Rethinking the role of immunity: lessons from *Hydra*

Thomas C.G. Bosch

Zoological Institute, Christian-Albrechts-Universität zu Kiel, Olshausenstr. 40, 24098 Kiel, Germany

The ability of multicellular organisms to detect and respond to microorganisms is fundamental and has ancient evolutionary origins. In this review, I evaluate our current understanding of the evolution of epithelial-based innate immunity in *Hydra*, an apparently simple animal that shares deep evolutionary connections with all animals, including humans. I highlight growing evidence that the innate immune system with its host-specific antimicrobial peptides and rich repertoire of pattern recognition receptors has evolved in response to the need for controlling resident beneficial microbes rather than to defend against invasive pathogens. These findings provide new insight into how developmental pathways beyond those associated with the immune system, such as stem cell transcriptional programs, interact with environmental cues such as microbes.

Animal evolution is intimately linked to the presence of microbes

The interaction of bacteria with animal hosts is an interesting crossover topic of increasing importance [1,2]. Historically, bacteria are seen as pathogens. A significant leap forward in scientific understanding of the natural forces and risk factors affecting the patterns of illness and death came with Louis Pasteur’s discovery of the link between germs and disease [3,4]. This led the way for Robert Koch to demonstrate in 1876 that a microorganism was the cause of an infectious disease. In the subsequent years, numerous microorganisms were identified as the causative agents of important human diseases; and bacteriologists, microbiologists, and immunologists have continued to focus on bacteria as pathogens for more than 100 years. This approach has led to enormous insights in the battle between the invading harmful microbes and the host, as well as enabled the development of efficient strategies to fight infections.

Bacteria have existed from very early in the history of life on Earth. They inhabit every environment on the planet. In fact, most bacteria are not harmful to plants or animals, and many of them are beneficial, playing a key ecological role. Bacteria fossils discovered in rocks date from at least the Devonian Period, and there are convincing arguments that bacteria have been present since early in the Precambrian Era, approximately 3.5 billion years ago [5]. Because animals diverged from their protistan ancestors some 3 billion years after bacterial life originated, and as much as 1 billion years after the first appearance of eukaryotic cells [6–8], relationships of animals with bacteria were likely to be already in existence when animals first appeared near the end of the Proterozoic Eon. Animal evolution, therefore, is intimately linked to the presence of microbes.

Eukaryotic cells *per se* appear to be the descendents of separate prokaryotic cells that joined together in an endosymbiotic event, with mitochondria being the direct descendents of a free-living bacterium that was engulfed by another cell [9,10]. Moreover, all animals, ranging from simple invertebrates to primates, are host to complex microbial communities [11–13] and, therefore, must be considered a meta-organism comprised of the macroscopic host and synergistic interdependence with bacteria, archaea, fungi, and numerous other microbial and eukaryotic species [14].

Living in the Ediacaran oceans, *Porifera* (sponges) and *Cnidaria* (jellyfish, corals, and hyroids) evolved early during the phylogeny of multicellular animals (Metazoa) [15–18] (Figure 1). Cnidarians not only are among the earliest known phyletic lineages to form natural symbiotic relationships with bacteria and eukaryotes [19–21] but also possess most of the gene families found in Bilaterians and have retained many genes that have been lost in *Drosophila* and *Caenorhabditis elegans* [22–26]. For this reason, these ‘basal metazoans’ allow us to gain insights into the very early evolution of biological modules that may be involved in innate immune defenses.

In this review, I discuss the evolution and characteristics of the innate immune system in the early branching metazoan *Hydra*. I review recent findings that provide a new perspective on the relationship between bacteria and animal cells, and propose, in the context of the evolutionary time scale of bacterial and animal emergence, that innate immune system with its host-specific antimicrobial peptides and rich repertoire of pattern recognition receptors has evolved in response to the need for controlling resident beneficial microbes rather than to defend against invasive pathogens.

The innate immune system in *Hydra*

*Hydra*, like all other early emerging metazoans, has developed an effective innate immune system to detect and...
control microbial colonization [27,28]. *Hydra* uses two types of receptors and signaling pathways for microbial recognition – Toll-like receptors (TLRs) with MyD88 as a signal transducer, and nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs). Engagement of these receptors leads to the rapid induction of protective programs, for example, the induction of antimicrobial peptides (AMPs), or the elimination of the infected cell by means of apoptosis.

