

# Value of the *Hydra* model system for studying symbiosis

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**ABSTRACT** Green *Hydra* is used as a classical example for explaining symbiosis in schools as well as an excellent research model. Indeed the cosmopolitan green *Hydra* (*Hydra viridissima*) provides a potent experimental framework to investigate the symbiotic relationships between a complex eumetazoan organism and a unicellular photoautotrophic green algae named *Chlorella*. *Chlorella* populates a single somatic cell type, the gastrodermal myoepithelial cells (also named digestive cells) and the oocyte at the time of sexual reproduction. This symbiotic relationship is stable, well-determined and provides biological advantages to the algal symbionts, but also to green *Hydra* over the related non-symbiotic *Hydra* i.e. brown hydra. These advantages likely result from the bidirectional flow of metabolites between the host and the symbiont. Moreover genetic flow through horizontal gene transfer might also participate in the establishment of these selective advantages. However, these relationships between the host and the symbionts may be more complex. Thus, Jolley and Smith showed that the reproductive rate of the algae increases dramatically outside of *Hydra* cells, although this endosymbiont isolation is debated. Recently it became possible to keep different species of endosymbionts isolated from green *Hydra* in stable and permanent cultures and compare them to free-living *Chlorella* species. Future studies testing metabolic relationships and genetic flow should help elucidate the mechanisms that support the maintenance of symbiosis in a eumetazoan species.

**KEY WORDS:** *Hydra viridissima*, symbiosis, zoochlorellae, endosymbiont, adaptations

## The force of symbiosis in evolution

Symbiosis is defined as a long-term, intimate relationship between two or more species belonging to different phyla with one of them at least being a eukaryotic organism (Margulis and Sagan, 2002). In the case of endosymbiosis, one of the organisms lives inside the cells of the other. Symbiotic events play an important role under stress conditions as they promote associations where one organism can acquire novel metabolic capability from its partners (Douglas, 1994). More generally as a direct consequence of symbiosis one partner can gain new form, new function or new metabolic capability from the other (Smith, 2001). Most often symbiosis is described as a mutualism (Latin, mutuari – to lend, to borrow) that corresponds to an association that is beneficial for each member and favors the establishment of a new ecological fitness (Douglas, 1994). Indeed when none of the partners can occupy an ecological niche alone, the association becomes obligate and new niches that would otherwise be inaccessible become populated (Chapman and Alliegro, 2007; Schmitt *et al.*, 2007).

Symbiotic associations provide selective advantages that promoted the evolution of multicellularity and the origin of biodiversity

(Margulis and Sagan, 2002). Symbiosis, successful when the genetic interactions between the host and symbiont are harmonic, is considered a dominant mechanism to induce quick evolutionary changes at the quantitative and qualitative levels as well as the emergence of new species (Rossinck, 2005; Rodriguez *et al.*, 2006). Symbioses are the rule and not the exception in eukaryotes as evidenced by the eukaryote genomes that result from symbiogenetic events, with large parts incorporated from symbiotic organisms (Margulis, 2001). In spite of its huge impact on ecology and evolution, our understanding of the mechanisms leading to symbiotic associations is limited. Hence green *Hydra* provides a fascinating framework to study these mechanisms.

## *Hydra* as a model of symbiotic host

### Historical aspects

In ancient myths *Hydra* (Greek, Hýdra) was the daughter of a 100-headed giant Typhon and halfwoman-halfsnake Ehydna, a monster with a snake-like body and nine dragon heads. It lived in a swamp by the town of Lern on the coast of Argolian bay. People were helpless against it, because out of each cut head two new

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heads would arise, and one of the heads was immortal. *Hydra* is an island in Aegean Sea as well as one of the most distant galaxies (Zamarovský, 1985). In the XVIII century, Abraham Trembley first observed the symbiotic green *Hydra*. Indeed he noted a very robust regenerative potential (Trembley, 1744; Lenhoff and Lenhoff, 1986). He named them *polyps*, a term that had been used ever since. But the mythological description also inspired the first scientists who classified animal species to subsequently name these polyps “*Hydra*” (e.g. *Hydra viridissima* Pallas, 1766).

