

UNDERSTANDING THE INTRACELLULAR NICHE IN CNIDARIAN-SYMBIODINIUM SYMBIOSES: PARASITES LEAD THE WAY

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SYMBIOSIS
CORAL
CNIDARIAN
SYMBIODINIUM
PHAGOCYTOSIS
HOST-MICROBE INTERACTION

ABSTRACT. – Most scleractinian corals and many other cnidarians host intracellular photosynthetic dinoflagellate symbionts. The symbionts contribute to host metabolism and skeletogenesis to such extent that this symbiosis is well recognized for its contribution in creating the coral reef ecosystem. However, the significance of this animal-microeukaryote association as the only widespread infection of animal cells by a mutualistic microeukaryote has yet to be explored. This is in stark contrast to the abundance of parasitic micro-eukaryotic infections of animals cells, including *Plasmodium*, *Toxoplasma*, *Leishmania*, and *Entamoeba*. Coral symbiologists can learn from the many analogous systems which are much better understood – that is, pathogenic infections of animal cells by microbes. The key areas to examine include the initial recognition event, invasion or uptake into a host cell, manipulation of the host cell response, replication within the host cell, and responses to the host's immune response. Examples of these better-studied systems offer new ideas and approaches for understanding and investigating the creation and composition of the intracellular niche in the cnidarian-dinoflagellate interaction.

Overview of the Cnidarian-Symbiodinium symbiosis

Coral reef ecosystems are well known as highly biodiverse ecosystems that are hotspots of productivity in otherwise relatively low productivity marine environments. Within these ecosystems, corals grow and secrete organic and carbonate skeletal material at rates that can produce vast stands of dense coral growth, as well as accumulation of carbonate sediments that form the reef (Hoegh-Guldberg *et al.* 2007, Venn *et al.* 2008). Thus it is the corals themselves that provide both the trophic underpinnings and three-dimensional structure of the coral reef. The rate of coral growth and carbonate production is a function of a symbiosis with dinoflagellates (photosynthetic unicellular eukaryotes) that release photosynthetically-fixed organic carbon to the host cells in which they live (Muscattine *et al.* 1958). The animal host, by housing photosymbionts within cells of its gastrodermal tissue, ensures a steady supply of reduced organic carbon that can be used to fuel metabolic requirements (Falkowski *et al.* 1984) and to create conditions that enhance the deposition of carbonate skeletal material that ultimately becomes the rock substrate of the reef (Muscattine *et al.* 2005). This producer-inside-a-consumer organization also reduces the loss of nutrients to the external environment, thus enhancing nutrient availability to both partners (Yellowlees *et al.* 2008).

This “holobiont” of animal host and algal symbiont is not simply a product of each partner independently, but rather should be considered as a complex network of interactions between the two partners, and interactions of the partners/holobiont with environmental variables (e.g., temperature, light, nutrient levels, salinity) as well as

complex microbial communities that live on the coral surface/mucus layer (Yokouchi *et al.* 2006, Wegley *et al.* 2007, Dinsdale *et al.* 2008). The initiation of the symbiotic association, the regulation of the established association, and the mechanistic underpinnings of symbiosis breakdown are all active areas of investigation in this field.

Scleractinian corals are not the only hosts to *Symbiodinium* – a number of species of other cnidarians host symbiotic dinoflagellates (octocorals, sea anemones, zoanthids, scyphozoans, and hydrozoans) (Muller-Parker *et al.* 2001), as well as a number of other invertebrates and protists. However, by far, the most common host to dinoflagellates are the cnidarians, particularly tropical species.

Some areas of research that have been gaining momentum in this field are investigations of the underlying genomic, molecular, and cellular processes that contribute to the host-symbiont interaction in cnidarians both in the “normal” healthy state and in the diseased state characterized by infection by pathogenic microbes, or stressor-induced bleaching (Coffroth *et al.* 2005, Edge *et al.* 2005, Coffroth *et al.* 2006, Perez *et al.* 2006, Rodriguez-Lanetty *et al.* 2006, Dunn *et al.* 2007, Forêt *et al.* 2007, Leggat *et al.* 2007, Van Oppen 2007, Wegley *et al.* 2007, DeSalvo *et al.* 2008, Dinsdale *et al.* 2008, Marhaver *et al.* 2008, Schwarz *et al.* 2008, Weis *et al.* 2008). However, an aspect of the cnidarian-dinoflagellate symbiosis that has generally not been explored is that this mutualism represents one of only a few examples of a mutualistic relationship between an animal cell and a unicellular eukaryote (a microeukaryote). By far, the majority of microeukaryotes that interact with animal cells are not mutualists,

but rather parasites. Parasitic microeukaryotes include the apicomplexans (for example *Plasmodium*, *Toxoplasma*, and *Babesia*), the trypanosomes (for example *Leishmania*), and parasitic amoebae (for example, *Entamoeba*).