**Antimicrobial peptides as host-derived regulators of the microbiota**

AMPs are known as prominent effector molecules of the innate immune system in vertebrates and invertebrates, where they act by disrupting the structure or function of the microbial cell membranes [29]. To date, three families of potent AMPs have been identified in *Hydra*: the hydramacin, periclin, and arminin families of peptides [27,30]. Species-specific variability and constitutive high-level expression have made the arminin peptide family, in particular, an excellent candidate for investigating the role of AMPs in shaping the host-specific microbiota of *Hydra*.

Bacteria in *Hydra* are specific for any given species [11]. Closely related *Hydra* species, such as *Hydra vulgaris* and *Hydra magnipapillata*, are associated with a similar microbial community. 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 [31].

To examine the impact of arminin function on the resident microbial population in *Hydra*, and to broadly interfere with the host’s arminin expression, we generated transgenic *Hydra vulgaris* (AEP) polyps that expressed a hairpin cassette containing arminin antisense and sense sequences fused to a reporter gene [31]. The resulting double-stranded RNA (dsRNA) triggered the RNA interference (RNAi) machinery, which led to a 97% decrease in the endogenous arminin transcript. A tissue extract of arminin knockdown polyps showed a 50% decrease in bactericidal activity. For functional analysis, germfree control and arminin-knockdown polyps were generated and subsequently recolonized by foreign bacterial consortia provided by co-cultivation with other *Hydra* species, such as *Hydra oligactis* or *Hydra viridissima*. Four hundred and fifty-four pyrosequencing trials revealed [31] that arminin-deficient *Hydra vulgaris* (AEP) polyps have a decreased ability to select suitable bacterial partners from a pool of foreign potential colonizers, because they are colonized differently than control polyps, which select for bacterial types partially resembling their native microbiota. These findings suggest that AMPs shape the stable associated microbiota, acting as host-derived regulators of microbial diversity, rather than unspecific bactericides.

**The ancestral function of TLR-signaling**

TLRs are conserved throughout animal evolution, but appear to serve different functions in different model organisms [32,33]. The Toll pathway was initially identified to be essential in early embryonic development in *Drosophila* [34]. In addition to its essential crucial role in the establishment of the dorsal–ventral axis, *Drosophila* Toll-1 was shown to be involved in muscle development [35] and heart formation [36]. Later, it was discovered that Toll-1-signaling in *Drosophila* also contributes to defense reactions against bacteria as well as to antifungal defense by regulating, among others, the expression of the antifungal peptide drosomycin [37,38]. In addition, Toll-5 was recently shown to be involved in the control of the immune response in respiratory epithelia in *Drosophila* [39]. All other *Drosophila* Toll family members (Toll-2–9) act in embryonic and larval development and seem to have no clear *in vivo* function in immunity [40]. Other invertebrates such as the nematode *Caenorhabditis elegans* lack central proteins of the canonical TLR-signaling cascade [41]). Only one Toll-homologue termed TOL-1 was identified in *C. elegans* [42]. The fact that TOL-1 mutants show strong developmental defects, despite the fact that mutants for the putative signaling cascade do not show any developmental abnormalities, led to the belief that TOL-1 in *C. elegans* might function as a cell–cell adhesion protein in neurons [41]. By contrast, the vertebrate homologues of Toll, the TLRs, are predominantly pattern recognition receptors (PRRs) of the innate immune system and are involved in the elimination of pathogens and controlling commensal colonization [43–45].

The wide range of known functions of TLR-signaling, from development in insects and cell adhesion in nematodes to immunity in vertebrates, prompted us to search for the putative ancestral function of TLR-signaling. Using the basal metazoan *Hydra* we simply asked: which function – regulation of embryonic development or immune defense – was first in evolution? To address this question, we performed a MyD88 loss-of-function study in *Hydra vulgaris* (AEP). A stable transgenic *Hydra* line with reduced expression level of the universal adapter protein MyD88 was generated by RNAi-induced knockdown, and microarray analyses were performed to identify effector genes downstream of the TLR-signaling cascade [46]. In parallel, we analyzed the gene expression profile of germfree animals to directly investigate the connection between TLR-signaling and bacterial recognition. We found that more than 75% of the MyD88-responsive transcripts appeared to be also...
altered in germ-free polyps, indicating that expression of these genes is responsive to bacterial recognition. Reduced expression of MyD88 also impacted susceptibility to infection by the pathogenic bacterium *Pseudomonas aeruginosa* strain PA14 [46]. This finding indicates that bacterial recognition is an ancestral function of TLR-signaling and that this process contributes to both the host-mediated recolonization by commensal bacteria as well as to defense against bacterial pathogens.

