synergistic interdependence with bacteria, archaea, fungi, viruses, and numerous other species including algal symbionts

Holobiont: the cnidarian host organism and all of its symbiotic algae and stably associated microbiota

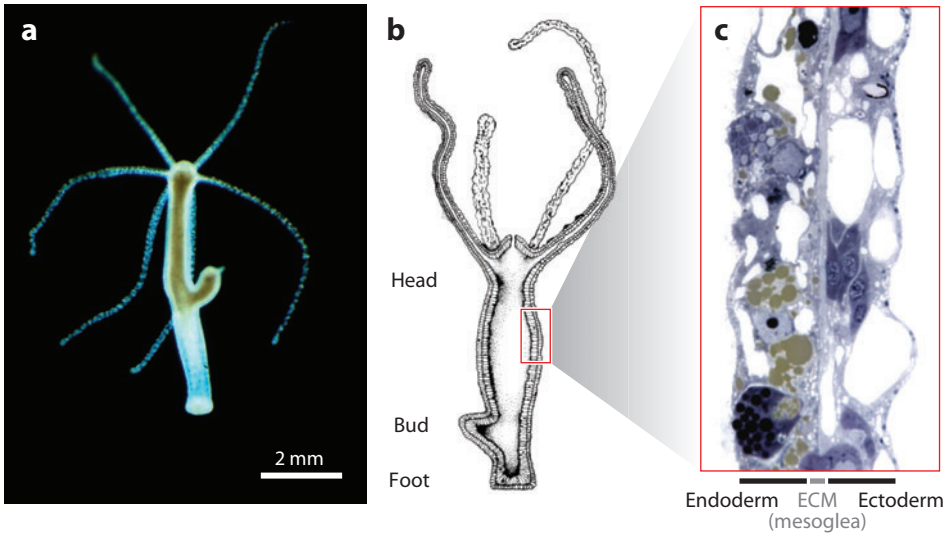

Figure 2

Morphology and histology of *Hydra*. (*a*) *Hydra oligactis* polyp. (*b*) Schematic drawing of a *Hydra* polyp. (*c*) Detailed view of a histological, longitudinal section of *Hydra* tissue. Abbreviation: ECM, extracellular matrix.

HYDRA AS A MODEL HOST

Symbiotic interactions between *Hydra* and photosynthetic algae had been the subject of research for decades because they not only provide insights into the basic toolkit necessary to establish symbiotic interactions, but also are of relevance in understanding the resulting evolutionary selection processes (e.g., 70, 74, 100). *Hydra* belongs to the phylum Cnidaria, which is a sister group to the Bilateria (24, 82). As one of the most basal representatives of eumetazoans (**Figure 1**), Cnidaria are characterized by true tissues connected by tight junctions; sensory, nerve, and muscle cells; a gastric cavity; and a blastoporus. In contrast to the triploblastic Bilateria, Cnidaria are diploblastic and possess one oral-aboral axis and a radially symmetrical body. The characteristic and synapomorphic feature of all cnidarians is the cnidocyte (nematocyte) (98). Cnidaria are subdivided into five classes: Anthozoa, Staurozoa, Scyphozoa, Cubozoa, and Hydrozoa (24). Within the Hydrozoa, the species *Hydra viridissima*, which forms a symbiotic association with unicellular *Chlorella* sp. algae (38), is located at the base of the phylogenetic tree followed by *Hydra oligactis*, *Hydra carnea*, and the laboratory strain of *Hydra vulgaris* (strain AEP) (60) (**Figure 1**). *Hydra vulgaris* and *Hydra magnipapillata* build the most derived group within the hydrozoans.

The *Hydra* polyp has a very simple body plan consisting of two monolayered epithelia: the ectoderm on the outside and the endoderm surrounding the gastric cavity (**Figure 2**). Both epithelial layers are connected by an extracellular matrix termed mesoglea, which provides stability and elasticity to the polyp (**Figure 2**). The adult *Hydra* has about 100,000 cells, which correspond to three independent stem cell lineages (i.e., the ectodermal epithelial cells, the endodermal epithelial cells, and the interstitial cells) whose differentiation behavior has been studied intensively during the past few decades (10, 12, 15, 25). A network of signaling pathways allows the three stem cell lineages to coordinate growth rates and to maintain tissue homeostasis (41). *Hydra* primarily reproduces asexually by budding. The budding frequency is tightly linked to the feeding conditions (17) and amounts to 0.3 to 0.8 buds per polyp per day. In addition to budding, *Hydra* can also

reproduce sexually (18, 58, 59). Environmental cues, e.g., temperature decrease (59) or starvation (17), induce the formation of gametes from germ line precursors. The asexual mode of reproduction by budding, which requires tissue consisting of stem cells with continuous self-renewal capacity, is also the reason behind *Hydra*'s remarkable immortality (6, 61, 71).

For analytical purposes, *Hydra* is a premier model organism, which in the laboratory is propagated and cultured in plastic or glass dishes at 18°C in *Hydra* medium with an artificial day-night rhythm of 12 hours and larval stages of *Artemia salina* as food source. With a 3.5-day life cycle, each animal produces genetically identical progeny, facilitating the establishment and maintenance of large populations of animals that can be housed in refrigerator-sized incubators. *Hydra* lacks any exoskeleton and is nearly transparent, greatly simplifying in vivo tracing of cell behavior and characterization of gene expression patterns. In addition, rich molecular resources including genome sequences are available (95). The *H. magnipapillata* genome, for example, consists of about 1 Gb of sequences and contains about 20,000 genes (23). Genome sizes vary among *Hydra* species (106). *H. oligactis* possesses the largest genome (1,450 Mbp), followed by *H. carnea* (1,350 Mbp), *H. vulgaris* (1,250 Mbp), and *H. circumcincta* (1,150 Mbp). The smallest genome (380 Mbp) is found in *H. viridissima*, the only symbiotic species within the genus *Hydra*. Differences in genome size correlate remarkably well with differences in size of the chromosomes, whereas the number of chromosomes ($2n = 30$) is identical in all species examined (106). Genome sequencing of *H. magnipapillata* has uncovered that as much as 50% of the whole genome is composed of transposable elements (23). In addition to the *H. magnipapillata* genome, a large set of expressed sequence tags from various *Hydra* species is available at **http://www.compagen.org/**. Additional genome and transcriptome sequences from related Cnidaria species such as *Acropora* (93) and *Nematostella* (66, 82) shed new light on the ancestral gene repertoire and show that Cnidaria have retained many ancestral genes that have been lost in *D. melanogaster* and *C. elegans* (41, 51, 67, 82, 99). Because the genome organization and genome content of Cnidaria are remarkably similar to that of bilaterians, these animals offer unique insights into the content of the genetic toolkit present in the cnidarian-bilaterian ancestor. Transgenic *Hydra* can be easily generated by embryo microinjection (105), allowing functional analysis of genes that control development (11) and immune reactions (31) and in vivo monitoring of host-microbe interactions.

Similar to other animals, each *Hydra* polyp is a metaorganism consisting of the animal and its associated endogenous microbiota as well as—at least in some species—obligate symbiotic algae (**Figure 3**). Thus, a long-term persistence of mutualistic associations is prevalent in a complex system comprising three or more associates including algae, bacteria, and viruses (13, 14). For analytical purposes a recent important breakthrough was the ability to produce and culture germ-free *Hydra* (30). Because most of the resident bacteria species of *Hydra* can be cultured independently from the host, the availability of sterile *Hydra* allows researchers to study the symbiotic relationship between *Hydra* and one or more of the resident bacteria one at a time. Because other recent reviews cover the relationship between *Hydra* and *Chlorella* algae (14), here I attempt to focus on *Hydra*-bacteria interactions and emphasize some parallels to host-microbe interactions in vertebrates.

