

# What Hydra Has to Say About the Role and Origin of Symbiotic Interactions

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**Abstract.** The *Hydra* holobiont involves at least three types of organisms that all share a long coevolutionary history and appear to depend on each other. Here I review how symbiotic algae and stably associated bacteria interact with the *Hydra* host and where in the tissue they are located. In particular I discuss the role of Toll-like receptor (TLR) signaling in maintaining *Hydra*'s species-specific microbiota. I also discuss studies in *Hydra viridis* and its symbiotic *Chlorella* algae which indicate that the symbiotic algae are critically involved in the control of sexual differentiation in green *Hydra*. Finally, I review the state of “omics” in this tripartite association and the fact that the functioning of this holobiont is also a tale of several genomes.

## The *Hydra* Holobiont—A Tale of Several Symbiotic Lineages

Inter-species interactions in the freshwater polyp *Hydra* (Fig. 1A) between symbiotic algae and host cells have been the subject of research for decades because they not only provide insights into the basic “tool kit” necessary to establish symbiotic interactions but also are relevant in understanding the resulting evolutionary selection processes (e.g., Muscatine and Lenhoff, 1963, 1965a, b; Pool, 1979; Thorington and Margulis, 1981; O'Brien, 1982). It is now becoming evident that in *Hydra* a long-term persistence of mutualistic associations is prevalent not only in two-party interactions of polyp and symbiotic algae but also in more complex systems comprising three or more associates including bacteria and viruses (Fig. 1B). Thus, the study of inter-species interactions in *Hydra* and other cnidarians may be a paradigmatic example of a complex community that influences the host's health and development.

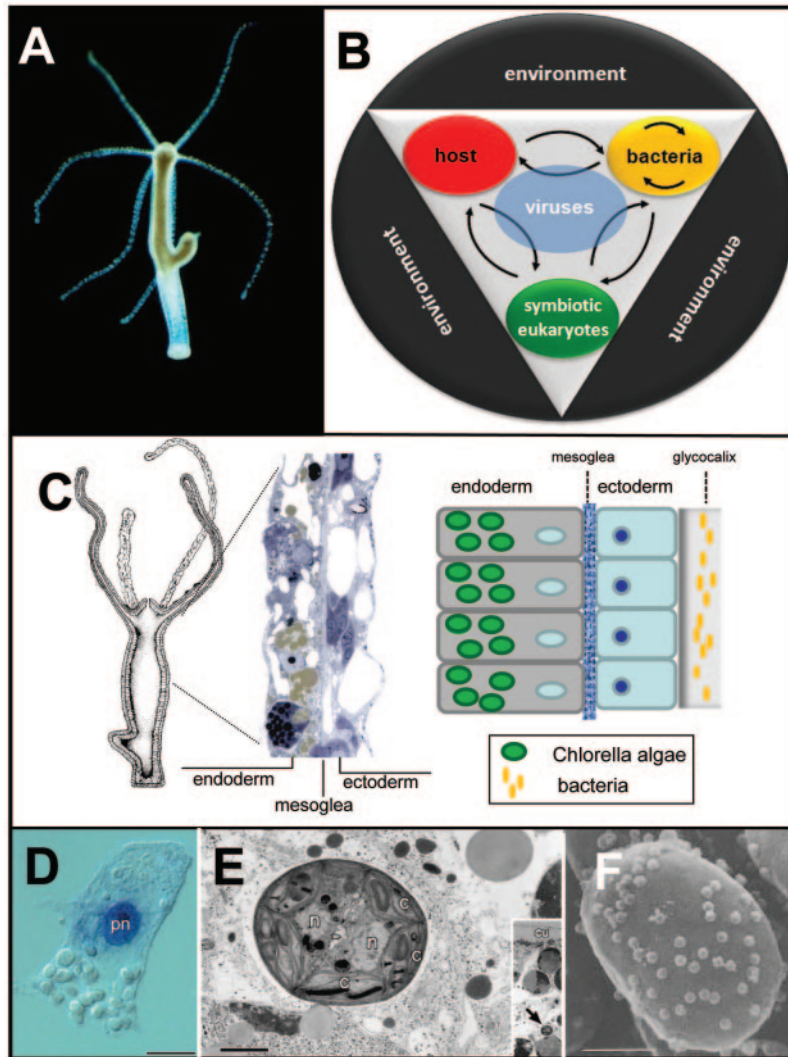
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Bacteria in *Hydra* (predominantly Proteobacteria) are specific for any given species, indicating that the *Hydra* epithelium actively selects and shapes its bacterial community (Fraune and Bosch, 2007, 2010) and that these stably associated microbes not only play an important role in the lives of their polyp hosts but also are important in their evolution (Bosch, 2012). Rosenberg and colleagues have argued recently that the holobiont (the host and its symbiotic microbiota) should be considered a unit of selection in evolution (Rosenberg *et al.*, 2007, 2009; Zilber-Rosenberg and Rosenberg, 2008). The holobiont, or “metaorganism,” concept (see also Bosch and McFall-Ngai, 2011) considers the dynamic communities of bacteria on epithelial surfaces as an integral part of the functionality of the organism itself. In this review I show that innate immune mechanisms play a crucial role in the *Hydra* holobiont and that *Hydra* provides an efficient, genetically tractable model system for the molecular dissection of these interactions.

