Investigating the Role of Lectin Proteins in Coral-Algal Symbiosis using *Aiptasia* as a Model System

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Abstract

Cnidarian-dinoflagellate interactions are a key component of the coral reef ecosystem. Corals and other cnidarians take up dinoflagellate algae from the environment to participate in a mutualistic relationship, or symbiosis. Once ingested, the algae provide their host with energy-rich compounds in exchange for protection and a source of unfixed carbon.

Genomic analysis of *Aiptasia*, a model organism for coral, reveals many proteins that are differentially expressed between symbiotic and aposymbiotic anemones. Among these are several lectins, a class of sugar-binding proteins often used in cell recognition. We hypothesize that a subset of these lectins are related to the recognition of compatible algal partners. If a secreted lectin were involved in symbiont recognition, we would anticipate selective binding towards known compatible or incompatible algae.

In this experiment, we investigated a possible method of symbiont recognition by testing the selective binding of CTL1, a secreted lectin expressed by *Aiptasia sp.,* towards different strains of *Symbiodinium*, a dinoflagellate. To test this, we mixed purified FLAG-CTL1 with fixed *Symbiodinium* cultures, then performed Immunofluorescence using Mouse Anti-FLAG as a primary antibody and Alexa Fluor-488 as a secondary antibody. We assayed lectin binding qualitatively using microscopy and quantitatively using flow cytometry. Additionally, we Western blotted the cells and supernatant to detect the presence of CTL1.

While CTL1 expressed by *Pichia* yeast did bind selectively to incompatible algal strains, the binding was very heterogeneous and species-dependent, leaving us with inconclusive data. We believe that CTL1 binding is heavily dependent on the age of the algal cells being bound – host *Symbiodinium* uptake may be limited to certain algal growth stages.

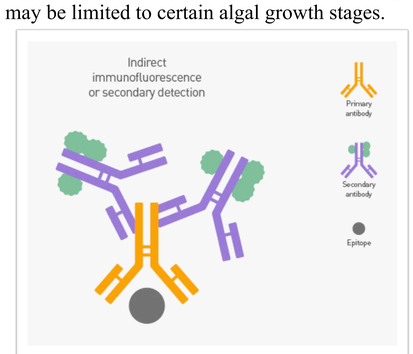

Fig. 1: Fluorescent antibodies bind onto an epitope, allowing for detection of the protein. Image credit to Thermo Fisher Scientific.

Introduction

Aiptasia sp. is a globally distributed sea anemone that hosts endosymbiotic *Symbiodinium* algae, much like its relatives, the stony corals. In contrast to corals, *Aiptasia* is well suited for laboratory culture, where it reproduces both sexually and asexually, can be maintained in an aposymbiotic (dinoflagellate-free) state, and is easily reinfected by a variety of *Symbiodinium* strains.

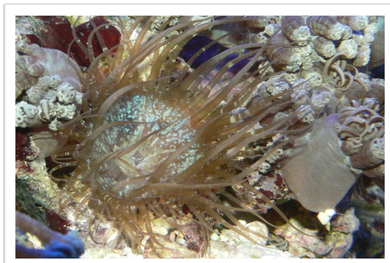

Fig. 2: An *Aiptasia* polyp.

Corals and *Aiptasia* must be able to differentiate between beneficial algae and hostile pathogens when looking for cells to ingest. This signal may be conveyed by lectins, a class of sugar-binding proteins often used in cell recognition.

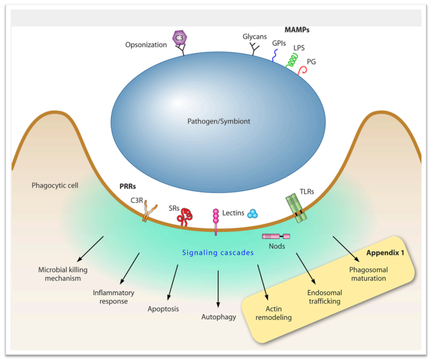

Fig. 3: Lectins on the cell surface interface with a phagocytic cell.

Hypothesis

If a lectin were involved in the detection of a compatible symbiont, we hypothesize it would selectively bind to known compatible or incompatible strains of algae.
In this experiment, we tested the selective binding of CTL1, a secreted lectin expressed by *Aiptasia sp.*, towards various symbiotically compatible and incompatible strains of *Symbiodinium*, a dinoflagellate.

Previous Work

CTL1 expressed from a bacterial vector with a GST-tag appears to show selective binding towards incompatible algal strains.
This had not been tested with a eukaryotic vector, which would more accurately replicate posttranslational modifications within *Aiptasia*.

