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To be an Egg or a Sperm, That is the Question — Sperm are Produced in the Ovary

Minoru TANAKA Associate Professor
Laboratory of Molecular Genetics for Reproduction,
National Institute for Basic Biology

Gene *foxl3* determines the sex of germ cells

Sexuality is present in many animal species. Various phenomena that attract us, such as the differences in morphology and actions of males and females, are complemented by the sex of the organism. The ultimate biological purpose of sex is to produce either eggs or sperm and to create offsprings, the gene combination of which differs from that of their parents.

Egg and sperm are generated from germ cells; the two cell types are generated from a single germ cell type. In other words, a germ cell must determine its destiny to develop into an egg or a sperm (sex determination). In many vertebrates including mammals, sex determination begins in a somatic cell (sustentacular cell) that is different from the germ cell. The sex-determining gene on the Y-chromosome is expressed in a sustentacular cell in the gonad. Other cells are affected by this expression and masculinized one by one, and consequently, the testis is developed. This phenomenon also occurs in medaka. It is known that a germ cell is affected by sex determination and by the differentiation of the surrounding cells for development into either an egg or a sperm (Fig. 1). However, the mechanism by which a germ cell determines the sex of an organism has not been elucidated so far. Although this is a fundamental matter in reproduction, both the mechanism of sex determination and the existence of such a mechanism in a germ cell have not been elucidated. Our recent study using medaka demonstrated that a unique mechanism exists in the germ cell and that a gene called *foxl3* functions as the genetic switch for sex determination in the germ cell. When the genetic switch was artificially triggered in our study, germ cells were masculinized even inside the ovaries (Ref. 1).

Mechanism of sex determination in germ cells — Discovery of a new molecular mechanism necessary in gametogenesis

How was *foxl3* found? The mammalian testis contains germline stem cells that developed a system to continuously supply sperm to the testis. Our laboratory revealed that germline stem cells existed in the ovary of medaka (Ref.2). However, the sex of these germline stem cells was not determined in the ovary. Transplant experiments between male and female medaka suggested that these germline stem cells were sexually undifferentiated or the sex of these germline stem cells was not fixed. When meiosis begins, sexual differences in morphology and gene expression are markedly observed in the germ cells. In other words, if sex determi- nation occurs in a germ cell, it must be somewhere between the formation of the germline stem cell to the initiation of meiosis. We thoroughly analyzed gene expression in germ cells at every differentiation stage in male and female medaka and found a difference between the two in the expression of a gene encoding for the transcription factor possessing the forkhead domain, *foxl3*, which occurs before the initiation of meiosis in germ cells. In other words, the gene expression continued only in the germ cells of female medaka (Fig. 2).

When mutant medaka, in which *foxl3* did not function, was produced using the transcription activator-like effector nuclease (TALEN) method, spermiogenesis began in female larval fish. On the basis of histological and gene expression analyses, we found that the gonad of this female mutant was an ovary. The shapes of the fins, which exhibited the secondary sex characteristics of a female, enabled us to conclude that the body s environment for internal secretion was of the female type.

When sperm were collected from the ovary of this female mutant and artificial insemination was performed using the collected sperm, eggs were fertilized, development began, and normal medaka of the next generation possessing fertility were obtained. This meant that in addition to sex determination caused by the somatic cells, a mechanism existed that independently determines sex in the germ cells. Once the genetic switch for this mechanism was turned on (masculinization), the germ cell changed to a sperm, although it is inside a female medaka. In the normal scenario, when the fish are determined to have the male sex, the expression of *foxl3* is inhibited in the germ cells following the sex determination in the somatic cells. As a result, germ cells change to develop into a male, and spermiogenesis is performed. This process was also confirmed in our study.

It was also revealed that meiosis and gametogenesis occurred normally and germline stem cells are normally established in the *foxl3* mutant. This meant that the mechanism underlying sex determination is clearly different from the molecular mechanism essential for gametogenesis in germ cells, and the molecular mechanism for determining the sex of the germ cells is a new discovery essential in creating gametes.

References

1. Nishimura *et al.*, *Science* (2015) 349, 328-331.
2. Nakamura *et al.*, *Science* (2010) 328, 1561-1563.

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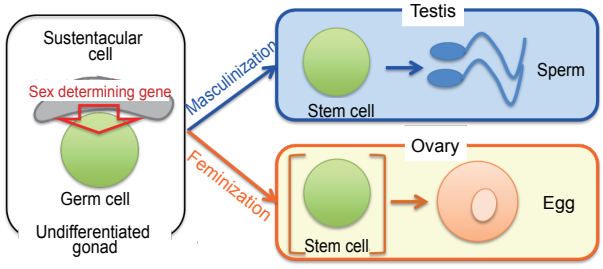

Fig. 1
The gonad consists of somatic and germ cells. It is believed that sex is determined in the somatic cells, and consequentially, the sex of germ cells is determined. In medaka, the ovary contains germline stem cells. However, no typical stem cells exist in the ovary of mammals. Therefore, an ovum is thought to be created without passing through a germline stem cell in mammals.