## Localization and Interaction of Symbiotic Algae and Stably Associated Bacteria With *Hydra*

In the green species *Hydra viridis*, the *Chlorella* symbionts are located in endodermal epithelial cells (Fig. 1C). A single endodermal epithelial cell usually contains 20 to 40 algae (Fig. 1D). Each alga is enclosed by an individual vacuolar membrane (Fig. 1E) resembling a plastid of eukaryotic origin like the complex plastids of Chlorarachniophytes at an evolutionary early stage of symbiogenesis (Habetha *et al.*, 2003). Proliferation of symbiont and host is tightly correlated (McAuley, 1981, 1985). Although it is not yet known how *Hydra* controls cell division in symbiotic *Chlorella*, the pioneering studies by Rahat and Reich in the 1980s (e.g., Rahat and Reich, 1983, 1984; Rahat, 1985) showed that there is a great deal of adaptation and specificity in this symbiotic relationship. Symbiotic *Chlorella* strain A99 is unable to grow outside its polyp host, indicat-



**Figure 1.** The *Chlorella* holobiont. (A) A *Hydra oligactis* polyp. (B) Multicellular organisms are metaorganisms comprising the macroscopic host and its synergistic interdependence with bacteria, archaea, fungi, and numerous other microbial and eukaryotic species including algal symbionts. (C) Localization of symbiotic algae and bacteria in *Hydra* epithelium. (D) Phase contrast micrograph of a single macerated endodermal epithelial cell containing symbiotic algae in the basal part below the nucleus (stained blue). Scale bar, 5  $\mu\text{m}$ . From Habetha *et al.*, 2003, with permission from Elsevier. (E) Electron micrograph of a symbiotic *Chlorella* within embryonic tissue of *Hydra viridis* strain A99. The endosymbiont is in the process of mitosis, so that two nuclei and several plastids are visible in this section. The inset shows the localization of the alga (arrow) within the embryo. Scale bar, 1  $\mu\text{m}$ . cu, cuticle, n, algal nucleus; c, chloroplast. Reprinted from Habetha *et al.*, 2003, with permission from Elsevier. (F) *Chlorella* with phytoviruses. Photo: Reprinted from Meints *et al.*, 1984, with permission from Elsevier.

ing loss of autonomy during establishment of the intimate symbiotic interactions with *Hydra* (O'Brien, 1982; Habetha *et al.*, 2003). The photosynthetic symbionts provide nutrients to the polyps in the form of maltose or glucose-6-phosphate, enabling *H. viridis* to survive periods of starvation (Lenhoff and Muscatine, 1963; Muscatine, 1965; Roffman and Lenhoff, 1969; Cook and Kelty, 1982; Huss *et al.*, 1993/94). Symbiotic algae can be removed from *H. viridis* polyps experimentally by various means (Jolley and Smith, 1978; Pardy, 1983; Muscatine, 1983). During sexual

reproduction of the host, *Chlorella* algae are translocated into the oocyte, giving rise to a new symbiont population in the hatching embryo to ensure transmission of the symbiotic algae from generation to generation (Hamann, 1882; Muscatine and McAuley, 1983; Campbell, 1990). Symbiotic algae have a severe impact on sexual reproduction in *H. viridis* by promoting oogenesis but not spermatogenesis (Habetha *et al.*, 2003; Habetha and Bosch, 2005). This is similar to findings in symbiotic anthozoans where in both a scleractinian coral and in a soft coral, loss of symbionts is