The *Hydra* Host Actively Shapes the Colonizing Microbiota

Bacteria are an important component of the *Hydra* holobiont (**Figure 4**). The 36 identified bacterial phylotypes represent three different bacterial divisions and are dominated by the phyla *Proteobacteria* and *Bacteroidetes* (35, 36). Disturbances or shifts in any of these partners can compromise the health of the whole animal (32). Because *Hydra* have been cultivated for tens of years under standard conditions at constant temperature and identical food, it came as a surprise that examinations of the microbiota in different *Hydra* species kept in the laboratory for more than 20 years under

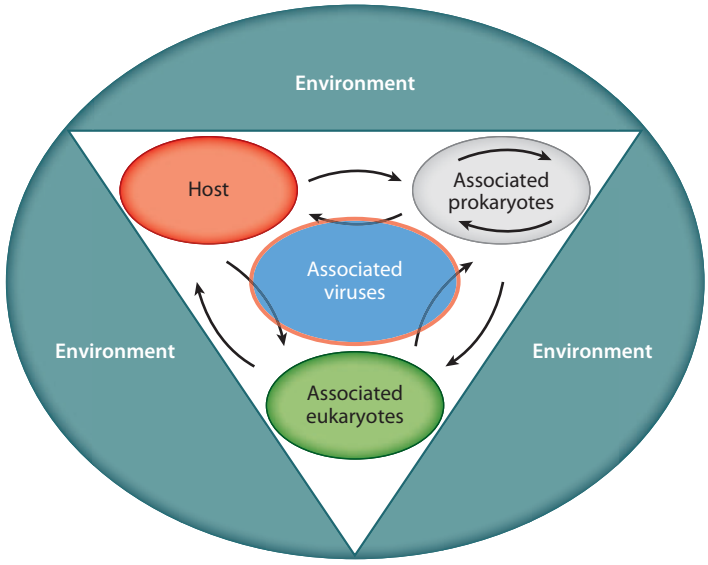

Figure 3

Multicellular organisms are metaorganisms or holobionts composed of the macroscopic host and synergistic interdependence with bacteria, archaea, viruses, fungi, and numerous other microbial and eukaryotic species including algal symbionts.

controlled conditions revealed an epithelium colonized by a complex community of microbes, and that individuals from different species but cultured under identical conditions differed greatly in their microbiota (**Figure 4**). Even more astonishing was the finding that individuals living in the wild were colonized by a group of microbes similar to those found in laboratory-grown polyps, pointing to the maintenance of specific microbial communities over long periods. Bacteria in *Hydra*, therefore, are specific for any given species (35) (**Figure 4**). Closely related *Hydra* species such as *H. vulgaris* and *H. magnipapillata* are associated with a similar microbial community. *H. oligactis*, the most basal *Hydra* species analyzed so far (40), is associated with the most distinct microbial community compared to the other *Hydra* species (**Figure 4*a***). In line with this, comparing the phylogenetic tree of the *Hydra* species with the corresponding cluster tree of associated bacterial communities reveals a high degree of congruency (**Figure 4*b***). This finding strongly indicates that distinct selective pressures are imposed on and within the *Hydra* epithelium.

Factors Influencing Microbial Colonization of *Hydra* Epithelia

Whether bacteria can colonize a given epithelium is determined by many ecological factors including the availability of nutrients, host immune responses, and the competition between strains of the same or different species of bacteria for attachment space. By profiling the assembly of the microbiota on *Hydra* epithelium up to 15 weeks posthatching, we (30) recently observed distinct and reproducible stages of colonization: High initial variability and the presence of numerous different bacterial species are followed by the transient preponderance of the bacterial species that later dominate the adult microbiota. At the end of the colonization process there is a drastic decrease of diversity. Applying a mathematical model allowed us to make two interesting and falsifiable predictions (30). First, assembly of a stable microbiota seems to require the transient preponderance of an initial member of the bacterial community, which after a characteristic decay finally

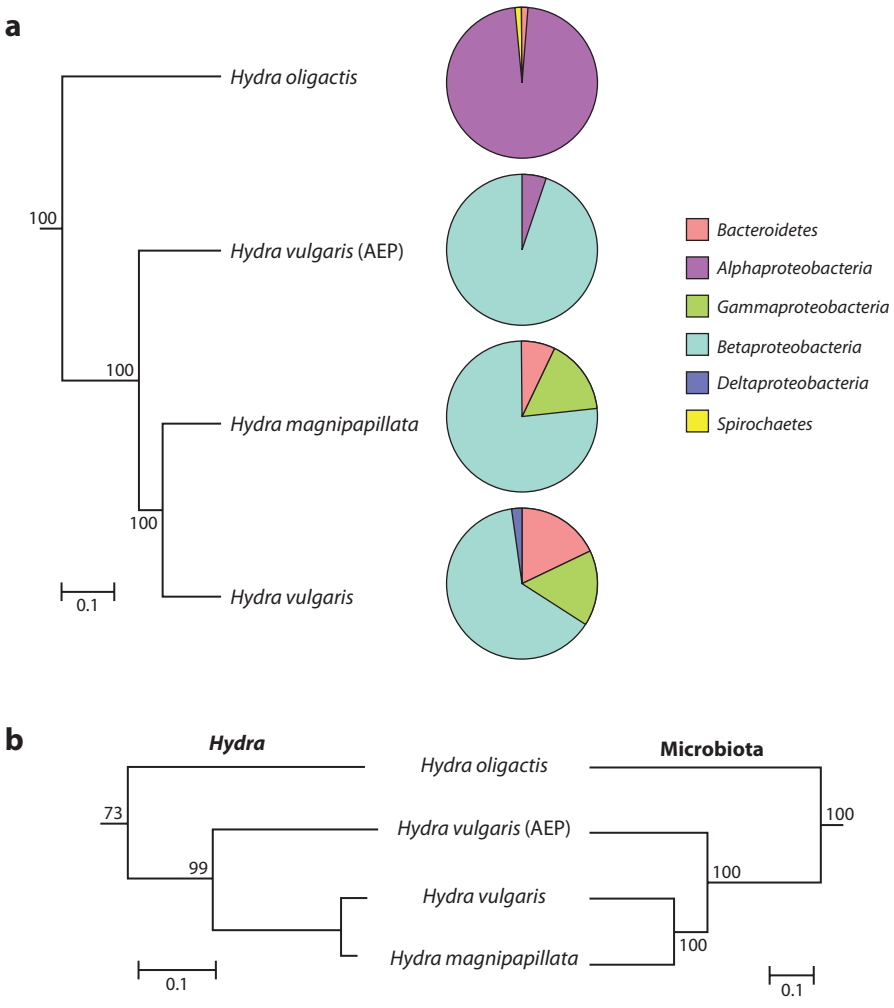

Figure 4

Hydra polyps are colonized by species-specific microbiota. (*a*) Bacterial communities identified from four different *Hydra* species. (*b*) Comparison of the phylogenetic tree from *Hydra* and the environmental cluster tree of the corresponding microbiota. Scale bar: distance in UniFrac units.

becomes the stable and most abundant component of the community. Second, deterministic and most likely host-derived factors appear to be necessary to restrict strongly fluctuating dynamics in the bacterial population (30). The observations suggest that both frequency-dependent bacteria-bacteria interactions and host factors such as components of *Hydra*'s innate immune system are shaping the colonizing microbial composition (30).

COMPONENTS OF THE INNATE IMMUNE SYSTEM CONTROL MICROBIAL COLONIZATION

In the absence of an adaptive immune system, *Hydra* has developed an effective innate immune system to detect and eliminate nonself cells and to survive in a potentially hostile environment.

Did this immune system evolve to keep out harmful organisms, or should we regard this ancient immune system "like a bouncer at a nightclub, trained to allow the right microbes in and kick the less desirable ones out" (102)? As outlined above, microbes have shaped the evolution of animals for millennia. I suggest on the basis of numerous observations in *Hydra* that immune systems evolved as much to manage and exploit beneficial microbes as to fend off harmful ones.

