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Commentary

Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis

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Summary

Cnidarian bleaching is a breakdown in the mutualistic symbiosis between host Cnidarians, such as reef building corals, and their unicellular photosynthetic dinoflagellate symbionts. Bleaching is caused by a variety of environmental stressors, most notably elevated temperatures associated with global climate change in conjunction with high solar radiation, and it is a major contributor to coral death and reef degradation. This review examines the underlying cellular events that lead to symbiosis dysfunction and cause bleaching, emphasizing that, to date, we have only some pieces of a complex cellular jigsaw puzzle. Reactive oxygen species (ROS), generated by damage to both photosynthetic and mitochondrial membranes, is shown to play a central role in both injury to the partners and to inter-partner communication of a stress response. Evidence is presented that suggests that bleaching is a host innate immune response to a compromised symbiont, much like innate immune responses in other host–microbe interactions. Finally, the elimination or exit of the symbiont from host tissues is described through a variety of mechanisms including exocytosis, host cell detachment and host cell apoptosis.

Key words: coral bleaching, symbiosis, coral, *Symbiodinium*, oxidative stress, reactive oxygen species, nitric oxide, apoptosis.

Introduction

Symbioses between a variety of marine invertebrates, including Cnidarians, and unicellular photosynthetic algae are common in the marine environment. One of the most ecologically significant of these mutualisms is that between Cnidarian stony corals (Order Scleractinia) and protozoan dinoflagellates in the diverse genus *Symbiodinium,* which together form the trophic and structural foundation of the coral reef ecosystem (Dubinsky, 1990). Cnidarian bleaching is a collapse of this interaction – a dysfunction of the symbiosis (Douglas, 2003). The ecological implications of coral bleaching are far-reaching. Bleaching can have a large variety of negative consequences specific to corals as well as many that impact the reef ecosystem as a whole (Hoegh-Guldberg et al., 2007; Hughes et al., 2003). This commentary will cover the very proximate cellular events that occur within the Cnidarian–dinoflagellate partnership that ultimately lead to loss of symbionts from host tissue and cause bleaching. We are increasingly gaining a full grasp of the early events that occur in the symbionts but still lack a deep understanding of the distal events in host tissues that ultimately lead to the response.

The cellular basis of Cnidarian–dinoflagellate symbioses

The Cnidarian–dinoflagellate partnership is centered around nutritional exchange; the dinoflagellate symbionts translocate a majority of their photosynthetically fixed carbon to the host in exchange for inorganic nitrogen, phosphorus and carbon from the host, in addition to a high light environment and refuge from herbivory (Venn et al., 2008; Yellowlees et al., 2008). In the case of Scleractinian corals, the symbiosis is also closely tied to the ability of the corals to deposit their massive calcium carbonate skeletons that form the reef structure. The host Cnidarians have a very simple two-tissue-layer body plan. They harbor the unicellular symbionts intracellularly in vacuoles (symbiosomes) within cells in the inner or gastrodermal tissue layer (Yellowlees et al., 2008). Such an intimate cellular relationship involves regulatory crosstalk between partners that allows the interaction to persist. This inter-partner communication includes: (1) the ability to recognize specific host–symbiont combinations; (2) the ability of symbionts to colonize host cells; (3) the corresponding ability of hosts to tolerate the presence of the symbiont; and (4) adaptations for mutual transport and exchange of nutritional resources (Douglas, 1994).

Symbiodinium can exhibit very high rates of photosynthesis in the high light environment of clear tropical reef waters and therefore it generates large quantities of dissolved oxygen (Lesser, 2006). Oxygen in high concentrations can form reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$) and superoxide (O_2^-) (Lesser, 2006). ROS causes major cellular damage including oxidizing membranes, denaturing proteins and damaging nucleic acids (Lesser, 2006). Both partners of the symbiosis have considerable adaptations for managing ROS to prevent cellular damage (Lesser, 2006; Merle et al., 2007; Richier et al., 2005). For example, both partners express, in high quantities, an unusually broad array of ROS handling enzymes including catalase, ascorbate peroxidase and multiple isoforms of superoxide dismutase. These enzymes act in concert to convert ROS back to oxygen and water. We will see that a key piece of the bleaching cascade occurs when this adaptive ROS-handling response becomes overwhelmed during stress.

Definition of coral bleaching and its ecological consequences

Symbiodinium cells are golden brown due to the presence of light harvesting and photosynthetic pigments in their chloroplasts. Healthy corals harbor millions of *Symbiodinium* per square centimeter of tissue and therefore have this same golden brown hue. Coral bleaching is so called because of the loss of color from host tissues, which reveals the underlying white limestone skeleton (Fig. 1). This loss of color is most often due to symbiosis dysfunction, which is the loss of symbionts from host tissues (Douglas, 2003). (It is sometimes due to bleaching of algal pigments, inhibition of pigment synthesis or a combination of pigment change and symbiont loss.)