### Ecological aspects

*Hydra* is very suitable for experimental research given its rapid reproduction time and extreme regenerative potential (Kalafatić *et al.*, 2001). However, little is known about the free-living populations. *Hydra* inhabit shallow freshwater lakes and calm, slow-moving water attached to periphyton on rocks, branches or on submersed vegetation. With any stimulus they contract and afterwards relax. They are usually regarded as sessile, they can detach from a substrate by producing a basal bubble that enables them to float and passively move with the water current. Occasionally *Hydra* that are photopositive, can be found in plankton. Thanks to this adaptation *Hydra* can avoid deteriorating environmental conditions (lack of prey and/or low O<sub>2</sub> pressure) and increase the possibility of reaching more suitable conditions in another location. *Hydra* can be extremely good indicators of environmental changes and are widely used in ecotoxicological research (Kalafatić, 1987; Kalafatić and Kopjar, 1995; Beach and Pascoe, 1998; Kovačević *et al.*, 2001; Arkhipchuk *et al.*, 2005), also see in this issue the reviews by Quinn *et al.*, 2012; Rachamin and Sher, 2012. However in the recent years, as a result of changes in aquatic habitats, *Hydra* can show different periodical appearance in known localities (Kalafatić *et al.*, 2003) and could also be found in polluted habitats.

### Phylogenetic aspects

*Hydra* is a freshwater polyp that belongs to Cnidaria, one of the first eumetazoan phylum that, given its phylogenetic position, provides useful model systems for comparative research in the

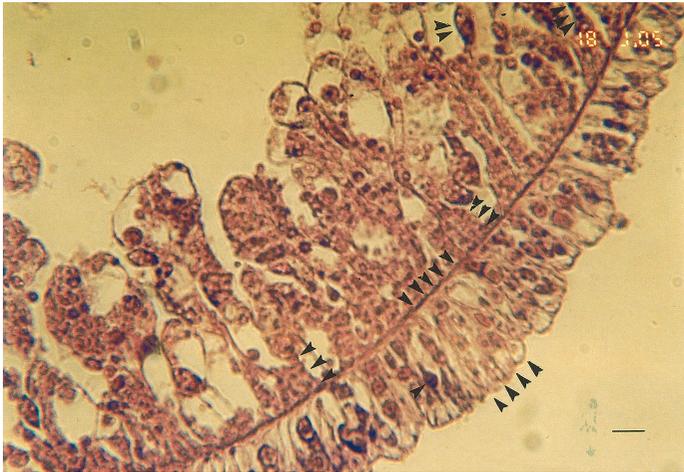


Fig. 1. *Hydra viridissima* Pallas, 1766. Bar, 1 mm.

field of evolution (Galliot and Schmid, 2002; Technau and Steele, 2011). Within Cnidaria *Hydra* belongs to the Hydrozoa class, order Hydroida, family Hydridae. The Hydrozoa class comprises 2700 species placed in five orders and Hydroida is the largest, including about 2000 species (Holstein and Emschermann, 1995). Shultze (1914, 1917) initiated the first phylogenetic studies on *Hydra* species by subdividing them into three groups: besides the *Hydra* genus (i.e. *Hydra littoralis*, *Hydra vulgaris* at that time named *Hydra attenuata*), he grouped the green *Hydra* in the genus *Chlorohydra* and the oligactis *Hydra* (characterized by a long stalk) in the genus *Pelmatohydra*. Today the latin name *Hydra viridissima* is commonly used for green *Hydra* (*viridissima* meaning “very green”), but in the past the names *Hydra viridis* and *Chlorohydra viridissima* were frequently used. Recent detailed phylogenetic analyses that included most *Hydra* species over the world showed that they can be divided into four groups (Kawaida *et al.*, 2010; Martinez *et al.*, 2010): the *viridissima* group, the *braueri* group as gracile *Hydra*, the oligactis group and the *vulgaris* group. The last three groups do not carry algal endosymbionts and are known as brown *Hydra* (Campbell, 1987, 1989). All *Hydra* species are monophyletic but the *viridissima* group diverged first and the brown groups derive from a common ancestor. Two clades of *Hydra*, green and *vulgaris*, are found on all continents whereas the *braueri* and oligactis groups are restricted to the northern hemisphere. Within the *Hydra* genus, *H. viridissima* has the smallest genome, which might result from symbiotic interactions and/or adaptive changes to environment (Johnston *et al.*, 1996; Zacharias *et al.*, 2004). Four species of green *Hydra* have been described *H. viridissima*, *H. hadleyi*, *H. plagiodesmica* and *H. sinensis*. However it is not clear, whether those are really separate species (Grayson, 1971).