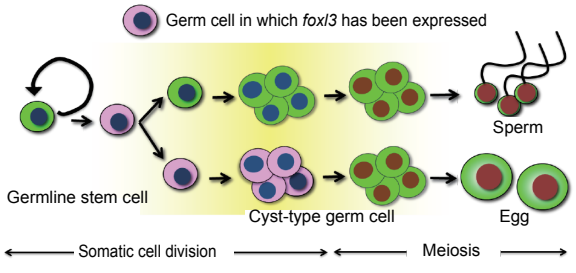

Fig. 2
The expression of *foxl3* begins in a germline stem cell. In male medaka, the expression disappears in a stem cell immediately after hatching out. In female medaka, the expression continues until the stem cell changes to a cyst-type cell. However, when meiosis begins, the expression disappears even in female medaka. Therefore, we concluded that *foxl3* is a gene that is expressed at the initial stage of gametogenesis in the germ cells of female medaka only.

Ovary and testis sharing a common functional structure

The sperm in the ovary of the *foxl3* mutant were produced in the region called the "germinal cradle" , which exhibited the function of the germline stem cell niche that is found in the ovary. This niche region exists in the extremely thin germinal epithelium on the boundary between the stroma where follicles develop and mature, and the ovarian cavity where mature eggs are released (the left part of Fig. 3 and Ref. 1). In the ovary of the *foxl3* mutant, this germinal epithelium is filled with sperm and expands, as there is no exit. The germinal epithelium exhibits a tissue image as if the ovarian stroma was filled with sperm (the right part of Fig. 3).

In general, the tissue of the ovary appears to be entirely different from that of the testis. In other words, ovarian development and testis development are thought to be separate organic differentiations. However, the "germinal cradle" found in medaka and the seminiferous tubule in the testis can be considered to possess a common structure on the basis of histomorphological and gene expression analyses, and this common structure contains sexually undifferentiated germline stem cells. In our study, we found that the sex of the germ cells was actually determined in this common structure, where the eggs and sperm were produced as well.

This fact provides a new perspective that the mature ovary and testis function as organs not only to simply control gametogenesis, but also to continuously determine the sex of germ cells. In other words, the ovary and testis determine the sex of the germline stem cells to develop into females or males, respectively.

The research on sex determination began to describe various phenomena and then advanced to elucidate molecular mechanisms underlying these phenomena. These molecular mechanisms not only determine the sex of germ cells but also secure the sexuality of the organism (if a germ cell does not become an egg, it becomes a sperm, and vice versa). Indeed, these molecular mechanisms exhibit the characteristics of sex determination.

In order to efficiently perform studies on sex determination, where transgenic individuals are produced, various genetic markers are obtained, and the sex differentiation of cells and tissues are analyzed, the use of medaka as a bioresource is indispensable.

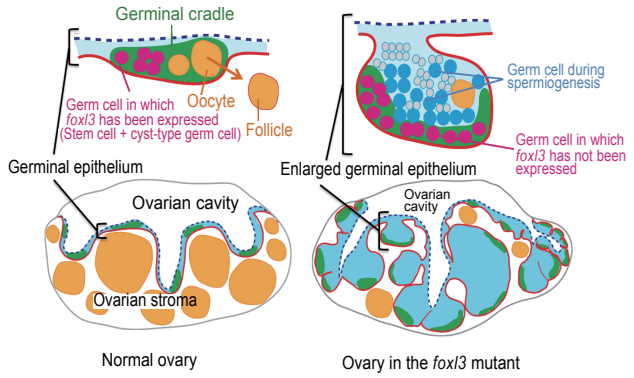

Fig. 3 Original drawing created by Toshiya Nishimura is amended.
Left drawing: Normal ovary. The "germinal cradle" structure in the germinal epithelium corresponds to the seminiferous tubule in the testis. This "germinal cradle" is the niche region where germline stem cells that express *foxl3* exist. Oogenesis begins in this region. A germ cell that has reached the meiotic prophase stage exits from the "germinal cradle" to the ovarian stroma, where it becomes a follicle.
Right drawing: Ovary of the *foxl3* mutant. Because the *foxl3* does not function, germ cells begin spermatogenesis. Sperm cannot exit in the germinal epithelium; therefore, the germinal epithelium is enlarged and begins to occupy the ovarian stroma.