Bleaching is a stress response to environmental perturbation. It can be caused by a myriad of stressors including changes in salinity, high visible and/or ultraviolet radiation, increased sedimentation and nutrients or pollutants, such as heavy metals (Coles and Brown, 2003). However, it is now well accepted that widespread bleaching in nature is a result of elevated sea surface temperatures associated with global warming in combination with high solar radiation (Hoegh-Guldberg et al., 2007; Hughes et al., 2003). Coral–dinoflagellate symbioses are near their maximal thermal tolerance limit. Even moderate increases of 1–2°C can result in reef-wide bleaching events especially when combined with seasonal high visible and/or UV radiation (Hoegh-Guldberg et al., 2007). These conditions have resulted in almost ocean-wide bleaching events such as the 1997-1998 El Nino Southern Oscillation event that resulted in massive bleaching of reefs across the Indian Ocean and some of the Western Pacific (Hoegh-Guldberg et al., 2007). The coral skeleton is an extremely efficient light gathering structure that greatly enhances light harvesting by absorbing light scatter (Enriquez et al., 2005). This light field enhancement is now thought to have negative effects in bleaching corals, with reduced pigmentation, where it serves to further elevate already-high solar radiation levels (Enriquez et al., 2005; Franklin et al., 2006).

The ability of corals to tolerate and adapt to environmental stress and change is an area of great interest and is the topic of other recent reviews (Coles and Brown, 2003; Douglas, 2003; Venn et al., 2008). There is evidence that both partners of the symbiosis have a considerable capacity to tolerate stress by employing protective mechanisms such as increased heat shock proteins expression, protective pigments and increased expression of antioxidant enzymes. There is ample evidence that tolerance varies within a population or between species (Fig. 1) of either or both hosts and symbionts (see reviews above). Whether differential tolerance will translate into differential survival and adaptation to climate change is an open question that is the subject of active research. The adaptive bleaching hypothesis, the idea that bleaching is a deliberate strategy that allows corals to swap their symbionts as an adaptation to a changing environment, has generated considerable disagreement in the field (Baker, 2001; Goulet, 2006; Hoegh-Guldberg et al., 2002). Symbiont types in corals have been sampled across a variety of spatial and temporal scales (Goulet, 2006). However, to date, there are only a few examples of symbiont shuffling in the wild (Berkelmans and van Oppen, 2006; Jones et al., 2008), and it is still unknown if the specific host–symbiont combinations found in nature can change with time and do so rapidly enough to keep pace with the changing conditions associated with climate change.

The ecological consequences of bleaching have been covered extensively in other reviews so they will only be touched on here. Widespread bleaching can lead to coral mortality. Even corals that recover exhibit decreased growth, fecundity (Coles and Brown, 2003) and increased susceptibility to disease (Rosenberg et al., 2007). Indeed the explosion in the observation of novel coral diseases in the last decade can be attributed to increased coral stress and bleaching (Rosenberg et al., 2007). This increased coral mortality and decreased fitness can have disastrous consequences such as reef degradation and even the collapse of the coral reef ecosystem. Even relatively pristine, unstressed reefs in the Pacific have lost 2% of coral cover per year in the last decade (Bruno and Selig, 2007). This sobering description of reef decline provides incentive to understand the underlying cellular events that drive the bleaching response.

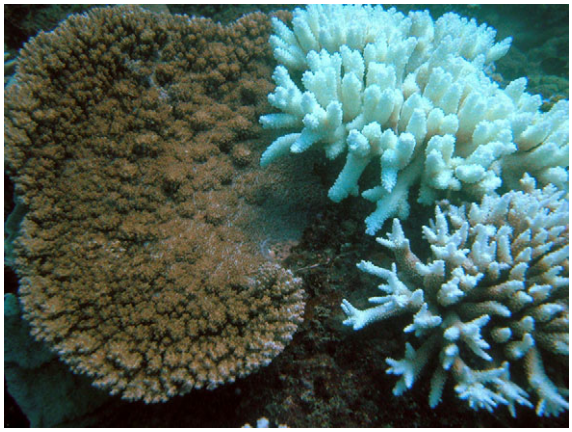

Fig. 1. Bleached and unbleached corals at Great Keppel Island on the Southern Great Barrier Reef in January 2002. The coral on the left appears brown and healthy whereas the colonies on the right are partially (lower right) and fully (upper right) bleached. This differential bleaching response between different coral species is commonly observed and not fully understood. Photograph courtesy of O. Hoegh-Guldberg, Centre for Marine Studies, University of Queensland.

