Characterization of tentacle branching morphogenesis in the jellyfish *Cladonema pacificum*

Akiyo Fujiki[1] Ayaki Nakamoto[1] Gaku Kumano[1]

1 Research Center for Marine Biology, Asamushi, Graduate School of Life Sciences, Tohoku University

To understand how tissues are shaped is a fundamental subject in the field of developmental biology. Among many tissue shaping events in animal development, we are particularly interested in branching morphogenesis. This study focuses on branching morphogenesis of the medusa tentacles of the jellyfish, *Cladonema pacificum*. One of the synapomorphic characters in the family *Cladonematidae* is that the medusa tentacles are branched with branches having nematocyst knobs and those having adhesive organs for landing. Therefore, studying tentacle branching mechanisms of *Cladonema pacificum* and other jellyfish species would provide us a useful insight into the evolutionary process of the acquisition of a novel morphological trait. In addition, since jellyfishes are not well-studied organisms and have simple cell constitutions, studying their branching morphogenesis might reveal a novel mechanism which might not be discovered in other branching phenomena such as those in the trachea of the fly, the mammalian lungs and angiogenesis of vertebrates. In this study, through analyses of the branching process of the *Cladonema pacificum* medusa tentacles, we try to obtain new knowledge to understand basic mechanisms of branching morphogenesis. As a first step toward this goal, we described how the tentacles are branched as well as how the branches with nematocyst knobs and adhesive organs form differently.

To characterize the process of tentacle branching, we tracked the same tentacles during a period of one month starting from day 0, when small tentacles appeared on medusa buds. We distinguished individual tentacles by using the shape of radial canals as references and also by marking them with fluorescent dyes. The small tentacle appeared at day 0 extended outward and formed nematocyst knobs at day 2 or 3 when a new branch formed at the proximal region of the main (extended) tentacle. As the main tentacle extended further after day 3, additional branches formed continuously at the regions proximal to the older branch on the main tentacle. These newly-formed branches always extended inward to the center of the medusa body and gained adhesive function when their lengths became longer than approximately 300 µm. However, to our surprise, these adhesive branches were subsequently transformed to those with nematocyst knobs in the order from the oldest and the number of the adhesive branches was kept in two. That is, when a new branch formed at the proximal region of the main tentacle, the branch of the two-branch older changed its adhesive function into a stinging function with nematocysts. By day 14, three or four branches with nematocyst knobs including the main tentacle were generated. After day 14, the fate transformation continued to occur but the number of the adhesive branches kept at the proximal region became three. Taken together, we found that new branches form at the proximal region of the main tentacles and are sent toward the distal ends as the main tentacles extend. We also found that the identity of the branches changes in the process of tentacle extension and that the number of adhesive branches is kept in a few. These results are interesting from the perspective of cell fate control during branching morphogenesis.

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