The nature and mechanisms of interactions of apicomplexan parasites with their hosts cells has been an active area of research and a SCOPUS search for publications on just one of the apicomplexans (*Plasmodium*) returns over 38 780 hits, whereas a search for “*Symbiodinium* OR zooxanthella” returns 1337. Dinoflagellates, apicomplexans, and ciliates are sister taxa all belonging to the Alveolata (Cavalier-Smith 1999), and while they diverged hundreds of millions of years ago (Berney *et al.* 2006), it is possible that there are parallels between the interactions of apicomplexans or dinoflagellates with their host cells, especially if there are cellular components or processes that are shared between Alveolates. The origin of the apicoplast in the Alveolates, a relict chloroplast that no longer retains photosynthetic capacity but remains essential for viability, is somewhat unclear. While it is clear that the both dinoflagellate chloroplast and the apicomplexan plastid were acquired by a secondary endosymbiosis with a eukaryotic photosymbiont, it is unclear whether there has been a single, or multiple independent, acquisition events. Arguments for either a red algal or green algal origin of the apicoplast plastid have been put forward (Blanchard *et al.* 1999, Okamoto *et al.* 2008). Recent discovery, however, of a photosynthetic apicomplexan living as a symbiont within corals provides strong evidence that there is a single plastid origin in the dinoflagellate/apicomplexan clade, not independent acquisitions of plastids, and that ancestor of the apicomplexans was a mutualistic symbiont (Moore *et al.* 2008).

In addition to the abundance of microeukaryotic parasites, there are other animal-microbial systems that may provide insights into the coral-dinoflagellate symbiosis. For example, there are pathogenic bacteria that, at least at first glance, appear to interact with their host cells in ways similar to cnidarian-*Symbiodinium* interactions. Like *Symbiodinium*, the pathogenic *Mycobacterium tuberculosis* is taken into host cells by phagocytosis and resist maturation of the phagosome. In contrast to *Symbiodinium*, however, the processes by which mycobacteria successfully exploit the host phagosome is a topic of active research (a SCOPUS search for “mycobacteri*” returns 79,243 hits).

Given the abundance of information on the nature, mechanisms, and outcomes of parasitic and pathogenic animal-microbial associations, it seems likely that coral symbiologists might benefit from an understanding of some of the ways in which other systems function. The goal of this paper, therefore, is to review selected examples of parasitic/pathogenic infections of animal cells, to consider what we might learn from understanding those systems in more detail, and to provide examples of where the study of host-symbiont interactions in cnidarians can go in the future.

Apicomplexan infections of animal cells: *Toxoplasma*

Apicomplexans are obligate intracellular parasites that cause several devastating human diseases including malaria (*Plasmodium*), Toxoplasmosis, and Babesiosis, as well as veterinary and wildlife diseases. Apicomplexans have evolved a variety of life-history strategies for completing their life cycle, including transmission between multiple hosts (Roos 2005, Mital *et al.* 2008, Ravindran *et al.* 2008). In one of the simpler examples (reviewed in Kim *et al.* 2004 and Kim *et al.* 2008), *Toxoplasma gondii* infects a single definitive host, a feline, within which it sexually reproduces in the intestinal tract. Parasites then enter the environment as “oocysts” in fecal material. Naïve animals become infected by ingesting oocysts from the environment. In the gut of this secondary host, *Toxoplasma* develops from the oocysts into an invasive stage (called the “tachyzoite”) that enters nucleated host cells and disseminates through the body of the host. Under immune pressure, a quiescent stage (bradyzoite) develops that can persist within the host indefinitely. If the infected host is ingested by a cat, the sexual cycle can occur to produce infective oocysts. Or, if the infected host is ingested by a naïve host, the quiescent bradyzoites can switch to the tachyzoite stage and infect the new host. Humans can be infected either by oocysts that contaminate food, or by tachy/bradyzoites that they ingest from undercooked meat infected with *Toxoplasma*. An overview of *Toxoplasma*’s subcellular structure and lytic cycle (described below) is illustrated in Fig. 1.

Invasion and manipulation of host cells

Apicomplexans invade their host cell using a complex of specialized secretory organelles located throughout the cytoplasm (dense granules), or at the apical tip of the cell (rhoptries and micronemes). When the parasite attaches to the host cell, these secretory organelles undergo a regulated secretion of proteins that assemble into an invasion complex on the host cell that interacts with the parasite’s internal actin-myosin based motor. Micronemes secrete proteins that facilitate adhesion to the host cell and contribute to building the “moving junction” of the invasion complex (Carruthers *et al.* 2007). Rhoptries secrete proteins that are utilized in construction of the parasitophorous vacuole membrane PVM that encloses the parasite within the host cell, or are targeted into the host cytoplasm or nucleus where they interact with signaling pathways to manipulate the host response (Bradley *et al.* 2007, Saeij *et al.* 2007, Boothroyd *et al.* 2008, Cesbron-Delauw *et al.* 2008). The dense granules secrete proteins which associate with the PVM and also play roles in creating the intracellular compartment in which *Toxoplasma* will live (Braun *et al.* 2008).