The events that lead to Cnidarian bleaching will be described as a single narrative but, in fact, the developing story is being uncovered by the study of many different coral–*Symbiodinium* and anemone–*Symbiodinium* partnerships (usually the anemone *Aiptasia* spp.) as well as studies of symbionts in culture. This illustrates the point that the study of Cnidarian–dinoflagellate symbiosis cell biology is a comparative field that has not traditionally taken a conventional model-systems approach. The idea of focusing, in the future, on a few experimentally tractable model associations that could speed progress in understanding symbiosis and bleaching has received recent attention by the coral biology field (Weis et al., 2008). In the following sections, it will be shown that we know: (1) a lot about the early stages of stress and ROS production in symbionts; (2) very little about the middle stages of cellular signaling cascades that trigger bleaching in the host; and (3) some about the final stages of the cellular mechanisms resulting in loss of symbionts.

1. Heat and light stress cause symbionts to produce high concentrations of ROS

It is now clear that ROS play a central role in temperature- and solar radiation-induced bleaching. Evidence of high ROS concentrations in heat- and light-stressed symbionts is a first clue that ROS plays a role in bleaching (Franklin et al., 2004; Lesser, 1996). How are ROS generated? Fig. 2 summarizes the events occurring in the symbiont that are thought to start the bleaching

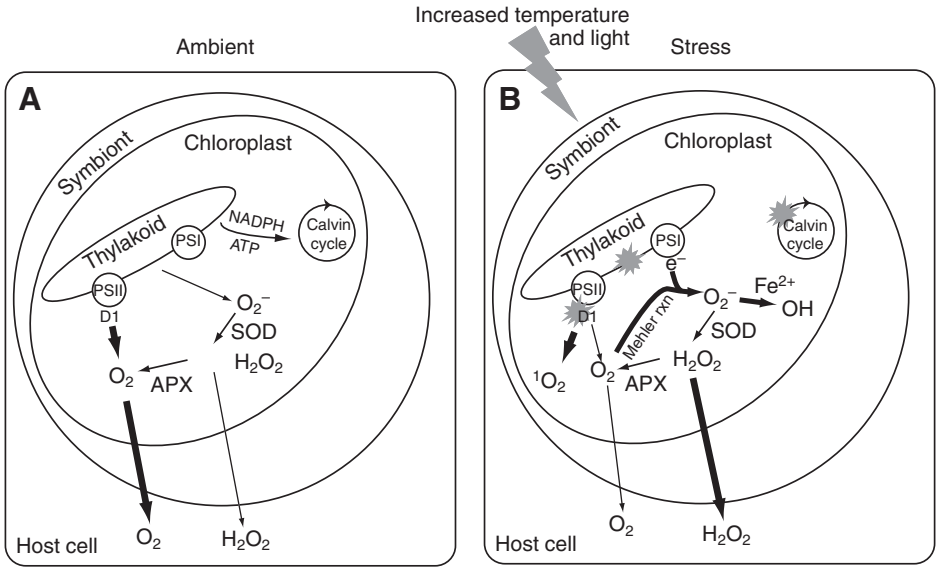

Fig. 2. Schematic representation of oxygen handling pathways in *Symbiodinium* resident in host cells under ambient (A), and elevated temperature and light (B) conditions. Under ambient conditions, the photosynthetic apparatus, consisting of photosystem II (PSII) and photosystem I (PSI) on the thylakoid, operates normally and produces large quantities of oxygen that diffuse into the host. ROS that are produced are converted back to oxygen with superoxide dismutase (SOD) and ascorbate peroxidase (APX). Under stressed conditions, damage to the photosynthetic apparatus occurs in at least three places (depicted as 'flashes' in the figure): the D1 protein in PSII; in the Calvin cycle; and on the thylakoid membranes. This damage acts to generate large amounts of ROS in the form of singlet oxygen ($^1O_2$) and superoxide (O_2^-) that overwhelm the oxygen-handling pathways. O_2^- is converted to both the most highly reactive hydroxyl radical (·OH) and the more stable and highly diffusible hydrogen peroxide (H_2O_2), which can move into host tissues. Figure adapted from Venn and colleagues (Venn et al., 2008).

process. The story begins in the symbiont chloroplast – the site of high rates of photosynthesis in the intact association. These complex photosynthetic events have been extensively reviewed recently (Lesser, 2006; Venn et al., 2008).

