The World of Autophagy as Seen through Yeast —The Intracellular Recycling System—

Yoshinori Ohsumi

Thank you very much for the kind introduction. I was truly surprised to receive the undeserved honor of the Kyoto Prize in Basic Sciences. I feel all the more honored and humbled to know that I am the first Japanese person to receive the prize in the field of Life Sciences since it was awarded in 1992 to the late Dr. Yasutomi Nishizuka for whom I have enormous respect.

I am essentially the kind of person who finds great delight in shaking test tubes and observing things through microscopes, and so far I have faithfully followed my interests as a researcher. At the same time, however, I feel that I myself am truly in the midst of the history of science for mankind. I often spoke to my students if I would still have been drawn to study autophagy in yeast had I lived in an inaccessible land. I believe that science is a form of social existence, and as such it cannot be separated from the times that I have lived through. People tend to believe that scientific advancement has been made by a small number of outstanding scientists, but I believe that science is an edifice of knowledge carved out by the whole of humankind throughout its long history.

Now, I would like to take some time to look back and tell you a little about myself.

I was born in 1945, six months before the Second World War came to a close, and so I have quite literally grown along with Japan in its postwar history. I was the youngest child in my family, with one brother and two sisters. Partly because of the poor food situation back in those days, I was apparently a very weak boy. My mother contracted tuberculosis soon after the end of WWII, and was bedridden for a long time when I was small; however, it was our good fortune that she was able to make a miraculous recovery after imported antibiotics became readily available. Because of this, I came to learn such names as streptomycin and PAS, or para-aminosalicylic acid, without having the slightest idea of what they were.

In my neighborhood there were many farmhouses, and I spent my childhood in the heart of remaining rich natural environment, playing among the rivers, mountains, and beaches. I was keen on insect collecting and would often look up at the starry canopy at night, feeling a strong yearning for outer space. In junior and senior high school, I think that I was a rather serious-minded student. I was a member of the chemistry club at my senior high school, and enjoyed mixing up many kinds of chemicals.

Both my father and grandfather were employed at universities, and so I had relatively few hurdles to stop me from seeking a career as a researcher in natural science. In

retrospect, my parents' expectations also had an implicit influence on me. Besides, I had no talent at all in art, literature, or sports, and so it might be more appropriate to say that I simply did not have much in the way of career options.

With that said, I believe that I have followed a very narrow path to become the person I am today, guided by various chance meetings and encounters with various people.

When I was studying to enter college, I was interested in chemistry. As I got to know this subject better after taking chemistry classes in senior high school, however, I became obsessed with the thought of taking on the challenge of a slightly younger discipline. After considerable indecision, I finally chose to enter the then newly established Department of Basic Science at the University of Tokyo's College of Arts and Sciences. It was there that I met Dr. Kazutomo Imahori, who evoked a strong desire in me to pursue a career as a researcher in molecular biology.

The years spent in my master's course coincided with the dawn of molecular biology. As a student, I was very fortunate to have witnessed the establishment of the central dogma and learn about one genetic code after another being decoded. My very first research theme was the functional analysis of ribosomes, which are the machineries of protein biosynthesis in *E. coli*. Ever since that time, the idea that protein synthesis is the very foundation of cells has been firmly entrenched in my mind.

For the doctoral course, I chose to study colicin, a protein that instantly stops protein synthesis in *E. coli*. Partly because my supervisor Dr. Akio Maeda moved there, I transferred the place of research to the then newly established Department of Biophysics at Kyoto University's Faculty of Science from the second year of my doctoral course. One year after moving there, I married Mariko, who was two years my junior at the Department, and so my short stay in Kyoto has become an unforgettable memory for me.

In the year after our marriage we were blessed with our first son. My wife quit her doctoral course and found a position at the Mitsubishi Kasei Institute of Life Sciences upon its establishment. So I decided to return to Tokyo to continue with my research under the tutelage of Dr. Imahori until I earned my degree. However, I had a difficult time finding a job, and so my wife supported the household while I searched for work and studied as a research student at the University of Tokyo's Faculty of Agriculture.