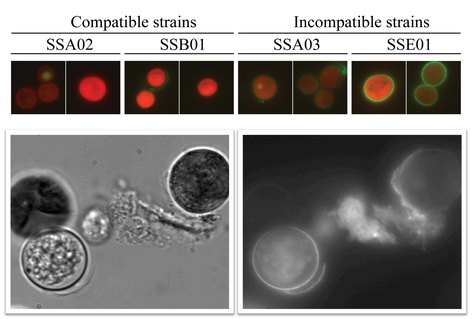

Fig. 4: CTL1 binds to SSE01, an incompatible algal strain. Note the 'rings' of CTL1 visible on the GFP image of the lighter colored cell.

Methods & Materials

Cells from SSA01 & SSE01 (incompatible algal strains) and SSA03 and SSB01 (compatible strains) were fixed in 1% PFA and immersed in media containing dilute purified FLAG-CTL1 secreted from *Pichia* yeast.
We then performed Immunofluorescence using Mouse Anti-FLAG and Alexa Fluor-488 as primary and secondary antibodies respectively. We assayed lectin binding qualitatively using microscopy and quantitatively using flow cytometry. Additionally, we Western blotted the cells and supernatant to detect the presence of CTL1.

As it became apparent that algal growth stage played a large role in CTL1 binding, cultures in different growth stages were isolated and run through the same trials.

Results

CTL1 does seem to bind strictly to incompatible algal hosts, as indicated by microscopy and flow cytometry data. The extent of this binding varies significantly, even within samples, and seems to vary directly with cell age. These measurements are complicated by cell autofluorescence within the GFP channel and unusual deviations within the flow cytometry readings.

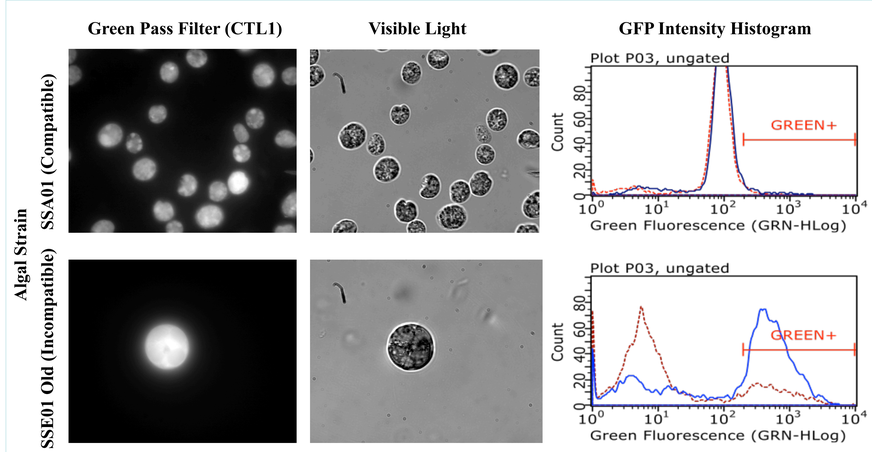

Fig. 5: Cells can be seen fluorescing in the GFP channel. While hard to see with the naked eye, clear binding differences between strains can be seen on the fluorescence intensity histogram, with control cells depicted in red and the CTL1-bound cells in blue.

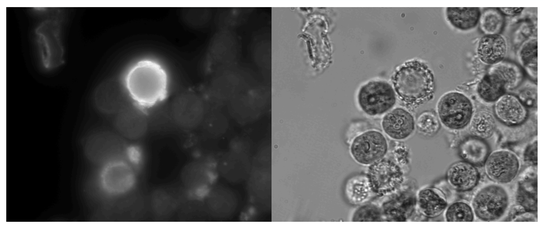

Fig. 6: A bright 'ring' of CTL1 can be seen on a SSA03 cell. Note the trend that fluorescence varies based on cell age – cells within the youngest, flagellated, life stage do not glow as much as older coccoid cells. Coccoid husks like the one shown fluoresce extremely brightly, indicative of high levels of CTL1 binding.

Conclusions

While CTL1 expressed by a eukaryotic vector did seem to bind selectively to incompatible algal strains, the binding was very heterogeneous and species-dependent, leaving us with inconclusive data.

In addition, CTL1 binding seems to depend heavily on cell stage. Cell wall composition changes as a cell ages – we conjecture that the formation of a symbiosis may be limited to certain algal growth stages, a factor not subject to much research until now. We plan to continue this line of inquiry by isolating cell samples from different life stages and testing their CTL1 affinity and compatibility as symbionts.

Acknowledgements

Lisl Esherick, for first identifying CTL1 and cloning it within a prokaryotic vector.
Sophie Cowez, for donating several algal samples.
Lorraine Ling, for being an incredible mentor.
All members of the Pringle Lab, for their help.
The Gordon and Betty Moore Foundation, for making this project possible.