Toll-Like Receptors and MyD88

The innate immune system of *Hydra* relies on a limited set of germ line–encoded receptors to recognize danger signals. These pattern recognition receptors sense invariant molecular signatures that either are present in microbes [microbe-associated molecular patterns (MAMPs), e.g., lipopolysaccharides or unmethylated CpG DNA] or are derived from endogenous sources (e.g., extracellular heat shock proteins or oxidatively modified proteins) and attest profound cellular damage (52, 97). Engagement of these receptors leads to a fast induction of protective programs, e.g., the induction of antimicrobial peptides or the elimination of the infected cell by means of apoptosis. For microbial recognition, *Hydra* uses two types of receptors and signaling pathways, the Toll-like receptors (TLRs) with MyD88 (myeloid differentiation factor 88) as signal transducer (**Figure 5**) and the nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs).

TLR function in *Hydra* is realized by the interaction between a leucine-rich repeat (LRR)-domain-containing protein and a Toll/interleukin-1 receptor (TIR)-domain-containing protein lacking LRRs (2, 3, 16) (**Figure 5**). Coexpression of both membrane proteins is linked to antimicrobial peptide induction in vivo, and heterologous overexpression of the two *Hydra* proteins in mammalian cell lines leads to a sensitization to the MAMP flagellin, supporting the hypothesis that the epithelium represents the ancient system of host defense (16). MAMP-triggered immunity in *Hydra* originates from the cytoplasmic TIR domains of TLRs (16, 42). In signal transduction, the conserved adaptor MyD88 has been identified in *Hydra* as well as in related cnidarians such as *Nematostella* (42, 68) and appears to be an essential component for the activation of innate immunity. MyD88 possesses the TIR domain in the C-terminal portion and a death domain in the N-terminal portion. MyD88 associates with the TIR domain of TLRs. Upon stimulation, MyD88 recruits interleukin-1 (IL-1) receptor-associated serine/threonine kinase (IRAK) to TLRs through interaction of the death domains of both molecules. IRAK is activated by phosphorylation and then associates with TRAF6, leading to the activation of two distinct signaling pathways and finally to the activation of JNK and NF-κB (42).

Is the TLR pathway involved in maintaining specific host-microbe interactions? Does it affect the mechanisms and routes by which functionally diverse bacteria colonize their host? To gain direct insight into these questions, we performed MyD88 (**Figure 5**) loss-of-function experiments in combination with microarray-based gene expression screening and 16S rRNA gene sequencing to detect changes in both the *Hydra* transcriptome and the composition of the associated microbiota (31). The patterns of differentially regulated host genes as well as changes in the bacterial colonization process and pathogen susceptibility in MyD88-knockdown polyps strongly indicate TLR signaling has a role in sensing and managing microbes (31). Thus, not only are TLRs the long-sought cell-surface receptors that recognize common microbial features such as bacterial cell wall components (e.g., flagellin), but their role in controlling the resident microbiota could date back to the earliest multicellular organisms, as humans and *Hydra* share the molecules involved in the TLR signaling cascade.

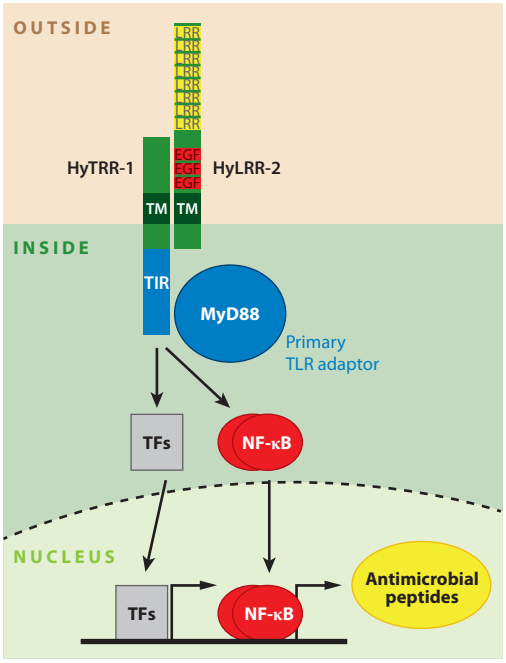

Figure 5

Innate immune recognition in *Hydra* by Toll-like receptor (TLR) signaling. In *Hydra* recognition of bacteria is mediated by an intermolecular interaction of HyLRR-2 as receptor and HyTRR-1 as signal transducer (16). The HyTRR-1 molecule contains a Toll/interleukin-1 receptor (TIR) domain, a transmembrane domain, and an extracellular domain lacking any specific domain structure. The HyLRR-2 gene encodes a transmembrane protein carrying up to eight TLR-related LRR domains in its N-terminal region in addition three EGF domains. Upon activation, the receptor recruits primary adaptor molecules such as MyD88 to engage downstream signaling pathways including NF-κB. Activation of this receptor complex then triggers the innate immune response, which involves the production of antimicrobial peptides. Abbreviations: MyD88, myeloid differentiation factor 88; TM, transmembrane; TFs, transcription factors; LRR, leucine-rich repeat; EGF, epidermal growth factor; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells.

The NLR Family

Microbes evading membrane-bound TLR receptors or specifically invading epithelial cells encounter another line of recognition defense inside the host cell, the intracellular nucleotide-binding and oligomerization domain (NOD)-like receptor (NLR) family (89). There is an unexpectedly large and complex NLR repertoire in *Hydra* (53). In a systematic survey of the NACHT and NB-ARC domain genes in existing expressed sequence tag and genome data sets of early-branching metazoans, we (53) observed that the *Hydra* and Cnidaria NACHT/NB-ARC complements include novel combinations of domains and that the number of one specific domain [NB-ARC and tetratricopeptide repeat (TPR) containing] in *Hydra* is particularly large (53). In addition, surveying the *Hydra* genome allowed the identification of a number of potential NLR-interacting proteins. One of these, a caspase containing a death domain, interacted with a *Hydra* NLR-like protein in vitro (53). Thus, a broad repertoire of NLRs seems to be involved in the recognition of conserved microbial components in *Hydra*. The evolutionary conservation of the NLRs underlines their significance in host-microbe interactions.

Antimicrobial Peptides Driving Evolution of the Holobiont

Antimicrobial peptides (AMPs): low-molecular-weight proteins with broad-spectrum antimicrobial activity against bacteria, viruses, and fungi

Crohn's disease: an inflammatory bowel disease (IBD) resulting in swelling and dysfunction of the intestinal tract with highly altered microbial communities

Antimicrobial peptides (AMPs) are known as prominent effector molecules of the innate immune system that often get secreted in response to external stimuli. Do AMPs, in addition to their killing activity against pathogens, have key regulatory functions in host-microbe homeostasis as the driving force that leads to changes in microbiota composition?

To investigate whether the ectotopic expression of a single AMP may affect the number and composition of the colonizing microbiota at the ectodermal epithelial surface, we (33) generated transgenic *Hydra* expressing the AMP periculin1a in ectoderm epithelial cells. A comparison of the bacterial load of these transgenic polyps with that of wild-type control polyps revealed not only a significantly lower bacterial load in transgenic polyps overexpressing periculin1a but also drastic changes in the bacterial community structure. By analyzing the identity of the colonizing bacteria, we (33) showed that the dominant *Betaproteobacteria* decreased in number, whereas *Alphaproteobacteria* were more prevalent in cells. Thus, overexpression of periculin not only decreases the number of associated bacteria but also alters bacterial composition. With the transgenic polyps overexpressing periculin, we apparently have created a new holobiont that is different from all investigated *Hydra* species (33). From these results we assume that specific associations between hosts and bacteria are a result of bacterial adaptation to different AMP repertoires of different host species. Evolutionary changes in the AMP repertoire of host species, therefore, are expected to lead to changes in the composition of the associated bacterial community. Future efforts will be directed toward analyzing the fitness-related performance of this new phenotype under different environmental conditions.