Then, toward the end of 1974, at the recommendation of Dr. Imahori I decided to study at Nobel Prize laureate Dr. Gerald M. Edelman's laboratory at The Rockefeller University in the U.S. My first time living abroad was an eye-opening and enjoyable experience for me. As far as my research went, however, I was confounded by the huge gap between *E. coli*, which I had become accustomed to dealing with, and mice. Without having had any adequate

preparation, I went through some very difficult times. I managed to establish a system for the *in vitro* fertilization of mice, but I was not certain about what to do next. And so, in my final year at Dr. Edelman's laboratory, I decided to make an about-face and work with my colleague Mike Jazwinski to study the initiation mechanism of DNA synthesis in yeast, which had been applied to pioneering work on the cell cycle. This was indeed a major change in my research theme, but it was also how I first encountered yeast.

Yeast is a tiny unicellular organism just 5 microns in diameter, but they have been used for brewing *sake* and many other types of alcohol, thus bringing joy to humanity since the days of antiquity. At the same time, the minuscule yeast cells have nearly the same set of characteristics that make up the cells of our own bodies and, as such, have made tremendous contributions to the elucidation of fundamental issues in life sciences and complex vital phenomena. Yeast is also a very attractive research material in that it offers various research advantages, such as its established genetics and the accumulation of a vast body of information on it. So fascinating is yeast that I have never grown tired of dealing with it, even after 35 years.

As the year 1977 drew to a close, Dr. Yasuhiro Anraku, who was then at the Laboratory of Botany of the University of Tokyo's Faculty of Science, kindly offered me a position as his assistant professor despite my lack of research achievements. I thus duly accepted his offer and returned home. The main research theme at Dr. Anraku's laboratory was the transport mechanism of *E. coli*. After much thought, I decided to work on vacuolar membranes which are found in yeast cells. Back in those days, this research subject was attracting very little attention.

I am not the kind of a person who likes to compete, and so I had always wanted to do something that no one else was doing. This credo of mine has remained intact to this day. It is true that research and competition are inseparable, but I simply cannot find much meaning in competing to become the first in something. Rather, I believe that the greatset joy for scientists is to initiate the development of a world that no one has ever seen before.

This is an image of yeast cells taken with an electron microscope (Fig. 1). In the cytoplasm, we can see membrane-enclosed structures called organelles. They are the nuclei, endoplasmic reticulum, and Golgi apparatus. This large structure enclosed by membrane is a vacuole, which accounts for quite a large portion of the cell. In those days, vacuoles were regarded as the garbage dumps of cells and attracted little attention. Another reason why I decided to study vacuoles was that, when I was isolating nucleus at The Rockefeller University, I noticed that the vacuoles were isolated as a pure white layer in the uppermost part of the centrifuge tube.

During a period of about ten years at the Faculty of Science, I was able to demonstrate for the first time that the vacuoles transport amino acids and ions via V-ATPase and play an important role in maintaining intracellular homeostasis. I used to cultivate several dozen liters of yeast to isolate vacuoles and analyze a small amount of refined vacuolar membranes, a persistent effort process.

Having been afforded the chance to assume a position as associate professor at the Department of Biology of the University of Tokyo's College of Arts and Sciences in 1988, I set up what I believed to be the smallest lab ever. At that time, I was already 43 years old, and I thought that it was one of the last few chances available for me to take on a new research theme. After assuming that office, I can still vividly recall telling the first department seminar class that yeast's degradation mechanism in the vacuole would become the main pillar of our research.

