Chapter 8
Where Simplicity Meets Complexity: *Hydra*, a Model for Host–Microbe Interactions

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Abstract For a long time, the main purpose of microbiology and immunology was to study pathogenic bacteria and infectious disease; the potential benefit of commensal bacteria remained unrecognised. Discovering that individuals from *Hydra* to man are not solitary, homogenous entities but consist of complex communities of many species that likely evolved during a billion years of coexistence (Fraune and Bosch 2010) led to the hologenome theory of evolution (Zilber-Rosenberg and Rosenberg 2008) which considers the holobiont with its hologenome as the unit of selection in evolution. Defining the individual microbe–host conversations in these consortia is a challenging but necessary step on the path to understanding the function of the associations as a whole. Untangling the complex interactions requires simple animal models with only a few specific bacterial species. Such models can function as living test tubes and may be key to dissecting the fundamental principles that underlie all host–microbe interactions. Here we introduce *Hydra* (Bosch et al. 2009) as such a model with one of the simplest epithelia in the animal kingdom (only two cell layers), with few cell types derived from only three distinct stem cell lineages, and with the availability of a fully sequenced genome and numerous genomic tools including transgenesis. Recognizing the entire system with its inputs, outputs and the interconnections (Fraune and Bosch 2010; Bosch et al. 2009; Fraune and Bosch 2007; Fraune et al. 2009a) we here present observations which may have profound impact on understanding a strictly microbe-dependent life style and its evolutionary consequences.

The Basal Metazoan Model Organism *Hydra* Enters the Genomic Era

Hydra belongs to one of the most basal eumetazoan phylum, the Cnidaria which are a sister taxon to all Bilateria. *Hydra* represents a classical model organism in developmental biology which was introduced by Abraham Trembley as early as 1744 (Trembley 1744). Because of its simple body plan, having only two epithelial layers (an endodermal and ectodermal epithelium separated by an extracellular matrix termed mesogloea), a single body axis with a head, gastric region and foot, and a limited number of different cell types, *Hydra* served for many years as model in developmental biology to approach basic mechanisms underlying de novo pattern formation, regeneration, and cell differentiation.

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The genome of *Hydra magnipapillata* is relatively large (1,300 Mb) (Chapman et al. 2010). Since up to 40% of the whole genome is composed of transposable elements (Chapman et al. 2010), this was interpreted as "a very dynamic genome" in which recombination events might occur even without sexual recombination. Whether this, in combination with horizontal gene transfer and trans-splicing, allows the immortal, constantly regenerating and asexually proliferating polyps to quickly adapt to changing environmental conditions, remains a matter of debate. In addition to the *Hydra magnipapillata* genome, a large set of expressed sequence tags (ESTs) is available at www.compagen. org (Hemmrich and Bosch 2008). Adding to the relatively rich data sets available in *Hydra*, in recent years, additional genome and transcriptome sequences have become available from related basal metazoans such as corals and *Nematostella,* shedding new and bright light on the ancestral gene repertoire (Putnam et al. 2007; Rast et al. 2006; Srivastava et al. 2008). The accumulated data show that Cnidaria posses most of the gene families found in bilaterians (Putnam et al. 2007; Kusserow et al. 2005; Kortschak et al. 2003; Miller et al. 2005) and therefore have retained many ancestral genes that have been lost in *D. melanogaster* and *C. elegans* (Miller et al. 2005; Technau et al. 2005). Since the genome organization and genome content of Cnidaria is remarkably similar to that of morphologically much more complex bilaterians, these animals offer unique insights into the content of the "genetic tool kit" present in the Cnidarian–bilaterian ancestor.

For analytical purposes, an important technical breakthrough in studies using basal metazoans was the development of a transgenic procedure allowing efficient generation of transgenic *Hydra* lines by embryo microinjection (Wittlieb et al. 2006). This not only allows functional analysis of genes controlling development and immune reactions, but also in vivo tracing of cell behavior.

Hydra Has an Effective Innate Immune System

The tube-like body structure of *Hydra* resembles in several aspects the anatomy of the vertebrate intestine with the endodermal epithelium lining the gastric cavity, and the ectodermal epithelium providing a permanent protection barrier to the environment (Fig. 8.1a, b). In *Hydra*, epithelial cells in both layers are multifunctional having both secretory and phagocytic activity (Bosch et al. 2009; Bosch and David 1986). A combined biochemical and transcriptome analysis approach revealed that in *Hydra,* most innate immune responses are mediated by epithelial cells (Bosch et al. 2009; Jung et al. 2009). Although there are no motile immune effector cells or phagocytes present in *sensu stricto*, endodermal epithelial cells not only contribute to digestion and uptake of food, but also are able to phagocytose bacteria present in the gastric cavity (Fig. 8.1c). Within the endodermal layer, gland cells contribute to innate immune reactions by producing potent antimicrobial serine protease inhibitors (Augustin et al. 2009a). Some cnidarians have a remarkable capability of regeneration. In *Hydra*, for example, gross damage to the tissue is quickly repaired due to the presence of continuously proliferating stem cells (Bosch 2007). Since cells infected or damaged by pathogens, such as bacteria or fungi, are quickly removed by apoptosis (Bosch and David 1986) and replaced by non-infected cells, this enormous regeneration capacity may be considered an additional arm of the innate immune defense.

