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VIRUSES - IMPORTANT REGULATORS IN THE METAORGANISM *HYDRA*

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SUMMARY

Hydra is not only associated with host specific bacteria, but also with a host specific viral community. The viral community is composed of eukaryotic and prokaryotic viruses (phages) infecting both *Hydra* and its associated bacteria. The observation of persistent viral infections in *Hydra* implies a cross-talk between eukaryotic viruses and their host. The virome of *Hydra* encodes viral structural and replication associated proteins, but also genes that interact with genetic processing and cellular regulation of their host suggesting that viruses interfere with the innate immune system and modulate *Hydra*'s functions. The bacteriophage population identified by virome sequencing is dominated by phages infecting Gammaproteobacteria and Betaproteobacteria. However, bacteriophages are not only present in their lytic lifecycle, we could also identify prophages in the genomes of *Hydra* associated bacteria. Reactivation of this hidden phage community may serve as internal regulator of host associated bacterial community or function as weapon to control bacterial colonization from the bacterioplankton community. Regarding the diversity, abundance and genetic repertoire of *Hydra* associated viruses we expect viruses to be important regulators of metaorganisms.

INTRODUCTION

All multicellular organisms are associated with a host specific bacterial community. This close association between bacteria and its host is beneficial for both partners and forms a functional unit termed "metaorganism" (*Deines* et al., 2017). Selection of host specific microbiota and the control of bacterial community composition are essential for the stability of the metaorganism. The freshwater polyp *Hydra* is an ideal model organism to study general principles of host-microbe interactions regarding selective colonization and the role of the innate immune system in establishing and maintaining the holobiont composition (*Schröder* and *Bosch*, 2016). *Hydra* features as basal metazoan only an innate immune system to detect and interact with microbes (*Bosch*, 2013). Surprisingly these basal control mechanisms enable *Hydra* to maintain its host specific bacterial community. Even under laboratory culture conditions for over 20 years *Hydra* maintains its specific bacteria and features a similar bacterial community to individuals living in the wild (*Fraune* and *Bosch*, 2007; *Franzenburg* et al., 2013). Bacterial communities are intensively studied and regarded as important part of holobionts. Compared to bacteria very little is known about viruses although they