### Anatomy and cellular organization

The *Hydra* body plan is a cylinder shaped around an apical-basal axis with an apical region at one end named head, formed of 5-7 tentacles placed at the basis of the hypostome (the dome surrounding the mouth opening) and at the basal end, the foot formed of a basal disc (Fig. 1). The *Hydra* anatomy is organized as two continuous myoepithelial layers, the endoderm and the ectoderm separated by an acellular layer named mesoglea composed of an extra-cellular matrix (see in this issue the review by Sarras, 2012). The mesoglea makes possible the transportation of nutrients as well as the cellular migration during regeneration (Žnidarić, 1970). If by chance the mesoglea is broken, then the epithelial layers are in direct contact (Kalafatić *et al.*, 1994) and the migration of cells is facilitated (Breslin-Spangenberg and Eakin, 1962). Located between the ectodermal myoepithelial cells are the interstitial cells (i-cells) (Fig. 2) that provide progenitors for the non-epithelial cell types, i.e. the neurons, the mechano-sensory cells named cnidocytes (highly abundant in the tentacles (Burnett, 1973)), the gland cells and the germ cells. The gland cells, also named zymogene cells (located in endoderm) as they contain granules filled with the digestive enzymes that make extracellular digestion possible (Burnett, 1973), have the potential to transdifferentiate into mucous cells and i-cells and this transdifferentiation process participates in the regeneration of the damaged body parts (Žnidarić, 1971; Siebert *et al.*, 2008). Both the epithelial and the interstitial stem cells permanently self-renew and *Hydra* thus displays the characteristics of an embryo (Bosch, 2009, and see



**Fig. 2.** Histological view of the cellular tissue in *Hydra viridissima*. The outer layer named ectoderm, is formed of epithelial cells (4 arrowheads) and interstitial cells (single arrowhead). The mesoglea (5 arrowheads) separates the ectoderm from the inner layer named endoderm or gastroderm, formed of zymogene cells (2 arrowheads) and myoepithelial cells that contain endosymbiotic algae (3 arrowheads). Bar, 20  $\mu$ m.

in this issue David, 2012; Hobmayer *et al.*, 2012). Green *Hydra* (*Hydra viridissima*) contains in its gastrodermal myoepithelial cells the endosymbiotic unicellular green algae and forms a stable symbiosis (Fig. 2).

### Zoochlorellae, the *Hydra* endosymbiotic green algae

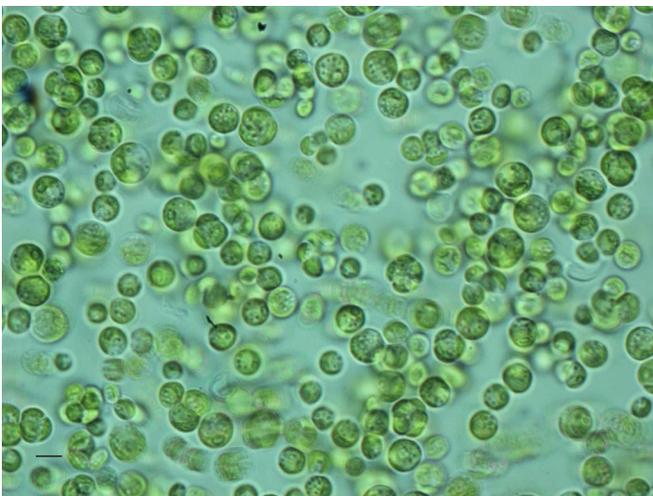
#### *Chlorella* as endosymbiont and as free-living organism

Endosymbiotic algae in freshwater invertebrates are commonly named zoochlorellae. The term zoochlorellae was given by Brandt (1882) who described the green bodies - coccoid unicellular *Chlorella*, which includes also endosymbiotic algae. A few years later Beijerinck (1890) used the same term as a synonym

