

# Towards a better understanding of the mechanisms of coral bleaching

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Coral reefs are among the richest and the most diverse biological ecosystems on Earth. The persistence, the resilience and the ecological success of reef-building corals in tropical oligotrophic waters relies on the symbiosis between cnidarians and photosynthetic dinoflagellates of the genus *Symbiodinium*. This association allows the transfer of highly energetic compounds and an efficient recycling of growth-limiting nutrients (CO<sub>2</sub>, nitrogen and phosphorus) in the oligotrophic environment that are tropical seas (Yellowlees et al. 2008). During last decades coral reefs had to face several large-scale bleaching events compromising their survival (Oliver et al. 2009). This phenomenon linked to anthropogenic climate change and El-Niño events is triggered by warming ocean temperatures in combination with high levels of solar irradiance (UV and/or visible radiations; Lesser (2011)). In these conditions, the mutualistic relationship between host cells and their intracellular symbiotic *Symbiodinium* sp. becomes unstable and results in the loss of the algae by the host organism (Weis 2008).

## The causes of coral bleaching

It has been shown at the end of the 80's that elevated solar radiation and temperature impair the photosynthetic activity and induce an oxidative stress in symbiotic cnidarians and in cultured *Symbiodinium* (Lesser et al. 1990). Since then a great deal of evidence has been collected, progressively lifting the veil on the cellular mechanisms leading to the disruption of the symbiosis. It has generally been found that the initial step of bleaching is very likely linked to photosynthesis in *Symbiodinium*. Indeed, thermal and light stress were reported to cause photoinhibition and damages to the chloroplast and the photosynthetic apparatus in at least three ways. (1) The photosystem II (PSII) and the associated D1 protein are damaged at a rate exceeding the normal repair mechanism (e.g., Hill et al. (2011)) or the repair mechanisms itself is inhibited by heat stress (Takahashi et al. 2009). (2) The Calvin-Benson cycle is compromised under these conditions, through the inactivation of ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco), the enzyme responsible for CO<sub>2</sub> fixation (Jones et al. 1998; Lilley et al. 2010). (3) The thylakoid membrane integrity of chloroplasts can be directly compromised by heat and high light. All of these ways are inter-related, may act in concert and result in the build-up of excess energy, ultimately leading to the generation of reactive oxygen species (ROS) (Suggett et al. 2008; Krueger et al. 2014). Overall this led to the establishment of the hypothesis that ROS and oxidative stress play a significant role in the bleaching phenomenon. Indeed, ROS produced in excess could cause major cellular damages in *Symbiodinium* and host cells. These include membrane oxidation (Tchernov et al. 2004), protein denaturation (Downs et al. 2000), damage to nucleic acids (see Lesser (2006) for review) and can trigger a cellular signaling cascade leading to cell death (Dunn et al. 2004; Richier et al. 2006; Bouchard and Yamasaki 2009) and/or the expulsion of the symbiotic algae by the host (Gates et al. 1992; Weis 2008).

## Getting a better insight into the photosynthetic mechanisms in *Symbiodinium*

Phylogenetic studies conducted these last two decades have revealed that the genus *Symbiodinium* is delineated into nine lineages or clades (A-I), each comprising multiple strains or species (Pochon and Gates 2010). Different *Symbiodinium* phylotypes can exhibit a wide range of physiological responses to environmental variations and stress (e.g. Krueger et al. (2014)), thus allowing their coral hosts to occupy a wide range of environmental niches and also significantly contributing to their persistence under change (Howells et al. 2012). Therefore, the host specificities along with the external environmental conditions act as the driving forces responsible for particular pairings between both partners. Light is likely the most important of these environmental factors and recent studies showed that the vertical distribution of certain coral species could be explained by their association with symbionts adapted to the particular light regime experienced by the host. These adaptations may originate, among other, from a less sensitive PSII repair mechanism (Takahashi et al. 2009) and peculiar regulation mechanisms of the photosynthetic activity (e.g. Reynolds et al. (2008)). In the natural environment the coral holobiont has to cope with significant daily variations in light intensities that sometimes exceed *Symbiodinium* photosynthetic capacity (such as during thermal stress). This implies the existence of regulatory mechanisms that mitigate the excitation pressure when the light absorbed is in excess of that required for CO<sub>2</sub> assimilation. This can be