Now, before I move on to talk about the main subject of this lecture, which is autophagy in yeast, I would like to take some time to go over the process of intercellular protein degradation. Vital activities are fundamentally supported by the functions of a superior polymer called protein. Proteins are linear polymers in which 20 different types of amino acid are bonded together in a specific sequence that is determined by RNA into which the base sequence encoded in DNA has been transcribed. Each protein is folded into a unique three-dimensional structure and performs its own specific function. Because protein synthesis is an issue concerning gene expression, many researchers are still working on this high road of molecular biology. Meanwhile, the process of degradation was long considered passive and thus not very important, and consequently attracted little attention. However, some pioneering research had already proven that proteins are degraded after their inherent life span.

At the University of Tokyo's College of Arts and Sciences, I used to begin my lectures for freshmen by asking them to calculate how many red blood cells are produced within their bodies in just one second after providing them with some basic numbers that they could work with. A very simple math calculation will give the answer of three million cells per second. And the number of hemoglobin red proteins that carry oxygen within red blood cells produced in a single second is an astonishing 10 to the power of 15, that is, 1,000 trillion. Viewed from the outside, our bodies do not seem to change very much on a daily basis, but the amazing fact is that almost all of the proteins that constitute our bodies are completely replaced in a matter of two to three months.

In school, we have all learned about proteins as one of the essential nutrients. The proteins that we take in from our everyday consumption of food does not function as it is;

rather, it is degraded into its constituent amino acids before being absorbed into the body to become the materials for synthesizing new proteins. It is believed that we synthesize about 300 to 400 grams of proteins in a single day. Now, what this means is that the same amount of proteins are degraded, but at the same time the amino acids produced from the protein in our meals fall far short of synthesizing that amount. One can easily understand then that the amino acids produced as a result of degradation of the proteins making up our bodies are recycled to synthesize new proteins.

This phenomenon becomes even more remarkable when an individual is in a state of starvation where certain nutrient has been depleted. We sometimes come across newspaper articles about persons who got lost at sea or in the mountains and managed to survive for many days with only water. Clearly, the human body can survive by degrading its own proteins to synthesize the necessary proteins.

As organisms often do not have constant access to food in the natural world, it is reasonable to think that the acquisition of a mechanism enabling them to survive periods of hunger must have been a key step in their evolution. The degradation of proteins is thus one of the most essential functions of living organisms.

Concerning this mechanism of cells degrading their own proteins, Nobel Prize-winning scientist Christian de Duve discovered organelles that contain proteases more than half a century ago while working on animal cell fractionation and dubbed them lysosomes. A group of scientists at The Rockefeller University, among others, later used electron microscopes to reveal how cells transport their cytosolic components to lysosomes. By sheer coincidence, this year marks the fiftieth anniversary of Dr. de Duve's naming of the process as "autophagy," with "auto" meaning "self" and "phagy" meaning "to eat." While I was at The Rockefeller University, Dr. de Duve and I happened to be in the same building, but at the time I never dreamed that I would investigate the process of autophagy in the future.

However, autophagy did not assume center stage in the field of biological studies, and not much progress was made for many years. Meanwhile, another pathway of intracellular degradation—the ubiquitin-proteasome pathway—was discovered, followed by a series of findings that revealed its key role in controlling various physiological functions, which in turn led to a broader recognition of the importance of the degradation process. In contrast, the mechanism of degradation by lysosomes remained shrouded in mystery for a long time. One of the reasons, I presume, is that autophagy can only be detected by electron microscopes, which require professional skills, although it is a phenomenon that can be observed in most of our tissues and organs.

Turning back to my own story, I began thinking that I would like to elucidate the

degradation function of vacuoles. As you can see on this slide (Fig. 2), and as its name suggests, a vacuole is a membrane-bound organelle without any internal structure that maintains an internal acidic pH. Because vacuoles contain a variety of proteases, they were regarded as organelles homologous with lysosomes. Biological membranes are the cell's most basic structures which compartmentalize a cell. It may appear that using membranes to isolate dangerous proteases from the cytoplasm where vital activities occur is a wise strategy; however, when degradation progresses inside that membrane, everything boils down to the question of what is to be transported beyond the membrane barrier, and through what mechanism. That is to say, degradation within lysosomes/vacuoles is always accompanied by an alteration of membranes. This is both a fascinating and complicated phenomenon.

