Artificial Induction of Organelle Formation in a Living Cell

Shouhei KOBAYASHI

Artificial devices, such as a semiconductor chip, become downsized and highly-integrated from year to year, and the size of such device will reach to the micrometer-order in the near future, which is comparable to the size of a cell. Thus we assume a cell, the minimum functional unit of a life, as a new target for ICT research. Here we introduce methodology to introduce artificial materials into living cells and to investigate cellular responses such as organelle formation around the materials by microscopy.

1 Introduction

In preparation for the coming advanced information society, great expectations are being held for the development of not only optical and electronic devices that enable rapid information processing, but also bio-inspired ICT that complements or even outperforms such devices[1]. In the near future, advances will be made in the miniaturization and high integration of semiconductor chips and other artificial devices, and they are expected to perform satisfactorily at sizes in the range of micrometers. Given this situation, it is thought that ICT research into cells, the smallest functional unit of life (ranging in size from several μm up to around 100 μm), is set to rapidly become top priority. The importance of research fusing healthcare and ICT, stepping beyond the framework of traditional ICT, has actually been advocated in Japan since 2011[2].

To stay one step ahead of this trend, the Cell Biology Group of the Bio ICT Laboratory has so far developed methods of artificially assembling organelles by introducing artificial materials with a variety of properties into living cells, the smallest functional unit of life, to examine how they respond (Fig. 1a)[3]. Gaining free control over the formation of organelles, which govern cell functions, by implanting artificial materials into cells will enable controlling the communication of information within or between cells (Fig. 1b). It will also be useful in developing tools to control cell functions. Instead of simply making use of the properties of artificial materials introduced into cells, if we are able to make use of characteristics unique to the cells themselves (e.g., autonomous ability for self-organization) to render special functions to the artificial materials in the intracellular environment, it will become an extremely powerful tool in cell ICT research.

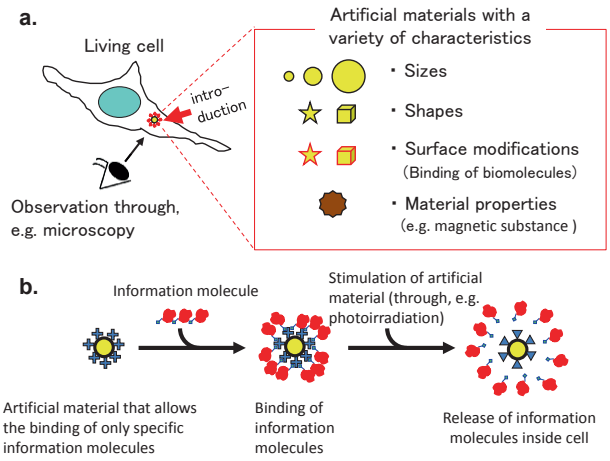

Fig. 1 Introducing artificial material into a cell and controlling cell functions
a. Introducing artificial material into a cell and analyzing its response
b. Image of controlling intracellular information using artificial material

2 Manipulating the formation of organelles using artificial materials

Developing a method of controlling cellular functions using artificial materials introduced into living cells is a search for rules on the conditions necessary for communication between cells and artificial materials, or in other words how cells recognize what characteristics of artificial materials as "information." As for how artificial materials can be introduced into cells, a lot of research has already been carried out so far in the field of drug delivery. However, many such materials were designed for the

purpose of delivering drugs to diseased areas, and their size was limited to around 50 to 200 nm. This is smaller than the spatial resolution of normal fluorescence microscopes, not allowing confirmation of what happens to introduced materials within the cells. It is also difficult to introduce artificial materials with diameters of over 1 μm into cells, with the exception of phagocytes (cells that specialize in engulfing and digesting materials, such as macrophages). This meant that very little was known about how cells respond to such materials.

Thus, we have studied about the rules on how cells recognize artificial materials by introducing a variety of artificial materials of different sizes and surface properties into non-phagocytic HeLa cells (a type of cultured human cell), and analyzing their responses (Fig. 1a). Below we will report on the method of introducing polystyrene beads (artificial beads) of over 1 μm in diameter into cells. We will also report on our results obtained so far, and future developments, with a focus on the formation of special organelles around the artificial beads. For more details of each experiment, refer to the original paper[4] and explanatory article[5].