TLR function in Hydra is realized by the interaction of a leucine-rich repeat (LRR) domain containing protein with a Toll/Interleukin-1 receptor (TIR)-domain-containing protein lacking LRRs [27]. By expressing the combination of *Hydra* HyLRR-2 lacking the TIR domain together with HyTRR-1 in human HEK293 cells, we demonstrated that recognition of bacterial molecules, such as flagellin, is mediated by an intermolecular interaction between HyLRR-2 as receptor and HyTRR-1 as signal transducer [27]. This receptor complex activation then triggers the innate immune response, which involves the production of species-specific antimicrobial peptides such as periculin [27].

These observations show that recognition of bacteria is an ancestral function of TLR-signaling, which is important for the selection and maintenance of resident microbiota, and contributes to defense against bacterial pathogens. Similar to Hydra, in mice, endocrine, goblet cells, and enterocytes of the intestinal epithelium express a range of Toll-like receptors to sense the presence of microbes [47]. Studies in knockout mice demonstrate that TLR signaling in the epithelium has a profoundly beneficial role in maintaining the resident microbiota [47]. Therefore, our findings in Hydra suggest that the role of the TLR pathway in controlling the resident microbiota could date back to the earliest multicellular organisms.

**A large and complex NLR repertoire in Hydra**

Microbes evading Hydra’s membrane-bound TLR receptors encounter another line of recognition defense inside the host cell, the NLR family of receptors [48–50]. Interestingly, there is an unexpectedly large and complex NLR repertoire in Hydra [51]. In a systematic survey of the NACHT and NB-ARC domain genes in existing expressed sequence tag (EST) and genome data sets, we observed that the *Hydra* genome has 290 putative NBD loci falling into two large groups: 130 of the NACHT domain type and 160 of the NB-ARC domain type [51]. The *Hydra* and Cnidaria NACHT/NB-ARC complements include novel combinations of domains. The number of one specific type [NB-ARC and tetratricopeptide repeat (TPR)-containing] in *Hydra* is particularly large [51]. Although these observations clearly show that NLRs are ancient genes, their immune function and role in cytoplasmic defense remains to be determined. Upon activation, NLRs recruit several supporting proteins that are necessary to form large molecular complexes, for example, the inflammasome comprised of NLRP1 or NLRP3 monomers, apoptosis-associated speck-like protein containing a CARD (ASC) [52], and caspase 1 [53,54]. Surveying the *Hydra* genome has allowed the identification of a number of potential NLR-interacting proteins. One of these, a caspase containing a DEATH domain, was shown to interact with a *Hydra* NLR-like protein in vitro [51]. Therefore, as in vertebrates, 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.

**Microbial colonization of Hydra epithelia**

All animals, ranging from simple invertebrates to primates, are host to complex microbial communities [11–13]. Microbial colonization appears to be an essential step in vertebrate ontogeny, contributing to the maturation of the immune system and gut development [55–57]. 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. Despite the importance of understanding the factors that control bacterial colonization in animals, however, the complexity and inaccessibility of the microbial niches of the vertebrate gut make it desirable to use a simple animal model for understanding the basic principles of the colonization process. We, therefore, explored the assembly of bacterial communities in *Hydra*. Our goal was to obtain mechanistic information about specific host–bacterial interactions and to elucidate the rules that determine how large and diverse bacterial populations colonize a newly born animal.

By profiling the assembly of the microbiota on *Hydra* epithelium up to 15 weeks post-hatching, we observed [58] 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 falsifiable predictions [58]. 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 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. 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. The observations also indicate that the bacterial community within an animal is not static, but constantly changing as part of a micro-evolutionary process, and that the process of bacterial colonization is a complex phenomenon where the system’s dynamics cannot be explained by merely adding the properties of its constituents. With these insights, we can now focus on identifying the underlying mechanistic processes and design experiments to address questions such as:

(i) Is the remarkable variability at the onset of the assembly process host-controlled and ontogenetically fixed, or a stochastic event?