At the molecular level *Hydra* recognizes "Microbial Associated Molecular Patterns" (MAMPs) with the help of the TLR signaling pathway. The Toll-like receptor in *Hydra*, functions as a co-receptor with the MAMP recognizing Leucin-rich repeats and the signal transmitting TIR domain on two separated but interacting proteins (Bosch et al. 2009) (Fig. 8.2). Homologous sequences to nearly all other components of the TLR pathway were identified (Bosch et al. 2009) including NFkB (unpublished) (Fig. 8.2). RNAi knock down experiments with Hydra TLR showed a drastic reduction of antimicrobial activity in the knock down tissue compared to the wild type, which makes it apparent that antimicrobial activity relies directly on the activation of the TLR cascade
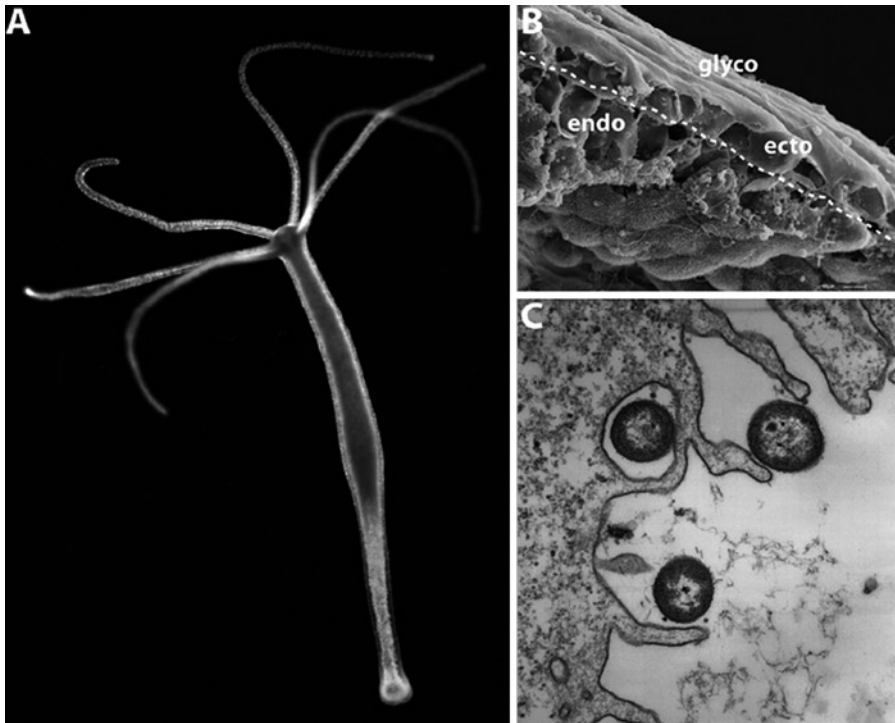
Fig. 8.1 Cnidarians are diploblastic animals. (**a**) Live image of *Hydra oligactis* (Photo by S. Fraune). (**b**) Raster electron micrograph showing the ectodermal (*ecto*) and endodermal epithelium (*endo*); which are separated by an extracellular matrix (mesoglea – *dashed line*); a true mesoderm is missing. The apical part of *Hydra* ectodermal epithelial cells is covered by a glycocalix layer (glyco) (Photo by F. Anton-Erxleben). (**c**) Transmission electron micrographic of endodermal epithelial cells phagocyte bacteria from the gastric lumen (Panel C modified from Bosch et al. 2009)

(Bosch et al. 2009) (Fig. 8.2). In addition to recognizing MAMPs at the cell membrane, intracellular recognition of bacteria in *Hydra* is mediated by an unexpected large number of cytosolic NOD-like receptors (Lange et al. 2011). We have proposed elsewhere (Lange et al. 2011) that upon their activation and dimerisation apoptosis as an evolutionary old mechanism in immune defence might be initiated (Lange et al. 2011).

Prominent effector molecules downstream of the conserved TLR cascade are antimicrobial peptides (AMPs). Up to now we have isolated four families of antimicrobial peptides including the Hydramacins, Arminins, Periculins and serine protease inhibitors of the Kazal type (Bosch et al. 2009; Jung et al. 2009; Augustin et al. 2009a, b; Fraune et al. 2010). Interestingly, all antimicrobial peptides isolated so far are present in endodermal tissue only. That supports the view that the endodermal epithelium surrounding the gastric cavity is especially endangered by the regular uptake of food, and that hydra's AMPs contribute to the chemical defense properties of this layer – similar to AMPs in the human small intestine. Periculins and Arminins are made as precursors. To activate them, a negatively charged N-terminal domain is cleaved and the highly positively charged C-terminal domain is released (Bosch et al. 2009; Augustin et al. 2009b). In the Periculin family, this cationic C-terminal region is rich in cysteines, indicating that this domain requires a distinct three dimensional structure for activity. In addition to endodermal epithelial cells, Periculin is also expressed in female germ cells and used for maternal protection of the embryo (Fraune et al. 2010).