Patients with Crohn's disease (7) often have strongly reduced α-defensin expression and drastically altered endogenous microbiota (104). Moreover, mice with abnormally strong expression of human α-defensin-5 (DEFA5) and mice lacking an enzyme required for the processing of mouse α-defensins show significant changes in intestinal microbiota composition (91). These findings support the view that epithelia-derived AMPs may represent an important regulatory mechanism that shapes the composition of epithelial microbiota.

In the same way that microbial communities are expected to be specific to certain parts of a body, they are also dynamic in time. To understand the temporal dynamics in *Hydra*-microbe interactions, we first investigated the establishment of the microbiota during oogenesis and embryogenesis. Early embryonic stages in *Hydra* are colonized by a limited number of microbes (33). During embryogenesis the composition and number of bacterial colonizers changes. For example, *Curvibacter*-related *Betaproteobacteria* are present only in late developmental stages and the prevalent phylotype in adult polyps, whereas they appear to be absent in the early embryo. Thus, early developmental stages have a microbiota that is clearly distinct from that of later developmental stages.

The differential colonization is reflected in differences in antimicrobial activity. *Hydra* embryos are protected by a maternally produced AMP of the periculin peptide family, which controls the establishment of the microbiota during embryogenesis. Beginning with the gastrula stage, *Hydra* embryos express a set of periculin peptides (periculin 2a and 2b), which replace the maternally produced periculin peptides 1a and 1b. This shift in the expression within the periculin peptide family represents a shift from maternal to zygotic protection of the embryo (33, 34). In adult *Hydra* polyps, additional AMPs including hydramacin (16) and arminin (2) contribute to the host-derived control of bacterial colonization.

Most of the AMP genes identified in *Hydra* as well as in other animals have no homology to sequences in other species and therefore are classified as taxonomically restricted genes. An informative example in *Hydra* is the periculin family of peptides mentioned above. Analysis of

the deduced amino acid sequence of periculin-1 and the charge distribution within the molecule revealed an anionic N-terminal region and an eight-cysteine residue containing a cationic C-terminal region. No identifiable orthologs were found in any sequence database. Periculin-1 has a strong bactericidal activity and is expressed in the endodermal epithelium as well as in a subpopulation of ectodermal interstitial cells (16). As discussed elsewhere (48), each animal species contains a significant number of such orphan genes encoding potent AMPs. For example, *Aurelia aurita*, one of the most common jellyfish, contains the novel 40-amino-acid AMP aurelin (77, 92). Similarly, the antibacterial immune response gene encoding diptericin is restricted to insects of the order Diptera (44, 54, 55), and the 11-kDa metal ion-binding S100 protein psoriasin (65) controls the microbiota composition in epithelia in mammals but not in other animals.

Taxonomically restricted host defense molecules appear to represent an extremely effective chemical warfare system to shape the colonizing microbiota while coping with specific environmental challenges. An important future line of inquiry in the evolutionary study of immunology, therefore, is to examine how these nonconserved components of the innate immune system have helped organisms adapt to their specific environments or ecological niches. Individual AMPs usually are active against a broad range of bacteria. Another important question, therefore, is how the cocktail of AMPs produced in a given host establishes the host-specific microbiota.

WHAT ARE MICROBES FOR?

The intimacy of the interaction between host and microbiota, as well as the high evolutionary pressure to maintain a specific microbiota, points to the significance of this interkingdom association and implies that hosts deprived of their microbiota should be at a disadvantage. To investigate the effect of absence of microbiota on *Hydra*, we have produced *Hydra* polyps devoid of any bacteria (30). Although no morphological differences were observed in comparison to control polyps (i.e. polyps which contain bacteria), we discovered that *Hydra* lacking bacteria suffer from fungal infections that do not occur in normally cultured polyps (S. Franzenburg, S. Fraune & T.C.G. Bosch, unpublished data). Future efforts directed toward isolating the active substances from these bacteria may eventually lead to the development of novel antimycotics.

In Cnidaria as well as in a large number of invertebrate and vertebrate species, microbes also provide signals for multiple developmental steps (reviewed in 64) (see sidebar Microbes as Partners in Animal Development). One of the most pervasive examples of microbial impact in animal

MICROBES AS PARTNERS IN ANIMAL DEVELOPMENT

Ample evidence indicates that microbes play a role in providing signals for multiple developmental steps in both invertebrates and vertebrates. Microbes can be essential for a range of developmental functions, including promotion of larval growth rate and body size in invertebrates, postembryonic maturation and renewal of the gut epithelium in invertebrates and vertebrates, development and activation of the immune system, and normal brain development in mammals. In the squid *Euprymna scolopes*, a complex organ forms during embryogenesis that facilitates subsequent colonization by the symbiont *Vibrio fischeri*. The induction of settlement and metamorphosis of many marine invertebrate larvae is contingent upon induction by exogenous morphogenetic cues, many of which are produced by bacteria associated with a particular environmental surface. This discovery has led to a new understanding of the biology, one that reflects strong interdependencies that exist between these complex multicellular organisms and their associated microbes.

development is in the induction of settlement and metamorphosis of many marine invertebrate larvae (39). This transition from the larval to the adult morphotype is dependent upon induction by exogenous morphogenetic cues, many of which are produced by bacteria associated with a particular environmental surface. *Hydractinia*, for example, a marine colonial cnidarian frequently found in the North Sea, commonly covers shells inhabited by hermit crabs. Fertile colonies, male and female, produce eggs and sperm, respectively, and within less than three days the fertilized egg develops into a mature planula larva. A mature larva is a larva that is able to metamorphose into a polyp, but under sterile laboratory conditions it never does. Rather, it will die as it is unable to take up food (29, 55, 103). To continue its development, a mature larva needs an external trigger, which appears to be a lipophilic substance provided in the natural habitat by certain sedentary bacteria of the genus *Alteromonas*. The mechanisms by which *Hydractinia* sense bacteria-derived environmental cues to form colonies and to reproduce may provide crucial insights into the genetic and developmental foundations of life cycles, but little is known about their natural history or biochemistry. These and numerous other observations challenge the traditional view of animal development as an autonomous process directed by the genome. We must rethink development, at least in part, as an orchestration of both animal-encoded ontogeny and interkingdom communication (64).

LINKING TISSUE HOMEOSTASIS TO MICROBIOTA COMPOSITION

Little is known about how epithelial homeostasis affects microbial community structure. To decipher putative links between epithelial homeostasis and species-level bacterial phylotypes, we (32) made use of a mutant strain of *Hydra* that has temperature-sensitive interstitial stem cells. Two weeks after temperature treatment, when the tissue still contained all epithelial cells but lacked interstitial cells and also had a reduced number of neurons and gland cells, the bacterial composition changed drastically (32). Bacteria of the *Bacteroidetes* group showed a drastic increase in abundance, whereas bacteria of the *Betaproteobacteria* decreased in abundance. Thus, changes in epithelial homeostasis significantly alter the microbial community, implying direct interaction between epithelia and microbiota (32). What are the mechanisms underlying this previously unrecognized link between tissue homeostasis and microbiota? How do host tissue and microbe community remain balanced over space and time?