As such, I had no clue whatsoever as to where I should begin in finding an answer to this question.

In dealing with this question, I tried thinking about when dramatic degradation takes place in yeast. When external sources of nitrogen have been depleted, yeast induces meiosis to form four spores. I thought that this process of remodeling must necessitate a major degradation of its own constituents, and that starvation is the first key point.

Vacuoles are the only organelles in yeast that can be observed using optical microscopes, and so I was always watching yeast through a microscope. When fluorescence microscopes were in their infancy, not many researchers want to look inside yeast using an optical microscope. Looking through the microscope, one can see that each and every cell has a unique tale to tell, and I still believe in the merits of microscopic observation. Such being the case, microscopic observation provided the second key point.

At a certain stage, I came up with the idea that if I were to use cells whose vacuoles lacked proteases, during the state of starvation I would be able to see under a microscope what was being sent to vacuoles without degradation of what was being sent. Fortunately, American geneticist Elizabeth W. Jones had isolated a series of yeast mutant strains whose vacuoles were deficient in various enzymes and donated them to Yeast Genetic Stock Center. I lost no time writing a letter to request those strains, which I then transplanted into a nitrogen-free starvation culture medium.

The difference was just as clear as I had expected—even more so, in fact. I found that fast moving spherical structures gradually accumulated inside of the vacuoles. These structures that moved actively inside of yeast cells with a lack of motility were intriguing and impressive, and I spent hours watching them. I then became convinced that what I was witnessing was a hitherto unknown phenomenon. And it is this observation that determined

the future course of my research, and marked the starting point of my studies on autophagy.

Then, the superlative electron microscopic observations by Dr. Misuzu Baba subsequently made it possible to comprehend an outline of this phenomenon in a form whose beauty would be hard for anyone to deny. Thus, I was able to present a schematic depiction of autophagy in yeast more than 20 years ago. Aside from the fact that vacuoles are far larger than lysosomes, the process was the same as that of the autophagy previously known in animal cells, which demonstrated that yeast would provide a favorable autophagy model system.

The next and most important development was the application of genetics to the autophagy research, which was made possible by taking advantages that yeast system has for the researcher. In order to understand the phenomena of autophagy at the molecular level, it was deemed most efficient to locate idiovariation related to autophagy and then identify the genes involved in that process. To do this, one had only to choose autophagy-defective mutants; however, the problem was that no one knew what would become of cells if they were autophagy defective. In other words, there was no way to go about selecting autophagy-defective mutants. Such being the case, we decided to use accumulation of autophagic bodies as a reference to select defective mutants, and so we intentionally caused the mutation of cells, transplanted them one by one into the starvation culture medium, and observed the autophagic bodies. Through this process, we were able to produce the first and only autophagy-defective mutant from among more than a thousand cells, and we named it *apg1* (later, renamed to *atg1*).

I thought that a greater variety of genes must be involved, and so I began wondering if I could find more autophagy related genes in a more comprehensive way. The only *atg1* mutant that was successfully produced had the unique property that it would begin dying from the second day after being placed in a starvation condition. Under the assumption that this was attributable to defective autophagy, we first gathered many mutants that died easily under starvation conditions and picked the autophagy-defective mutants from among them. At that time, we were able to isolate some 100 mutants in one shot and identify 14 gene mutations as a result of genetic analysis. The fact that we were able to identify many genes in a comprehensive manner at the outset was critically significant to subsequent research activities. This series of genetic analysis was made possible in about one year's time thanks to the splendid work of Miki Tsukada—my first graduate student.

The next task was to isolate each *ATG* gene involved in autophagy using the mutants gained thus far, and then investigate what protein was involved.