2.1 Method of introducing artificial beads to living cells

In general, there are two main methods of introducing artificial materials into living cells. One is the physical method of making a hole in the cell membrane to directly inject the materials into the cytosol, such as microinjection or electroporation. The other is the chemical method of inducing cells to take up the materials making use of the cell's natural mechanism (endocytosis), by coating the surface of the materials with a special lipid, as represented by the liposome method. These methods have their advantages and disadvantages, but we used the chemical method because it does not require any special equipment, it can be duplicated easily, and there are few limits on the size of materials that can be introduced. Specifically, we coated the surface of beads with a transfection reagent (a reagent used widely for introducing DNA to cells) that contains a cationic lipid, which we sprinkled onto cells cultivated on a culture dish. The beads were then taken up by the cells through endocytosis. At this stage, the artificial beads exist in a compartment circumscribed by a lipid membrane (a membrane formed by invagination of the cell membrane), known as the endosome as shown in Fig. 2. The efficiency of beads uptake by cells is dependent on the combination of the cells, beads and transfection reagents used. By using this method we have so far succeeded in demonstrating that artificial beads of up to 3 μm can be introduced efficiently even into non-phagocytic cells, to which substances of over 1 μm were formerly considered difficult to introduce[4].

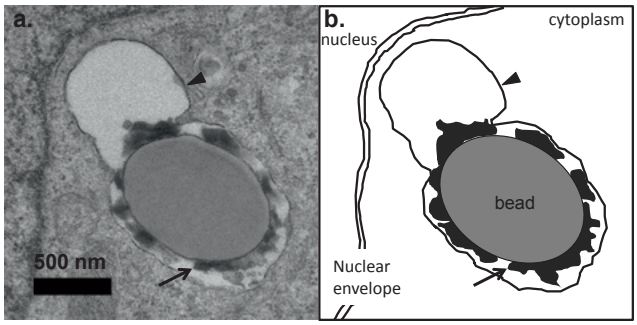

Fig. 2 (a): Electron micrographs of a bead that has been taken up by a cultured human cell through endocytosis
(b): A schematic diagram of the image
The substance with high electron density around the bead (arrow) is a lipid used to introduce the bead into the cell. The arrowhead is pointing to the endosome membrane.

Artificial materials introduced into cells in this way undergo different processes depending on their properties. In the next section we will discuss autophagy, the field our research is most advanced in.

2.2 The breakdown system of cells: Autophagy

Autophagy[6] is one of the degradation systems in cells. Cells break down cellular factors that they no longer have any use for through autophagy, in order to recycle necessary substances and maintain homeostasis, or respond to external environmental changes (e.g., nutrient starvation). Figure 3 shows an outline of autophagy. First, a special membrane structure known as an isolation membrane forms within the cytoplasm in response to a stimulus such as nutrient starvation. Next, this membrane becomes extended until it envelops the targeted area to form a double-membrane structure known as the autophagosome (regarded as a type of cell organelle). In the

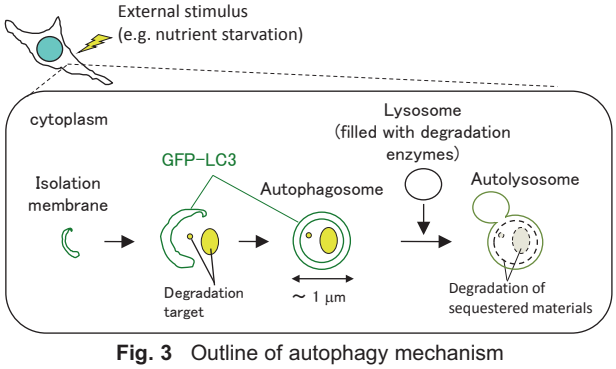

Fig. 3 Outline of autophagy mechanism

end the substance isolated by the autophagosome and contained within its inner membrane is broken down through the fusion between the outer membrane of the autophagosome and lysosome (autolysosome), a vesicle containing a hydrolytic enzyme. In this way, autophagy is a special mechanism that induces a particular function (degradation in this instance) by formation of a new organelle (autophagosome in this instance). However, it was not possible to predict when and where autophagy would begin using the former method of triggering autophagy through, e.g., nutrient starvation. This meant that it was difficult to carry out a detailed analysis of the series of processes from the formation of a single autophagosome to its fusion with a lysosome.

2.3 Triggering autophagy using artificial beads

We used HeLa cells stably expressing GFP-LC3[7], a protein that is a fusion of LC3, an autophagosome membrane-specific protein, and green fluorescent protein (GFP), to analyze the formation of autophagosomes by artificial beads[4] (Fig. 4). Using artificial beads enables observations to be limited to the area around the beads, allowing detailed analyses of the accumulation of GFP-LC3 signals (formation of autophagosomes). First, beads coated with a commercially available transfection reagent (such as Effectene) that contains a cationic lipid are loaded onto HeLa cells. The beads are taken up by the cells via endocytosis (Fig. 4). At first, they are entrapped in the endosome membrane. At this stage the inside of the endosome is maintained in an acidic state (with a pH of around 5 to 6). This means that pH indicating fluorescence marker pre-bound onto the surface of the beads, which emits red fluorescence only in an acidic environment, will enable ascertainment of which beads are inside the acidic endosome from their color (Fig. 4a). Next we observed the time-lapse fluorescence of beads taken up by living cells, as shown. We observed the significant concentration of GFP-LC3 signals around the beads over time (Fig 4b). Furthermore, live imaging-associated correlative light and electron microscopy; live CLEM[8] revealed that the membrane structures typical of autophagy (autophagosomes) were formed at the area corresponding to GFP-LC3 fluorescence around the beads. Moreover, the results of detailed analyses showed that the formation of autophagosomes around beads begins approximately 5 minutes after the loss of pH-indicating marker fluorescence (indicating that the endosome membrane around the beads was broken down and the beads were exposed to the cytosol with neutral pH).