(ii) Does the assembly process vary between related host species?
(iii) Is there a close connection between tissue integrity and colonization?

**Hydra links stem cell proliferation to innate immunity and microbiota composition**

As always, the unexpected is the most fascinating. First hints that there is indeed a relationship between regulators of tissue homeostasis and the associated microbiota came from efforts to uncover the molecular logic behind *Hydra*’s unlimited life span. *Hydra* seems to be one of few organisms who escape from aging, and at least in the laboratory, may be considered as potentially immortal (see Box 1 for background information on *Hydra*’s lifestyle and biology). Using an unbiased transcriptome analysis, we found that the transcription factor forkhead box O (FoxO) is strongly expressed by all three stem cell lineages in *Hydra*, whereas it is absent in differentiated cells [59]. By gain-of-function and loss-of-function analysis we subsequently could show that FoxO is indeed a critical component of the mechanisms controlling stem cell behavior in immortal *Hydra* [59]. In mammals, FoxO1 is essential for the maintenance of ESC self-renewal and pluripotency [60], whereas FoxO3a is critical regulator of stem cell homeostasis in neural [61], leukaemic [62], and hematopoietic stem cells (HSCs) [63].

**Box 1. *Hydra* as a model host**

*Hydra* belong to the phylum Cnidaria which is a sister group of the Bilateria (see Figure 1 in main text). 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 radial symmetrical body. The *Hydra* polyp has a very simple body plan consisting of two monolayered epithelia, the ectoderm at the outside, and the endoderm surrounding the gastric cavity (see Figure 2 in main text). Both epithelial layers are connected by an extracellular matrix termed mesogloea, which provides stability and elasticity to the polyp. Adult *Hydra* have approximately 100,000 cells, corresponding to cells of three independent stem cell lineages (i.e., the ectodermal epithelial cells, the endodermal epithelial cells, and the interstitial cells) whose differentiation behavior has been intensively studied during the past few decades. A network of signaling pathways allows the three stem-cell lineages to coordinate growth rates and to maintain tissue homeostasis [26]. *Hydra* primarily reproduces asexually by budding. The asexual mode of reproduction by budding, which requires a tissue consisting of stem cells with continuous self-renewal capacity, is also the reason for *Hydra*’s remarkable immortality.

For analytical purposes, *Hydra* is a premier model organism, which is propagated and cultured in the laboratory 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 a 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 facilitating *in vivo* tracing of cell behavior and characterization of gene expression patterns. Additionally, rich molecular resources, including genome sequences, are available. Because the genome organization and genome content of *Cnidaria* is remarkably similar to that of Bilaterians, these animals offer unique insights into the content of the ‘genetic tool kit’ present in the Cnidarian–Bilaterian ancestor. Transgenic *Hydra* can be easily generated by embryo microinjection [89], allowing functional analysis of genes controlling development and immune reactions, as well as *in vivo* monitoring of host-microbe interactions.

Likewise, foxO3a knockdown in bone marrow cells and neural stem cells (NSCs) in mice leads to a reduction of colony-forming cells and decrease in stem cell frequencies [61,63], clearly demonstrating a role of FoxO factors in stem cell maintenance. At the same time, there are strong data implicating FoxO in maintaining immune homeostasis in mammals. In the mouse, knocking down foxO3a perturbs cytokine production, induces NF-κB activation, hyperactivates T cells, and causes spontaneous lympho-proliferation, resulting in severe inflammation in several tissues [64,65]. Other studies showed that FoxO influences the innate immune system by regulating the activity of antimicrobial peptides. In *Drosophila* that are overexpressing dFoxO, direct binding of dFoxO to the drosomycin regulatory region leads to an induction of AMPs synthesis [66].