Elevated temperature and high irradiance can cause photoinhibition and damage the chloroplast and photosynthetic apparatus in at least three inter-related ways that act in concert to start the bleaching cascade: (1) The D1 protein is part of the water-splitting complex in photosystem II resident in the thylakoid membranes. It can be viewed as the Achilles Heel of the photosynthetic apparatus in general because it is easily destabilized (Ohad et al., 1994). There is, however, an active system of repair that, under normal conditions, keeps the complex functional (Ohad et al., 1994). During elevated temperature in *Symbiodinium,* the D1 protein becomes damaged and this damage outpaces the normal repair mechanism (Warner et al., 1999). There is even evidence that heat damages the repair mechanism itself (Takahashi et al., 2004). D1 damage results in a backup in excitation energy and the dysfunction of photosystem II (Warner et al., 1999). (2) It has also been suggested that the dark reaction of photosynthesis is compromised by heat and light such that carbon fixation declines (Jones et al., 1998). The site of damage may be ribulose bisphospate carboxylase oxygenase (Rubisco), the enzyme responsible for primary carboxylation (Lesser, 1996). This results in reduced consumption of ATP and NADPH coming from the light reactions (referred to as sink limitation) that in turn leads to the dysfunction of photosystem II *via* backup of excitation energy as described above (Jones et al., 1998; Venn et al., 2008). (3) Heat and high light also directly damage the thylakoid membranes (Tchernov et al., 2004). This causes energetic uncoupling of electron transport in both photosystems. As a result, the photosynthetic apparatus continues to generate electrons but stops making ATP and NADPH.

The build up of electrons by any or all of the mechanisms described above is thought to ultimately lead to the generation of multiple ROS in the symbiont. Excess electrons reduce O_2, in the Mehler reaction in photosystem I (instead of reducing $NADP^+$), to produce a highly reactive ROS, superoxide (O_2^-) (Tchernov et al., 2004), which can be reduced by superoxide dismutase (SOD) to a less reactive, but still damaging, and more stable hydrogen peroxide (H_2O_2) (Jones et al., 1998; Lesser, 2006). H_2O_2 can react with ferrous iron (Fe^{2+}) to form the most reactive ROS, a hydroxyl radical (·OH). In addition, excess electrons can react photochemically with pigments and O_2 to ultimately generate highly reactive singlet oxygen ($^1O_2$) (Lesser, 2006), which further exacerbates the problem by damaging, and reacting with, other D1 proteins and bleaching pigments in the photosynthetic apparatus in the thylakoids (Venn et al., 2008).

As the concentration of ROS increases with photosynthetic dysfunction, the antioxidant defense system in place in the symbiont, such as the enzymes SOD and ascorbate peroxidase, becomes overwhelmed and cannot detoxify the ROS and it begins to accumulate (Franklin et al., 2004; Lesser, 1996). Therefore, ROS can proceed to further damage photosynthetic membranes, as described above, in an escalating positive feedback loop (Lesser, 2006). Furthermore, ROS begin to diffuse into the host tissue where the damage continues and ultimately leads to bleaching (see below).

2. The signaling events in hosts that lead to bleaching are only partially understood

With an understanding of the events occurring in the symbiont during elevated temperature and light in hand, let us now turn to the events in the host. Does the presence of high ROS levels and symbionts compromised by stress trigger the host cell to bleach? Is the host similarly damaged by elevated temperature and light? What are the cellular mechanisms by which either host cells remove