Fig. 8.2 Molecular components of the pathways involved in the *Hydra* epithelial host defence system (Panel modified from Bosch et al. 2009)
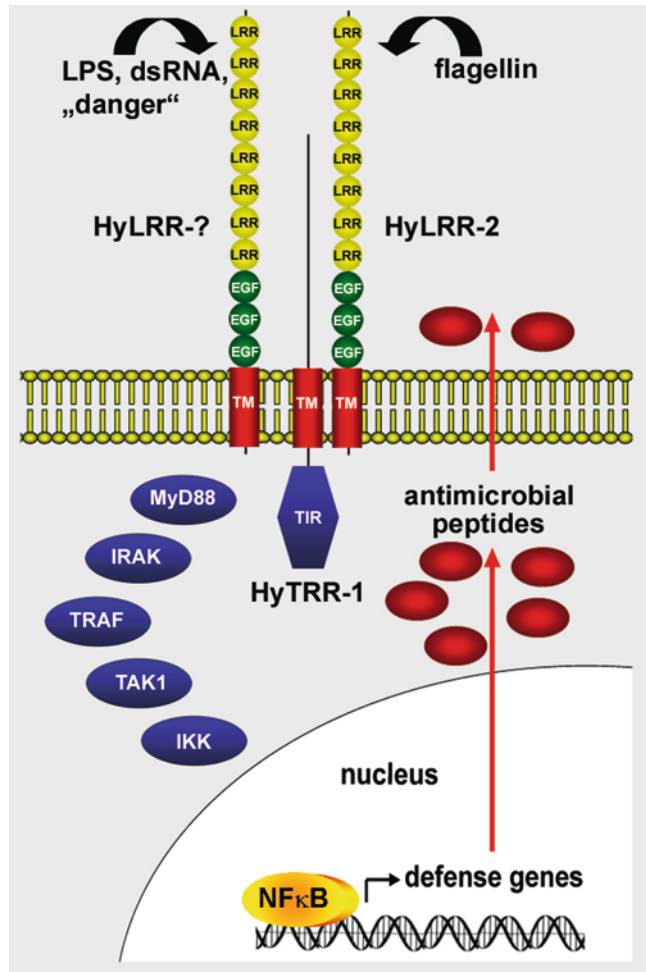
Arminins are characterized by a positively charged and variable C-terminus that includes the last 31 amino acids (pI of 12.1) (Augustin et al. 2009b). When used in liquid growth inhibition assays, the C-terminal part of Arminin 1a is capable of killing a large number of antibiotic resistant bacteria including methicillin resistant *S. aureus* (Fig. 8.3) and vancomycin resistant strains of *E. faecalis* and *E. faecium* (Augustin et al. 2009b).

Hydramacins have no precursor form. They are made as single peptides with an N-terminal signal peptide which is followed by an eight cysteine containing cationic C-terminal part. The peptide seems to be highly active mostly against gram negative bacteria also including human pathogenic multi resistant bacteria (Bosch et al. 2009). The three dimensional structure is characterized by an unusual arrangement of cationic and hydrophobic amino acid residues. The cationic amino acids form a central ring that is flanked by two hydrophobic parts. Based on a 3D model we hypothesize that bacteria attach to hydramacin to form large aggregates (Jung et al. 2009). The model is supported by an observation that after application of Hydramacin-1 bacteria aggregate, precipitate and finally die. Electron microscopic pictures show that these bacteria die within intact membranes (Jung et al. 2009). We assume that the aggregation processes may induce some kind of programmed cell death in bacteria (Engelberg-Kulka et al. 2005, 2006). If true, it might also explain