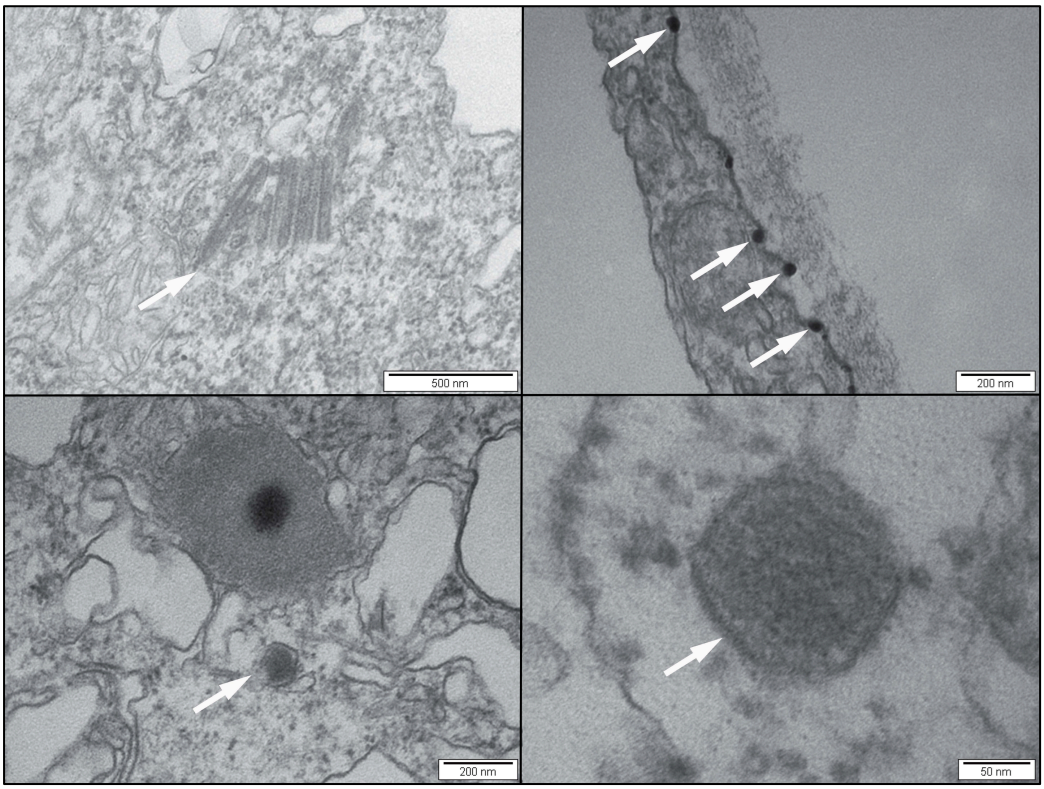

Figure 1: Virus-like particles within *Hydra* tissue. Transmission electron micrographs of ultrathin sections of *Hydra* stained with uranyl acetate. Baculovirus replication (top-left), accumulation of virus-like particles at the ectodermal epithelial layer (top-right), virus-like particles featuring morphological similarity to Phycodnaviridae (bottom).

are most abundant and highly diverse (*Suttle*, 2007). It is well documented that viruses have a huge impact on population dynamics and are important regulators within ecosystems (*Brussaard*, 2004). However, since viruses are genetically diverse lacking genes that could be targeted by universal primers and the majority cannot be propagated by culturing, both composition and function of the viral community within metaorganisms resisted analysis (*Reyes* et al., 2012). In this article we summarize recent knowledge about the composition and function of viral communities in the basal metazoan *Hydra*.

VIRUSES OF *HYDRA*

Hydra is not only associated with a specific bacterial community they are also associated with a host-specific viral community (*Grasis* et al., 2014). The viral community is composed of eukaryotic viruses that can be expected to infect *Hydra* and a diverse bacteriophage community that might interact with the associated bacterial community. Surprisingly, *Hydra* as basal metazoan is already associated with viruses that are known as causative

agents of infective disease in vertebrates, like Herpesviridae and Poxoviridae, but features also viruses that infect invertebrates, like insect viruses of the family Baculoviridae and even plant viruses of the family Phycodnaviridae. However, these viruses are not causing noticeable disease symptoms in *Hydra*, so that a persistent or chronic viral infection can be proposed. Observed shifts within the viral community composition under environmental stress conditions suggest changes in host-viral interactions or the presence of latent viral infection of *Hydra*. During latency viral genome persists as nucleic acid either integrated or as episome in the nucleus of host cells. Stress exposure, such as temperature, toxins or UV-radiation switch this viral life cycle to a lytic stage and new virons are produced (*Traylen* et al., 2011; *Kenney* and *Mertz*, 2014).

The presence of virus-like particles (VLPs) within the tissue of *Hydra* could be confirmed by transmission electron microscopy (TEM). Microscopic investigation of *Hydra* tissue supports virome sequencing data and the view that viral replication in *Hydra* is balanced or in a controlled mode of viral replication. Baculovirus replicate in *Hydra* cells (Figure 1) and are released via budding from the ectodermal epithelial cell layer (*Deines* et al., 2017). Apart from Baculoviruses VLPs with different morphology could be observed in close proximity to the ectodermal membrane (Figure 1). The observation that VLPs are released to the environment via controlled budding together with an apparent sporadic appearance of VLPs within *Hydra* tissue, e.g. VLPs with morphological similarity to Phycodnaviridae (Figure 1), indicate that several viruses have established a chronic viral infection. In a metaorganismic view this points to a homeostatic relation between virus and *Hydra*. In this balanced state viruses are still able to replicate but in a controlled manner to ensure that host expenses for viral replication do not become detrimental. This suggests a cross-talk between both partners.

FUNCTION OF VIRUSES WITHIN *HYDRA*

Hydra is permanently associated with a host specific viral community. To establish persistent viral infections, viruses have to evade the host immune defence so that viral infections are not entirely cleared. In *Hydra* viral infections can be chronic with a continuous proliferation of virons or lysogenic phages (*Deines* et al., 2017). However, in both cases some viral genes are active and interact with their host and modulate *Hydra's* functions. Viruses have developed a variety of different mechanisms to escape host immune response (*Christiaansen* et al., 2015; *Kang* and *Kieff*, 2015), while the host has coevolved to control viral infections (*Klotman* and *Chang*, 2006; *Iwasaki* and *Medzhitov*, 2015). Virus-host interactions influence cellular pathways and host metabolism (*Goodwin* et al., 2015; *Powdrill* et al., 2016). This functional remodelling of the host *Hydra* is not limited to active viral replication; also viruses in latency remain active and cross-talk with their host. Baculoviridae interfere with cellular pathways of their host during latency (*Davis* et al., 2015). Several genes are transcribed and manipulate immune system, metabolism and cell-cycle (*Monteiro* et al., 2012). Viral infection and functional manipulation may only affect some cells and the im-