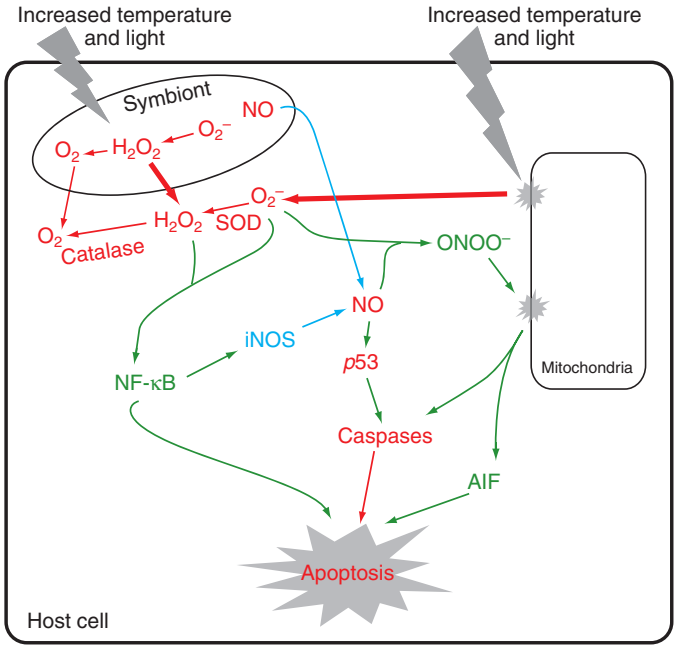

Fig. 3. Model of cell signaling pathways in the host cell that lead to bleaching by host cell apoptosis. Generation of ROS in the symbiont is described in Fig. 2. Although oxygen-handling pathways including superoxide dismutases (SOD) and catalase are present, they become overwhelmed by the high concentrations of ROS. In one pathway, high concentrations of superoxide (O_2^-), generated from host mitochondrial membrane damage (depicted as a 'flash' in the figure), and hydrogen peroxide (H_2O_2), coming from both symbiont and host, trigger the activation of the innate immunity gatekeeper transcription factor NF-κB. It, in turn, activates apoptosis directly and/or induces the expression of inducible nitric oxide synthase (iNOS) that produces nitric oxide (NO). In another pathway, NO is produced directly by the symbiont and diffuses into the host. NO combines with O_2^- to form highly reactive peroxynitrite ($ONOO^-$) that damages the mitochondrial membrane (depicted as a 'flash' in the figure). This damage releases potent pro-apoptotic molecules such as apoptosis inducing factor (AIF) and cytochrome *c* (not shown) that activate caspases, the proteases responsible for carrying out apoptosis. In another pathway, NO activates *p*53, a pro-apoptotic transcription factor, which in turn activates caspases and apoptosis. There is direct evidence in Cnidarian–dinoflagellate symbioses for pathways depicted in red, indirect evidence for those depicted in blue and evidence only in other metazoans and host–microbe interactions for those depicted in green. See text for details.

symbionts or symbionts exit host cells? Once again, as there are, at present, only partial answers to these questions, there is strong evidence that oxidative stress plays a critical role in the host bleaching cascade. Oxidative stress functions in animal systems in modulating cell death/cell survival pathways (Martindale and Holbrook, 2002) and in innate immune responses to microbes (Fang, 2004). Studies in Cnidarian–dinoflagellate symbioses are suggesting that ROS and reactive nitrogen species are functioning in these roles during the bleaching response. A cellular model for the events described below is shown in Fig. 3.

ROS concentration and production in host tissues

In addition to ROS leaking from damaged symbionts (Lesser, 2006; Tchernov et al., 2004), it is produced directly from host cell mitochondrial damage resulting from elevated temperature and light (Dykens et al., 1992; Nii and Muscatine, 1997). The host does mount an antioxidant response (e.g. Merle et al., 2007; Richier et al., 2006) but the response is insufficient to handle the high concentrations of ROS produced and the result is damage to host DNA (Lesser and Farrell, 2004), proteins and membranes (Richier et al., 2005).

Nitric oxide, inter-partner signaling and a role for host innate immunity in bleaching

The reactive nitrogen species nitric oxide (NO) may play a pivotal role in the bleaching cascade. NO acts as both a cytotoxic and a signaling molecule in animals including during host–pathogen interactions (Fang, 2004). It can react with O_2^- to form the potent and highly diffusible oxidant peroxynitrite, $ONOO^-$ (Pacher et al., 2007). In the symbiotic anemone, *Aiptasia pallida*, NO levels in host tissues increase dramatically in response to elevated temperature or inhibition of symbiont photosynthesis by blockage of photosystem II (Perez and Weis, 2006). Furthermore, the addition of NO to anemones at ambient temperature causes bleaching (Perez and Weis, 2006).

The original source of the NO, whether it comes from host, symbiont or both, is not yet clear. Perez and Weis (Perez and Weis, 2006) provide evidence that NO is produced in the host by the induction of nitric oxide synthase (NOS), which catalyzes the conversion of arginine, NADPH and O_2 to NO, citrulline and $NADP^+$. It is hypothesized that high ROS in host cells trigger an innate immune response in the host by induction of the innate immune gatekeeper transcription factor NF-κB (Sadikot et al., 2004) that goes on to induce NOS and leads to high NO (Fang, 2004). This is a common innate immune pathway observed in other animals, which leads to downstream elimination of invading microbes by various mechanisms including host programmed cell death or apoptosis (Kumar et al., 2004; Pacher et al., 2007). However, two other studies found high concentrations of NO in cultured or freshly isolated symbionts with elevated temperature (Bouchard and Yamasaki, 2008; Trapido-Rosenthal et al., 2001). This provides evidence that NO is a direct signaling molecule between the partners of symbiosis that could initiate a bleaching cascade. Whatever the source of NO is in host tissues, the direct target of NO and subsequent events, which cause loss of symbionts remain unknown. Again, based on studies of other systems, Perez and Weis hypothesize that NO and O_2^- combine to form $ONOO^-$ that causes mitochondrial membrane damage, which, in turn, causes the release of potent pro-apoptotic molecules that initiate an apoptotic cascade (Pacher et al., 2007). NO could also initiate the apoptotic cascade through induction of pro-apoptotic *p*53 (Brune et al., 1999). Testing of these models awaits future empirical study. This topic of apoptosis as a cause of symbiont loss, including the presence of *p*53 and caspases (Fig. 3), will be revisited in the next section.