Given the small size of my laboratory, I expected that it would take quite some time to

deal with 15 such genes; however, thanks to the generous cooperation of Dr. Mariko Ohsumi (my wife) and the members of her laboratory at a private university, and also the achievement of sequencing of the entire yeast genome, we were able to identify almost all of the *ATG* genes in an unexpectedly short period of time. Nevertheless, because all of the *ATG* genes thus identified were new, we were unable to infer their functions from their possible amino acid sequences, which forced us to grope blindly in the dark for some time.

Then, a turning point came in 1996 when I was offered a faculty position at the National Institute for Basic Biology in Okazaki City, Aichi Prefecture. I was already 51 at the time and, if it were now, when younger researchers are preferentially given posts as professors, it would have been quite impossible. The National Institute for Basic Biology offers the best environment imaginable for conducting basic research. Because I was privileged to have my research staff there, I decided to pursue parallel research in animal autophagy—the leading area for such research—and invited Dr. Tamotsu Yoshimori to serve as an associate professor and Dr. Takeshi Noda and Dr. Yoshiaki Kamada as assistant professors. The following year saw the addition of Dr. Noboru Mizushima as a postdoctoral researcher, and soon other postdoctoral researchers and graduate students began to assemble from across the country, making ours a large and vibrant laboratory. Some graduate students then began research into the autophagy of higher plants. While taking advantage of the superior facilities of the Institute, everyone at my laboratory came together as one to form a team unparalleled in the world, with its members busy working on yeast, animals, and plants under the keyword of autophagy.

As is usually the case with any research work, ours did not progress in a linear pattern, and one striking discovery after another was made in just the first few years that followed. Unfortunately, I do not have time to give you a detailed account, but it was a very fruitful period when the functions of *ATG* genes were elucidated in a short period. In retrospect, it was also a highly exciting time when we were able to see at long last the real substances of proteins coded by *ATG* genes. These research findings brought autophagy research into the spotlight practically overnight and, as the number of related papers shown in this graph illustrates (Fig. 3), autophagy research achieved explosive development.

Based on these outcomes, Dr. Yoshimori and Dr. Mizushima went on to commence analyzing the *ATG* genes of higher animals. We were also leading the world in the analysis of Arabidopsis *ATG* genes. The fact that *ATG* genes gained from yeast are almost completely preserved in higher animals and plants indicates that autophagy is a fundamental mechanism acquired during the initial emergence stage of eukaryotic cells.

Mice whose entire bodies expressed protein that fuses fluorescent protein with LC3,

which is a protein homolog of yeast's Atg8, played a determining role in visualizing autophagy, while Atg5 knockout mice were an important key in investigating autophagy-defective individuals. Such mice were distributed to laboratories throughout the world, leading to considerable contributions to their researches.

As I have been discussing, the process of identifying autophagy related genes, beginning with yeast, brought about a complete change in the quality of autophagy research. This is because technological progress during that period made it possible to manipulate genes in higher animals and plants freely, which in turn enabled analysis of the physiological roles of autophagy in cells and individual organisms from a diverse range of species. Researchers around the world began studying autophagy, and gradually discovered its relation to various pathological conditions through the roles of autophagy including elimination of proteins and their aggregates that are harmful to cells, qualitative and quantitative control of mitochondria and other organelles, protection against bacterial infection, embryogenesis, and antigen presentation, in addition to the role of simply overcoming starvation. Recent years have seen a number of reports on its relation to such conditions, including cancer and neurodegenerative diseases like Alzheimer's disease. These are just some example cases where achievements by our laboratory led to the explosive spread of the autophagy research on a global scale. Because autophagy is the most fundamental function of any cell, I am sure that its relation to various physiological functions will become increasingly clear. Having said that, autophagy is a new research field still in its infancy, and so much more experimentation needs to be conducted before we will be able to understand its extremely diverse physiological functions. Perhaps what we are doing today could be likened to describing a huge elephant while being only able to touch various parts of its body with our eyes closed.

