Development of immunity in basal metazoans

***Dr Mauricio Rodriguez-Lanetty** from Florida International University (FIU), is currently conducting research that focuses on immunological priming in corals and anemones, a process by which an animal can resist pathogens through repeated, non-lethal exposure. As corals are at risk due to climate change, this is an important project to help further our understanding of their immunology. The project also includes an outreach programme to motivate minority students to pursue science as a career.*

Coral reefs are one of the most important ecosystems on the planet as they support a wide and diverse array of organisms and human activities. Unfortunately, however, they are also one of the most vulnerable.

Corals are categorised as anthozoans, a class shared by anemones, and are made up of small, colonial animals which come together to create reefs. As coral tissues contain symbiotic algae, they can only live within a "goldilocks zone", where the waters are suitably warm and where the algae receive enough light to photosynthesise and support their coralline hosts. Climate change has reduced the size of the area habitable by corals due to ocean warming, and has caused further issues that include ocean acidification and an increased prevalence of coral diseases. As such, swathes of reef are being lost around the world due to bleaching (when symbiotic algae leave their coral host).

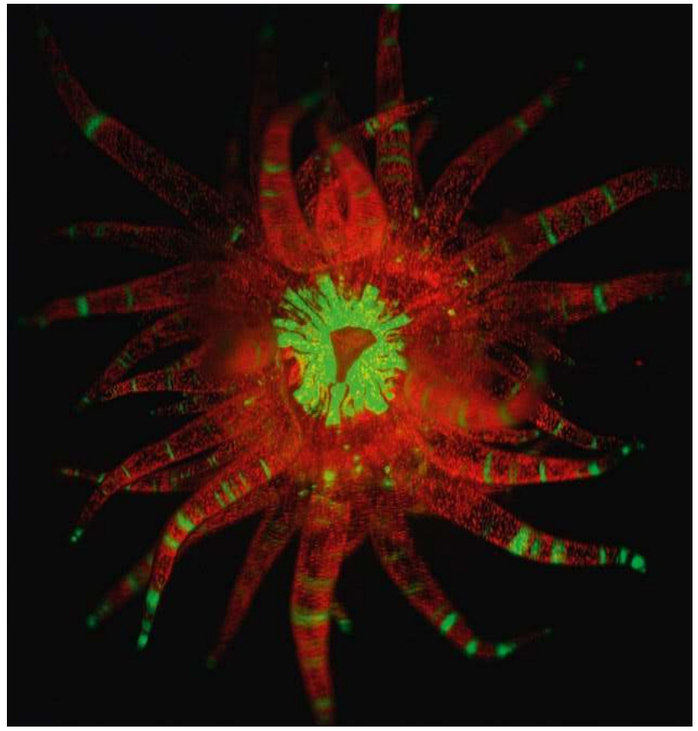
It is imperative that we find ways to improve corals' resilience against worsening environmental conditions and the range of problems they can cause. Dr Mauricio Rodriguez-Lanetty is currently leading a programme to research immunological priming in anthozoans and determine if these animals can be inoculated against disease. His programme will also provide educational and research experiences to high school, undergraduate, and postgraduate students to inspire the next generation of scientists.

CORAL DISEASE

Not only are corals very visually appealing, attracting thousands of tourists every year, they provide numerous services to local populations. They act as nurseries for fish, support fisheries, and protect coastlines from facing the full force of storms. They are also home to thousands of animals and are areas of high biodiversity. As such, it is vital that we find ways to protect them against the changing environment.

One recent problem faced by corals is the prevalence of pathogens, which has been exacerbated by climate change. As corals are very long-lived (some can survive for hundreds of years), it is expected that they will encounter the same pathogens at several points during their lives. Therefore, it is believed that, through repeated low-level exposure, corals may build up a resistance against disease-causing pathogens. However, this area of science is highly under-researched.

Research suggests that immunological priming could have developed much earlier in the evolutionary tree



ANEMONES AS MODELS

To further our understanding of disease resistance in corals, Dr Rodriguez-Lanetty studies the immune response of anemones as a model system. Anemones are easier to cultivate in laboratories, where they also have symbiotic algae and undergo bleaching, making them suitable replacements for coral species in research.