**Table 1***A compilation of frequently used terms*

Term	Definition
Symbiosis	A long-term association between different species from which all participating organisms benefit.
Microbiota	The microorganisms that typically inhabit body surfaces covered by epithelial cells and are exposed to the external environment. The microbiota represents a complex, dynamic, and diverse collection of different species of bacteria. Microbial community members can be identified by sequence comparison to known bacterial 16S rRNA gene sequences.
Metaorganism	An association composed of the macroscopic plant or animal host and synergistic interdependence with bacteria, Archaea, fungi, viruses, and numerous other microbial eukaryotic species including algal symbionts. The term “metaorganism” defines a superordinate entity that is applicable to all kinds of interdependent associations and is not constrained to specific taxonomic groups such as “holobiont” (usually used for cnidarians) or “superorganism” (used for social insects such as ants).
Holobiont	The cnidarian host organism and all of its symbiotic algae and stably associated microbiota.

correlated with a drastically reduced reproductive output (Michalek-Wagner and Willis, 2001). Very little is known about the underlying genetics and molecular basis that enables *Chlorella* to survive and proliferate within the vacuoles of *H. viridis* and controls the interaction between the partners. We are exploring the impressive capabilities of the *Hydra* holobiont by asking a range of questions. By what mechanisms does the *Hydra* host recognize its specific algal partner? What are the influences of symbiotic algae on developmental processes of the *Hydra* host and how is the symbiont population maintained in balance over the host's lifetime, such that neither does the symbiont overgrow the host nor does the host eliminate the symbiont? Do the difficulties of growing symbiotic *Chlorella* outside their host cells reflect the fact that the endosymbionts have transferred some of their genetic material to the nuclear genome of *Hydra*?

Beside photosynthetic algae, bacteria are another important component of the *Hydra* holobiont (Fig. 1B). The 36 identified bacterial phylotypes represent three bacterial divisions and are dominated by Proteobacteria and Bacteroidetes (Fraune *et al.*, 2007, 2010). In the *Hydra vulgaris* strain Basel, Bacteroidetes are represented by two phylotypes (or operational taxonomic units, OTUs) and  $\beta$ -Proteobacteria by seven phylotypes including Polynucleobacter as the most abundant bacterium. In *H. vulgaris* strain AEP, Bacteroidetes are represented by five phylotypes and  $\beta$ -Proteobacteria by nine phylotypes, which include the abundant *Curvibacter* bacterium. In *Hydra oligactis*, the majority of

phylotypes belong to the  $\beta$ -Proteobacteria (Rickettsiales). *Hydra*'s stably associated bacteria are almost exclusively associated with the ectodermal epithelium and in particular with the glycocalyx layer (Fig. 1C; unpubl. data). The glycocalyx represents an array of highly diverse glycoproteins and glycolipids expressed on the membrane of epithelial cells. This complex mucous layer may not only provide a food source for bacteria but also selectively enhance the survival of specific bacterial species due to the composition of the mucus. Since an intact glycocalyx is required to prevent infection with, for example, pathogenic *Saprolegnia ferax* spores (Augustin, Hahn, and Bosch, unpubl. data), *Hydra*'s glycocalyx appears to represent the front-line defense barrier between the external environment and the epithelial tissues. Different *Hydra* species are host to different microbiota. Remarkably, *Hydra* living in the wild in their native habitats are colonized by a composition of microbes similar to that of the *Hydra* polyps cultured under controlled laboratory conditions for extended periods of time (Fraune and Bosch, 2007). Thus, the *Hydra* host appears to selectively shape its bacterial community. Genetic factors of the host apparently outweigh environmental influences in determining microbial surface colonization. What are the specific factors that come into play? And how does epithelial homeostasis affect microbial community structure? One factor almost certainly involved is the tissue architecture of the host. We showed that eliminating distinct cell types from the epithelium subsequently causes significant changes in the bacterial community of *Hydra* (Fraune *et al.*, 2009). When compared with controls, animals lacking neurons and gland cells showed reduced abundance of  $\beta$ -Proteobacteria accompanied by a significantly increased abundance of a Bacteroidetes bacterium. This previously unrecognized link between cellular tissue composition and microbiota demonstrates that there is a direct interaction between epithelia and microbiota. How then does the host sense the microbes?

