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# Shedding light on the mechanism of development and evolution of organisms using the sea squirt (ascidian, tunicate)

Making the most of state-of-the-art experiment methods and techniques to promote research

In bioscientific research projects, the outcome of each project can vary largely depending on “what subject one chooses.” When he was a graduate student, Assistant Professor Kohji Hotta of the Department of Biosciences and Informatics encountered a marine organism known as the “sea squirt (ascidian, tunicate).” Ever since then, he has focused on research using sea squirts. We asked Dr. Hotta about his inexhaustible interest in the sea squirt and the latest results of his study of this organism.

**Sea squirt — an organism called a “relative of *Homo sapiens*”**

Do you happen to know a marine organism called the sea squirt? Sea squirts can be found in calm sea areas where currents are mild, such as canals and yacht harbors. Some varieties are cultivated as seafood. When sticking to craggy surfaces, sea squirts just look like plants, but they are actually an animal that has a heart and preys on plankton.

Asked about his impression of this sea animal, Dr. Hotta says, “Maybe because I have a yearning for plants.” The reason? – Whereas sea squirt larvae that have just been hatched from eggs look like tadpoles and swim around in the water wagging their tails, they lose the tail and muscles that were used to move the tail when grown up, and dare to choose a stationary lifestyle. Another feature that makes the sea squirt resemble a plant is that it is capable of producing cellulose.

“The sea squirt is an intriguing animal with a number of distinctive characteristics. For example, it may interest you to know that if you follow the sea squirt’s path of evolution, you will know that it is one of the relatives of vertebrates, including *Homo sapiens*.” In the process of forming its tail, a sea squirt larva extends its tail by making a “notochord” inside the body, which will extend the body. We human beings also have the notochord (later to be replaced by the vertebra). This is why the sea squirt can be identified biologically as a relative of *Homo sapiens*. As such, if we can find points in common between the sea squirt and *Homo sapiens* in their processes of development (from the egg to the forming of the basic body), it will shed light on the most primordial

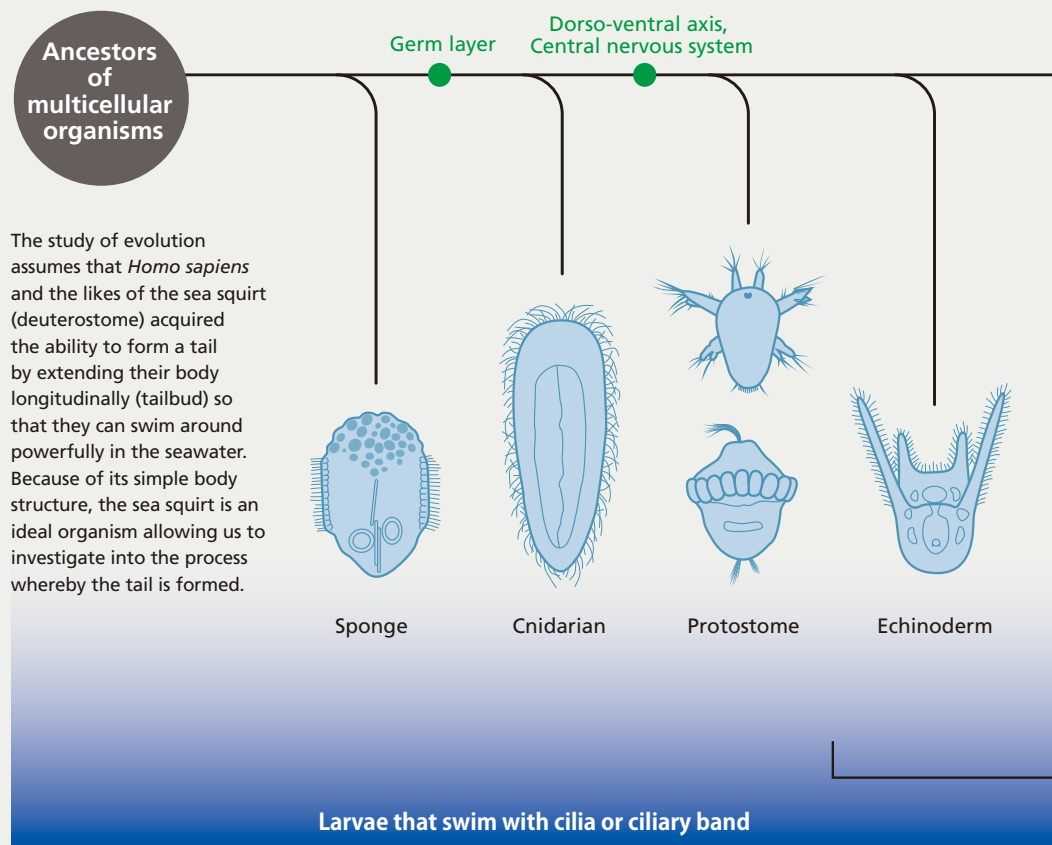
structure of organisms. This is the significance of research being undertaken into development of organisms using the sea squirt as a model. As is pointed out “the process of ontogeny repeats that of phylogeny,” the development process of a given animal looks as if the animal is following the process of its evolution. So it is safe to say that the study of development will lead to the elucidation of the evolution process.

