Ohsumi Frontier Science Foundation

## Seeking the true nature of "stem cells" that support the continual sperm production

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### [About my research]

The average human male's testes produce 100 million sperm per day for more than 60 years. "Sperm stem cells" propagate to produce the numerous sperm, but some of them remain as stem cells to prevent their loss (Fig. 1). Some calculations predict that human sperm stem cells may divide more than 1,000 times during their lifetime. It is not at all an easy task to maintain a constant number of stem cells (called self-renewal) while continuing to produce sperm (called differentiation) for such a long period. We can say that the testis is an excellent factory that combines productivity, sustainability, and accuracy.

How is the balance between sperm stem cell differentiation and self-renewal maintained? This question has attracted many researchers. However, finding and characterizing sperm stem cells is not easy, as the stem cells are very rare. Based on the microscopic observations of tissue preparations conducted in the 1950s, new techniques including gene and protein expression analysis and gene knock-out using mice flourished from the end of the last century to the present century. Transplanting the sperm stem cells into the testis of other mice to retore spermatogenesis was achieved in 1994; culturing the sperm stem cells was made in 2003. It has been generally believed to be true that stem cells are tethered to a fixed place, called a "niche", and that when a stem cell divides one stem cell and one differentiated cell are always produced (Fig. 2). Indeed, it is a simple, beautiful, and reasonable concept.

Fig. 1

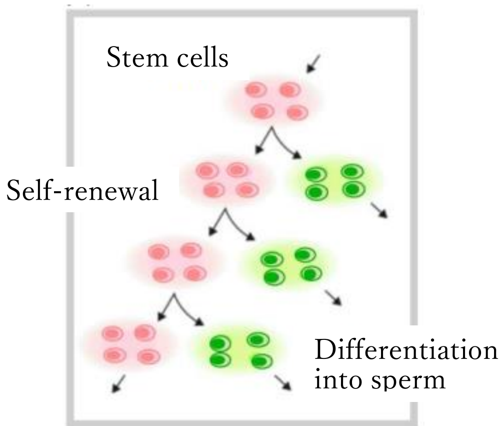
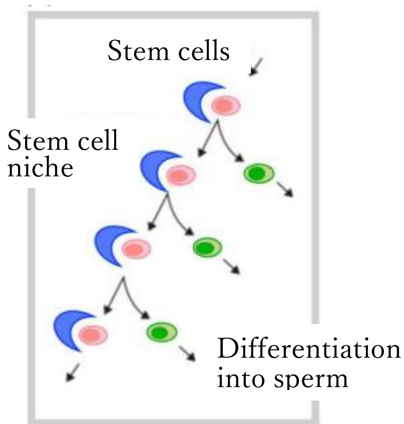



Fig. 2



However, these studies did not directly examine the stem cell behavior in the testis. Fixation (freezing time) and staining were inevitable, if you wanted to look into the testis with its normal architecture. On the other hand, to explore the functions of living stem cells, each cell had to be separated individually. This was a big dilemma in studying time-living organisms, especially multicellular ones. I thought it was essential to track the behavior of every stem cell in the testis over time, so I conducted two experiments. One was "intra-vital live imaging" – using the green fluorescent protein (GFP), for which Osamu Shimomura et al. won the Nobel Prize (2008), I was able to visualized the testicular stem cells in live. Furthermore, I succeeded in continuously observing and filming fluorescent stem cells in the live mouse testis. The other is the "pulse labeling" experiments – by marking stem cells at a certain time point, I could successfully trace the fate of their progeny.

These experiments revealed the behavior of sperm stem cells over time, which was surprisingly unrestrained. They did not stay in the same place but actively moved throughout the testis (Fig. 3). The division pattern was also inconsistent, giving rise to two stem cells, two differentiated cells, or one stem and one one differentiated cells. The fate of each stem cell was disparate and different from the authentic image of stem cells. However, as a population, the stem cells maintained a precise balance between self-renewal and differentiation (Fig. 4). In other words, while individual cells behaved randomly, collectively, they held a stable order without descending into chaos. Moreover, this system has also revealed that spermatogenesis can be flexibly regenerated when the testes are damaged.