A potential answer to these questions recently came from a completely unexpected direction. In an unbiased search for factors maintaining stem cell self-renewal and thereby controlling longevity of *Hydra*, transcription factor FoxO was strongly expressed in all three stem cell types but silent in terminally differentiated cells (11). FoxO's well-documented function in regulating life span of other organisms led us to speculate that in *Hydra* FoxO might be a key driver for the continuous self-renewal capacity of stem cells. To assess this directly, we performed gain- and loss-of-function experiments. Overexpression of FoxO in the interstitial stem cell lineage increased proliferation of stem cells (11). Silencing of FoxO in epithelial cells influenced the delicate balance between stem cells and differentiated cells by increasing numbers of cells going into terminal differentiation, accompanied by a considerable slowdown of population growth rate (11). FoxO downregulation also caused drastic changes in the expression level of the above-mentioned AMPs arminin, hydramacin, and periculin2b. Hydramacin and periculin2b were strongly upregulated, whereas the level of arminin was downregulated by 50% in FoxO knockdowns (11). In addition, in silico analysis revealed multiple FoxO-binding sites on the promoter sequences of the three AMPs. These unanticipated observations might indicate that FoxO-dependent transcriptional programs control the synthesis of AMPs, and thereby the microbiota composition, suggesting a direct link between

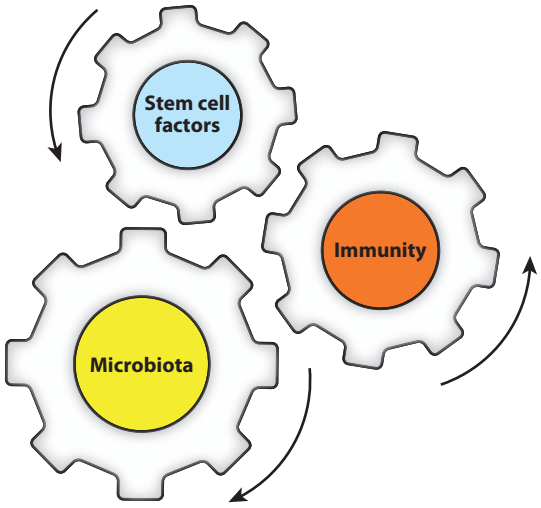

Figure 6

In the *Hydra* holobiont, beneficial microbes represent a major factor whose activities are linked to both tissue homeostasis, illustrated as stem cell factors, and immunity.

tissue homeostasis and the microbiota (11) (**Figure 6**) (see sidebar A Role for FoxO Stem Cell Transcription Factors in Microbial Colonization?).

The link between FoxO and components of the innate immune system is of particular interest because aging processes in humans result in impairment of both innate and adaptive immunity (immunosenescence) as well as in a proinflammatory status (inflammaging) (71). The observations in *Hydra* (11) indicate that the role of FoxO in life span regulation is highly conserved, suggesting that longevity is dependent on two major factors, the maintenance of stem cell functionality and maintenance of immune homeostasis.

A ROLE FOR FoxO STEM CELL TRANSCRIPTION FACTORS IN MICROBIAL COLONIZATION?

Forkhead box-O (FoxO) transcription factors have been implicated in conferring increased life span and stress resistance in flies and worms and are an important component of the genetic signatures of human exceptional longevity (28, 45, 47, 56, 71, 90) due to their key role in maintenance of adult stem cells (11, 69, 78, 83, 101, 107). Given the stable association of animals with a specific microbiota, we do not understand how epithelial tissue homeostasis and microbes are connected. Are FoxO proteins involved? FoxO proteins have been implicated in the regulation of the innate immune system in invertebrates and vertebrates. dFOXO overexpression in *Drosophila*, for example, leads to an induction of antimicrobial peptide (AMP) synthesis by direct binding of dFOXO to the drosomycin regulatory region (8). And FoxO3a knockout mice display inflammation in several tissues (57, 94). In *Hydra*, FoxO is involved in both regulating stem cell behavior and controlling the expression level of a number of AMPs (11). Because microbial colonization is controlled by AMPs (33), stem cell transcription factor FoxO seems to have significant roles in controlling the microbiota composition, suggesting a direct link between tissue homeostasis and the microbiota.

THE *HYDRA* HOLOBIONT IN THE FACE OF A CONSTANTLY CHANGING ENVIRONMENT

Individuals, populations, and species cope with environmental changes by adapting physiologically through responses that are immediate and reversible. How? It is well known that organisms may respond by phenotypic plasticity, genetic adaptation, movement (range shifts), or extinction. Two lesser explored factors are the effects of transposable elements (TEs) and the impact of changes in biotic interactions and community structure on species' survival.

Two features make *Hydra* well-equipped for surviving in a constantly changing environment and for maintaining evolutionary flexibility. First is the large genome (about 1,000 Mb in *H. magnipapillata*); 50% of the whole genome consists of transposable elements (TEs) (23). Geneticist Barbara McClintock suggested in the mid-1980s that TE activity could be a response to challenges to the genome (62). Several decades later, advances in high-throughput sequencing technologies made it clear that TEs, once categorized as junk DNA, can influence genomic function by increasing the coding and noncoding genetic repertoire of the host. Today, we know that the activity of TEs can be induced by environmental factors and in particular by stresses (22, 27, 80). In this way, by increased TE mobility, the large number of TEs in *Hydra* (23) might be key elements for new genetic variability in the face of changing environmental conditions.

Second, the associated microbiota of *Hydra* (**Figure 4**) might change in response to environmental conditions. The impact of different environmental conditions on the bacterial community in *Hydra* has been demonstrated empirically by culturing polyps, which were taken from the wild, for two months under standard laboratory conditions (35). Thereafter, we compared the associated bacteria with the bacteria from polyps taken directly from the wild. Culturing of polyps from the wild under laboratory conditions involves a change in culture temperature, culture medium, and food source (35). These changes have significant effects on the composition of the bacterial community. For example, whereas one bacterial phylotype belonging to the *Alphaproteobacteria* could be identified as the most dominant species in long-term culture, in polyps from the wild and two months after the shift to the laboratory, this bacterium was present only in relatively low abundance (35). Other bacterial species completely disappeared from the tissue owing to the change in culturing conditions.

Thus, *Hydra* is not only associated with species-specific bacteria but also responds to changes in the environment with changes in the bacterial community (35). The *Hydra* holobiont, therefore, appears to be a dynamic system characterized by functional redundancy and fast adaptations to altered environmental conditions.

This view is conceptualized by the holobiont concept: Rosenberg et al. (87) proposed in 2007 that animals adapt rapidly to changing environmental conditions by altering their associated microbiota. Depending on the variety of different niches provided by the host, which can change with developmental stage, diet, or other environmental factors, a more or less diverse microbial community can be established within a given host species. Because this, for example, may provide corals with resistance against certain pathogens, enabling them to adapt much faster to novel environmental conditions than by mutation and selection, host-microbe interactions may be considered as significant drivers of animal evolution and diversification (87).

This hypothesis is supported by at least three observations: (*a*) Corals are associated with diverse microbiota (20, 86); (*b*) the associated microbiota change in response to environmental stress (79, 85) or seasons (50); and (*c*) corals can develop resistance against pathogens even though they lack adaptive immune responses (84). The *Hydra* model, due to the limited number of bacterial phylotypes present and the ability to culture germ-free polyps, provides an ideal system to examine further the adaptive role of microbes.

CONCLUDING REMARKS

As sister group to the Bilateria, Cnidaria is an important phylum providing key insight into the ancestry and evolution of immune reactions. I have reported that in the cnidarian *Hydra* the beneficial microbiota resembles an ecosystem that is essential to the development, protection, and overall health of its host and that the host's role far outweighs other environmental factors in molding the composition of the microbiota. AMPs appear to be key factors for host-bacteria coevolution. Why and exactly how the different components of the innate immune system evolved remain to be understood. On the basis of observations in transgenic polyps with an altered AMP repertoire or silenced TLR/MyD88 activity, I have argued that *Hydra*'s innate immune systems evolved to control the resident beneficial and coevolved microbes rather than fight pathogens. Recent experiments also have identified stem cell transcription factor FoxO as a critical regulator of both stem cell behavior and immune maintenance in *Hydra*, suggesting that longevity is dependent on two major factors: maintenance of stem cell functionality and maintenance of immune homeostasis. Finally, and perhaps most importantly, I have argued that the dynamic relationship between symbiotic microorganisms and environmental conditions results in the selection of the most advantageous holobiont (**Figure 3**). Switching its microbial partners may allow *Hydra* as well as all other animals to adapt to changing environmental conditions much more rapidly than via mutation and selection.

In sum, *Hydra* is a valuable model for exploring not only the basis of interkingdom communication but also the role of bacterial signaling in animal development. Findings derived from the in vivo context of the *Hydra* model may also provide one of the simplest possible systems to address questions of how a stable host-microbe community is established and remains balanced over time. Further work is needed to elucidate the bacteria-derived signals that trigger transcriptional responses in the *Hydra* holobiont. Important areas that require further study include extensive characterization of the signaling networks that govern the holobiont and the cell types in which they function.