In 2009, I assumed the post of professor at the Tokyo Institute of Technology. My laboratory has consistently dealt with autophagy in yeast and made constant efforts to reveal the functions of Atg proteins with a focus on their molecular mechanisms, namely, the system by which membranes known as autophagosomes surround and isolate parts of the cytoplasm. I will avoid going into further detail here, as things would get rather technical. At any rate, having been involved in the research of yeast autophagy for a quarter of a century, I think that it is safe to say that I have learned a great deal, but at the same time I am keenly aware that many fundamental mysteries still remain.

That said, I feel very fortunate to have been involved in such an extensive research subject.

Before I finish, I would like to share some of my thoughts based on my personal history

that I described to you earlier. During this time of the year, one of the biggest attractions of Kyoto is its crimson autumn foliage. For the coming winter, proteins such as green color chlorophyll needed for photosynthesis are degraded into amino acids to be collected from the leaves into the trunk before the leaves fall. Accessory pigments which are not degraded and left in the leaves create vivid colors. This is what we see as beautiful autumn color of leaves. Plants are thus degrading their proteins to prepare for the following spring. As I have mentioned, a marvelous recycling system, of which autophagy is a part, has been created in cells. Organisms never waste precious resources without good reason, and degradation is a process essential for the creation of new life.

Turning to the present state of humankind, I have become strongly aware of the difficult problems that we are now faced with. The hard facts in the wake of the earthquake, tsunami, and nuclear accident that struck East Japan brought me to the painful realization that the foundations of our livelihood are not as firm as we might like to think. Planet Earth has an enormous circulatory system of matter. Organisms form a stable ecosystem as they closely interrelated with each other in the food chain. We have long believed that this planet is highly resilient.

When I think of human society, up until several decades ago when I was a small boy, people used to live in harmony with the natural surroundings in their respective regions. Recently when I studied again the history of Japan during the Edo period, I was profoundly surprised to know that people in Edo, or present-day Tokyo, had formed a wonderful eco-friendly society some 200 or 300 years ago when it was already one of the largest cities in the world. It left a strong impression on me to know that our ancestors in this country were living a spiritually affluent lifestyle and achieving an ecological balance between production and consumption. Turning to our present situation, the reality is that per capita energy consumption in this country has risen to four tons of oil per year as the country has taken its place among the ranks of economic superpowers.

After the Industrial Revolution, progress in science and technology dramatically changed our lifestyles, making it possible for humans to create all sorts of artificial materials, including electronics and plastics. One prime example of this is nuclear power. So long as we engage in various kinds of production, we must always be aware of the efficient use and appropriate processing of resources. I believe there is something that we can learn from other organisms when we consider how we can live without placing an undue burden on the natural environment in order to ensure our survival into the distant future.

Allow me to finish my lecture by sharing my thoughts on basic research. I suspect that one of the reasons why degradation studies did not advance as smoothly as synthesis studies

was the vague assumption of many that, compared to synthesis, degradation is a passive process and thus is not involved in any important controlling function. When I began my autophagy studies, other researchers often asked me what autophagy was, and so I had to provide a lot of explanations. As mentioned earlier, I would venture to say that, whenever some research subject is "in fashion," scientists should leave the foregoers in that subject to pursue their studies, because whatever is in fashion today may not be so tomorrow. I hope that my words reach the ears of scientists in the younger generations. The important thing is, I believe, to continue doing whatever you find truly interesting, and to have a system that makes this possible.

Science is a means of realizing people's "desire to understand things." I think that it truly does not matter what motivates people to become involved in science. The simple desire to be of service to someone or something can be one of the common motives, but it would be very risky if the course of research were to be determined with excessive emphasis on creating something of instant service from a shortsighted perspective. In present-day Japan, and the whole world for that matter, there is a growing tendency to seek greater efficiency. Out of a wish to establish themselves as professional researchers, young scientists tend to do whatever is popular at the moment, with the result that everyone ends up being cast in the same mold. However, I am sure that there are still some who consider things logically and deeply, and others who can discover something just by making close observations of nature. I believe in the existence of a world that can only be seen by those who have taken a roundabout path. The question that we are being required to answer now is, "How can we create an environment where young students of basic science can develop properly?"