### ***Hydra*/Microbe Interkingdom Communication Involves TLR and NLR Signaling as well as Epithelial-Derived Antimicrobial Peptides**

The Toll-like receptor (TLR) system has been an archetype of our understanding of how invariant extracellular sensor structures may recognize threats induced by microbial assaults (Rakoff-Nahoum *et al.*, 2004; Iwasaki and Medzhitov, 2004; Fitzgerald and Chen, 2006). 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 (Bosch *et al.*, 2009; Augustin *et al.*, 2010). Co-expression of both membrane proteins is linked to antimicrobial peptide (AMP) induction *in vivo*, and heterologous overexpression of the two *Hydra* proteins in mammalian

cell lines leads to a sensitization to the microbial associated molecular pattern (MAMP). *Hydra* epithelial cells respond to MAMPs by cytoskeletal rearrangement and increased secretory activity of AMP (Bosch *et al.*, 2009; Augustin *et al.*, 2010). To date, three large families of AMPs—hydracin, periculin and arminin—have been identified in *Hydra* (Bosch *et al.*, 2009; Augustin *et al.*, 2009; Jung *et al.*, 2009; Augustin and Bosch, 2011). Some of the AMPs were found to control the bacterial colonization of both the early embryo and the adult polyp (Fraune *et al.*, 2010). Thus, antimicrobial peptides, until now known as gene-encoded key elements of innate immunity, apparently play a key role in the molecular communication between bacteria and host by regulating the composition of the colonizing microbiota.

In addition to the canonical TLR signaling cascade, early diverging metazoans such as the cnidarians have large and complex NLR repertoires (Lange *et al.*, 2011). NLR genes encode for cytosolic proteins that compose a trimodular domain structure, characterized by a central nucleotide-binding and oligomerization domain (NOD), and an N-terminal DEATH-fold-like effector binding domain. To understand the evolutionary origins of NLRs as epithelial sensors involved in maintenance of commensal diversity and defense against pathogens, we have set out to systematically survey the repertoires of NACHT and NB-ARC domain genes in *Hydra* (Lange *et al.*, 2011). Our phylogenetic analysis shows that in *Hydra* as well as in many other animal species, NLR gene expansions occurred (Lange *et al.*, 2011). Whether these cytosolic MAMP sensors play roles in species-specific adaptations to a variety of ecological niches or in maintaining a stable host-microbe community remains to be shown.

### The *Hydra* Holobiont—A Tale of Several Genomes

Novel computational tools and genomic resources such as the *Hydra magnipapillata* genome (Chapman *et al.*, 2010), the genome of a *Curvibacter* bacterial species that is stably associated with *Hydra magnipapillata* (Chapman *et al.*, 2010), and the *Chlorella* NC64 genome project (Blanc *et al.*, 2010), together with several large-scale expressed sequence tag (EST) projects (Kortschak *et al.*, 2003; Technau *et al.*, 2005; Miller *et al.*, 2007; Hemmrich *et al.*, 2007a, 2012) have brought a molecular perspective on the *Hydra* holobiont. The genome sequence of *H. magnipapillata* (Chapman *et al.*, 2010) revealed an unexpectedly high genetic complexity. We also sequenced the genome of a bacterial species in the *Curvibacter* genus that is stably associated with *Hydra*. The *H. magnipapillata* genome is large (1290 Mbp in size) (Zacharias *et al.*, 2004; Hemmrich *et al.*, 2007b; Chapman *et al.*, 2010) and contains about 20,000 protein coding genes. We found clear evidence for conserved genome structure between *Hydra* and other animals, including humans. This contrasts with organisms such