How many of these important answers will apply to vertebrates as well, and to what extent? Can the uncovered basic molecular machinery then be transferred to more complex organisms, providing conceptual insights into the complexity of host-microbe interactions in general? Insights gained from the study of host-microbe interactions in *Hydra* can be applied to understand human barrier disorders by describing a strictly microbe-dependent life style and the resulting evolutionary selection processes or advantages. The identification of genes responsible for human diseases affecting biological barriers (e.g., skin or intestinal mucosa) often does not in itself provide a clue to etiopathogenesis or therapeutic targets, as the interaction of a suite of genes in a complex system such as the human is difficult to understand. Likewise, the involved pathways that ultimately lead to the development of the disease phenotype are unclear. Searching for the evolutionary origin of the disease-causing genes and characterizing the variation in such genes under known evolutionary pressures may provide insights into the development of diseases in humans and identify new targets for therapy or prevention. By studying *Hydra* it might be possible to unravel the complex interplay of host-pathogen signaling cascades that are also relevant to human barrier organs and their microbiota.

SUMMARY POINTS

1. The freshwater polyp *Hydra* was developed as a model for studying innate immunity in 2009. In the absence of mobile phagocytes, effective innate immune responses in *Hydra* are mediated by the epithelium and are based on TLR signaling. A highly conserved TLR, MyD88, and NF-κB signaling cascade plays a central role in sensing microbes.

2. *Hydra* species are associated with species-specific microbiota, indicating that the host selectively shapes its bacterial community and suggesting that genetic factors of the host can outweigh environmental influences in determining microbial surface colonization.
3. Peptides with antimicrobial activity are major components of *Hydra*'s innate immune system. In addition to their role in protecting a host from overt pathogens, AMPs in *Hydra* have a key role in regulating the composition of the colonizing microbiota.
4. During assembly of the epithelia-associated microbiota, frequency-dependent bacteria-bacteria interactions and host-derived factors combine to influence the resulting pattern of microbial diversity.
5. FoxO-dependent transcriptional programs might be involved in controlling the synthesis of AMPs, indicating a direct link between tissue homeostasis and resident microbiota composition.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

My sincere thanks are to the members of my laboratory. I thank Anna Marei Böhm and Sebastian Fraune for help with the figures, and René Augustin for critically reading the manuscript. The work related to this review was supported in part by grants from the Deutsche Forschungsgemeinschaft (DFG) and by grants from the DFG Clusters of Excellence programs The Future Ocean and Inflammation at Interfaces.

LITERATURE CITED

1. Alegado RA, Brown LW, Cao S, Dermenjian RK, Zuzow R, et al. 2012. A bacterial sulfonolipid triggers multicellular development in the closest living relatives of animals. *Elife* 1:e00013
2. Augustin R, Anton-Erxleben F, Jungnickel S, Hemmrich G, Spudy B, et al. 2009. Activity of the novel peptide arminin against multiresistant human pathogens shows the considerable potential of phylogenetically ancient organisms as drug sources. *Antimicrob. Agents Chemother.* 53:5245–50
3. Augustin R, Fraune S, Bosch TCG. 2010. How *Hydra* senses and destroys microbes. *Semin. Immunol.* 22:54–58
4. Baliński A, Yuanlin S, Jerzy D. 2012. 470-million-year-old black corals from China. *Naturwissenschaften* 99(8):645–53
5. Barbeitos MS, Romano SL, Lasker HR. 2010. Repeated loss of coloniality and symbiosis in scleractinian corals. *Proc. Natl. Acad. Sci. USA* 107(26):11877–82
6. Baudisch A, Vaupel JW. 2012. Evolution. Getting to the root of aging. *Science* 338(6107):618–19
7. Baumgart DC, Sandborn WJ. 2012. Crohn's disease. *Lancet* 380(9853):1590–605
8. Becker T, Loch G, Beyer M, Zinke I, Aschenbrenner AC, et al. 2010. FOXO-dependent regulation of innate immune homeostasis. *Nature* 463(7279):369–73
9. Bevins CL, Salzman NH. 2011. The potter's wheel: the host's role in sculpting its microbiota. *Cell Mol. Life Sci.* 68:3675–85
10. Boehm AM, Bosch TCG. 2012. Migration of multipotent interstitial stem cells in *Hydra*. *Zoology* 115(5):275–82

11. Boehm AM, Khalturin K, Anton-Erxleben F, Hemmrich G, Klostermeier UC, et al. 2012. FoxO is a critical regulator of stem cell maintenance in immortal *Hydra*. *Proc. Natl. Acad. Sci. USA* 109(48):19697–702
12. Bosch TCG. 2009. *Hydra* and the evolution of stem cells. *BioEssays* 31(4):478–86
13. Bosch TCG. 2012. Understanding complex host-microbe interactions in *Hydra*. *Gut Microbes* 3:345–51
14. Bosch TCG. 2012. What *Hydra* has to say about the role and origin of symbiotic interactions. *Biol. Bull.* 223:78–84
15. Bosch TCG, Anton-Erxleben F, Hemmrich G, Khalturin K. 2010. The *Hydra* polyp: nothing but an active stem cell community. *Dev. Growth Differ.* 52(1):15–25
16. Bosch TCG, Augustin R, Anton-Erxleben F, Fraune S, Hemmrich G, et al. 2009. Uncovering the evolutionary history of innate immunity: the simple metazoan *Hydra* uses epithelial cells for host defence. *Dev. Comp. Immunol.* 33:559–69
17. Bosch TCG, David CN. 1984. Growth regulation in *Hydra*: relationship between epithelial cell cycle length and growth rate. *Dev. Biol.* 104:161–71
18. Bosch TCG, David CN. 1986. Male and female stem cells and sex reversal in *Hydra* polyps. *Proc. Natl. Acad. Sci. USA* 83:9478–82
19. Bosch TCG, McFall-Ngai MJ. 2011. Metaorganisms as the new frontier. *Zoology* 114:185–90
20. Bourne D, Iida Y, Uthicke S, Smith-Keune C. 2008. Changes in coral-associated microbial communities during a bleaching event. *ISME J.* 2:350–63
21. Broderick NA, Lemaitre B. 2012. Gut-associated microbes of *Drosophila melanogaster*. *Gut Microbes* 3(4):307–21
22. Capy P, Gasperi G, Biémont C, Bazin C. 2000. Stress and transposable elements: co-evolution or useful parasites? *Heredity* 85:101–6
23. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, et al. 2010. The dynamic genome of *Hydra*. *Nature* 464(7288):592–96
24. Collins AG, Cartwright P, McFadden CS, Schierwater B. 2005. Phylogenetic context and basal metazoan model systems. *Integr. Comp. Biol.* 45(4):585–94
25. David CN. 2012. Interstitial stem cells in *Hydra*: multipotency and decision-making. *Int. J. Dev. Biol.* 56(6–8):489–97
26. Domazet-Lošo T, Tautz D. 2008. An ancient evolutionary origin of genes associated with human genetic diseases. *Mol. Biol. Evol.* 25:2699–707
27. Fedoroff N. 2012. Transposable elements, epigenetics and genome evolution. *Science* 338:758–67
28. Flachsbart F, Caliebe A, Kleindorp R, Blanché H, von Eller-Eberstein H, et al. 2009. Association of *FOXO3A* variation with human longevity confirmed in German centenarians. *Proc. Natl. Acad. Sci. USA* 106(8):2700–5
29. Frank U, Leitz T, Müller WA. 2001. The hydroid Hydractinia: a versatile, informative cnidarian representative. *BioEssays* 23:963–71
30. Franzenburg S, Fraune S, Altrock PM, Künzel S, Baines JF, et al. 2013. Bacterial colonization of *Hydra* hatchlings follows a robust temporal pattern. *ISME J.* 7:781–90
31. Franzenburg S, Fraune S, Künzel S, Baines JF, Domazet-Loso T, Bosch TCG. 2012. MyD88-deficient *Hydra* reveal an ancient function of TLR signaling in sensing bacterial colonizers. *Proc. Natl. Acad. Sci. USA* 109(47):19374–79
32. Fraune S, Abe Y, Bosch TCG. 2009. Disturbing epithelial homeostasis in the metazoan *Hydra* leads to drastic changes in associated microbiota. *Environ. Microbiol.* 11:2361–69
33. Fraune S, Augustin R, Anton-Erxleben F, Wittlieb J, Gelhaus C, et al. 2010. In an early branching metazoan, bacterial colonization of the embryo is controlled by maternal antimicrobial peptides. *Proc. Natl. Acad. Sci. USA* 107:18067–72
34. Fraune S, Augustin R, Bosch TCG. 2011. Embryo protection in contemporary immunology: why bacteria matter. *Commun. Integr.* 4:369–72
35. **Fraune S, Bosch TCG. 2007. Long-term maintenance of species-specific bacterial microbiota in the basal metazoan *Hydra*. *Proc. Natl. Acad. Sci. USA* 104:13146–51**
36. Fraune S, Bosch TCG. 2010. Why bacteria matter in animal development and evolution. *BioEssays* 32:571–80

