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Meet the author

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See Kinoshita Y. et al. (2022) *Cytologia* 87, 17-22

What is your background and motivation for this experiment?

Currently, plants are an important key to realize a low-carbon economy and a resource recycling bioeconomy. Plants build their bodies by collecting carbon from CO2 released into the environment by photosynthesis. The plant biomass thus produced becomes an industrially usable material again. The development of technology for producing plant biomass with a high efficiency and low energy input is required. We are working on the growth physiology of algae, which have the highest growth rates among plants. There are two types of algae, unicellular microalgae and multicellular macroalgae, and microalgae with a high growth rate have been explored to optimize the economics of the biomass production process, subsequently less research effort has been prioritized to macroalgae which have relatively low growth rates. However, microalgae require much energy to be harvested from the liquid medium, and their industrial use is limited. On the other hand, macroalgae can be easily collected from liquid media in a net or similar structure. Highly efficient algal biomass production systems can be constructed if both advantages of micro- and macroalgae are utilized. From this point of view, our research theme is to elucidate a mechanism of switching between unicellular proliferation and multicellular proliferation of algae in order to improve the efficiency of algae biomass production.

Fig. 1. Young leafy multicellular plant of *Monostroma latissimum* cultured in the presence of the morphogens.

Is it possible to switch the growth mode of multicellular plants and make them proliferate in a unicellular state like microalga? In 2017, we discovered a phenomenon in which the somatic cells of the marine leafy multicellular alga

Gayralia oxysperma and the closely related species *Monostroma latissimum* proliferate exponentially like microalgae in a unicellular state. Normally, in the sea, both species grow in the form of thin blades with a single cell layer of several tens of centimeters in length (Fig. 1). It is known that morphogenesis-inducing factors (morphogens) such as thallusin produced by specific marine bacteria induce the development of the multicellularity of these algae. However, in laboratory culture with artificial seawater in the absence of morphogens both species lose their typical leafy morphology and make an amorphous aggregate of cells (Fig. 2). Previous studies have focused on the multicellularity induced by morphogens, so the cell masses that failed to develop multicellularization had been treated as "abnormal" cells and subsequently excluded from studies. I wondered what would happen if I continued to culture a small amount of the abnormal cell mass, so I added inorganic nutrients to the medium lacking morphogens and cultured it in a flask with aeration. Then, in about a week, the medium of the flask turned into a dense green suspension (Fig. 3). This is the moment when I first realized that a multicellular plant can vigorously proliferate exponentially in a unicellular state. I was surprised by their flexible growth potential.

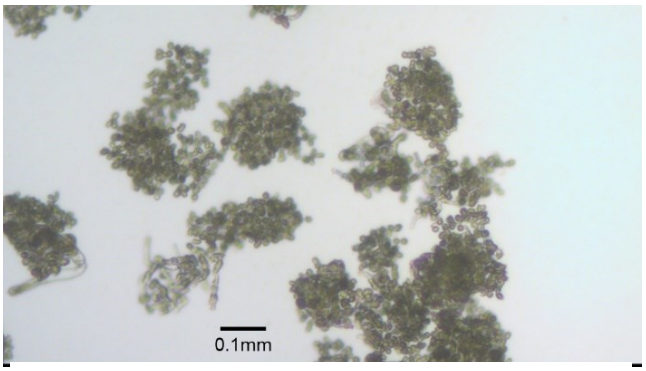

Fig. 2. Amorphous aggregates of *Gayralia oxysperma* cells cultured in the absence of the morphogens.

The present study was designed to investigate in more detail how this unicellular proliferation phenomenon occurs. In particular, we investigated the developmental potential of two forms of cells, blade cells and rhizoid cells, that are found in cell masses that have failed to become multicellular.

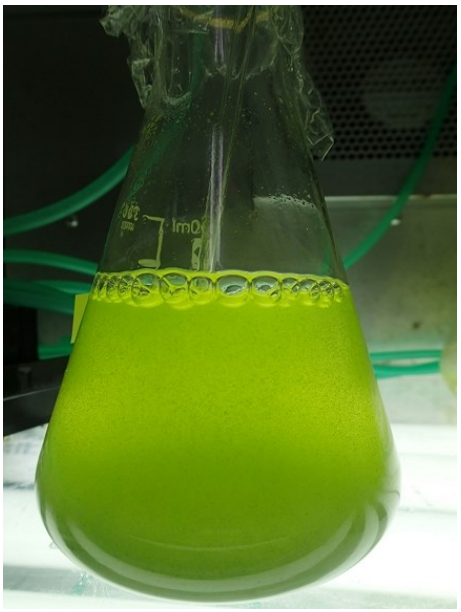

Fig. 3. Unicellular proliferation of multicellular Monostroma in 300mL flask.

What is the appeal and importance of *Gayralia oxysperma* as an experimental material?

Volvocales are well-studied as algae that model the evolution from single cell to multicellular organisms. However, it is not possible to isolate somatic cells from the multicellular bodies of the Volvocales and proliferate them as unicellular organisms like

Chlamydomonas which is a unicellular member of the Volvocales. On the other hand, *Gayralia oxysperma* is a multicellular plant that is easily induced to grow in a unicellular state by a simple method of culturing in a medium lacking morphogens. Using such a method, it is possible to isolate and examine the somatic or blade cells that have increased in this way one by one. Furthermore it is also possible to carry out comparative studies in the case of multicellular proliferation and the case of unicellular proliferation in a cloned cell population having the same genome. Such *Gayralia* and related species are expected to become new model organisms for studies on evolution, growth physiology, and developmental biology of multicellular plants.

What were your challenges or innovations in publication of this paper?

This is the first paper focusing on developmental ability of the cell masses that failed to develop multicellularization in *Gayralia*. Morphologically there are at least two types of the *Gayralia* cells. The round blade cells that make up the leaf part that occupies most of the multicellular form, and the elongated rhizoid cells that make up the small holdfast. These two types of cells were isolated from the cell mass and their developmental potential was examined. The blade cells divided to produce blade cells and/or rhizoid cells. This indicates that the blade cells retain totipotency. On the other hand, the rhizoid cells did not have ability to divide but only have the ability to elongate. Surprisingly, they continued to lengthen beyond 1 mm and some of them became a giant cell up to a maximum of 3 mm. Currently, we are investigating the limit of how far such cells will lengthen. The findings obtained in this study will contribute to the field of developmental biology of plant multicellularization, but may be industrially applicable. For example, cellulose nanofiber (CNF) is attracting attention as a next-generation industrial material for the bioeconomy, but if the giant rhizoid cells can be mass-produced, it could be used as a raw material for CNF. In addition, such millimeter-sized cells can be easily collected on a fine mesh, implying various potential bioindustrial applications.