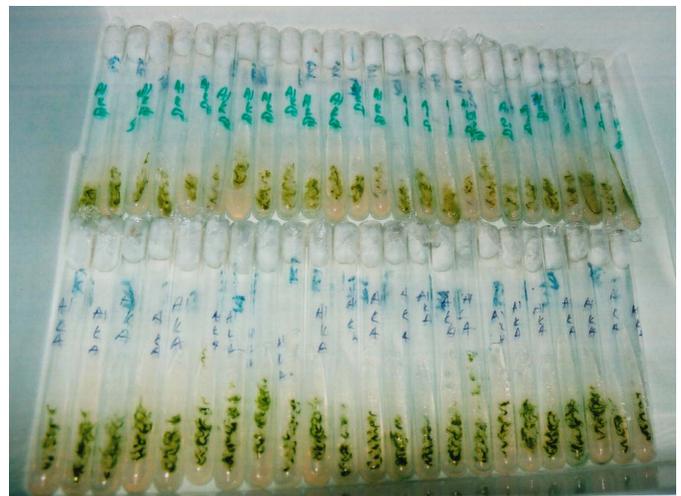
for the *Chlorella* genus, which he described as the “green balls”. Endosymbionts can also be micrococoid microalgae described as “little green balls” or “little round green things” (Callieri and Stockner, 2002). Today, zoochlorellae is used as a generic term that covers all green algae that form endosymbiotic associations in freshwater invertebrates. Among cnidarians, some anthozoans are symbiotic as corals that carry endosymbiotic zooxanthellae and the sea anemone *Anthopleura elegantissima* that hosts in parallel endosymbionts from the zoochlorellae and zooxanthellae groups (Verde and McCloskey, 2001).

Green algae are suitable test organisms due their small size, quick reproduction rate and easy maintenance in laboratory conditions (Lupi *et al.*, 1998; Babica *et al.*, 2006; Kovačević *et al.*, 2008). As a unicellular organism, *Chlorella*, which is one of the best-studied phototrophic eukaryote, is present as endosymbiont in protists and invertebrates but also as a free-living organism (Fig. 3) (Douglas, 1994). However up to recently the general idea was that it was not possible to successfully achieve the permanent and stable culture of endosymbionts independently from green *Hydra* as it is the case for free-living relatives (McAuley and Smith, 1982; Rahat, 1992; Huss *et al.*, 1993/1994; Friedl, 1997; Habetha and Bosch, 2005). But recently different species of endosymbionts have been isolated from green *Hydra* and cultured on the long term (Fig. 4) (Kovačević *et al.*, 2010a). However the isolation of endosymbiotic *Chlorella* from green *Hydra* was reported by Jolley and Smith (1978), although disputed by Habetha *et al.*, (2003) who stated that “the ability of *in vitro* culturing *Hydra* symbionts has long been debated and it is conceivable that earlier reports of long-term *in vitro* culture of symbiotic *Chlorella* (Jolley and Smith, 1978) were due to contaminations with free living algae as discussed elsewhere (Huss *et al.*, 1993/94).”

Isolated endosymbionts were characterized in cellular, biochemical and molecular analyses that identified several cytological morphometric parameters, the composition and isoenzyme activity for several enzymes (catalase, peroxidase, esterase) and their phylogenetic relationships using 18S rRNA as molecular marker



**Fig. 3 (Left).** Magnified view of the free-living *Chlorella kessleri* (Fott and Novak). [Kessler & Huss, 1992]. Bar, 5  $\mu$ m.



**Fig. 4 (Right).** Comparison of isolated endosymbionts from green hydra and related free-living species. (Upper row) A culture of endosymbiotic algae isolated from green *Hydra*, in which a different intensity of green color can be observed in comparison to a (lower row) culture of the free-living species *Chlorella kessleri*.

(Kovačević *et al.*, 2010 a,b). These results provide the conditions for systematic comparative analyses.

### Phylogenetic aspects

*Chlorella* is a polyphyletic genus belonging to Chlorophyta, the most diverse group of algae (Friedl, 1997; Huss *et al.*, 1999). Systematics of *Chlorella* appeared up to recently extremely complicated and ununified (Friedl, 1997; Pröschold *et al.*, 2011). Indeed algae within *Chlorella* genus are morphologically indistinguishable, because of their small size (often less than 5 µm), simple morphology and environmentally-induced plasticity (Trainor and Egan, 1991; Habetha *et al.*, 2003). These algae were systematized on their physiological, biochemical and macromolecular characteristics. Chemotaxonomy and comparative biochemical taxonomy were based on the determination of the substances that algae release in the medium or on the composition of the cell wall (Corre *et al.*, 1996; Takeda, 1996). Pyrenoid structure is a feature of taxonomic importance in defining species or genera for unicellular free-living or symbiotic green algae of the *Paramecium bursaria* (Nakahara *et al.*, 2003). The similar pyrenoid structures of endosymbiotic *Chlorella* in *Vorticella*, *Paramecium* and *Hydra* (Graham and Graham, 1978) suggested that these *Chlorella* species are evolutionary-related (Ikeda and Takeda, 1995). However recent molecular phylogenies proved quite unambiguously that endosymbiotic zoochlorellae are actually polyphyletic (Pröschold *et al.*, 2011). This recent study also showed, as previously suspected, that *Hydra viridissima* from different origins (here Europe and Israel) do not contain the same algae, *Chlorella*-clade algae in Europe, *Auxenochlorella*-clade algae in Israel. The general unsystematic name given to all endosymbiotic algae isolated from green *Hydra* and permanently cultured in a stable culture is *Chlorella zagrebiensis* (Kovačević *et al.*, 2010a).