done by dissipating the energy in excess and/or diverting electrons in excess in the photosynthetic apparatus. In addition to the linear electron flow (LEF) operating during oxygenic photosynthesis, alternative electrons flows (AEF) have been widely described in higher plants and microalgae. They include cyclic electron flow around Photosystem I (PSI-CEF) and oxygen reduction by photosynthetic electrons through various processes: the Mehler reaction, chlororespiration, photorespiration, and mitochondrion-dependent reoxidation of reducing equivalents (see Cardol et al. (2011) for review). In the current context of climate changes and their impacts on symbiotic cnidarians a better understanding of the AEF present and their roles in *Symbiodinium* is needed. Indeed, despite that the existence of Mehler reaction, chlororespiration, or PSI-CEF has been strongly suggested in many reports to date, the nature and the relative amplitude of these mechanisms is still a matter of debate in *Symbiodinium*. Therefore, the purpose of the study presented in the first publication of the “scientific contribution” was to investigate the amplitude of photosynthetic alternative electron flows to oxygen (chlororespiration, Mehler reaction, mitochondrial respiration), its light dependence and the occurrence of PSI cyclic electron flow in *Symbiodinium* strains belonging to different Clades (A1, B1 and F1). Joint measurements of oxygen evolution, PSI and PSII activities allowed us to demonstrate that PSI-CEF and chlororespiratory activities are low compared to the extent of the Mehler reaction which can account for up to 50% of maximum photosynthetic electron transfer rate in all strains. This mechanism in *Symbiodinium* plays a photoprotective role. It takes place under high light intensities when the LEF to CO<sub>2</sub> fixation saturates, thus acting as an efficient electron sink. By alleviating the excitation pressure over PSII and PSI, it might thus prevent photoinhibition and photodamages. By acting as an efficient electron flow, it also generates an extra proton gradient across the thylakoid membranes, without net synthesis of NADPH. Thus, it might promote the synthesis of extra ATP probably required for CO<sub>2</sub> fixation or other cellular reactions (Cardol et al. 2011).

#### **The dark side of the Mehler reaction**

Discovered in 1951, the Mehler reaction involves the direct reduction of O<sub>2</sub> by PSI and leads to the production of superoxide ion (O<sub>2</sub><sup>•-</sup>) (Mehler 1951). The generated ROS are rapidly converted into water thanks to the activities of the two chloroplast-associated enzymes, superoxide dismutase (SOD) and ascorbate peroxidase (APX) (together grouped into the Mehler Ascorbate Peroxidase or MAP pathway). The flow of electrons extracted from water at the PSII level to water produced by APX, is called the water-water cycle (WWC) (Asada, 1999). The Mehler reaction is at the basis of the sink-limitation model of coral bleaching proposed by Wooldridge (2009). Indeed, the O<sub>2</sub> reduction by PSI and the WWC possess only a finite capacity for protection beyond which the electron transport chain becomes over-reduced, significant damage to PSII occurs and the rate of ROS production may exceeds the capacity of the cellular antioxidant network.

Although several studies have pointed out the water-water cycle as being one of the primary mediators of the ROS-mediated process leading to coral bleaching (Suggett et al. 2008; Weis 2008), the impacts of environmental stress on the O<sub>2</sub> reduction by PSI and the associated ROS-detoxifying enzymes remained to be determined. Based on the results of the first study presented here (Roberty et al. 2014), I analyzed the impacts of an acute thermal and light stress (conditions known to induce bleaching) on the WWC in the model *Symbiodinium* strain A1 (2nd publication). We observed that high light treatment at 26°C resulted in the up-regulation of SOD, APX and GR activities and an increased production of ROS with no significant change in the amplitude of the Mehler reaction. Under high light and at 33°C, the Mehler reaction significantly increased relative to total electron transport (75% vs 50% at 26°C). This increase was concomitant with a two-fold increase in ROS generation compared to the treatment at 26°C, while enzymes involved in the WWC were largely inactivated. These data show for the first time that combined heat and light stress inactivate antioxidant capacities of the WWC, and suggests that its photoprotective functions are overwhelmed under these conditions.

This study also indicates that cnidarians may be more prone to bleach if they harbor *Symbiodinium* cells having a highly active Mehler-type electron transport, unless they are able to quickly up-regulate their antioxidant capacities (Roberty et al. 2015). Indeed ROS accumulating in the chloroplasts, and more especially H<sub>2</sub>O<sub>2</sub>, will diffuse into the host tissue where damages occur and where they trigger a cellular signaling cascade that will ultimately lead to cell death (Bouchard and Yamasaki 2009) and to the phenomenon of coral bleaching (Weis 2008). The resulting loss of symbionts by the coral host will have dramatic ecological consequences on the colony and over the entire reef ecosystem (Hoegh-Guldberg 1999).

#### **References**

- Bouchard JN, Yamasaki H (2009) Implication of nitric oxide in the heat-stress-induced cell death of the symbiotic alga *Symbiodinium microadriaticum*. *Marine Biology* 156:2209-2220
- Cardol P, Forti G, Finazzi G (2011) Regulation of electron transport in microalgae. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* 1807:912-918