The brown anemone, called *Exaiptasia pallida*, is used in the "CAREER: Exploring the Immunological Priming in a Basal Metazoan (Anthozoan)" programme. This species is found in the western Atlantic Ocean, sharing the same waters as Caribbean reefs. It is likely to encounter the same pathogens as corals in this area, making it susceptible to certain diseases that allow it to be used in place of corals in this research. By studying immunological priming in brown anemones, our understanding of the processes behind immunological defence in anthozoans, and the evolution of immunity in basal metazoans (species that diverged early on in evolution and have remained relatively unchanged since) will expand.

IMMUNOLOGICAL PRIMING

Immunological priming is a process by which the immune response of an organism is strengthened by repeated exposure to a pathogen. This is common in more advanced species, such as humans and other vertebrates, but research suggests that priming could have developed much earlier in the evolutionary tree.

In his initial research proposal for the CAREER programme, Dr Rodriguez-Lanetty hypothesised that sub-lethal exposure to a pathogen could result in anthozoans establishing a defensive response that they will be able to express more effectively when they encounter the pathogen again in the future.

To determine if this is the case, brown anemones were repeatedly exposed to *Vibrio coralliilyticus* – a bacterial species known to cause morbidity and bleaching in both corals and anemones. It was found that three days of exposure to the bacterium could be considered "sub-lethal", and anemones exposed to the pathogen twice were more likely to survive future encounters to it, than those naïve to it. This is an exciting finding that suggests learnt immune responses such as this are not limited to complex vertebrates. As a result, this has opened many doors for further research into the immunological processes of anthozoans.

Anemones exposed to the pathogen twice were more likely to survive future encounters to it than those naïve to it

Differences in proteins between unexposed and exposed anemones were also identified, suggesting that this defensive mechanism is controlled at a molecular level. The identified proteins were similar to genes associated with immune responses in other organisms. One in particular was similar to inotropic glutamate receptors (iGluR), which are associated with neurotransmissions in animals with a nervous system, and are common in exposed anemones. Again, using brown anemones as a model system, nine iGluR-like genes were identified and found to change when the animal was exposed to a pathogen. This is a particularly interesting finding, as it suggests these types of receptors are more widespread than previously thought, and that they originated as mechanisms for sensing cues early on in evolutionary history.

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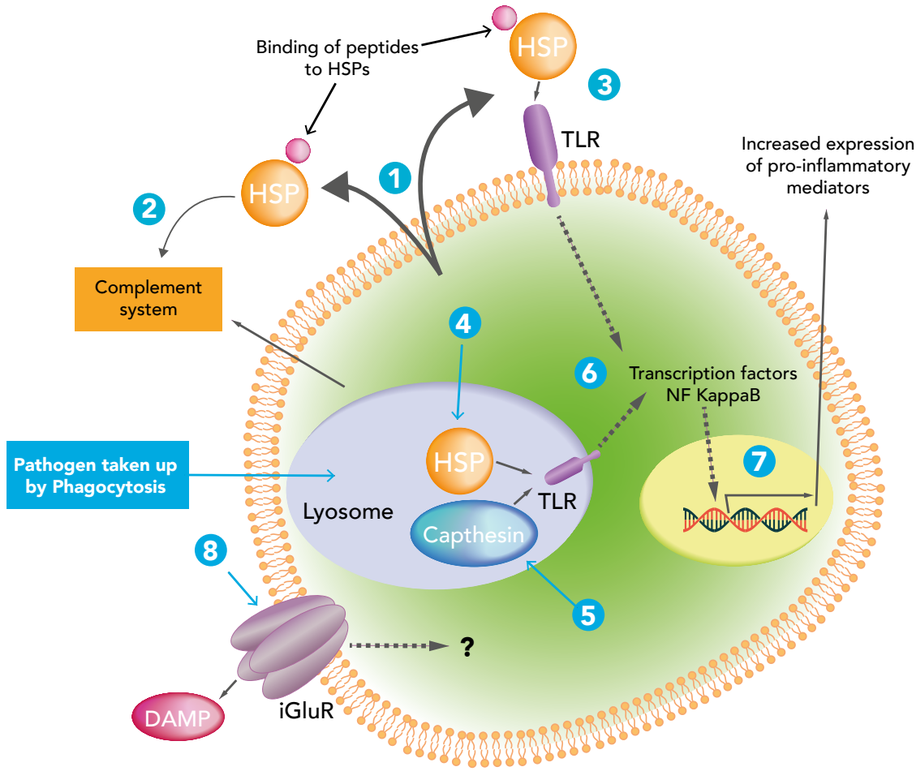