Fig. 8.3 Morphology changes of cArminin 1a treated *S. aureus* ATCC 12600. (**a**) Transmission electron micrograph of *S. aureus* (10^8 cells/mL) incubated with cArminin 1a for 1.5 h. (**b**, **c**) Magnification of two bacterial cells of (**a**); *arrows* point to the detachment of peripheral cell wall. (**d**) Transmission electron micrograph of *S. aureus* (10^8 cells/mL) incubated in 10 mM sodium phosphate buffer pH 7.4 for 1.5 h as negative control (intact cells). *Bars* represent 1 μm (Panel from Augustin et al. 2009b)
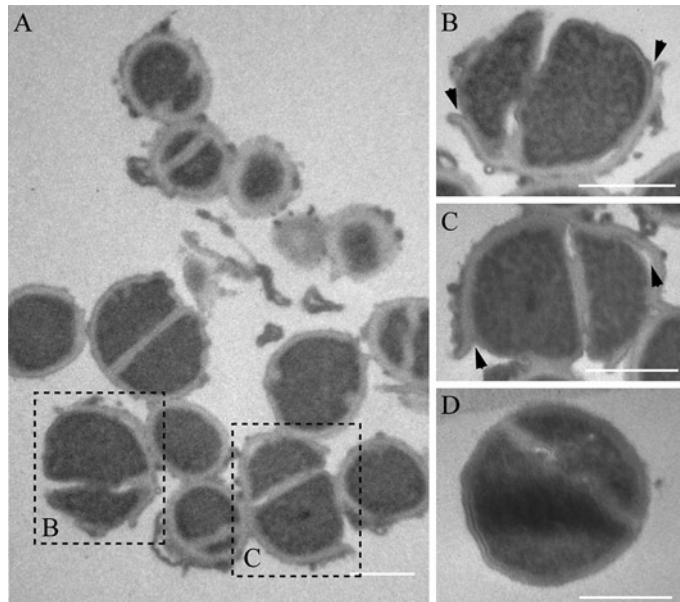
the very low doses at which Hydramacin-1 normally is active. Since human pathogens share little or no evolutionary history with Hydra-associated microbes and appear to be particularly vulnerable by hydra-antimicrobial molecules, antimicrobial peptides from such basal metazoans may provide interesting lead structures for a novel generation of antibiotics.

Taken together, *Hydra* has an effective innate immune system to interact with bacteria at the epithelial interface. The crucial question now is whether its main function is to keep out pathogens, or to allow the right community of microbes in.

Hydra and the Hologenome Theory of Evolution

In the traditional view of evolutionary biologists, the concept of individual selection posits that adaptation takes place on the level of individuals or genes. Wilson and Sober (1989) expanded this concept to the 'superorganism,' which considers selection on individuals (or genes), but additionally also on single- or multispecies communities. Based on the concept of the 'holobiont', Rosenberg and colleagues in 2007 proposed that organisms such as corals are able to 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 the developmental stage, the diet or other environmental factors), a more or less diverse microbial community can establish with a given host species (Zilber-Rosenberg and Rosenberg 2008). Since this may provide corals, for example, with resistance against certain pathogens (Rosenberg et al. 2007), enabling them to adapt much faster to novel environmental conditions than by mutation and selection, host–microbe interactions must also be considered as significant drivers of animal evolution and diversification.

To test theories regarding the assembly of tissue-associated microbial communities and to gain insights into the function of the microbiota of a phylogenetically ancient epithelium, we have started

Fig. 8.4 *Hydra* polyps are colonized by species specific microbiota. Bacterial communities indentified from different *Hydra* species (Panel modified from Fraune et al. 2009b)
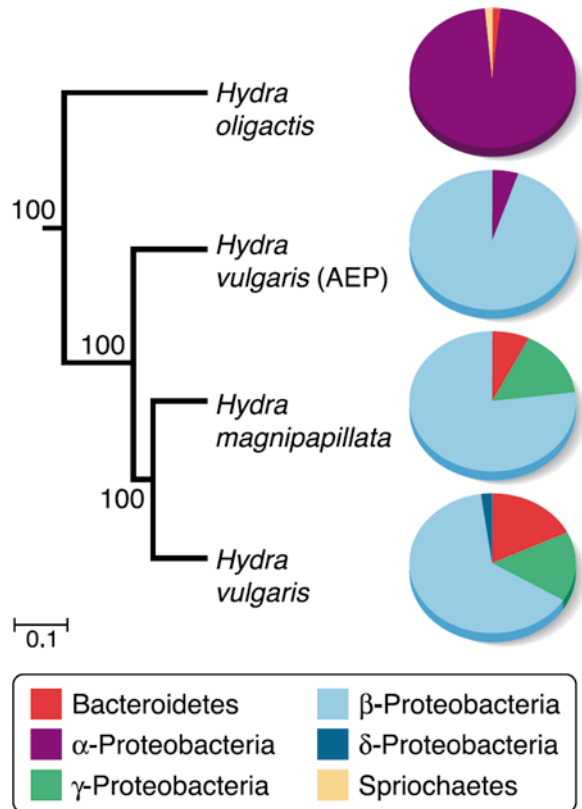
to characterize the microbiota of different *Hydra* species (Fraune and Bosch 2007). When analyzing different species we discovered that they differ greatly in their associated bacterial microbiota, although they were cultured under identical conditions. Comparing the cultures maintained in the laboratory for >30 years with polyps directly isolated from the wild revealed a surprising similarity in the associated bacterial composition. The significant differences in the microbial communities between the species and the maintenance of specific microbial communities over long periods of time strongly indicate distinct selective pressures within the epithelium (Fraune and Bosch 2007, 2010) (Fig. 8.4). The diversity of the bacterial communities is comparably low and includes less than 25 bacterial phylotypes. Most of these bacteria can be cultivated (Fraune and Bosch 2007) and, therefore, are a perfect tool to decipher the contribution from each partner to the *Hydra* holobiont.