35. Supports the idea that the host selects its microbiota and that genetic host factors outweigh environmental influences in determining microbial colonization.

37. Gilbert SF, Epel D. 2008. *Ecological Developmental Biology*. Sunderland, MA: Sinauer
38. Habetha M, Anton-Erxleben F, Neumann K, Bosch TCG. 2003. The *Hydra viridis/Chlorella* symbiosis. Growth and sexual differentiation in polyps without symbionts. *Zoology* 106(2):101–8
39. Hadfield MG. 2011. Biofilms and marine invertebrate larvae: what bacteria produce that larvae use to choose settlement sites. *Annu. Rev. Mar. Sci.* 3:453–70
40. Hemmrich G, Anokhin B, Zacharias H, Bosch TCG. 2007. Molecular phylogenetics in *Hydra*, a classical model in evolutionary developmental biology. *Mol. Phylogenet. Evol.* 44:281–90
41. Hemmrich G, Khalturin K, Boehm AM, Puchert M, Anton-Erxleben F, et al. 2012. Molecular signatures of the three stem cell lineages in *Hydra* and the emergence of stem cell function at the base of multicellularity. *Mol. Biol. Evol.* 29(11):3267–80
42. Hemmrich G, Miller DJ, Bosch TCG. 2007. The evolution of immunity: a low-life perspective. *Trends Immunol.* 28(10):449–54
43. Hughes DT, Sperandio V. 2008. Inter-kingdom signalling: communication between bacteria and their hosts. *Nat. Rev. Microbiol.* 6:111–20
44. Imler JL, Bulet P. 2005. Antimicrobial peptides in *Drosophila*: structures, activities and gene regulation. *Chem. Immunol. Allergy* 86:1–21
45. Jünger MA, Rintelen F, Stocker H, Wasserman JD, Végh M, et al. 2003. The *Drosophila* forkhead transcription factor FOXO mediates the reduction in cell number associated with reduced insulin signaling. *J. Biol.* 2(3):20
46. Keeling PJ, Palmer JD. 2008. Horizontal gene transfer in eukaryotic evolution. *Nat. Rev. Genet.* 9:605–18
47. Kenyon C. 2010. A pathway that links reproductive status to lifespan in *Caenorhabditis elegans*. *Ann. N. Y. Acad. Sci.* 1204:156–62
48. Khalturin K, Hemmrich G, Fraune S, Augustin R, Bosch TCG. 2009. More than just orphans: Are taxonomically restricted genes important in evolution? *Trends Genet.* 25(9):404–13
49. Knoll AH. 2003. *Life on a Young Planet*. Princeton, NJ: Princeton Univ. Press
50. Koren O, Rosenberg E. 2006. Bacteria associated with mucus and tissues of the coral *Oculina patagonica* in summer and winter. *Appl. Environ. Microbiol.* 72:5254–59
51. Kortschak RD, Samuel G, Saint R, Miller DJ. 2003. EST analysis of the cnidarian *Acropora millepora* reveals extensive gene loss and rapid sequence divergence in the model invertebrates. *Curr. Biol.* 13:2190–95
52. Kumar S, Ingle H, Prasad DV, Kumar H. 2013. Recognition of bacterial infection by innate immune sensors. *Crit. Rev. Microbiol.* 39:229–46
53. Lange C, Hemmrich G, Klostermeier UC, López-Quintero JA, Miller DJ, et al. 2011. Defining the origins of the NOD-like receptor system at the base of animal evolution. *Mol. Biol. Evol.* 28:1687–702
54. Lee JH, Cho KS, Lee J, Yoo J, Lee J, Chung J. 2001. Diptericin-like protein: an immune response gene regulated by the anti-bacterial gene induction pathway in *Drosophila*. *Gene* 271(2):233–38
55. Leitz T, Wagner T. 1993. The marine bacterium *Alteromonas espejiana* induces metamorphosis of the hydroid *Hydractinia echinata*. *Mar. Biol.* 115:173–78
56. Li Y, Wang WJ, Cao H, Lu J, Wu C, et al. 2009. Genetic association of *FOXO1A* and *FOXO3A* with longevity trait in Han Chinese populations. *Hum. Mol. Genet.* 18(24):4897–904
57. Lin L, Hron JD, Peng SL. 2004. Regulation of NF-κB, Th activation, and autoinflammation by the forkhead transcription factor Foxo3a. *Immunity* 21(2):203–23
58. Littlefield CL. 1984. The interstitial cells control the sexual phenotype of heterosexual chimeras of *Hydra*. *Dev. Biol.* 102(2):426–32
59. Littlefield CL, Finkemeier C, Bode HR. 1991. Spermatogenesis in *Hydra oligactis*. II. How temperature controls the reciprocity of sexual and asexual reproduction. *Dev. Biol.* 146(2):292–300
60. Martin VJ, Littlefield CL, Archer WE, Bode HR. 1997. Embryogenesis in *Hydra*. *Biol. Bull.* 192(3):345–63
61. Martínez DE. 1998. Mortality patterns suggest lack of senescence in *Hydra*. *Exp. Gerontol.* 33(3):217–25
62. McClintock B. 1984. The significance of responses of the genome to challenge. *Science* 226:792–801
63. McFall-Ngai M. 2007. Adaptive immunity: care for the community. *Nature* 445(7124):153
64. McFall-Ngai M, Hadfield MG, Bosch TC, Carey HV, Domazet-Lošo T, et al. 2013. Animals in a bacterial world: a new imperative for the life sciences. *Proc. Natl. Acad. Sci. USA* 110:3229–36