These early studies of NO have interesting implications for the dynamics of inter-partner communication during a shift from a healthy to a dysfunctional host–symbiont interaction. Host–microbe interactions are driven by the ability of the invading microbes to evade and control a host immune response, and the ability of the host to detect and destroy the pathogenic invaders (Gruenberg and van der Goot, 2006). Cnidarian–dinoflagellate mutualism could be viewed as a controlled infection whereby the symbionts successfully modulate the host's immune response; the symbionts are cloaked when in the host. It may ultimately be determined that bleaching is the removal of the cloak; the symbionts signal their presence by high ROS and/or high NO that trigger the host to initiate a response to eliminate them.

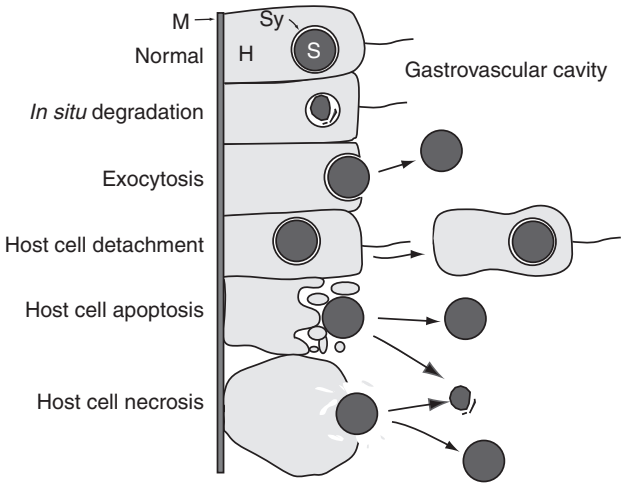

Fig. 4. Five different types of cellular mechanisms of symbiont loss from Cnidarian host tissues. The different types are discussed in the text from top to bottom. Symbionts lost by *in situ* degradation die or are killed in the host cell and are either digested or expelled (not shown). Symbionts lost *via* exocytosis are expelled free within the gastrovascular cavity. In host cell detachment, symbionts are lost when whole host cells with their symbionts still resident within, become detached from the mesoglea and surrounding cells and are released into the gastrovascular cavity. Host cells undergoing apoptosis, shrink and form multiple apoptotic bodies and in the process release viable or degrading symbionts into the gastrovascular cavity. Host cells dying by necrosis, swell and burst, releasing their contents, including symbionts (either viable or degrading) into the gastrovascular cavity. Normal host cells (H) are anchored to the acellular mesoglea (M). Symbionts (S) are contained with host vacuoles or symbiosomes (Sy). Figure adapted from Gates and colleagues (Gates et al., 1992).

3. Symbionts can be eliminated from or exit host cells *via* several mechanisms

While the cellular cascade of events in the host that leads from high ROS to Cnidarian bleaching is just beginning to be unraveled, there are a variety of studies on the ultimate cellular mechanisms that cause the loss of symbionts from host tissues. This section will describe the diverse array of cellular mechanisms that have been observed and proposed (Fig. 4). A similar list of mechanisms was first proposed by Gates and colleagues (Gates et al., 1992) and has been covered in other reviews (Douglas, 2003; Lesser, 2004). However, there is still no agreement on how these mechanisms figure into the larger environmental picture of bleaching. For example, it is largely unknown: (1) which mechanism(s) is(are) prevalent in nature, especially as a function of elevated temperature and light; (2) whether different mechanisms occur due to differing types and/or degrees of the stress; (3) how occurrence of these different types differs between host and/or symbiont taxa; and (4) how and if different mechanisms interact, for example by shared pathways, co-occurrence or through time.

Evidence for the cellular mechanisms resulting in bleaching has largely come from histological analyses of bleaching events in nature, as well as during experimentally induced bleaching, usually by hyperthermic stress. You will see that most of the accounts are cellular snapshots; neither the dynamic processes that cause them nor how they are interrelated, either mechanistically or temporally, are fully understood. Perez reviews all of these mechanisms and develops hypotheses for how they occur based on cellular studies in other systems (Perez, 2007).

In situ degradation of symbionts

Microscopic examination of corals bleaching in nature (Ainsworth and Hoegh-Guldberg, 2008; Brown et al., 1995) and corals and anemones subjected to hyperthermic stress (Dunn et al., 2004; Franklin et al., 2004; Strychar et al., 2004) reveal profiles of symbionts that are apparently degrading within host cells. There are at least two possible mechanisms of *in situ* degradation. The first is that the symbionts themselves are dying and degrading from the effects of ROS. In all cases, morphological profiles of symbionts that are consistent with cells dying *via* programmed cell death (PCD) are described. PCD has been described in unicellular organisms (Ameisen, 2002) and shares many common features with apoptosis, a form of PCD in animals that is described in detail below. Dunn and colleagues, and Strychar and colleagues also identify symbiont cells under severe stress that appear to be undergoing necrosis, a form of uncontrolled cell death (also described below) (Dunn et al., 2004; Strychar, et al., 2004).