Proposed model of Heat Shock Proteins (HSP), Capthesin and Glutamate Receptor (iGluR) roles in cnidarian molecular defense priming: (1) HSP are up regulated and some are extracellularly secreted where bind to peptides and act as DAMPs; (2) as DAMPs, HSP help with a faster activation of the innate complement system, and/or (3) interact and cause a quicker activation of outer host cell membrane TLRs; (4) intracellularly, up-regulated HSP proteins can be delivered into lysosomes in which they can also interact and activate endosomal cell membrane TLRs; (5) higher production of Capthesin are delivered into lysosomes in which they can also interact and activate endosomal cell membrane TLRs; (6) activated TLRs either from the outer membrane or endosomal membranes will trigger cell signaling pathways that will converge in the activation of transcription factors (likely NF-kappa β) that will ultimately induce the expression of immune-related genes (7) resulting in the production of potential pro-inflammatory molecules; (8) Higher expression of iGluR expressed on the outer membrane will also facilitate a faster sensing of potential DAMPs upon secondary exposure of pathogens. Adapted from a figure originally published in *SCIENTIFIC REPORTS* under the Creative Commons CC BY 4.0 license.

Based on these findings, future research will assess whether host responses to repeated infectious agent exposure are pathogen-specific, and how symbiotic microbes respond to immunological priming. These are exciting areas that with further improve our understanding of disease resistance in anthozoans.

CAREER DEVELOPMENT

Inspiring future generations is a vital part of the scientific process. An important component of the CAREER programme is outreach to students from high school to postgraduate levels, and particularly to minorities who are underrepresented in science. This is highly important as it encourages those that may not pursue careers in science to consider entering the field.

By 2050, 60% of the US' population growth is expected to originate from the Hispanic community, however, Hispanic people are the least likely to enter higher education. For this reason, the educational aspect of the programme focuses on engaging Hispanic students and other minorities.

Conducting a summer science programme entitled "Aventura Cientifica", Dr Rodriguez-Lanetty hopes to motivate minority high school students to consider scientific research as a career. This is inspired by his own experiences, attributing a large part of his success to the positive role models he encountered throughout school and extra-curricular activities.

Aventura Cientifica is a four-week long experience held at Florida International University that will involve high school students in subjects included in the CAREER programme, improving their confidence and appreciation for science. This is vital to ensure that people from all backgrounds have opportunities to advance their interests in science and ultimately pursue a career in research. It will also enable the future generation to continue protecting the environment, which will become increasingly more important over the coming years.

Behind the Bench

Dr Mauricio Rodriguez-Lanetty

E: rodriguezlanetty@gmail.com; rodmauri@fiu.edu **T:** +1 337 254 7136 **W:** http://imageslab.fiu.edu
W: http://biology.fiu.edu/portfolio/mauricio-rodriguez-lanetty/

Research Objectives
Dr Rodriguez-Lanetty's research programme will elucidate the specificity, memory and molecular basis of the defence response of corals upon repetitive encounters with pathogens. Understanding how the immune system of these organisms responds to pathogens may offer insights into the resilience of these ecosystems, and potentially may inform remediation of them.

Funding
National Science Foundation (NSF)

Collaborators
The work and experiments of cnidarian immunology is being conducted in collaborations with Biologists from Florida International University. Graduate students: Tanya Brown and Ellen Dow. Postdoctoral Fellow: Dr Anthony Bellantuono.