- Downs CA, Mueller E, Phillips S, Fauth JE, Woodley CM (2000) A molecular biomarker system for assessing the health of coral (*Montastraea faveolata*) during heat stress. *Marine Biotechnology* 2:533-544
- Dunn SR, Thomason JC, Le Tissier MDA, Bythell JC (2004) Heat stress induces different forms of cell death in sea anemones and their endosymbiotic algae depending on temperature and duration. *Cell Death and Differentiation* 11:1213-1222
- Gates RD, Baghdasarian G, Muscatine L (1992) Temperature stress causes host-cell detachment in symbiotic cnidarians - implications for coral bleaching. *Biological Bulletin* 182:324-332
- Hill R, Brown CM, DeZeeuw K, Campbell DA, Ralph PJ (2011) Increased rate of D1 repair in coral symbionts during bleaching is insufficient to counter accelerated photo-inactivation. *Limnology and Oceanography* 56:139-146
- Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. *Marine and Freshwater Research* 50:839-866
- Howells EJ, Beltran VH, Larsen NW, Bay LK, Willis BL, van Oppen MJH (2012) Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature Clim Change* 2:116-120
- Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U (1998) Temperature-induced bleaching of corals begins with impairment of the CO<sub>2</sub> fixation mechanism in zooxanthellae. *Plant Cell and Environment* 21:1219-1230
- Krueger T, Becker S, Pontasch S, Dove S, Hoegh-Guldberg O, Leggat W, Fisher PL, Davy SK (2014) Antioxidant plasticity and thermal sensitivity in four types of *Symbiodinium* sp. *Journal of Phycology* 50:1035-1047
- Lesser MP (2006) Oxidative stress in marine environments: Biochemistry and physiological ecology. *Annual Review of Physiology* 68:253-278
- Lesser MP (2011) Coral bleaching: causes and mechanisms *Coral reefs: an ecosystem in transition*. Springer, pp405-419
- Lesser MP, Stochaj WR, Tapley DW, Shick JM (1990) Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation, and temperature on the activities of protective enzymes against active oxygen. *Coral Reefs* 8:225-232
- Lilley RM, Ralph PJ, Larkum AWD (2010) The Determination of Activity of the Enzyme Rubisco in Cell Extracts of the Dinoflagellate Alga *Symbiodinium* sp. by Manganese Chemiluminescence and its Response to Short-term Thermal Stress of the Alga. *Plant, Cell & Environment* 9999
- Mehler AH (1951) Studies on reactions of illuminated chloroplasts: I. Mechanism of the reduction of oxygen and other hill reagents. *Archives of Biochemistry and Biophysics* 33:65-77
- Oliver JK, Berkelmans R, Eakin CM (2009) Coral bleaching in space and time. In: van Oppen MJH, Lough JM (eds) *Coral Bleaching: Patterns, Processes, Causes and Consequences*. Springer, Berlin Heidelberg, pp21-39
- Pochon X, Gates RD (2010) A new *Symbiodinium* clade (Dinophyceae) from soritid foraminifera in Hawai'i. *Molecular Phylogenetics and Evolution* 56:492-497
- Reynolds JM, Bruns BU, Fitt WK, Schmidt GW (2008) Enhanced photoprotection pathways in symbiotic dinoflagellates of shallow-water corals and other cnidarians. *Proceedings of the National Academy of Sciences of the United States of America* 105:13674-13678
- Richier S, Sabourault C, Courtiade J, Zucchini N, Allemand D, Furla P (2006) Oxidative stress and apoptotic events during thermal stress in the symbiotic sea anemone, *Anemonia viridis*. *Febs Journal* 273:4186-4198
- Roberty S, Bailleul B, Berne N, Franck F, Cardol P (2014) PSI Mehler reaction is the main alternative photosynthetic electron pathway in *Symbiodinium* sp., symbiotic dinoflagellates of cnidarians. *New Phytologist* 204:81-91
- Roberty S, Fransolet D, Cardol P, Plumier JC, Franck F (2015) Imbalance between oxygen photoreduction and antioxidant capacities in *Symbiodinium* cells exposed to combined heat and high light stress. *Coral Reefs* 34:1063-1073
- Suggett DJ, Warner ME, Smith DJ, Davey P, Hennige S, Baker NR (2008) Photosynthesis and production of hydrogen peroxide by *Symbiodinium* (Pyrrhophyta) phylotypes with different thermal tolerances. *Journal of Phycology* 44:948-956
- Takahashi S, Whitney SM, Badger MR (2009) Different thermal sensitivity of the repair of photodamaged photosynthetic machinery in cultured *Symbiodinium* species. *Proceedings of the National Academy of Sciences of the United States of America* 106:3237-3242
- Tchernov D, Gorbunov MY, de Vargas C, Yadav SN, Milligan AJ, Hagblom M, Falkowski PG (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proceedings of the National Academy of Sciences of the United States of America* 101:13531-13535

- Weis VM (2008) Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *Journal of Experimental Biology* 211:3059-3066
- Wooldridge SA (2009) A new conceptual model for the warm-water breakdown of the coral-algae endosymbiosis. *Marine and Freshwater Research* 60:483-496
- Yellowlees D, Rees TAV, Leggat W (2008) Metabolic interactions between algal symbionts and invertebrate hosts. *Plant Cell and Environment* 31:679-694.