To decipher putative links between epithelial homeostasis and species-level bacterial phylotypes, we made use of mutant strain sf-1 of *Hydra magnipapillata* which has temperature sensitive interstitial stem cells (Fraune et al. 2009a). Treatment for a few hours at the restrictive temperature (28°C) induces quantitative loss of the entire interstitial cell lineage from the ectodermal epithelium, while leaving both the ectodermal and the endodermal epithelial cells undisturbed. Intriguingly, 2 weeks after temperature treatment, when the tissue was lacking not only all interstitial cells as well as nematoblasts and most nematocytes, but in addition also had a reduced number of neurons and gland cells, the bacterial composition began to change drastically. Thus, changes in epithelial homeostasis causes significant changes in the microbial community, implying a direct interaction between epithelia and microbiota.

Antimicrobial Peptides – Key Factors for Host–Bacteria Co-evolution

What is the driving force that leads to changes in microbiota composition? Because of their obvious ability to influence bacterial life, promising candidate molecules are AMPs. To investigate whether the ectopic expression of an AMP may affect the number and composition of the colonizing microbiota at the ectodermal epithelial surface, transgenic *H. vulgaris* (AEP) expressing Periculin1a in ectoderm epithelial cells were generated. Comparing the bacterial load of these transgenic polyps with wild type control polyps revealed not only a significantly lower bacterial load in transgenic polyps overexpressing Periculin1a but also, unexpectedly, drastic changes in the bacterial community structure. Analyzing the identity of the colonizing bacteria showed that the dominant β-Proteobacteria decreased in number, whereas α-Proteobacteria were more prevalent. Thus, overexpression of Periculin causes not only a decrease in the number of associated bacteria but also a changed bacterial composition (Fraune et al. 2010) (Fig. 8.5). With the transgenic polyp overexpressing periculin we have created a new Holobiont which is different from all investigated hydra species. Future efforts will be directed towards analyzing the performance of this new Holobiont phenotype under different environmental conditions. According to the hologenome theory of evolution, changing the microbial community is one relatively rapid way of adapting to novel environmental conditions.

## Bacteria Deprived Holobionts Have to Suffer

The intimacy of the interaction between host and microbiota, as well as the strong evolutionary pressure (Fraune and Bosch 2007) (Fig. 8.4) to maintain a specific microbiota, points to the significance of the interkingdom association, and implies that hosts deprived of their microbiota should be handicapped. To investigate the effect of absence of microbiota in *Hydra* we recently produced gnotobiotic polyps which are devoid of any bacteria. While morphologically no differences could be observed with control polyps, *Hydra* lacking bacteria suffer from fungal infections unknown in normally cultured polyps (René Augustin, Sören Franzenburg, Julia Hahn, personal observation). Thus, the beneficial microbes associated with *Hydra* appear to produce powerful anti-fungal compounds. Additional support for a fungal defense function of the bacteria associated with *Hydra* comes from analyzing the pathogenicity of oomycetes (*Saprolegnia* spec.). Many *Saprolegnia* species cause economic and environmental damage due to their ability to infect a wide range of plants and animals (Phillips et al. 2008) causing saprolegniosis. Under standard culture conditions, *Saprolegnia* does not infect *Hydra*. Although zoospores are capable of attaching to the glycocalyx (Fig. 8.1b), germination appears to be inhibited. Under experimental conditions involving perturbations such as tissue dissociation and reaggregation in antibiotic-containing water, zoospores do not only attach, but also germinate, infect and destroy the animals completely (to be published). Thus, severe defects in the epithelial barrier and the absence of an intact microbial community facilitate germination of the spores. When different cultivable bacterial strains from hydra's microbiota were tested for their ability to inhibit germination of the spores, we identified three strains with strong inhibitory activity (to be published). Future efforts will be directed towards isolating the active substances from these bacteria which may lead to the development of novel antimycotics.

The "Green" *Hydra* – A Tripartite Interplay

In addition to uncovering mechanisms involved in host–bacteria interkingdom communication, the green species *Hydra viridissima* offers the possibility to investigate the interaction, not only between the eukaryotic host and the microbes, but also the relationship between the host, microbes