The parasite moves into the host cell surrounded by membrane that contains both host and parasite-derived

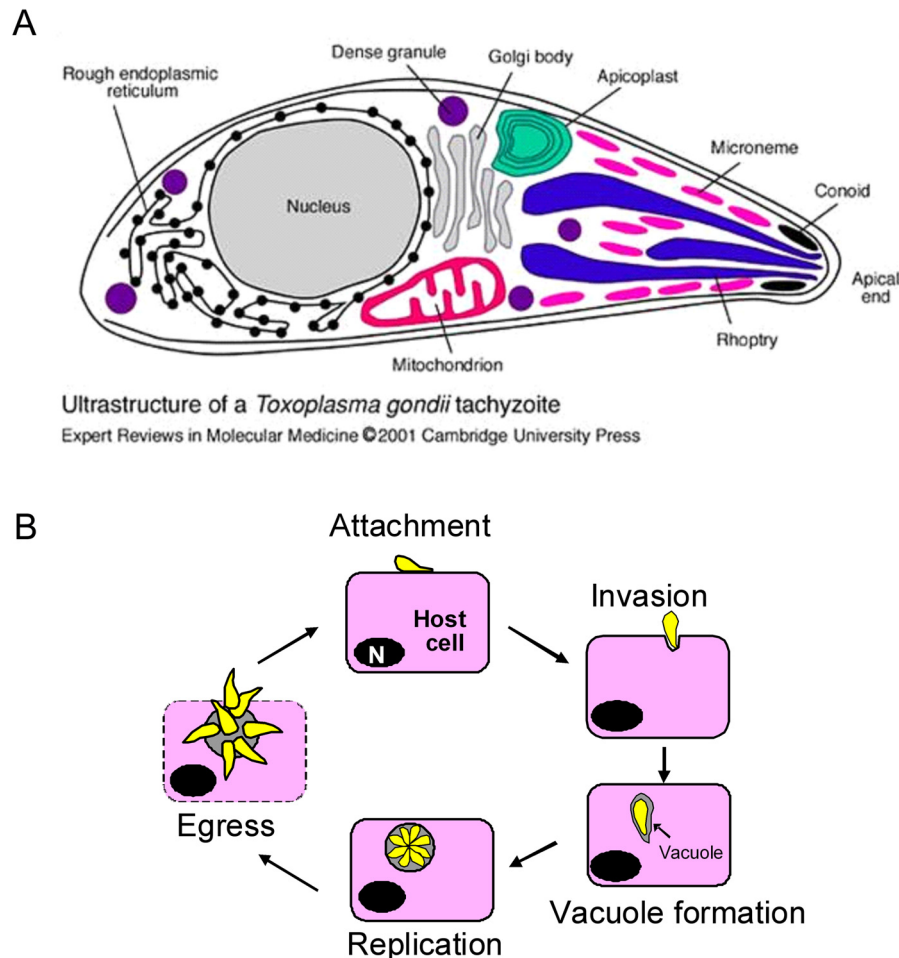


Fig. 1. – The ultrastructure and intracellular niche of *Toxoplasma gondii*. The ultrastructure of this parasite (A) reveals complex and structured arrangements of specialized secretory organelles containing proteins that the parasite targets to the host cell to penetrate the host cell, modify the host membrane that encloses it, and manipulate the host response. Also shown is the apicoplast, a relict plastid that has lost its photosynthetic genes, but retains genes that are essential for parasite viability. The lytic cycle (B) involves host cell invasion to form the parasitophorous vacuole (PV), replication by mitosis within the PV, egression from the PV and host cell, and invasion of a naïve host cell. Courtesy of Gustavo Arrizabalaga.

materials (Ravindran *et al.* 2008), including contents from the apical organelles or dense granules of the parasite. The parasite further remodels the host cell through recruitment of host organelles, such as ER and mitochondria, to the PVM (Mercier *et al.* 2005, Coppens *et al.* 2006, Martin *et al.* 2007). Thus although the parasite is surrounded by a membrane (the PVM), it is functionally and structurally distinct from a phagosome. There it replicates asexually until the host cell has been overrun by parasites (still contained within the PVM). The parasites then exit the host cell to identify and invade naïve host cells.

Microbial infections of animal cells via phagocytosis: mycobacteria

A common point of entry by microbes into host cells is by taking over the phagocytic cells which internalize them with the intention of destroying them. For example, the bacteria mycobacteria, *Salmonella*, and *Legionella*, and

the protozoan parasite *Leishmania* all employ variations on this mechanism. While some microbes are able to withstand that harsh acidic and proteolytic conditions of the mature phagolysosome, most microbes that are taken into host cells by phagocytosis employ a diversity of tricks for either circumventing or overcoming the maturation of the phagosome into a microbicidal compartment. Because *Symbiodinium* gains entry into cnidarian cells via phagocytic uptake, it is worthwhile to review some aspects of phagocytosis and then focus on selected mechanisms employed by one group of pathogens, mycobacteria, to overcome destruction by phagocytosis.