The second possible mechanism is that the host is actively destroying the symbionts and ultimately digesting or expelling them. This could represent more evidence of a host innate immune response to compromised symbionts. The Rab GTPases are important regulators of vesicular trafficking including in professional phagocytes such as macrophages (Schwartz et al., 2007). Recent studies on the differential localization of three Rab homologs in *Aiptasia pulchella* symbiont-containing-host cells in healthy hosts compared with stressed hosts suggest that stress results in changes in lysosomal maturation and targeting of symbiosomes for fusion with lysosomes presumably resulting in symbiont digestion (Chen et al., 2005). In addition, there is early evidence in *Aiptasia pallida* that autophagy, a cellular pathway that controls organelles, vacuoles and tissue homeostasis, could be playing a role in bleaching (Dunn et al., 2007). It too could result in digestion of unwanted symbionts and is described in more detail under the discussion of apoptosis below.

Exocytosis of symbionts

There is also evidence of exocytosis of symbionts from stressed *A. pulchella* and corals. Apparent exocytotic profiles and free symbionts in the host gastric cavity have been observed in bleaching animals (Brown et al., 1995; Steen and Muscatine, 1987). Furthermore, cellular studies examining cytoskeletal assembly and intracellular Ca^{2+}, two cellular components required for exocytosis, point towards active exocytosis during bleaching in the coral *Acropora grandis* (Fang et al., 1997; Huang et al., 1998).

Host cell detachment

Other studies document the release of living whole-host cells, with symbionts still inside, from heat- and cold-stressed bleaching corals and *A. pulchella* (Brown et al., 1995; Gates et al., 1992). Attempts to link a cytoskeletal collapse with intracellular Ca^{2+} fluxes that could lead to detachment were unsuccessful (Sawyer and Muscatine, 2001). Mechanisms driving this phenomenon, therefore, await further investigation. Host cell detachment might be a downstream event that follows host cell death (described below) and resulting tissue destabilization and disintegration.

Apoptosis

Apoptosis is one form of programmed cell death found in the Metazoa. It is a highly conserved, critical process in tissue

morphogenesis and homeostasis and in the elimination of damaged or infected cells (Raff, 1998). It is characterized by an orderly, proscribed set of events that leads to cell death. Once the cascade is initiated, morphological alterations include cell shrinkage, fragmentation of DNA, condensation of chromatin and the formation of apoptotic bodies that contain packaged cellular debris. Apoptosis in vertebrates is highly complex and includes initiation and execution stages, which involve multiple different and interacting pathways. Initiation can be triggered by either extrinsic or intrinsic signals. The centerpiece of the cascade is the caspases, a family of proteases that carry out the majority of the cell death process (Raff, 1998). Caspases and other apoptosis genes, are present in Cnidarians (Cikala et al., 1999; Dunn et al., 2006; Richier et al., 2006), and apoptosis is an area of active research in the study of metazoan evolution (David et al., 2005).

Studies have shown evidence of apoptosis of symbiont-containing gastrodermal cells in thermally- and high light-induced bleaching anemones (Dunn et al., 2004; Richier et al., 2006) and corals (Lesser and Farrell, 2004) using a variety of measures of apoptosis. These include increases in: (1) caspase activity; (2) DNA fragmentation, determined through both histological staining and by gel electrophoresis; and (3) the number of apoptotic cells in tissue – those with increased vacuolization, apoptotic bodies and condensed nuclei. There are at least two possible causes for apoptosis initiation that require further investigation. The first, originally proposed by Dunn and colleagues is that apoptosis acts to mitigate tissue damage from ROS and thereby maintain tissue homeostasis by deleting damaged cells (Dunn et al., 2004).

The second possibility returns me to my above discussion of ROS, NO signaling and apoptosis (Fig. 3): it is that apoptosis acts as part of an innate immune response that recognizes symbionts damaged by oxidative stress and seeks to remove them by host cell suicide. Apoptosis plays a major role in the host innate immune response to invading microbes (James and Green, 2004). If pathogenic microbes fail to evade host innate immune defenses, the host often removes the pathogen by apoptotic host cell death. Conversely, some pathogens retard their removal *via* host apoptotic cell death by controlling host anti-apoptotic signaling mechanisms (James and Green, 2004). One intrinsic pathway of apoptosis initiation involves the release of pro-apoptotic molecules from the mitochondria, including apoptosis inducing factor (AIF) and cytochrome *c*, which goes on to activate caspases (Chipuk and Green, 2008; Lorenzo et al., 1999). This can be achieved by either damage to the mitochondrial outer membrane, for example by reactive oxygen or nitrogen species (as described above) (Brune et al., 1999) or through the specific actions of apoptosis regulatory proteins (members of the bcl-2 family) on the membrane (Chipuk and Green, 2008). Another intrinsic pathway involves the upregulation of the pro-apoptotic *p*53 transcription factor that promotes apoptosis initiation (Evan and Littlewood, 1998). There is evidence in corals that *p*53 protein levels increase in thermally stressed corals (Lesser and Farrell, 2004). Now that there is evidence of high NO, *p*53, the presence of caspases and increased caspase activity with elevated temperature, further studies are need to firmly demonstrate the pathways that they participate in to cause bleaching (Fig. 3).