### Regulation of symbiosis in green *Hydra*

#### Natural regulation of symbiosis in green *Hydra*

Symbiosis in green *Hydra* can be described as a classical school example for explaining symbiosis as well as a potent model system for scientific investigations. The green *Hydra-Chlorella* association that belongs to the group of phycozoans (Pardy, 1983), has been intensively studied since the 1960s and adaptations and specificities in this symbiotic relationship have been widely described (McNeil, 1981; Müller-Parker Pardy, 1987a; Rahat, 1991; Zacharias *et al.*, 2004; Kovačević *et al.*, 2005). Freshly collected *Hydra* contains a larger number of symbiotic algae than cultured *Hydra*; similarly algae in nature are larger and contain more chlorophyll than algae in cultured *Hydra*. In a single gastrodermal myoepithelial cell, one can count up to 20 algae in animals maintained in culture conditions (Dunn, 1987; Holstein and Emschermann, 1995) but over 50 in freshly collected animals (Müller-Parker and Pardy, 1987b). Algae are located in the basal part of the gastrodermal myoepithelial cells, regularly placed in columns close to the mesoglea (Pool and Muscatine, 1980) (Fig. 2).

All the endosymbiotic algae found in a single host are clones (Douglas, 1994) and the constant density of algae within the host is maintained, but Dunn (1987) suggested some mechanisms coordinating the growth of algae and the growth of host cells. Indeed algae can divide when the *Hydra* cells are dividing (McAuley, 1980), but the host and algal mitotic index is closely coordinated only for small *Hydra* strains. As polyps increase in size, this 'tight

link' between host and algal mitosis breaks down (Bossert and Dunn, 1986). Bossert and Dunn provided experimental evidences and evolutionary rationale to explain the need for mechanisms that control algal growth independently of 'coordinated mitosis'. The host can control the size of the endosymbiont population by different mechanisms, either by modulating the algal cell cycle, or by expelling the algae outside of the cell, or by digesting the algae (Douglas and Smith, 1984; Dunn, 1987; Douglas, 1994; Baghdasarian and Muscatine, 2000; Fishman *et al.*, 2008). Expulsion of algae is possible to achieve experimentally. Digestion of algae can be observed when polyps are starved (Dunn, 1987). In asexual conditions of reproduction (i.e. budding), algae are transferred through buds from generation to generation (Pool and Muscatine, 1980). During sexual development, algae are incorporated in the oocyte and the eggs can contain up to 1000 algae. Finally algae can also get into the host by vector-herbivorous zooplankton (Rahat, 1985; Douglas, 1994).

#### Symbiosis versus non-symbiosis in green *Hydra*

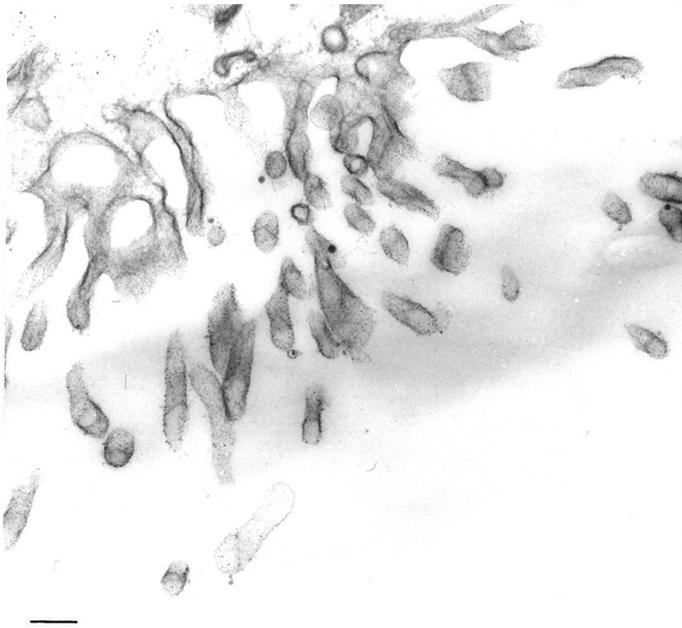
The symbiotic relationship in green *Hydra* is firm, stable and well determined; it represents a biological advantage over the non-symbiotic, brown *Hydra* (Kovačević *et al.*, 2007b, 2009). However there is some plasticity in symbiosis: *Hydra* that forms endosymbiotic relationship with algae during a certain period, can expel them and continue living on without endosymbionts. Such *Hydra* are named aposymbiotic; this state can be transient as aposymbiotic *Hydra* can be repopulated with algae. When an aposymbiotic strain of green *Hydra* becomes infected with free-living *Chlorella*, a stable symbiosis forms, but different from the original symbiosis as now algae form clusters (Rahat and Reich, 1984).