Fig. 8.5 Periculin1a controls bacterial colonization. (**a**) Expression constructs for generation of transgenic *Hydra*. (*Upper*) Construct containing periculin1a including signal peptide fused in frame at the 5′ end and periculin1a lacking signal peptide at the 3′ end of EGFP. (*Lower*) Control construct with EGFP driven by 1,386-bp actin 5′ flanking region. (**b**, **c**) Confocal micrographs of single transgenic ectodermal cells of (**b**) *Hydra vulgaris* (AEP) EGFP:periculin1a polyp (notice peptide localization in vesicles) and (**c**) *Hydra vulgaris* (AEP) EGFP control polyp (notice EGFP localization in the cytoplasm). (**d**, **e**) In vivo images of (**d**) transgenic polyp *Hydra vulgaris* (AEP) EGFP:periculin1a and (**e**) control polyp *Hydra vulgaris* (AEP) EGFP. EGFP protein is green; actin filaments are red. (**f**) PCR of genomic DNA amplifying bacterial 16S rRNA genes, equilibrated on *Hydra* actin gene. The number of bacterial 16S rRNA genes associated with transgenic *Hydra vulgaris* (AEP) EGFP:periculin1a polyps is significantly reduced. (**g**) Comparison of bacterial composition associated with transgenic *Hydra vulgaris* (AEP) EGFP:periculin1a polyps and control (WT) polyps (Panel from Fraune et al. 2010)
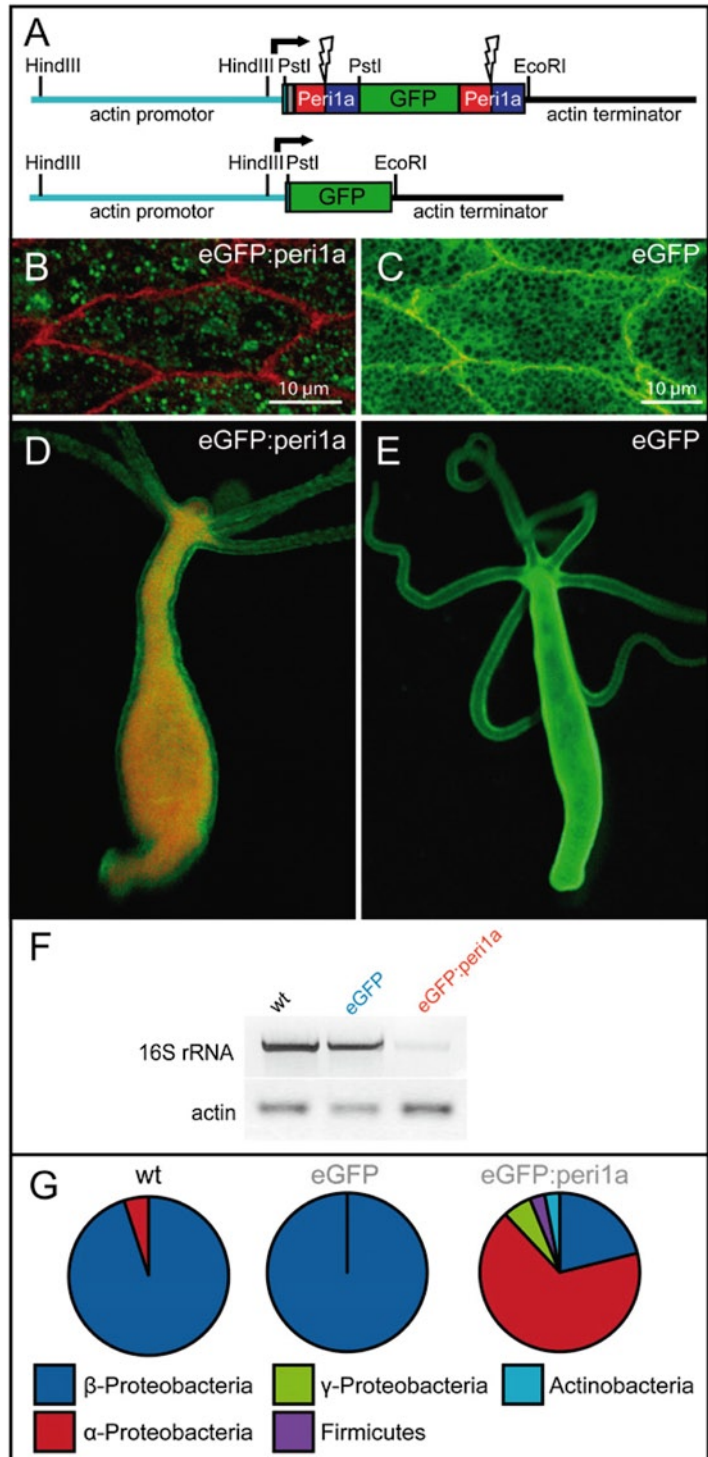
and a eukaryotic symbiont (Chlorella algae). *Hydra viridissima* forms a stable symbiosis with the intracellular green algae of the *Chlorella* group (Muscatine and Lenhoff 1963) and has been a classical model system for investigating the benefits of the symbiosis between algae and *Hydra*. The symbionts are located in endodermal epithelial cells. Each alga is enclosed by an individual vacuolar membrane resembling a plastid of eukaryotic origin at an evolutionary early stage of symbiogenesis (O'Brien 1982). Proliferation of symbiont and host is tightly correlated. The photosynthetic symbionts provide nutrients to the polyps enabling *Hydra* to survive extended periods of starvation (Muscatine and Lenhoff 1963; Thorington and Margulis 1981). Symbiotic *Chlorella* is unable to grow outside the host, indicating a loss of autonomy during establishment of the intimate symbiotic interactions with *Hydra*. During sexual reproduction of the host, *Chlorella* algae are translocated into the oocyte, giving rise to a new symbiont population in the hatching embryo (Habetha et al. 2003). The molecular mechanisms involved in the recognition and/or toleration of the algal symbiont are not yet known. Currently we are taking a transcriptomic approach using microarray technology to identify hydra genes involved in the onset and control of the symbiosis. Future efforts are directed towards uncovering if and how the algal symbiont influences the bacterial community.