Bio
Dr Mauricio Rodriguez-Lanetty is an Associate Professor at Florida International University (FIU, Miami). After obtaining a bachelor degree in Biology in Venezuela, Dr Rodriguez-Lanetty achieved a PhD in Marine Studies from the University of Queensland (Australia) and is now the Principal Investigator of the IMAGES lab at FIU.

Contact
Dr Mauricio Rodriguez-Lanetty
Associate Professor and Director
QBIC Programme (Quantifying Biology in the Classroom)
Department of Biological Sciences
Florida International University
11200 SW 8th St; Miami, FL 33199
USA

Q&A

What first sparked your interest in corals?
Coral reefs encompass one if not the most productive and diverse ecosystems within the marine realms, housing hundreds of thousands of species from single-cell microorganisms to spectacular colourful fish vertebrates. What is incredible, is that a symbiotic association between reef-building coral hosts and photosynthetic microalgae living inside the corals powers the foundation of such ecological success. The mysteriously intricate cellular communication of these mutualistic associations in which both host and symbionts benefit from each other, caught my research interest early on during my career as scientist. Unfortunately, coral reef ecosystems are in decline because of the threats of local man-made disturbances and global climate factors such as ocean warming and acidification. Many maladies linked to these stressors such a coral bleaching and diseases are on the rise. My current motivation is to find solutions and remedies to mitigate the problems currently affecting corals.

What must be considered when using anemones as proxies for studying corals?
Coral species are very slow-grower organisms that on average, extend their calcareous skeleton a few centimetres per year, and consequently, this poses a challenge to set up well replicated experiments that can be conducted in a reasonable timeframe to study how corals function and how they respond to biotic and abiotic factors around their changing environments. Hence, a surrogate model to study corals has been in need. Over the last ten years, several of us in the field of coral biology have proposed and are actively working using the sea anemone *Exaiptasia pallida*, as a model organism to study corals. The important features considered in this selection were attributed to the fact that these anemones engage symbiotically with similar microorganisms as corals do and therefore, make them good proxies to study coral biology and ecology. Furthermore, these anemones are fast growers and easy to culture in captivity and in laboratory conditions. A great advantage of this system is that clonal populations of anemones can be raised from a dozen of individuals to a population of hundreds in a matter of a months in the lab. All these features make the anemones the right 'laboratory mouse' to study corals.

How similar are the immune responses of anemones to our own immunological priming mechanisms?
In many cellular and molecular aspects, there are considerable differences between the immune system of anemones and those from humans, however, we have also learned they share more similarities that we once considered the long evolutionary history separating anemones from us.

Anemones, and all invertebrates lack of the cellular components of the adaptive immune system, such as T cell and B cells (Lymphocytes), has led to the assumption that evolutionary early diverged invertebrates do not have the capability to develop a trained or acquired immunity. Nevertheless, our recent phenomenological findings, in concordance with results from insects, have revealed that the defence system of sea anemones display some degree of acquired immunity with a mechanism yet to be fully understood. Also, anemones and humans share many components of first line of defence, the innate immune system, composed of conserved Pathogen Recognition Receptors (PRRs) that sense and distinguish large groups of Pathogen-Associated Molecular Patterns (PAMPs) found on many infectious microbial pathogens.

Is there much crossover between the pathogens encountered by anemones and corals?
We do not know yet the degree in which natural populations of sea anemones are affected by the same pathogens causing infections on corals, and this type of research exploration is currently being undertaken. Having said that, we have shown that many identified infectious pathogens affecting coral species also cause disease sign development on *E. pallida* anemones, which adds strength to our model system for studying cnidarian immunology.

How successful has the outreach programme been so far?
The educational summer science-experience workshop (Aventura Cientifica) tailored for high school students has been very successful. We have run it twice with a total of 40 students that have already participated in the programme. We even have some students continuing their science involvement after completing the workshop either in their high schools or in University research laboratories. Our plan is to expand and enlarge the participation of students over the next three remaining years of the programme.