as *Drosophila* and the roundworm *Caenorhabditis elegans* in which gene order has been shuffled extensively during evolution. In spite of the fact, however, that *Hydra* belongs to the phylogenetically oldest eumetazoan lineage, this organism certainly is not a “living fossil”; its genome contains a rather unique combination of ancestral, novel (see Khalaturin *et al.*, 2009), and “borrowed” (*e.g.*, via horizontal gene transfer) genes, similar to the genomes of other animals. Nuclear horizontal gene transfer (HGT) is rare in multicellular eukaryotes. The *Hydra flp* gene, whose homologs are in the genome of *Trichomonas* protists (Steele *et al.*, 2004; Dana *et al.*, 2012), may represent an example of such a rare occurrence. Interestingly, the *H. magnipapillata* genome is about three times larger than that of *Hydra viridis* (which contains algal symbionts of the *Chlorella* group) (Zacharias *et al.*, 2004; Hemmrich *et al.*, 2007b). Transposon expansion and a large amount of repetitive DNA appear to be the major factors contributing to the large genome size of *H. magnipapillata* (Chapman *et al.*, 2010). Thus, there have been dramatic changes in genome size in the *Hydra* lineage, and the question arises why the only symbiotic species within this genus, *H. viridis*, has a 3-fold smaller genome than the nonsymbiotic species (Zacharias *et al.*, 2004). Is the small genome size in *H. viridis* a consequence of the symbiotic interaction with *Chlorella* algae? Interestingly, in *H. viridis* the size of certain cell types including the interstitial cells is conspicuously smaller than in *Hydra* species that are not associated with *Chlorella* algae. It is known that by modulating DNA content, animals can fine-tune metabolic rates via the intermediate of cell size (Vinogradov, 1995, 1997; Gregory and Hebert, 1999; Gregory, 2002). Since small cell sizes appear to require small genomes, it is tempting to speculate that the contribution of symbiotic *Chlorella* algae to the metabolism of *H. viridis* polyps (Habetha *et al.*, 2003) explains not only the small cell size but also the small genome.

Sequence assembly of the genome of the most abundant bacterium that is stably associated with *H. magnipapillata* yielded eight large contigs that span a total of 4 Mb and represent an estimated 98% of the bacterial chromosome of a novel *Curvibacter* species belonging to the family Comamonadaceae (order Burkholderiales) (Chapman *et al.*, 2010). About 60% of annotated *Curvibacter* genes have an ortholog in another species of Comamonadaceae. Notably, the *Curvibacter* sp. genome encodes nine different ABC transporters putatively involved with sugar transport, compared to only one or two in other species of Comamonadaceae, possibly reflecting an adaptation to life in the sugar-rich glycocalyx layer of the *Hydra* epithelium.

The recent analysis of the complete genome of a *Chlorella* sp. NC64A (Blanc *et al.*, 2010) has also produced a number of unexpected findings. This *Chlorella* strain is able to infect *H. viridis* endothelial cells, but in contrast to *Chlorella* strain A99 does not reside permanently in the

*Hydra* cells (Habetha *et al.*, 2003). Since *H. viridis* and *Chlorella* NC64A can be cultivated separately, this symbiosis is considered facultative in laboratory conditions. Since *Chlorella* algae are also host for a family of large double-stranded DNA viruses that are found in freshwater throughout the world (Fig. 1F), the genomic analysis of *Chlorella* promises insights into both algal symbioses and algal-viral interactions. A total of 9791 protein-encoding genes were predicted in the *Chlorella* NC64A genome (Blanc *et al.*, 2010). Surprisingly, these include a number of genes encoding known meiosis-specific proteins, despite the fact that *Chlorella* NC64A has long been assumed to be asexual (Grimsley *et al.*, 2010). The genomic analysis also led to unexpected insights with respect to genes involved in *Chlorella* cell wall metabolism. The *Chlorella* cell wall contains glucosamine polymers, such as chitin and chitosan, instead of the cellulose and hemicelluloses that make up the cell walls of land plants. However, the *Chlorella* NC64A genome does not contain homologs of plant genes involved in synthesis of cellulose or hemicelluloses. Much to the surprise of the sequencing consortium, such homologs were found in *Chlorella* DNA viruses, leading to the hypothesis that components of *Chlorella* chitin metabolism could have been acquired *via* lateral gene transfer from a virus. This so-called “chlorovirus” (Blanc *et al.*, 2010) is itself endowed with chitinase, an enzyme capable of specifically breaking down chitin. By this means the virus has secured exclusive use of its *Chlorella* host against other viruses, which are incapable of piercing through the *Chlorella* cell wall. With so many unexpected insights coming from the sequence of the *Chlorella* sp. NC64A genome, we now eagerly await the genome analysis of *Chlorella* sp. A99, the obligate symbiont of *H. viridis*.