As isolation was described initially by Jolley and Smith (1978), different endosymbiotic algal partners can now be isolated from green *Hydra* and survive in culture outside the host, providing the conditions to compare in culture symbiotic and free-living species (Kovačević *et al.*, 2010a). By testing the survival of *Chlorella* endosymbionts recently isolated from their host (i.e. green *Hydra*), we observed that depending on the growth-period in culture, these algae modify their morphology from coccoidal to cenobial, with transitional forms also present (Kovačević *et al.*, 2010b). For example Boraas *et al.*, (1998) found that if a phagotrophic flagellate predator is added to a culture of the unicellular alga *Chlorella vulgaris*, then *Chlorella* forms clusters of tens to hundreds of cells and after 10 to 20 generations 8-celled colonies predominate. *Chlorella* test (Kovačević *et al.*, 2008) with aluminum showed dramatic differences in the growth rate of the endosymbiotic algae isolated from green *Hydra* when compared to the growth rate of the free-living *Chlorella kessleri* species: 36,7 % viability for the *C. kessleri* cells versus 3.3% for the endosymbiotic algae isolated from green *Hydra* (data not published). These results indicated that isolated algae exhibited lower viability and were less adapted to unfavorable conditions than their free-living relatives.

### Symbiogenesis and host-endosymbiont exchanges

#### Entry into the host, formation and maintenance of the symbiosome

The interaction between the algal surface and the host membrane is responsible for the recognition of two partners. Once inside the host organism, algae stimulate the production of endocytotic



**Fig. 5. Microvilli of gastrodermal myoepithelial cells.** In the gastric cavity, algae are integrated into the host cells by induction of microvilli. Bar, 300 nm.

microvilli (Fig. 5) at the apical side of gastrodermal cells. Specific proteins from the algal surface stimulate phagocytosis and unspecific ingestion of algae occurs. Subsequently the algae are placed in symbiotic vacuoles that are protected from phagolysosomal digestion (Pool and Muscatine, 1980; Lee and Reyes, 2006). Digestive cells of green *Hydra* use the elements of the cytoskeleton for separating the symbionts from the organelles where intracellular digestion takes place (Kalafatic and Kopjar, 1995).

Each endosymbiont is enclosed within a membrane structure named symbiosome (Douglas, 1994). In control *Hydra* the symbiosomes and perialgal spaces are narrow and well defined, whereas in case of disturbed symbiosis (Kalafatic *et al.*, 2001) they can be widened and merged into larger ensembles that contain the remains of perialgal spaces and symbiosomes (Kovačević *et al.*, 2007a). Only in rare cases the symbiont is in a direct contact with host cytoplasm (Smith, 1979) but the merging of the perialgal spaces seems to be very important for keeping the algae viable and for subsequent symbiosis reassembly (Kovačević *et al.*, 2007a). Indeed the main paradoxical constraint of symbiosis in *Hydra* is to keep the content of the symbiosome, i.e. the algae, impermeable to the enzymatic activity of the vacuoles of the epithelial cells that act as digestive cells! In the symbiosome algae are protected from merging with lysosomes and have the possibility of reproduction (Rahat, 1992).