Mycobacterium tuberculosis is a gram positive bacteria that infects and resides within animal cells. It is the causative agent of tuberculosis, and thus infects millions of people worldwide. Its primary site of infection are lung or GI tract macrophages, which are professional phagocytes of the immune system. The interaction of mycobacteria with its phagosome is illustrated in Fig. 2.

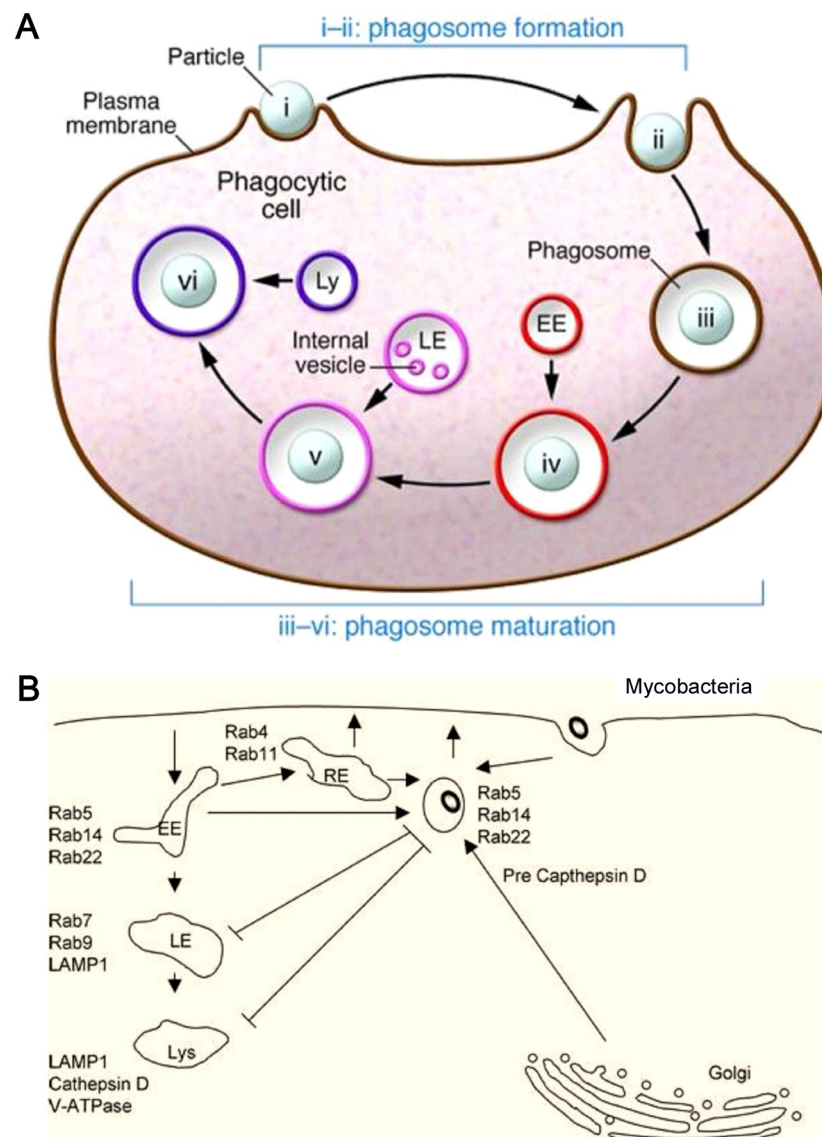


Fig. 2. – Phagosome maturation and its arrest by mycobacteria. (A) Phagosome formation and maturation: i, particle engagement; ii, phagocytic cup formation; iii, nascent phagosome; iv, early phagosome; v, late phagosome; vi, phagolysosome. As the phagosome matures, it undergoes multiple sequential interactions with early endosomes (EE), late endosomes (LE), and lysosomes (Ly). (B) Arrest of phagosome maturation by mycobacteria. During a typical progression of phagosomal maturation, phagosomes mature into a phagolysosome via interactions with endocytic and lysosomal organelles (EE, early endosome, LE, late endosome, RE, recycling endosome, Lys, lysosome), with delivery of lysosomal constituents, via a biosynthetic trafficking pathway from trans-Golgi network to phagosomes. Autophagic membrane may be involved. In contrast, *M. tuberculosis* phagosomes do not mature past the Rab5 stage. Rabs are small GTP-binding proteins that control intracellular trafficking and supervise the maintenance of specific organellar identity. Reprinted with permission from (A) Steinber *et al.* 2008 and (B) Brumell and Scidmore 2007.