Conclusion and Perspectives

Here we present the fresh water polyp *Hydra* as a model to study host–microbe interaction in the context of a superorganismic organization. Molecular tools, including transgenesis as well as rich genomic and transcriptomic resources, make *Hydra* a valuable system that can be manipulated experimentally. Because of its simplicity in body structure, and the exclusive reliance on the innate immune system of epithelia, the maintenance of epithelial barriers can be investigated in the absence of the adaptive immune system and other immune-related cell types and organs. The uncovered basic molecular machinery can be related to more complex organisms, including man, since nearly all known molecules involved in innate immunity are present in *Hydra*. Moreover, due to the relatively simple microbial community of only few bacterial phylotypes (most of which can be cultured in vitro), the influence of the microbiota under healthy and disease conditions can be dissected. This might also help to decipher why bacteria under certain conditions change from commensal to pathogenic state. An evolutionary approach to innate immunity considering the co-evolution between *Hydra* and its microbial community (Fraune and Bosch 2007) may also explain why *Hydra* AMPs belong to the most powerful peptides against human pathogenic bacteria. Human associated bacteria share little or no evolutionary history with *Hydra*-associated microbes and therefore lack any adaptation to hydra peptides. Basal metazoan animals, therefore, not only provide conceptual insights into the complexity of host–microbe interactions, but may also have considerable potential as source for novel antimicrobial compounds.

References

Augustin R, Siebert S, Bosch TC (2009a) Identification of a kazal-type serine protease inhibitor with potent anti-staphylococcal activity as part of Hydra's innate immune system. Dev Comp Immunol 33:830–837

Augustin R, Anton-Erxleben F, Jungnickel S, Hemmrich G, Spudy B, Podschun R, Bosch TC (2009b) Activity of the novel peptide arminin against multiresistant human pathogens shows the considerable potential of phylogenetically ancient organisms as drug sources. Antimicrob Agents Chemother 53:5245–5250

Bosch TC (2007) Why polyps regenerate and we don't: towards a cellular and molecular framework for Hydra regeneration. Dev Biol 303:421–433

Bosch TCG, David CN (1986) Immunocompetence in Hydra: epithelial cells recognize self-nonself and react against it. J Exp Biol 238:225–234

Bosch TC, Augustin R, Anton-Erxleben F, Fraune S, Hemmrich G, Zill H, Rosenstiel P, Jacobs G, Schreiber S, Leippe M, Stanisak M, Grotzinger J, Jung S, Podschun R, Bartels J, Harder J, Schroder JM (2009) Uncovering the evolutionary history of innate immunity: the simple metazoan Hydra uses epithelial cells for host defence. Dev Comp Immunol 33:559–569

Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T, Rattei T, Balasubramanian PG, Borman J, Busam D, Disbennett K, Pfannkoch C, Sumin N, Sutton GG, Viswanathan LD, Walenz B, Goodstein DM, Hellsten U, Kawashima T, Prochnik SE, Putnam NH, Shu S, Blumberg B, Dana CE, Gee L, Kibler DF, Law L, Lindgens D, Martinez DE, Peng J, Wigge PA, Bertulat B, Guder C, Nakamura Y, Ozbek S, Watanabe H, Khalturin K, Hemmrich G, Franke A, Augustin R, Fraune S, Hayakawa E, Hayakawa S, Hirose M, Hwang JS, Ikeo K, Nishimiya-Fujisawa C, Ogura A, Takahashi T, Steinmetz PR, Zhang X, Aufschnaiter R, Eder MK, Gorny AK, Salvenmoser W, Heimberg AM, Wheeler BM, Peterson KJ, Bottger A, Tischler P, Wolf A, Gojobori T, Remington KA, Strausberg RL, Venter JC, Technau U, Hobmayer B, Bosch TC, Holstein TW, Fujisawa T, Bode HR, David CN, Rokhsar DS, Steele RE (2010) The dynamic genome of Hydra. Nature 464:592–596

Engelberg-Kulka H, Hazan R, Amitai S (2005) mazEF: a chromosomal toxin-antitoxin module that triggers programmed cell death in bacteria. J Cell Sci 118:4327–4332

Engelberg-Kulka H, Amitai S, Kolodkin-Gal I, Hazan R (2006) Bacterial programmed cell death and multicellular behavior in bacteria. PLoS Genet 2:e135

Fraune S, Bosch TCG (2007) Long-term maintenance of species-specific bacterial microbiota in the basal metazoan Hydra. Proc Natl Acad Sci USA 104:13146–13151

Fraune S, Bosch TC (2010) Why bacteria matter in animal development and evolution. Bioessays 32:571–580