The secretion of maltose is a unique feature of symbiotic *Chlorella* (Cernichiari *et al.*, 1969) and this secretion prevents the fusion of the symbiosome with lysosomes (Douglas, 1994; Hohmann *et al.*, 1982). The host/symbiont specificity is determined by ecological factors given by the (micro-)environment inside the *Hydra* phagosomes rather than by a recognition process (Huss *et al.*, 1993, 1994). Also, the pH is of importance for survival of the enclosed organism because it regulates the release of lysosomal enzymes in the phagosome (Rahat, 1992). Symbiotic algae are

acid-tolerant but below a specific pH, they start to secrete higher amounts of maltose (Kessler *et al.*, 1991). In standard and stable culture conditions, algae are most often located in the “quiet” part of the cell where no intensive intracellular digestion takes place (McAuley and Smith, 1982). However in other conditions the algae are found in the apical region where it appears that they are being digested (Dunn, 1987).

In the gastric region of green *Hydra* the secretory droplets are smaller, less dense and less numerous (Haynes, 1973) and the mid-gastric area never contains mucous cells (Burnett *et al.*, 1973). When gastrodermal myoepithelial cell membranes are damaged, algae fall out of the host cells, spread over the *Hydra* body and appear on places where they are usually absent (Kalafatic *et al.*, 2001; Kovačević *et al.*, 2001). Finally when it comes to the question of competitive survival, the stronger partner in this symbiotic relationship is the endosymbiont, i.e. the algae (Kovačević *et al.*, 2010c). As an example, in the symbiotic lichen that are maintained in unfavorable conditions, algae outgrow the fungus (Margulis and Sagan, 2002).

#### **Metabolic flow in symbiotic green *Hydra***

Symbiotic associations are not just the sum of the organisms, but dynamic entities where the metabolic activities are integrated with each other (Yellowlees *et al.*, 2008). In green *Hydra*, algae find their habitat; they are protected from excessive light and make use of the metabolites produced by *Hydra*, i.e. CO<sub>2</sub>, phosphates, nitrates and sulphates. Growth, division and number of algae produced in the host depend also of the amount of nitrogen as well as sulphur compounds that are supplied by *Hydra* (Douglas and Smith, 1984; McAuley, 1991; McAuley *et al.*, 1996). The *Hydra* host supplies amino acids that may form the primary source of nitrogen for algae. In addition this supply of amino acids is important for protein synthesis and further control of *Chlorella* cell division in green *Hydra*. Amino acids and possibly other metabolites are transported to the perialgal vacuoles where algae assimilate those compounds, process them and return them to the host (Cook, 1980; McAuley, 1986). Symbiotic algae differ from the free-living species by the amount of substance (carbohydrates, oxygen) that they release in the surrounding medium (Rahat, 1991). In fact about 40% to 80% of the photosynthesized products are directed to the host (Thorington and Margulis, 1980). In case of starvation, the light can compensate for the lack of nutrients and support the growth of green *Hydra* as symbionts continue to synthesize maltose from photosynthetically fixed carbon and export it to the host (Mews, 1980). In case of darkness, green *Hydra* grows slower because algae depend on host derivatives (Douglas and Smith, 1983). Similarly, in symbiotic *Paramecia* grown in the dark, some algae aggregate and degenerate. Algae can maximize the photosynthetic efficiency by acquiring the ability of phototrophic migration utilizing the host *Paramecia* as vehicles (Kadono *et al.*, 2004).

#### **Genetic flow in symbiotic green *Hydra***

Although green *Hydra* has been an issue of interest for centuries, the basis underlying a long-term symbiotic compatibility between *Hydra* and algae is still a pending issue (Rahat, 1992). Today it is assumed that horizontal gene transfer (HGT), which is opposed to vertical legacy from the ancestors (Eisen, 2000), indeed occurred between endosymbionts and hosts as a mechanism leading to gene acquisition. Genes acquired through HGT are transcribed and

transferred to the offspring (Pierce *et al.*, 2007). As a convincing evidence, *Hydra viridissima* acquired a plant-like ascorbate peroxidase that does not have any cognate gene in the animal kingdom but is expressed in the digestive cells and in the oocyte (Habetha and Bosch, 2005). Similarly in the green sea slug *Elysia chlorotica* that forms a symbiotic relationship with algal chloroplasts, algal nuclear genes involved in photosynthesis were identified in the host cell genome (Rumpho *et al.*, 2008). HGT might actually be a common process in cnidarians as it was recently identified for the acquisition by cnidarians of the subunit of bacterial poly-gamma-glutamate (PGA) synthase with consequences for the building of the nematocyst capsules in stinging cells (Denker *et al.*, 2008).