## Why does the tail grow?

“By having a tail, the sea squirt larva acquired an overwhelming driving force

unlike what it used to be as an organism drifting with cilia. It also became possible for the sea squirt larva to move on its own will thanks to the central nervous system that developed at the same time. Why did its tail take this shape? My focus of interest now is moving to this question,” remarks Dr. Hotta. With a model of a *Coina intestinalis* larva in his hand, he explains that the tail is a complex structure made up of various organs, such as the notochord, nerves and muscles, and it can take the normal tail shape only when these component organs have grown in a good balance.

Fifteen years have passed since Dr. Hotta first took up the study of the sea squirt, and he has used the best possible experiment methods available at the time. For Dr. Hotta, merely identifying “the genes that work when the tail grows” is not the real aim of his “Study of the Tail.” But he is more eager to thoroughly shed light on every aspect of the question of “What cellular behaviors are required



for the tail to grow into the final complete shape after the genes act?” He continues, “I must introduce a physical approach, modeling techniques and so on because I need to know what forces are exerted on the tail tip and how substances flow. Naturally I need the help of other people.” So, he is working together with researchers of other universities to identify methods of measuring forces that are exerted on the tail while collaborating with computer science specialists to develop a program to simulate the tail forming process.

### How nerves are formed?

Dr. Hotta also pays special attention to the process in which nerves of the sea squirt are formed. The sea squirt’s neural tube is made of cells arranged in a sheet form, which has been transformed into a tubular shape. One end of this neural tube grows larger to become the brain and the other end becomes the spinal cord, thus forming the nervous system capable of sensing light and controlling the tail movement. By illuminating sea squirt nerve cells using fluorescence, Dr. Hotta succeeded in capturing sheet-like cells transforming into a tubular shape. He intends to proceed to more detailed research.

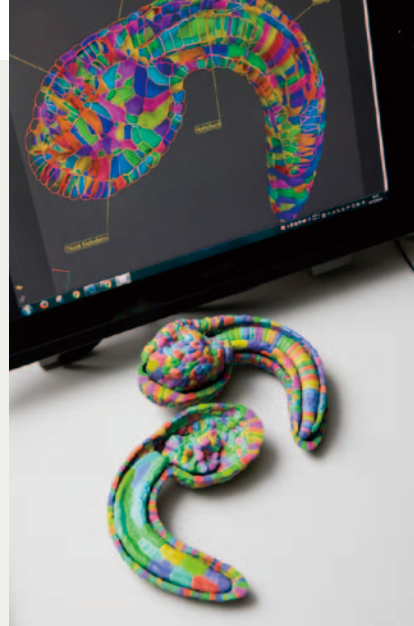
He adds, “With humans, an inborn deformity of the neural tube that cannot be closed (neural tube closure defects) is known, but its cause is not identified yet. We expect that our study will contribute much to the elucidation of

the mechanism of human neural tube defects.”

### Simple Is Best

“Many researchers unanimously say that the sea squirt is a truly marvelous organism. This is because the sea squirt larva is simplicity itself and beautiful – something consisting only of the key elements of vertebrate, eliminating all unnecessary elements. Yes, Simple Is Best when it comes to model organisms,” says Dr. Hotta. For example, a sea squirt *Ciona intestinalis* larva is only 200µm in length and very small, consisting of only 3,000 or so cells in total – small enough to be captured within the field of vision with a microscope, making it easy for us to observe. The small number of cells (3,000) also makes it easy for us to follow the behavior of cells in their developmental process. So we can shed light on how each of the organs constituting the tail grows. It is also possible to visually capture the process in which cells that number 32 in the beginning proliferate to approximately 300 by repetitive cell division. Furthermore, the small number of cells means reduced computational complexity, which allows us to simulate the tail forming process with a limited volume of observation data.

However, the sea squirt being an easy-to-observe organism does not necessarily mean that experiments are easy. “After an egg has been fertilized, we can observe the process in which the neural tube is formed in 7 to 11 hours. During three



By color-coding sea squirt’s tail bud cell by cell, we can know that it is made of a surprisingly small number of cells.

to four hours of this process, we cannot leave the microscope because we have to constantly keep the microscope in focus on the egg according to its growth.” Compared to the time required for development of other organisms, three to four hours are much shorter, but this duration is very long for us who have to keep looking into the microscope with concentration, requiring considerable patience. In addition, it is not always the case that we can obtain a desired experiment result because of the condition of the egg and various other reasons. On the other hand, the results of experiments – in the form of visuals or others – that have been obtained after such hard work are valuable and have been rated highly.

There are reasons for Dr. Hotta being able to capture good visuals. It is not only because the sea squirt is superb as a model organism, but also because Professor Kotaro Oka, who jointly operates this lab, emphasizes imaging and measuring technologies. Taking advantage of imaging technology, Dr. Hotta has created a sea squirt database known as “FABA” introducing the sea squirt’s stage of growth in 3D visuals, which is open to the public (<http://chordate.bpni.bio.keio.ac.jp/faba>). Dr. Hotta gave his name (“Hotta’s Stage”) to each stage of development – an expression of his sense of fun and attachment for his research. Incidentally, this “FABA” is now a world standard.

While studies of the sea squirt are being pursued worldwide, it seems certain that leading-edge research endeavors by Dr. Hotta and other Japanese researchers will continue to lead the world.

(Reporter & text writer : Akiko Ikeda)