Phagocytosis in macrophages

Macrophages are vertebrate immune cells that are specialized for recognizing, phagocytosing, and degrading microbial pathogens or parasites (Haas 2007). Innate immune receptors on macrophages include scavenger receptors, C-type lectins, integrins, and Toll-like receptors. These receptors physically interact with surface molecules of microbes, including lipopolysaccharides on Gram-negative bacteria, peptidoglycan and lipoteichoic acid on Gram-positive bacteria, and a variety of proteins,

glycans, and lipid moieties of parasites. The interaction of these receptor molecules with the surface molecules of microbes triggers phagocytosis, a receptor-mediated, actin-driven reorganization of the cytoskeleton and membrane structure that results in engulfment of the microbial particles into a membrane-bound phagosome.

Typically, particles that have been taken into a cell by phagocytosis are ultimately degraded. Thus phagocytosis consists of two processes: the formation of the phagosome, an intracellular vacuole that forms from the plasma membrane and contains the particulate matter as well as

the extracellular milieu, and the maturation of the phagosome into a microbicidal compartment (Fig. 2). During this maturation process, the phagosome fuses with a series of endosomes and lysosomes that deliver proteases and contribute to acidification of the phagolysosome; the end result is proteolytic cleavage, and degradation, of the phagocytosed particles (Vieira *et al.* 2002).

Aside from macrophages, which are specialized cells devoted to phagocytic destruction of particles, many other vertebrate and invertebrate cells are phagocytic and thus potentially susceptible to infection. Recent success in efforts to isolate the symbiosome compartment provide a way forward for these questions to be effectively addressed (Kazandjian *et al.* 2008).

Manipulation of host response: arresting phagosome maturation

Mycobacteria have taken advantage of the phagosome by re-directing its fate. Instead of progressing through the multiple stages of fusion with a series of vesicular organelles that would transform it into a phagolysosome, mycobacteria-containing phagosomes are arrested from undergoing maturation (Nguyen *et al.* 2005, Deretic *et al.* 2006). In so doing, the pathogen is protected from acidification and proteolytic degradation. One of the primary mechanisms by which it does so is by interfering with Rab-mediated progression of endosomal/lysosomal interaction. Rabs are small GTP-binding proteins that control intracellular trafficking and direct the progression and maintenance of organellar development. Rab5 is associated with early endosomes, Rab7 with late endosomes, Rab11 with recycling endosomes, and Rab9 with controlling anterograde trafficking from endosomes to the trans-golgi network. By preventing the phagosome from maturing past the Rab5 stage, mycobacteria effectively block the maturation process (Chua *et al.* 2004).

Mycobacteria also interfere with host production of nitric oxide, a highly reactive molecule that the host produces to cause severe damage to the pathogenic cells. In an actin-based localization process, inducible nitric oxide synthase is typically localized to phagosomes, but by an unknown mechanism, mycobacteria inhibit localization of iNOS to the phagosome and therefore reduce their exposure to nitric oxide (Miller *et al.* 2004). This can be overcome by application of LPS or other molecules that upregulate the host immune response and restore phagosome maturation.

Lipids are critical for phagocytosis, which makes them targets for pathogen-interference. Phosphoinositol (PI) is abundant in plasma membrane, and therefore the nascent phagosome. Phosphorylation of PI produces phosphatidylinositol 3-phosphate (PI3P), which is a membrane-trafficking lipid critical for phagosome progression. PI3P is generated on the membranes of early endosomes and phagosomes and its synthesis is temporally regulated

(Steinberg *et al.* 2008). Proteins that interact with PI3P are involved in the maturation process and thus interference with these PI3P-associated proteins results in a failure of the phagosome to undergo maturation. Mycobacteria-containing phagosomes have been shown to dissipate a key PI3P-binding protein (Hrs) and arrest phagosome maturation (Chua *et al.* 2004). Thus interference in lipid biosynthesis or modification is a key pathogen mechanism for controlling the host response.

Although mycobacteria suppress host cell apoptosis pathways, they also can utilize host cell apoptosis as a mechanism for exiting host cell, but appear to do so in manner that does not involve caspases, but rather by involvement of lysosomal proteases in a novel macrophage cell death pathway (Lee *et al.* 2006, O'Sullivan *et al.* 2007).

Dinoflagellate infection of animal cells: *Symbiodinium*

Much less is known about the cellular, molecular, and genomic underpinnings of the cnidarian-dinoflagellate association in comparison to the wealth of knowledge about host-parasite or host-pathogen interactions. A summary of the discussion below, which focuses on what we do know about the infection cycle of *Symbiodinium* with a typical cnidarian cell, is shown in Fig. 3.