65. Meyer JE, Harder J, Sipos B, Maune S, Klöppel G, et al. 2008. Psoriasin (S100A7) is a principal antimicrobial peptide of the human tongue. *Mucosal Immunol.* 1(3):239–43
66. Miller DJ, Ball EE. 2008. Cryptic complexity captured: The *Nematostella* genome reveals its secrets. *Trends Genet.* 24(1):1–4
67. Miller DJ, Ball EE, Technau U. 2005. Cnidarians and ancestral genetic complexity in the animal kingdom. *Trends Genet.* 21:536–39
68. Miller DJ, Hemmrich G, Ball EE, Hayward DC, Khalturin K, et al. 2007. The innate immune repertoire in Cnidaria—ancestral complexity and stochastic gene loss. *Genome Biol.* 8(4):R59
69. Miyamoto K, Araki KY, Naka K, Arai F, Takubo K, et al. 2007. Foxo3a is essential for maintenance of the hematopoietic stem cell pool. *Cell Stem Cell* 1(1):101–12
70. Muscatine L, Lenhoff HM. 1963. Symbiosis: on the role of algae symbiotic with *Hydra*. *Science* 142:956–58
71. Nebel A, Bosch TCG. 2012. Evolution of human longevity: lessons from *Hydra*. *Aging* 4(11):730–31
72. Nichols SA, Dayel MJ, King N. 2009. Genomic, phylogenetic, and cell biological insights into metazoan origins. In *Animal Evolution: Genes, Genomes, Fossils and Trees*, ed. MJ Telford, DTJ Littlewood, pp. 24–32. Oxford: Oxford Univ. Press
73. Nyholm SV, Graf J. 2012. Knowing your friends: Invertebrate innate immunity fosters beneficial bacterial symbioses. *Nat. Rev. Microbiol.* 10(12):815–27
74. O'Brien TL. 1982. Inhibition of vacuolar membrane fusion by intracellular symbiotic algae in *Hydra viridis* (Florida strain). *J. Exp. Zool.* 223(3):211–18
75. Ochman H, Worobey M, Kuo CH, Ndjango JB, Peeters M, et al. 2010. Evolutionary relationships of wild hominids recapitulated by gut microbial communities. *PLoS Biol.* 8(11):e1000546
76. O'Hara AM, Shanahan F. 2006. The gut flora as a forgotten organ. *EMBO Rep.* 7:688–93
77. Ovchinnikova TV, Balandin SV, Aleshina GM, Tagaev AA, Leonova YF, et al. 2006. Aurelin, a novel antimicrobial peptide from jellyfish *Aurelia aurita* with structural features of defensins and channel-blocking toxins. *Biochem. Biophys. Res. Commun.* 348(2):514–23
78. Paik JH, Ding Z, Narurkar R, Ramkissoon S, Muller F, et al. 2009. FoxOs cooperatively regulate diverse pathways governing neural stem cell homeostasis. *Cell Stem Cell* 5(5):540–53
79. Pantos O, Cooney RP, Le Tissier MD, Barer MR, O'Donnell AG, et al. 2003. The bacterial ecology of a plague-like disease affecting the Caribbean coral *Montastrea annularis*. *Environ. Microbiol.* 5:370–82
80. Piskurek O, Jackson DJ. 2012. Transposable elements: from DNA parasites to architects of metazoan evolution. *Genes* 3:409–22
81. Pradeu T. 2011. A mixed self: the role of symbiosis in development. *Biol. Theory* 6:80–88
82. Putnam NH, Srivastava M, Hellsten U, Dirks B, Chapman J, et al. 2007. Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science* 317:86–94
83. Renault VM, Rafalski VA, Morgan AA, Salih DA, Brett JO, et al. 2009. FoxO3 regulates neural stem cell homeostasis. *Cell Stem Cell* 5(5):527–39
84. Reshef L, Koren O, Loya Y, Zilber-Rosenberg I, Rosenberg E. 2006. The coral probiotic hypothesis. *Environ. Microbiol.* 8:2068–73
85. Ritchie KB, Smith WG. 1995. Carbon-source utilization of coral-associated marine heterotrophs. *J. Mar. Biotechnol.* 3:107–9
86. Rohwer F, Seguritan V, Azam F, Knowlton N. 2002. Diversity and distribution of coral-associated bacteria. *Mar. Ecol. Prog. Ser.* 243:1–10
87. Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I. 2007. The role of microorganisms in coral health, disease and evolution. *Nat. Rev. Microbiol.* 5:355–62
88. Rosenberg E, Sharon G, Zilber-Rosenberg I. 2009. The hologenome theory of evolution contains Lamarckian aspects within a Darwinian framework. *Environ. Microbiol.* 11(12):2959–62
89. Rosenstiel P, Philipp EE, Schreiber S, Bosch TCG. 2009. Evolution and function of innate immune receptors—insights from marine invertebrates. *J. Innate Immun.* 1:291–300
90. Salih DA, Brunet A. 2008. FoxO transcription factors in the maintenance of cellular homeostasis during aging. *Curr. Opin. Cell Biol.* 20(2):126–36
91. Salzman NH, Hung K, Haribhai D, Chu H, Karlsson-Sjoberg J, et al. 2010. Enteric defensins are essential regulators of intestinal microbial ecology. *Nat. Immunol.* 11:76–83

73. Addresses how symbionts might be distinguished from pathogens.

75. Provides evidence that in hominids host phylogeny can supersede environment in predicting community structure of colonizing microbiota of the intestine.

92. Shenkarev ZO, Panteleev PV, Balandin SV, Gizatullina AK, Altukhov DA, et al. 2012. Recombinant expression and solution structure of antimicrobial peptide aurelin from jellyfish *Aurelia aurita*. *Biochem. Biophys. Res. Commun.* 429(1–2):63–69

93. Provides an intriguing look into an ancient cnidarian genome.

93. Shinzato C, Shoguchi E, Kawashima T, Hamada M, Hisata K, et al. 2011. Using the *Acropora digitifera* genome to understand coral responses to environmental change. *Nature* 476(7360):320–23

94. Snoeks L, Weber CR, Wasland K, Turner JR, Vainder C, et al. 2009. Tumor suppressor FOXO3 participates in the regulation of intestinal inflammation. *Lab. Invest.* 89(9):1053–62
95. Steele RE, David CN, Technau U. 2011. A genomic view of 500 million years of cnidarian evolution. *Trends Genet.* 27(1):7–13
96. Stolarski J, Kitahara MV, Miller DJ, Cairns SD, Mazur M, Meibom A. 2011. The ancient evolutionary origins of Scleractinia revealed by azooxanthellate corals. *BMC Evol. Biol.* 11:316
97. Takeuchi O, Akira S. 2010. Pattern recognition receptors and inflammation. *Cell* 140(6):805–20
98. Tardent P, Holstein T. 1982. Morphology and morphodynamics of the stenotele nematocyst of *Hydra attenuata* Pall (Hydrozoa, Cnidaria). *Cell Tissue Res.* 224(2):269–90
99. Technau U, Rudd S, Maxwell P. 2005. Maintenance of ancestral complexity and non-metazoan genes in two basal cnidarians. *Trends Genet.* 21:633–39
100. Thorington G, Margulis L. 1981. *Hydra viridis*: transfer of metabolites between *Hydra* and symbiotic algae. *Biol. Bull.* 160:175–88
101. Tothova Z, Gilliland DG. 2007. FoxO transcription factors and stem cell homeostasis: insights from the hematopoietic system. *Cell Stem Cell* 1(2):140–52
102. Travis J. 2009. On the origin of the immune system. *Science* 324(5927):580–82
103. Walther M, Ulrich R, Kroiher M, Berking S. 1996. Metamorphosis and pattern formation in *Hydractinia echinata*, a colonial hydroid. *Int. J. Dev. Biol.* 40:313–22
104. Wehkamp J, Salzman NH, Porter E, Nuding S, Weichenthal M, Petras RE. 2005. Reduced Paneth cell α-defensins in ileal Crohn's disease. *Proc. Natl. Acad. Sci. USA* 102:18129–34
105. Wittlieb J, Khalturin K, Lohmann JU, Anton-Erxleben F, Bosch TCG. 2006. Transgenic *Hydra* allow in vivo tracking of individual stem cells during morphogenesis. *Proc. Natl. Acad. Sci. USA* 103(16):6208–11
106. Zacharias H, Anokhin B, Khalturin K, Bosch TCG. 2004. Genome sizes and chromosomes in the basal metazoan *Hydra*. *Zoology* 107(3):219–27
107. Zhang X, Yalcin S, Lee DF, Yeh TY, Lee SM, et al. 2011. FOXO1 is an essential regulator of pluripotency in human embryonic stem cells. *Nat. Cell Biol.* 13(9):1092–99
108. Zilber-Rosenberg I, Rosenberg E. 2008. Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol. Rev.* 32:723–35

Annual Review of Microbiology

Volume 67, 2013

Contents

Index

Errata

An online log of corrections to *Annual Review of Microbiology* articles may be found at http://micro.annualreviews.org/