To facilitate access to and analysis of genomic and transcriptomic data of the members of the *Hydra* holobiont, we have established a local bio-computational platform, compagen (Hemrich and Bosch, 2008). The sequence databases at compagen contain regularly updated sequences from sponges and cnidarians up to the lower vertebrates. In addition, compagen also provides already processed data such as assembled EST datasets or predicted peptides. The data sets compiled in compagen offer convincing proof for the view that Cnidaria share most of their genes with the Bilateria and that many human disease genes had already evolved in the common ancestor of the Cnidaria and Bilateria (Kortschak *et al.*, 2003; Technau *et al.*, 2005; Hemrich *et al.*, 2007a; Miller *et al.*, 2007; Domazet-Loso and Tautz, 2008).

### What *Hydra* Has to Offer

The results reviewed here provide compelling evidence for a complex cross-talk of an ancient epithelial barrier and the residing symbiotic algae and resident bacteria. I have

also shown that we are beginning to understand that the complex interaction between commensal communities of bacteria residing on the surfaces of *Hydra*'s epithelial barriers is required for innate defenses and maybe even for normal development. Despite these insights, however, mechanisms that mediate the interdependent and complex interactions within this holobiont, or metaorganism, are almost entirely unknown. How do close associations of organisms influence each other's fitness? How do the associated organisms coordinate their interactions at the molecular level? How do the underlying reactive genomes co-evolve? What is driving this symbiotic relationship? Do the microbes shape their environment or adapt to the host environment? Finally, only a few studies have focused on the “resilience” phenomenon, that is, the capacity of the microflora to regain homeostasis of diversity after environmental challenges such as infections. This phenomenon involves the process of “quorum sensing,” that is, the communication between microorganisms by specific ligands such as homoserine lactones, but also the creation of ecological niches for beneficial microbes by the *Hydra* epithelium. *Vice versa*, a requirement of tonic recognition of bacterial components through TLRs and NLRs seems to exist in order to create a normal regenerative capacity of the epithelium (Rosenstiel *et al.*, 2009).

Clearly, we are far from understanding the *Hydra* holobiont and the numerous interactions between *Hydra*, symbiotic algae, and stably associated bacteria and viruses. But recent technical advances with the potential of large-scale gene expression analysis and comparative genome assessments give good reason for hope that elucidating the molecular basis of one of the most remarkable animal-algae-microbe associations formed in millions of years of coevolution is possible in the near future. Novel high-throughput sequencing technologies will allow building comparative maps of the members of this tripartite holobiont together with an inventory of the associated bacteria in different populations. These data will provide a solid basis for systems biology approaches to understand the establishment, function, and collapse of the symbiotic interactions involved as well as a platform for understanding the response of a holobiont to environmental change.

Understanding the complex interactions maintaining holobionts in time and space certainly is a challenging task ahead of us. As shown here, *Hydra* appears to be a particularly suitable model organism for approaching these questions. Such questions, when addressed in an integrative and comparative context, are relevant not only for our understanding of the fundamental mechanisms controlling host-microbe interactions in the common ancestor of all animals, but also because answering them may contribute to the emerging concept of evolutionary medicine in order to identify novel targets for therapeutic augmentation of epithelial barrier function. Time will tell whether this ancient

tale of several genomes develops into a relevant human paradigm. But even without that, the *Hydra* holobiont for sure will help us understand ourselves and our position in nature.

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