Entry into host cells

Most cnidarians reproduce sexually to produce a motile planula-type larva, which undergoes settlement and metamorphosis into the polyp stage within hours to days. The majority of sexually-produced offspring from symbiotic cnidarians do not acquire symbionts directly from maternal sources via the oocyte. Instead, they acquire symbionts from the environment, either during the larval or young polyp stages. The source of the initial symbiont population to enter the host may be derived from populations shed from the maternal colony, or from the surrounding water. The mechanism by which *Symbiodinium* enters the cells of naïve hosts is most commonly by phagocytic uptake by gastrodermal cells (Fitt *et al.* 1983, Schwarz *et al.* 1999). However, as is the case for almost all other parasites/pathogens, there is not a single mechanism for entry into host cells. In cnidarians, there appear to be multiple mechanisms for infection that are either completely separate from phagocytic uptake, or work in conjunction with phagocytosis (Montgomery *et al.* 1995, Marlow *et al.* 2007).

The initial interaction between host and symbiont is likely mediated by several ligand/receptor interactions. During the initial onset of infection in a coral larval stage, it was determined that α -mannose, α -glucose, and/or α -galactose present on the surface of *Symbiodinium* engage receptors, such as lectins on the host cell (Wood-Charlson *et al.* 2006) and previous studies have also

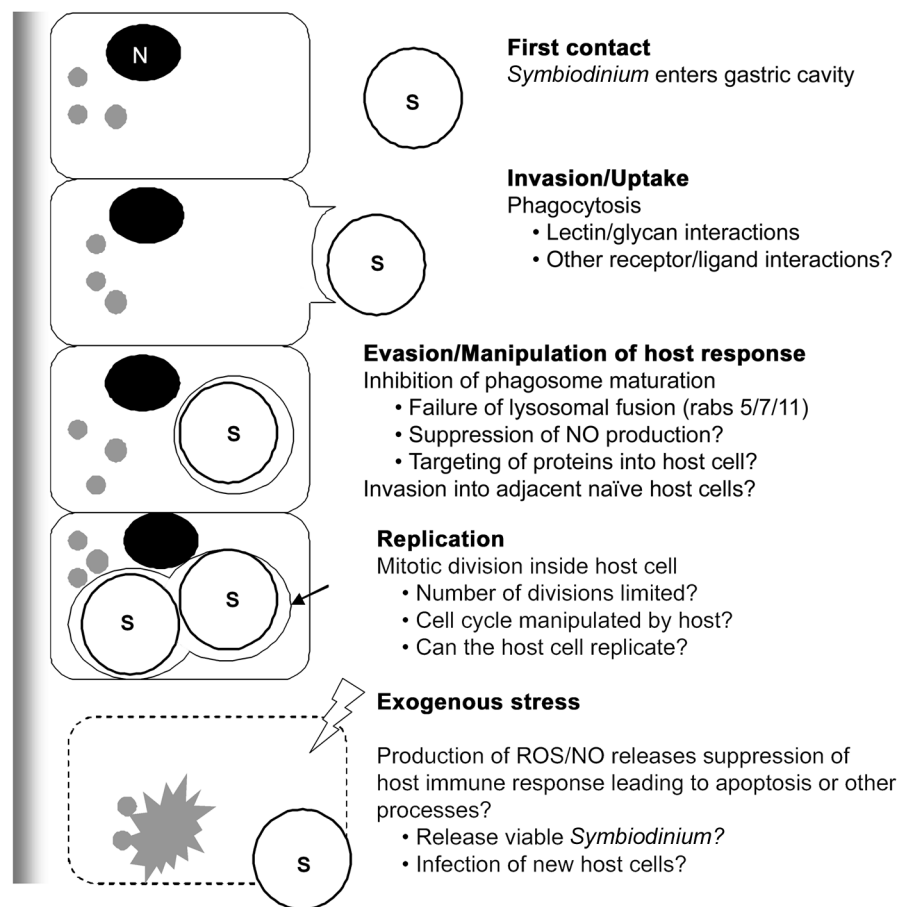


Fig. 3. – Model of the “lytic” cycle of *Symbiodinium*. *Symbiodinium* infects a new host cell via phagocytosis and replicates slowly (in some host species, no more than 2 or 4 symbionts reside within a single host cell). The effect of infection on host cell cycle is not known. Although photosynthesis results in cellular exposure to high levels of ROS, the host cell responds by production of high levels of oxidative stress-response proteins (SOD, Catalase, HSPs). However, under exogenous stressors (high UV, high temperature), the host cell produces a typical immune response, including production of nitric oxide. The production of ROS and NO is sufficient to damage host and/or symbiont cells resulting in exocytosis of symbionts or apoptosis, necrosis, or autophagy. In addition, intact host cells containing symbionts may detach from the ECM, and are removed from the host. These processes resemble a typical lytic cycle of intracellular parasites/pathogens: invasion or uptake by a host cell, evasion or manipulation of the host response, establishment of a compartment for replication, and exit from host cells under suboptimal conditions. S = *Symbiodinium* cell; Grey circles = endo/lysosomes; black ovals = host nucleus; arrow = symbiosome membrane.

implicated lectins-mediated recognition processes (Koike *et al.* 2004). While little more is known about the cell-cell molecular interactions, genomic and bioinformatic searches of genes in cnidarians reveals that cnidarians possess many components of the classical innate immune pathways, including proteins that might mediate cell-cell interactions during the onset of infection, including scavenger receptors, lectins, complement homologs, and TIR-domain proteins and toll-like receptors (Miller *et al.* 2007, Schwarz *et al.* 2008).