Similarly, silencing of FoxO activity in *Hydra* not only affects developmental and differentiation genes, but also changes the expression patterns of AMPs. Polyps where FoxO expression was reduced by RNAi showed significant changes in the expression of hydramacin, periculin, and aramin peptides [59]. In line with this, *in silico* analysis revealed multiple FoxO-binding sites on the promoter sequences of the corresponding genes. The continuously highly expressed AMPs of the aramin family appeared to be down-regulated, whereas the inducible AMPs hydramacin-1 and periculin-2b were up-regulated in foxO knockdown polyps [59]. The link between FoxO and components of the innate immune system indicates that, in *Hydra*, developmental pathways represented in this review by stem cell transcription factor FoxO are tightly coupled to innate immunity (Figure 2). This may be a common feature in plants and animals because stem cells in the shoot apical meristem of plants express high levels of a peptide controlling immune signaling and microbe interaction [67]. This is of particular interest because aging processes in humans are known to result in impairment of both innate and adaptive immunity (‘immunosenescence’) as well as in a pro-inflammatory status (‘inflammaging’) [68]. *Hydra* is one of the very few examples of animals that appear to be effectively immortal due to the asexual mode of reproduction by budding [69]. The *Hydra* perspective tells us that aging might be the consequence of both reduced stem cell function and altered innate immune defense. *Hydra* has much to teach us.

**Lessons from *Hydra***

**Complexity is the ancestral condition**

Since the turn of the 19th century, researchers have speculated about how our complex system of defenses arose [70,71]. To the present day, scientists are trapped by the prejudice that early emerging animals with their seemingly simple body plans have minimalistic developmental, behavior and physiological activities and, therefore, will provide simple answers. However, results from *Cnidaria* genome projects in *Nematostella* [25], *Hydra* [72], and *Acropora* [73] have overturned these prejudices drastically, revealing that the genomes of these simple invertebrates are unexpectedly complex and remarkably similar to those of vertebrates [22–25,74].

Genome sizes in *Hydra* are generally large, varying from 1450Mbp (*Hydra oligactis*) to 380Mbp in *Hydra viridissima* [75]. Cnidarian genomes, in addition to a large portion of
novel or ‘orphan’ genes [76], harbor many genes important for the development of key Bilaterian traits such as a mesoderm and bilaterality [77]. The complexity of developmental gene families [78–80], as well as the gene regulatory landscape [74] in Cnidaria, is similar to that found in vertebrates. Similar to development, innate immunity in Hydra [21,27] and other cnidarians [81] involves the activation of conserved signaling pathways, such as TLR and NLR signaling, but also the induction of ‘orphan’ genes (new genes that have no similarity with genes in other organisms; see Box 2) encoding cationic antimicrobial peptides that act by damaging microbial cell membranes. Taken together, because even the most ancient multicellular organisms employ sophisticated mechanisms for development and immune defenses, it now seems that complexity is the ancestral condition. More recently evolved invertebrates, such as worms and flies, have developed derived simplicity over evolutionary time, most likely due to extensive gene loss [22]. Our common ancestor apparently had all of the basic genetic components and a very similar genomic architecture to ourselves.

**The immune system—hardware for a functioning interspecies network**

Did the immune system evolve to defend against harmful pathogens? 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’