Fig. 3

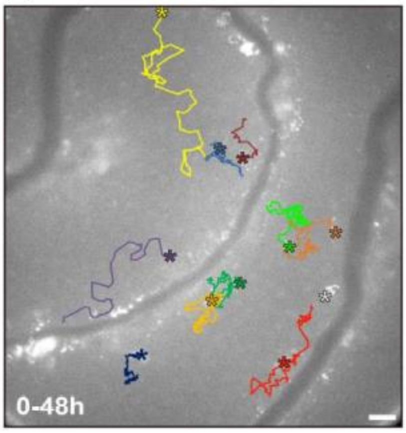



Fig. 4

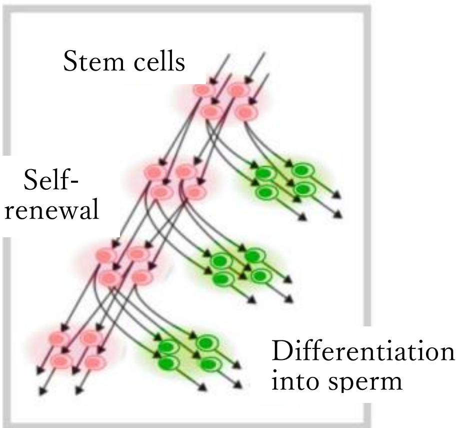



Fig. 3: An example of an intravital live imaging of sperm stem cells. The 48-hour trajectory of stem cells are Shown. The scale bar is 30 micrometers, reprinted from Cell Stem Cell (2014).

Organisms seem to be far ahead of the wisdom of researchers. Currently, I am trying to elucidate the mechanism by which "individual randomness" gives rise to "collective order and stability". This is an essential question not only for sperm stem cells but also for understanding the formation of multicellular organisms in general. Although I cannot intuitively understand the meaning of the erratic behavior of stem cells, I am fortunate to have collaborators who analyze them using mathematical statistics and biophysics. The simple behavioral principles that give rise to the complex behavior of stem cells and the molecular mechanisms responsible for this behavior are actively investigated now. I am repeatedly excited every time I see an unexpected aspect of stem cells.

Key publications: **Cell Stem Cell** 14, 658 (2014), **Science** 328, 62 (2010), **Cell Stem Cell** 7, 214 (2010), **Science** 317, 1722 (2007), **Dev Cell** 12, 195 (2007)

**[My basic science approach]**

I have continued my research according to my curiosity. I think that there are two types of researchers: those who "want to be able to do what cannot be done" and those who "want to know what is not known," and I am the latter. Living organisms are full of things that humans do not know (we know much less than what we don't, indeed), which are fascinating subjects for basic researchers. As many people say, the motivation for basic research comes down to the individual researcher's curiosity. "Basic research" is often contrasted with "applied research". However, I don't think that basic research is conducted to form the foundation for successful applied research. Rather, basic research means nothing other than "the research that genuinely pursues the mechanism of nature (or life)." This is philosophy, the "Ph" of Ph.D. meaning a doctor. In this sense, the term "basic research," which reminds us of building foundations, may not the best term. Although I cannot tell the best words, "academic research" or "pure research" are words with this in mind.

I have to confess that, when I started my research, I thought that basic research was sublime and superior than applied research. However, my thinking changed 180 degrees when I met a top-notch applied researcher who is also a respected friend of mine. He said, "If it is not useful, what is the point?" He is willing to do whatever is necessary to achieve his goal. Impressively, the results that he obtains are not only beneficial but also exceptional as basic research, and they perfectly capture the essence of living organisms. I now believe that really beneficial applied research can be made, only if you capture the essence of living organisms. Visa versa, only basic research that captures the essence of living organisms can lead to truly useful applications. Whether it is basic research or applied research, the essence of living organisms can only be discovered when a researcher is motivated to confront living organisms thoroughly.

There are many good things about being a basic researcher, but I will mention only one here. I have learned that there are always some people (though not many) in the world who find the discoveries that one person has worked so hard to make and found truly interesting to be just as interesting from the bottom of their hearts. With these people, you quickly become like longtime friends. It doesn't matter what country you are from or sometimes even what field of research you are in. It is a great pleasure to talk with such casual friends on various occasions, such as joint research projects and conferences, and I learn many things outside of research. I feel that science is a common language that connects us all.