Establishment and composition of the symbiosome

Upon entry into the phagosome of the host cell, the symbiont continues to reside within this membrane-bound compartment, the “symbiosome.” It appears that maturation of the phagosome is inhibited in host cells that have phagocytosed *Symbiodinium*, presumably because the phagosome fails to fuse with lysosomes (Fitt *et al.*

1983). This maturation arrest may result from symbiont-directed exclusion from the phagosome of the normal rab proteins that direct trafficking and fusion of endosomes and lysosomes (Chen *et al.* 2003, Chen *et al.* 2004, Chen *et al.* 2005). Ultimately multiple membranes accumulate around the symbiont, a phenomenon that appears to be unique to the coral-dinoflagellate association. The origin of the multiple membranes may be repeated cycles of ecdysis from *Symbiodinium* resulting in membrane shedding (Wakefield *et al.* 2001), but it is not known how much of the membrane is comprised of host vs symbiont-derived material, nor the lipid or protein components of this membrane. Given the recent progress in the understanding of phagosome biogenesis and maturation, as well as mechanisms employed by pathogens/parasites to manipulate these processes, a closer examination of phagocytic uptake of *Symbiodinium* is warranted, in particular the roles of lipid, protein, and glyco-conjugate components of the phagosome, endosomes, and lysosomes.

Manipulation of host response

Currently, aside from the nascent understanding of how *Symbiodinium* may be interfering with phagosome maturation, there is little known about how the symbiont manipulates the host cell. A recent microarray study that compared symbiotic with aposymbiotic individuals of the sea anemone *Anthopleura elegantissima* uncovered clues about the symbiotic host response to harboring intracellular symbionts, in particular alterations to the cell cycle and apoptosis regulation (Rodriguez-Lanetty *et al.* 2006). It is commonly recognized that in most host species, the majority of the volume of the host cell is taken up by *Symbiodinium*, making it likely that the presence of *Symbiodinium* must completely alter many host cell processes, transforming them essentially into not much more than living compartments from which some nutrition is derived.

A model for the coral bleaching response: Bleaching may represent release from symbiont-mediated suppression of host cells

The majority of the focus on understanding the mechanistic underpinnings of the symbiosis has focused on interactions of the holobiont with alterations to its regular environmental regime. Under suboptimal conditions, the symbiosis typically breaks down, with massive jettisoning of symbionts known as “bleaching”. This breakdown can be best characterized as a breakdown in the cellular mechanisms that allow the symbiont to reside stably within the host cell. For example, one of the major causes of bleaching is perturbation to the photosynthetic apparatus of the symbionts leading to accumulation of reactive oxygen species that result in oxidative stress (Tchernov *et al.* 2004, Lesser 2006). Oxidative stress, or other cellular stresses such as osmotic stress (Mayfield *et al.* 2007), may trigger pathogenic cellular responses, such as the production of nitric oxide by the host (Perez *et al.* 2006), that in turn may trigger cell death, either necrosis or apoptosis of host or symbiont cells (Dunn *et al.* 2007), or detachment of entire gastrodermal cells with their resident symbiont(s) (Gates *et al.* 1992, Sawyer *et al.* 2001). The end result is the loss of symbionts and associated gastrodermal cells, which at one extreme, results in coral mortality. At the other extreme, bleached corals may become repopulated with symbionts, either from retention of a small population of symbionts that repopulate the replaced gastroderm, or from free-living symbionts newly acquired from the environment (Coffroth *et al.* 2006, Jones *et al.* 2008).

The discovery that thermally-stressed hosts upregulate production of nitric oxide which then triggers “eviction” of symbionts (bleaching) (Perez *et al.* 2006) is strikingly similar to the mechanism utilized to overcome *Leishmania* infection of mammalian cells. *Leishmania* also sur-

vive in macrophages via inhibition of phagosomal maturation. However, application of compounds that upregulate nitric oxide in the host has the effect of restoring phagosomal maturation processes, leading to killing of the parasite (Winberg *et al.* 2007). Thus the bleaching response appears to mirror the innate immune response of animals to parasitic infection (Weis 2008).