Improve your productivity using Gmail features!

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Nowadays, it is difficult to live without emails, be it at your work life or private life. Productivity tends to suffer when we must constantly check our emails that are sent to us from different sources on a daily basis. In this article, I would like to recommend few features of Gmail, Google' s email service, that can help to improve your productivity.

Filters

Labels are used to organize emails in instead of folders. You can use Filters when you want to add processing rules to emails based on various criteria. To use Filters, perform the following actions:

1. Click the down arrow next to the Search box, where you can apply various criteria to filter emails.
2. Click "Create filter with this search>>" option to add an action against emails that match the search criteria.

Note: You can apply the same rule against existing matching emails, by selecting the checkbox next to "Also apply filter to matching conversations." You can change the matching criteria by clicking the Settings icon and select Settings option (Fig 1), then selecting the "Filters and Blocked Addresses" tab.

Archiving

Using the Archiving feature, you can remove emails from your inbox without deleting them. Archiving emails that are no longer required helps to focus on new and important emails. To archive an email, select the checkbox against the emails that are no longer required to be in your inbox and click the Archive button (Fig. 2).To refer to an archived email, click on the "All Mail" link from the list found on the left-hand side. You can also search for it using the search box. To archive certain kinds of emails that you receive every time, set "Skip the Inbox (Archive it)" as the action for a search criteria configured in the "Filters and Blocked Addresses."

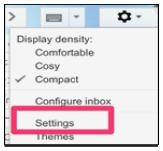

Fig. 1. Accessing Settings Option

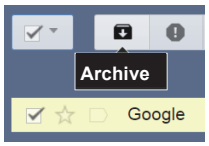

Fig. 2. Archiving

Multiple Inboxes

Have you ever wanted to view all your important emails in a separate location where you can always refer to them? In such cases, you can use the Gmail' s "Multiple Inboxes" feature. First, from the "Configure Inbox" menu item in the settings drop-down, select only the "Primary" tab to be displayed.Next, select "Labs" on the Settings page (Fig. 3-A). In the list of features, find "Multiple Inboxes" and change its setting from "Disabled" to "Enabled," then click on "Save Changes." When you go to Settings page, now you will find a new tab that has been added titled "Multiple Inboxes" (Fig. 3-B). You can enter a search keyword here and save the search result to be displayed in a separate inbox. Now you can access specific emails by a single click, while keeping an eye on your primary inbox.For example, add stars to emails first. Then, by using the search term "is:starred" for one of your multiple inboxes (Fig.3-C), you can create an inbox that lists all starred emails, even those that have been archived (Fig. 4). If you star all emails that you would like to revisit in the future, then the primary inbox can be used for accessing new emails, while another inbox will have all the starred emails. For details on how to configure search criteria, refer the link. (https://support.google.com/mail/answer/7190?hl=ja)

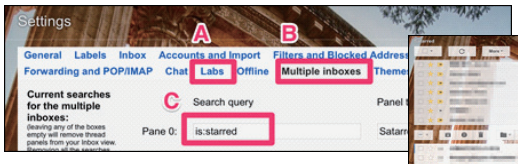

Fig. 3. Configuring Multiple

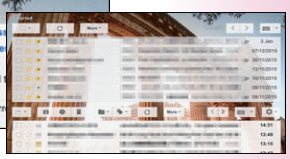

Fig. 4. Multiple Inboxes in use

I hope you have found this article useful. Gmail has many other useful features, using which you can make various customizations for a stress-free email experience.

(Haruka Kouyama,Genetic Resource Center)

Contact Address

Genetic Resource Center, National Institute of Genetics
1111Yata, Mishima-shi, Shizuoka 411-8540, Japan
Tel.: 055-981-6885 (Yamazaki)
E-mail： brnews@shigen.info

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Editor's Note

Recently, Japanese researchers have continuously published excellent research results using medaka. The laboratory of Associate Professor Tanaka, who kindly wrote this article for this month' s issue of BioResource Now! succeeded in demonstrating the existence of germline stem cells in the ovary of a vertebrate for the first time worldwide in 2010. Five years later, the laboratory successfully elucidated the molecular mechanism of determining the sex of germ cells. These are excellent outcomes indeed. I was surprised to hear that "functional sperm are produced in the ovary!" As Associate Professor Tanaka also described in the last part of his article, it is important that substantial resources exist for obtaining excellent research results.
This newsletter wraps up for the year with the best content. We will continuously make every effort to deliver various topics concerning bioresources to you. We wish you a good year ahead (Y. Y.).

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