Interestingly the first comparative transcriptomic analyses performed on symbiotic and aposymbiotic sea anemones (*Anthopleura elegantissima*) showed that we should not expect “symbiotic-specific” genes but rather “symbiotic-specific” genetic regulations that would provide favorable genetic background for the emergence and /or the maintenance of symbiosis (Rodriguez-Lanetty *et al.*, 2006). Therefore, beside highlighting the phylogenetic status of the green *Hydra*, high-throughput sequencing strategies performed on symbiotic and aposymbiotic *Hydra viridissima* will open routes to characterize the respective contributions of the host and the endosymbiont in the genetic flow, to identify candidate genetic regulations (similar or not to that identified in sea anemones), which would either promote the establishment of symbiosis, or provide the mechanisms to support its maintenance.

### Evolutionary and ecological impacts on symbiogenesis in green *Hydra*

One anticipates that some particular symbiotic association was necessary for promoting the first-evolved symbiotic relationships. It is proposed to see the (algal) symbiotic partner as a possible “trigger” for such evolutionary event. Indeed cnidarian-dinoflagellate associations have played a key role in the evolutionary radiation and biodiversity of cnidarian species (Rodriguez-Lanetty *et al.*, 2006). Was it also the case with the emergence of symbiosis in the green *Hydra*? We cannot exclude that *Hydra* might have selected several symbiotic partners during its evolution, some of them being lost and replaced by *Chlorella*. Indeed it has been reported that *Hydra viridissima* can also host bacteria (Thorington and Margulis, 1990) when some *Hydra magnipapillata* strain can host another type of algae *Symbiococcum hydrae* (Chlorosarcinate-type) (Rahat and Reich, 1989). In *Paramecium* it was shown that the types of the symbionts depend on the host lines (Hoshina *et al.*, 2005).

Only one species of algae is found in a given *Hydra*. Different strains of green *Hydra* can carry different species of algae, depending on the natural habitat of a particular strain of green hydra (Kovačević *et al.*, 2010a). Adaptations to various environmental conditions including the surrounding populations of algae in the environment might be the key in the selection of symbiotic partners. Also, microenvironmental features and microhabitat conditions are important, suggesting that the coccoid communities of similar habitats (lakes) can be quite different (Fawley *et al.*, 2004). *Chlorella* possibly competes for the host and when it finds its habitat within the *Hydra* cell, the other species cannot settle any more. As the result of preadaptations of both *Hydra* and endosymbiotic alga, changes were achieved. It is assumed that upon regular phagocytosis of green algae in gastroderm, green *Hydra* can

identify the endosymbionts.

During millions of years of coevolution, preadaptations and achieved mechanisms of symbiosis evolved, possibly through a first phase of parasitic relationships that secondarily became obligatory mutual and are today facultatively mutual (although possibly mutual during the entire life of the individuals (Kovačević *et al.*, 2010a)). Rahat (1991) proposed that contemporary algae in *Hydra* today are probably the descendants of different colonizers of *Hydra* cells: Some algae were competing intracellularly and those that made the best use of the vacuolar resources won the competition. On the other side, *Hydra* whose cells received a survival bonus or whose viability was not affected by the presence of algae, had an evolutionary advantage and prevailed.

### A look beyond

Although symbiosis in green *Hydra* attracted the interest of researchers since long ago, little is known about the molecular basis of survival of endosymbiotic algae and about the mechanisms controlling the interactions between both partners (Habetha and Bosch, 2005). Why some *Hydra* species can, when other species cannot form an endosymbiotic relationship with the unicellular algae? Why some algae can become symbionts and others not? The answer lays in preadaptations of symbionts and in host/symbiont specific interactions. So far HGT was clearly identified in several symbiotic species, although restricted to a unidirectional genetic flow, from the endosymbiont to the host. If confirmed on a larger scale, the HGT process could provide us with the main source for integrating new genes in existing genomes, a process that can be performed quickly, even though the transmission potential does not seem equal between all genes (Gogarten *et al.*, 2002).

Concerning the molecular actors that regulate the interactions between the host and the endosymbiont, the recent possibility to isolate and culture different algal endosymbiont species should help answering the following questions: what are the signals that trigger symbiosis? Is it possible to manipulate the endosymbionts to test their symbiogenetic potential in *Hydra*? Who is imposing the limiting constraints on the symbiogenetic process, the algal endosymbiont or the *Hydra* host? Finally the combination of these *in silico* and *in vivo* experimental settings should also help understand the environmental impact of various ecological niches on this symbiotic process. Sure there was never so exciting time to study symbiosis in *Hydra*!

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