Comparative “Infectomics” and Future Directions

The study of cnidarian-dinoflagellate symbiosis, long been studied for its ecology and physiology, is now poised at a new age of discovery that will be fueled by genomic, cell biology, and genetic approaches. Recent recognition of the importance of community-wide application of a tractable model system that is amenable to experimental manipulation as well as cell biology and genetic approaches (Dunn *et al.* 2007, Forêt *et al.* 2007, Weis *et al.* 2008) promises significant advances in this field. In addition to a model system, coral symbiologists would benefit from a more explicit awareness that mutualists have converged on many of the same strategies for exploiting their host cells as have parasites. Therefore, a more comparative approach would be very powerful by allowing us to transfer knowledge about the host-parasite relationship to formulate testable hypotheses about the coral-*Symbiodinium* relationship. A more complete understanding of some of the common pathways that pathogens travel in their interactions with their hosts, will allow coral symbiologists to develop testable hypotheses regarding some of the significant molecular components and pathways involved in the coral symbiosis (Table I).

Some future directions include:

1. *The coordination of the host and symbiont host cell cycles.* What happens to the host cell upon two or more rounds of symbiont cell division: does the the host cell divide, and if not, what happens to it? Do host cells undergo apoptosis and release viable symbionts or do they undergo necrotic cell death, or are they released from the endoderm as intact cells containing multiple symbionts? This requires a model system that allows for examination and manipulation of single host cells.

2. *Uptake into the host cell.* To what extent does *Symbiodinium* utilize the same mechanisms for manipulating the phagosome as do mycobacteria (manipulation of rabs and other molecules involved in phagosome maturation)? Do *Symbiodinium* ever engage in some kind of active invasion similarly to their apicomplexan relatives? While there is no evidence to support this currently, and no real evidence that *Symbiodinium* harbors any kind of an apical complex, it remains possible that *Symbiodinium* employs some apicomplexan-like mechanisms for interacting with their host cells. A genomic or transcriptomic dataset with deep coverage of the proteome may reveal genes previously thought to be apicomplexan-specific.

Table I. – Selected examples of known molecules and mechanisms involved in creation of the intracellular niches created by a parasite, a pathogen and a symbiont

	<i>Toxoplasma</i>	<i>Mycobacteria</i>	<i>Symbiodinium</i>
Site of host-microbe contact	Gastrointestinal tract	Gastrointestinal tract Lung	Gastric cavity
Entry into host cell	Active Invasion	Host-mediated Phagocytosis	Host-mediated Phagocytosis
Cell type infected	All nucleated cells	Phagocytes: Macrophage	Phagocytes: Gastrodermal cells
Example host recognition/ adhesion molecules	GAGs, sialic acid	Complement C3	Lectin
Intracellular niche	Non-phagosome vacuole (PVM)	Modified phagosome	Modified phagosome
Manipulation of host response	Manipulation of host signaling pathways	Phagosome maturation arrest	Phagosome maturation arrest
Exit from host	Motor-driven egress and lysis of host cell	Cytolysis apoptosis/necrosis	Apoptosis/Necrosis/ Autophagy/Lysis/ Host cell detachment

3. *The nature of the symbiosome.* What is the composition of the symbiosome membrane? Does *Symbiodinium* target proteins or lipids into the symbiosome membrane, or is the symbiosome entirely a host-derived compartment?

4. *Manipulation of the host response by Symbiodinium.* How actively do *Symbiodinium* manipulate their host cells? For example, do they synthesize proteins that they then target into the host cell/nucleus to manipulate the host cellular machinery or transcription?

5. *The nature of the cnidarian immune response.* As basal metazoans with clear homologs for many components of the metazoan innate immunity repertoire, cnidarians will have much to reveal about the evolution of metazoan innate immunity. Even more significant, however, is the ability to compare infection by mutualists (*Symbiodinium*) with infections by pathogenic microbes. This comparative approach might reveal intriguing insights into host response to microeukaryotes, which could illuminate our understanding of apicomplexan infections.

6. *The cellular basis of bleaching.* Over the past few years, there has been increasing recognition that there are multiple pathways by which the holobiont responds to thermal or other environmental stressors. It has recently

been proposed (Weis 2008) that these multiple pathways operate under a unified model in which bleaching is fundamentally an innate immune response, in which the symbiotic suppression of host immunity is breached by responses to environmental stress, resulting in cascading host cell responses, such as apoptosis, that result in destruction of the host and/or symbiont cells and therefore, the bleaching event.

While coral biologists are working to understand questions focusing on mediation and regulation of the host-symbiont interaction, especially as it relates to the alarming rates and extents of coral bleaching and disease questions, it is likely that we will find that *Symbiodinium* is mostly unremarkable in the strategies it uses to remain viable within the host cell. It is highly likely that we will discover that *Symbiodinium* primarily utilizes mechanisms that have already been described for other parasites or microbial pathogens. However, the remarkable feature of *Symbiodinium* is the mutualistic nature of the symbioses with its host, representing the only major infection of animal cells by a “friendly” microeukaryote. Thus *Symbiodinium* offers a truly insightful model with which to better understand the interactions of animal cell and their microeukaryotic inhabitants.

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