Take the progress of autophagy research as an example. It was only just recently that we began to see the possibility of it being of service in overcoming pathological conditions and maintaining good health. This in itself is a very pleasing thing to me, but I did not have any conviction that my research would lead to the elucidation of diseases when I first began. A single discovery can bring about dramatic progress in research, and I must say that the pace of such progress is very rapid today.

I think that it will be highly beneficial for the future of humankind if everyone in our society has a certain amount of mental "breathing room" and intellectual curiosity, not being spurred on only by immediate economic benefits.

I have been involved in autophagy research for 25 years and have encountered many obstacles and dramatic situations along the way. Modern biologists are required to have a good command of various research techniques, and no single person can hope to do

everything by him or herself. My research would not have made such great progress without the daily dedication of more than 80 staff members, postdoctoral researchers, graduate students, and technicians with whom I have had the pleasure of working over the past quarter of a century. I would like to take this opportunity to express my sincere gratitude to those many talented fellow researchers, for whom I am often envied, and I would like to share this honor with them.

I would also like to express my heartfelt appreciation to my supervisors, senior researchers, the University of Tokyo, the National Institute for Basic Biology, and the Tokyo Institute of Technology for their support of my research, and the Ministry of Education, Culture, Sports, Science and Technology for funding my scientific research.

I would like to thank my parents, siblings, and sons for their continued support. I am particularly grateful to my wife Mariko, who, despite my self-centered lifestyle as a researcher, has been devotedly taking care of our family while working at the same time.

Last but not least, I offer my deepest appreciation to the nominators and members of the Kyoto Prize selection organization, staff members of the Inamori Foundation, and President Kazuo Inamori for providing me with this superb opportunity to speak to such a wonderful audience. Thank you very much.

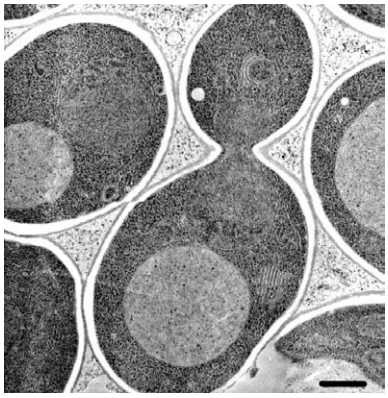

Fig. 1

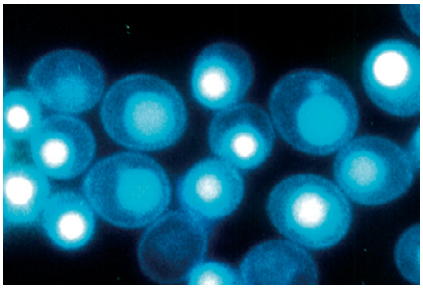

Fig. 2

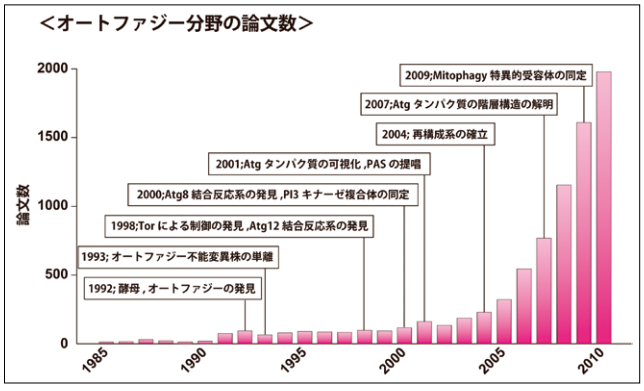

Fig. 3