pact on the entire individual is limited. Environmental stress conditions can change virus-host interactions and switch latent to lytic viral replication (*Traylen* et al., 2011) leading to an imbalance, uncontrolled viral replication and finally to the onset of disease.

BACTERIOPHAGES ASSOCIATED WITH *HYDRA*

Bacteriophage community accounts for approximately 60% of the total viral community of *Hydra* and is composed of Myoviridae, Podoviridae, Inoviridae and Siphoviridae (*Grasis* et al., 2014). Predicted host range of bacteriophages based on sequence analysis suggests that the largest proportion of *Hydra* associated phages infect Betaproteobacteria and Gammaproteobacteria (*Grasis* et al., 2014). It has to be pointed out that the phage population does not simply mirror the host specific bacterial community. The bacterial community is dominated by Betaproteobacteria accounting for more than 90% of the bacterial community while Gammaproteobacteria are underrepresented in the bacterial population of *Hydra*. In contrast bacteriophages infecting Beta- and Gammaproteobacteria were equally abundant. It can be expected that the observed difference between phage and bacterial population is caused by:

i) transient phages that originate from the surrounding water, adhere to *Hydra's* surface (*Barr* et al., 2014) and/or infect *Hydra's* associated microbiota or,
ii) resident phages originating from *Hydra's* associated bacterial community act as internal regulators of host specific bacterial community and downregulate Gammaproteobacteria by phage infection.

It is well known that phages are important regulators within bacterial populations (*Proctor* and *Fuhrman*, 1990; *Brussaars*, 2004; *Shapiro* et al., 2010) and we expect phages to play an important role in stabilizing and maintaining host specific bacterial community of the metaorganisms *Hydra*. First evidence that phages could be involved in controlling *Hydra* associated bacteria we got from a simple bacteria-bacteria interaction experiment (*Li* et al., 2015). In this experiment Li and colleagues analysed the interaction of the two main colonizers of *Hydra Curvibacter sp.* and *Duganella sp*. Surprisingly they observed a frequency-dependent, non-linear growth rate of *Duganella sp.*, which could not simply be explained by the presence of *Curvibacter sp.* (*Li* et al., 2015). For this reason we hypothesized that a phage could be hidden in form of a prophage within the genome of *Curvibacter sp.* and serves as third player in this interaction experiment. Screening the genome of both bacteria revealed the presence of a prophage signature in the genome of *Curvibacter sp.* Finally, we could prove that this phage is inducible and able to cross infect *Duganella sp*. This observation emphasizes that phages have a regulatory function within the host associated bacterial community and the occurrence of a hidden phage population, which is present in form of prophages in the genomes of associated bacterial community. Analogue to latent eukaryotic viral infections prophages are transcriptional active and able to modulate their bacterial host (*Mann* et al., 2015). This lysogenic conversion of bacteria increase their genetic repertoire by horizontal gene transfer and may change host bacterial interactions (*Madera* et

al., 2009). Being associated with a prophage can protect bacteria from phage infections by superinfection exclusion. Switching from a lysogenic to a lytic lifecycle can be advantages for the bacterium but also for the eukaryotic host. On one hand reactivated phages can serve as weapon against other bacteria and eliminate competitors on the other hand induction of prophages can function as internal regulation of host associated bacterial community.

CONCLUSION

Viruses are key components of metaorganisms, nevertheless their composition and function has been neglected. Regarding their diversity, abundance and genetic repertoire viruses have a huge impact on a cellular, organismic and population level. Under normal environmental conditions a homeostatic relation between viral community and their hosts can be expected. Due to their fast evolution and dependency on the host cell replication machinery cross-talk between eukaryotic viruses and their host has coevolved and fine-tuned the innate immune system, while host specific bacteriophages inherit regulatory function within the associated bacterial population.

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