There is early evidence that another form of cell death, autophagy, is involved in the bleaching process and that it is interlinked with apoptosis (Dunn et al., 2007). Autophagy is a catabolic pathway that degrades intracellular components *via* lysosomal degradation. Using autophagy, cells dispose of obsolete, excess or damaged parts, such as mitochondria, peroxisomes and regions of the Golgi, but the process can also result in cell death (Cuervo, 2004). Autophagy was originally described in organisms, ranging from yeast to humans, as a response to starvation but has since been shown to function in a variety of key cellular functions, including cellular differentiation, tissue remodeling, growth control and cellular defense against invasion (Cuervo, 2004). For example, autophagy plays a major role in the control of the bacterial parasite *Mycobacterium* in human macrophages (Gutierrez et al., 2004).

In *A. pallida*, Dunn and colleagues demonstrate that chemical induction of autophagy causes massive bleaching at ambient temperature suggesting that it can play a role in symbiont regulation (Dunn et al., 2007). Yet high temperature-induced bleaching is repressed only when both autophagy and apoptosis are inhibited simultaneously. From these data, it is hypothesized that there is an interconnectivity between the two forms of cell death, such that when one is inhibited, the other is induced. A similar see-saw connection between apoptosis and autophagy occurs in vertebrates (Boya et al., 2005). Testing of this hypothesis awaits future studies of auptophagy such as examination of expression and localization of the highly conserved autophagic genes and microscopic profiles of autophagic membranes around symbionts.

Necrosis

In contrast to apoptosis, necrosis is uncontrolled cell death that is most often triggered by extrinsic factors that cause the cell and its contents to swell, eventually causing rupture of the plasma membrane and release of cellular material (Wyllie et al., 1980). It lacks both the distinctive morphological signatures of apoptosis, such as condensed chromatin and apoptotic bodies, as well as a molecular signature such as execution by a suite of genes and pathways. Host cell necrosis, documented by morphological appearance of cells, has been described in the symbiont-containing gastrodermal cells of thermally stressed *A. pallida* (Dunn et al., 2004). In these studies, Dunn and colleagues subjected anemones to a range of elevated temperatures for varying amounts of time. There is a shift from apoptosis at the lower stress levels, i.e. moderate temperature stress and shorter duration, to necrosis at the more severe stress levels. This led to the hypothesis that apoptosis at moderate stress levels is acting to mitigate tissue damage from ROS and thereby maintain tissue homeostasis by deleting damaged cells but that this control is lost under severe stress where necrosis predominates.

Fate of released symbionts

Regardless of the mechanism of release, an obvious question is: are symbionts that are released during bleaching viable? Although there is some conflicting information from several studies, the answer increasingly seems to be no (reviewed by Hill and Ralph, 2007). Franklin and colleagues, as mentioned above, described degenerating symbionts with compromised photosynthetic machinery being released from bleaching corals in nature (Franklin et al., 2004; Franklin et al., 2006). Studies from corals, experimentally bleached with heat and light, showed that symbionts released early in the bleaching process initially appeared intact morphologically and displayed normal photosynthesis but that over a period of a few days, they declined (Hill and Ralph, 2007). Symbionts released later in the bleaching process were abnormal from the start.

Conclusions

In summary, the cellular mechanisms underlying Cnidarian bleaching are a complex set of interactions between the two

partners of the Cnidarian–dinoflagellate symbiosis as they react to environmental stress, especially elevated temperature and solar radiation. ROS in both partners, generated by impairment of multiple membranes and proteins, plays a central role in both causing damage in both partners and in inter-partner signaling. Evidence that both ROS and reactive nitrogen species initiate an innate immune response in the host is similar to responses found in other host–microbe interactions. This paves the way for further study of the cellular pathways involved in eliminating highly compromised symbionts from similarly stressed host tissues. I have participated in many conversations with colleagues about which partner is in control: are symbionts actively leaving hosts or are hosts actively expelling symbionts? I feel that we will ultimately determine that the reality lies somewhere in-between where stress changes the highly regulated inter-partner conversation that in turn tips the delicate balance from symbiosis health to dysfunction.

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