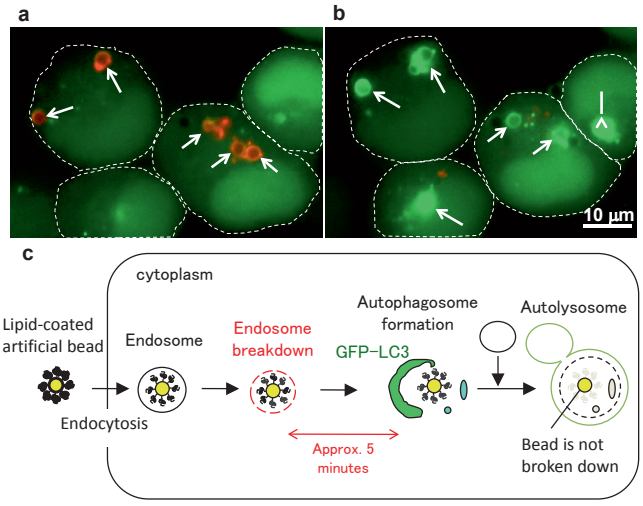

Fig. 4 Autophagy induced around the artificial bead

a. Taken approximately an hour after introduction of the beads (arrows). The green parts indicate GFP-LC3, a marker of autophagy. Red parts show fluorescence from pH-responsive dye (pHrodo) bound to the surface of the beads. pHrodo fluoresces a bright red color only in a strongly acidic environment. It is acidic inside the endosome, while the cytosol and culture medium are neutral. The fluorescence of pHrodo allows ascertainment of whether the beads are inside or outside the endosome.

b. Photo of cells taken approximately 30 minutes after a. Autophagy was induced around the beads escaped from the endosome (they have no red fluorescence).

c. Outline of how autophagy is induced around artificial beads. Important points to take note of are that endosome breakdown occurs before formation of the autophagosome, and autophagosome formation occurs only around the beads.

By introducing artificial beads into cells in this way, we succeeded in triggering the temporally and spatially limited formation of specific organelles (autophagosomes) around beads (Fig. 4c). One point worthy of note is the fact that using artificial beads has enabled the detailed analysis of when artificial materials taken up by cells through endocytosis escape from the endosome to be released into the cytosol, and what happens to them after that. In the future, we hope to clarify the characteristics of beads required to avoid autophagy by carrying out the same experiment upon altering the size of beads, and the type and quantity of biological molecules bound to their surface.

3 Conclusions

In this paper we reported on the state of fundamental cell ICT research into "what happens when cells come into contact with foreign artificial materials." Mainly two different approaches are needed to further build on this research in the future. One is the cell biology approach to gain a deeper understanding of cells. The autophagosome, which we successfully triggered the artificial formation of

this time, is related to the breakdown mechanism of cells. If we could discover a way of avoiding this mechanism, we could maintain artificial materials within living cells in a stable state without them being broken down. Moreover, if we could artificially create organelles with more complicated functions, such as a nucleus, by using other materials than those we used this time, we would not only be able to clarify the structure of that organelle, but we would also be able to apply the knowledge to artificial manipulation of cell functions and monitoring of the state of the cell. The second approach is to advance ways of measuring cellular responses using artificial materials. For example the scope of research could be expanded greatly by using artificial functional materials such as hollow beads that can selectively retain a variety of substances, magnetic substances whose position can be controlled by a magnetic field, and biodegradable materials that are broken down over time. This approach also includes controlling the timing of endosome breakdown around the beads taken up by endocytosis, or improving in the temporal and spatial resolving power of microscopes as the result of advances in correlative light and electron microscopy. Research involving not only biology, but also fusing it with other fields such as nanotechnology, organic and inorganic synthetic chemistry will become vital in achieving all of the above.

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Shouhei KOBAYASHI, Ph.D.

Senior Researcher, Bio ICT Laboratory,
Advanced ICT Research Institute
Cell Biology
skobayashi@nict.go.jp