Fraune S, Abe Y, Bosch TCG (2009a) Disturbing epithelial homeostasis in the metazoan Hydra leads to drastic changes in associated microbiota. Environ Microbiol 11:2361–2369

Fraune S, Augustin R, Bosch TCG (2009b) Exploring host–microbe interactions in hydra. Microbe 4:457–462

Fraune S, Augustin R, Anton-Erxleben F, Wittlieb J, Gelhaus C, Klimovich VB, Samoilovich MP, Bosch TC (2010) In an early branching metazoan, bacterial colonization of the embryo is controlled by maternal antimicrobial peptides. Proc Natl Acad Sci USA 107:18067–18072

Habetha M, Anton-Erxleben F, Neumann K, Bosch TCG (2003) The Hydra viridis/Chlorella symbiosis. Growth and sexual differentiation in polyps without symbionts. Zoology 106:101–108

Hemmrich G, Bosch TC (2008) Compagen, a comparative genomics platform for early branching metazoan animals, reveals early origins of genes regulating stem-cell differentiation. Bioessays 30:1010–1018

Jung S, Dingley AJ, Augustin R, Anton-Erxleben F, Stanisak M, Gelhaus C, Gutsmann T, Hammer MU, Podschun R, Bonvin AM, Leippe M, Bosch TC, Grotzinger J (2009) Hydramacin-1, structure and antibacterial activity of a protein from the basal metazoan Hydra. J Biol Chem 284:1896–1905

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 13:2190–2195

Kusserow A, Pang K, Sturm C, Hrouda M, Lentfer J, Schmidt HA, Technau U, von Haeseler A, Hobmayer B, Martindale MQ, Holstein TW (2005) Unexpected complexity of the Wnt gene family in a sea anemone. Nature 433:156–160

Lange C, Hemmrich G, Klostermeier UC, López-Quintero JA, Miller DJ, Rahn T, Weiss Y, Bosch TC, Rosenstiel P (2011) Defining the origins of the NOD-like receptor system at the base of animal evolution. Mol Biol Evol. 28(5):1687–702. Epub 2010 Dec 23

Miller DJ, Ball EE, Technau U (2005) Cnidarians and ancestral genetic complexity in the animal kingdom. Trends Genet 21:536–539

Muscatine L, Lenhoff HM (1963) Symbiosis of Hydra with algae. J Gen Microbiol 32:6

O'Brien TL (1982) Inhibition of vacuolar membrane-fusion by intracellular symbiotic algae in Hydra-viridis (Florida strain). J Exp Zool 223:211–218

Phillips AJ, Anderson VL, Robertson EJ, Secombes CJ, van West P (2008) New insights into animal pathogenic oomycetes. Trends Microbiol 16:13–19

Putnam NH, Srivastava M, Hellsten U, Dirks B, Chapman J, Salamov A, Terry A, Shapiro H, Lindquist E, Kapitonov VV, Jurka J, Genikhovich G, Grigoriev IV, Lucas SM, Steele RE, Finnerty JR, Technau U, Martindale MQ, Rokhsar DS (2007) Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. Science (New York, NY) 317:86–94

Rast JP, Smith LC, Loza-Coll M, Hibino T, Litman GW (2006) Genomic insights into the immune system of the sea urchin. Science (New York, NY) 314:952–956

Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I (2007) The role of microorganisms in coral health, disease and evolution. Nat Rev 5:355–362

Srivastava M, Begovic E, Chapman J, Putnam NH, Hellsten U, Kawashima T, Kuo A, Mitros T, Salamov A, Carpenter ML, Signorovitch AY, Moreno MA, Kamm K, Grimwood J, Schmutz J, Shapiro H, Grigoriev IV, Buss LW, Schierwater B, Dellaporta SL, Rokhsar DS (2008) The Trichoplax genome and the nature of placozoans. Nature 454:955–960

Technau U, Rudd S, Maxwell P, Gordon PM, Saina M, Grasso LC, Hayward DC, Sensen CW, Saint R, Holstein TW, Ball EE, Miller DJ (2005) Maintenance of ancestral complexity and non-metazoan genes in two basal cnidarians. Trends Genet 21:633–639

Thorington G, Margulis L (1981) Hydra-viridis – transfer of metabolites between Hydra and symbiotic algae. Biol Bull 160:175–188

Trembley A (1744) Mémoires, Pour Servir à l´Histoire d´un Genre de Polypes d´Eau Douce, à Bras en Frome de Cornes. Verbeek, Leiden

Wilson DS, Sober E (1989) Reviving the superorganism. J Theor Biol 136:337–356

Wittlieb J, Khalturin K, Lohmann JU, Anton-Erxleben F, Bosch TC (2006) Transgenic Hydra allow in vivo tracking of individual stem cells during morphogenesis. Proc Natl Acad Sci USA 103:6208–6211

Zilber-Rosenberg I, Rosenberg E (2008) Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. FEMS Microbiol Rev 32:723–735