---

**Box 2. AMPs shed light on orphan genes and their role in lineage specific adaptations**

The genes encoding antimicrobial peptides (AMPs) in Hydra have no identifiable orthologs in other species outside the genus Hydra. Therefore, they represent new or taxonomically restricted genes (TRGs). The hallmark of the signature of a new gene (or orphan gene) is that it arises at some time within the evolutionary lineage towards an extant organism, and has no similarity with genes in organisms that have split before this time [76,90–92]. This distinguishes orphan genes from genes that arise through full or partial duplication processes to form paralogous genes or gene families [93,94]. An informative example is the Hydra gene *periculin-1*, so-called due to its rapid response to a wide variety of bacterial and tissue ‘danger’ signals [27]. Analysis of the deduced amino acid sequence of *periculin-1* and the charge distribution within the molecule revealed an anionic N-terminal region and eight cysteine residues containing a cationic C-terminal region [27]. 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. Each animal species contains a significant number of such ‘orphan’ genes encoding potent antimicrobial peptides, which are involved in the defense of taxon-specific microbial pathogens, as well as in shaping the species-specific commensal microbiota [76]. Evolutionary changes in the AMP repertoire of host species, therefore, are expected to lead to changes in the composition of the associated bacterial community. The finding that arminin-deficient Hydra polyps have a decreased ability to select suitable bacterial partners from a pool of foreign potential colonizers [31] (see main text) strongly supports this assumption and shows that specific associations between hosts and bacteria are a result of bacterial adaptation to different repertoires on AMPs. Therefore, taxonomically restricted host defense molecules, such as AMPs, play a major role in lineage specific adaptations by shaping the colonizing microbiota, and therefore, contribute to evolutionary innovations. It has been shown previously in Hydra [95], fly [90], and mouse [96] models that *de novo* gene evolution and ‘orphan’ genes play an important role, not only in the innate defense system, but also in the creation of phylum specific novelities and in the generation of morphological diversity. Given that *de novo* evolution of transcripts and genes from intergenic non-coding sequences is a rather active process throughout all evolutionary times [97], and assuming that these taxon-specific genes enable organisms to adapt to changing conditions [76,98], it would be surprising if comparative analyses of RNAseq transcriptomes and genome wide data would not lead to the identification of large populations of novel genes that might reflect species-specific evolutionary events.
Numerous observations in Hydra indicate that immune systems evolved as much to manage and exploit beneficial microbes as to fend off harmful ones. Evidence for this view comes from the discovery that individuals from different species differ greatly in their microbiota, and that individuals living in the wild are colonized by microbiota similar to that in individuals grown in the lab, pointing to the maintenance of specific microbial communities over long periods of time [11, 13, 82]. As a result of the finding that interactions between animals and microbes are not specialized occurrences, but rather are fundamentally important aspects of animal biology [2, 14], and that AMPs and other components of the immune system are key factors for allowing the right microbes to settle and to kick the less desirable ones out [31, 83], the view of the role of the immune system has changed radically in the last decade.

It has been known for some time that aging is accompanied by profound changes in the immune system [84, 85]. This gradual deterioration of the immune system, also termed immune senescence [86], is characterized by a breakdown of the epithelial barriers of the skin, lungs, and gastrointestinal tract [87] as well as by changes in both the adaptive [88] and the innate immune system [68]. What was not known was how developmental pathways and mechanisms controlling tissue homeostasis in the adult are linked to components of the innate immune systems. Gain and loss of function analyses in Hydra have shown [61] that FoxO plays an important role in controlling the expression of antimicrobial peptides and immune functionality. This has at least shed some light on the age-old problem of how developmental pathways are linked to components of innate immunity.

Concluding remarks
Our work has shown that seemingly simple Hydra polyps provide us with important information in understanding the evolution of epithelial-based innate immunity. The work has contributed to a paradigm shift in evolutionary immunology: components of the innate immune system, with its host-specific antimicrobial peptides and a rich repertoire of pattern recognition receptors, appear to have evolved in early branching metazoans because of the need to control the resident beneficial microbes rather than because of invasive pathogens. Yet in spite of all these insights in an ultimately simple ‘holobiont’, we have still not been able to coherently integrate the accumulated abundance of information into a truly mechanistic understanding of host–microbe interactions. Questions to be addressed in the future include:

(i) What are the functional roles of all members of the Hydra holobiont?
(ii) How do bacteria contribute to the phenotypic stability of their hosts?
(iii) What effect does a changing environment have on microbial associates and the fitness of the holobiont?

Elucidating these issues will not only contribute to the understanding of host interactions with microbial communities in one of the simplest possible animal systems, but may also provide conceptual insights into the complexity of host–microbe interactions in general. I conclude that we have only begun to understand the true impact of microbiota on shaping host physiology and the emergence of new functions, including immunity.

Acknowledgments
I would like to thank the contributions of many students, postdocs, and co-investigators over the years, particularly to Rene Augustin and Sebastian Fraune, who initiated work on innate immunity in my lab. I thank Alexander Klimovich for support with the figures, Ralph V. Williams for helping to shape the manuscript, and Judith Ittner-Bosch for invaluable support. The work related to this review was supported in part by grants from the Deutsche Forschungsgemeinschaft (DFG).

References
26 Hemmrich, G. 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, 3267–3280
54 Franzenburg, S. et al. (2013) Bacterial colonization of Hydra hatchlings follows a robust temporal pattern. ISME J. 7, 781–790
59 Miyamoto, K. et al. (2007) Foxo3a is essential for maintenance of the hematopoietic stem cell pool. Cell Stem Cell 1, 101–112
88 Aw, D. et al. (2007) Immunosenescence: emerging challenges for an ageing population. Immunology 120, 435–446