The 2016 Nobel Prize in Physiology or Medicine

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The Nobel Prize in Physiology or Medicine was awarded to a Japanese scientist for the second year in a row for 2016. The laureate is Professor Yoshinori Osumi of the Tokyo Institute of Technology who received the award for the discovery of autophagy[1],[2]. Generally speaking, knowledge of autophagy is limited to researchers in biology related fields, therefore the achievement of Professor Osumi may not be understood immediately as compared to the work by Professor Satoshi Omura discovering ivermectin which prevents blindness in over 300,000 people every year[2].

The first observation of autophagy by Dr. Osumi occurred when budding yeast lacking a degradation enzyme contained in the vacuole were cultured in a nitrogen-free starvation medium[3]. The key was successful observation of cytoplasmic components which are normally decomposed by vacuolar enzymes with a visible light microscope. In this genetically defected yeast, autophagy was activated in the starvation state, but recycling was suppressed because there was no enzymes for decomposing cellular waste proteins.

"Auto" in autophagy means "self" in Greek and "phagy" means "eating." Therefore, the literal meaning of autophagy is "self-eating." Some people may think of the example of self-eating when an octopus eats its tentacles under stress. However, autophagy, which occurs in cells, is a function strongly conserved through all eukaryotes ranging from yeast to mammals[4]. As it is evidenced by the development and maintenance of eukaryotic organisms, autophagy is an essential metabolic function for maintaining life not only for surviving starvation. From the mechanism of autophagy which will be described later, we can observe autophagy is a metabolic mechanism only possible in a eukaryotic cell having organelles surrounded by lipid double bilayer.

Generally, it is known that the amount of protein that humans intake in a day is about 1 g per 1 kg of body weight. For example, a person weighing 60 kg intakes 60 g of protein per day. However, the amount of protein required daily is about for this same person is 200 g. Where does the remaining 140 g come from? The protein deficit is synthesized internally from the amino acids derived from proteins degraded by autophagy are the source for this synthesis. Autophagy is a mechanism to recycle mainly proteins but it can also be viewed as a mechanism for cleaning and reusing unnecessary proteins such as degraded, mistranslated and partially translated proteins. Although it is not a problem for cells having a short lifespan of several days such as small intestinal epithelial cells, cleaning of waste products is a very critical mechanism for cells having a long lifespan such as cranial nerves. Autophagy is an unnecessary function in prokaryotes having a short life cycle.

Cell differentiation is the process by which each organ is formed in embryogenesis of multicellular organisms. Autophagy also plays an important role in this differentiation. In order for the cells to differentiate and to fulfill new functions, it is necessary to perform substantial remodeling relevant to the environment. With further in autophagy research, it may be possible to develop drugs that control the recurrence of cancer caused by differentiation from resistant cancer stem cells.

Endocytosis is a mechanism to capture and degrade substances outside the cell while autophagy is a mechanism for degrading intracellular proteins and organs. There are mainly three types of autophagy: microautophagy, macroautophagy and chaperone-mediated autophagy. In microautophagy, a lysosome directly engulfs cytoplasm, and degrades and decomposes proteins. In chaperone-mediated autophagy, the protein recognized by the chaperone is transported through the lysosomal membrane and hydrolyzed. Of the three types of autophagy, macroautophagy is the one most widely studied, therefore "autophagy" usually refers to macroautophagy. Macroautophagy was the subject of the 2016 Nobel Prize in Physiology and Medicine. Figure 1 is a schematic diagram of the mechanism of autophagy[5]. In the first stage, the substances and organs to be decomposed are surrounded by lipid double bilayer membrane. This organelle is called the autophagosome. In the second step, the autophagosome fuses with lysosomes, and is called the autolysosome. Lysosomes contain various degrading enzymes such as proteolytic enzymes, where proteins are decomposed into amino acids, sugars into monosaccharides, and nucleic acids into nucleosides. The product of degradation is released to the cytoplasm by the transporter present in the autolysosomal membrane and reused.

Over 40 types of proteins have been identified as factors responsible for autophagy[6]. These proteins are named as Atg proteins taking three letters from "Autophagy." The major analytical tools for the study of autophagy are optical microscopes, fluorescence microscopes and electron microscopes. However, elucidation of the detailed mechanism at the molecular/atomic level requires a high resolution

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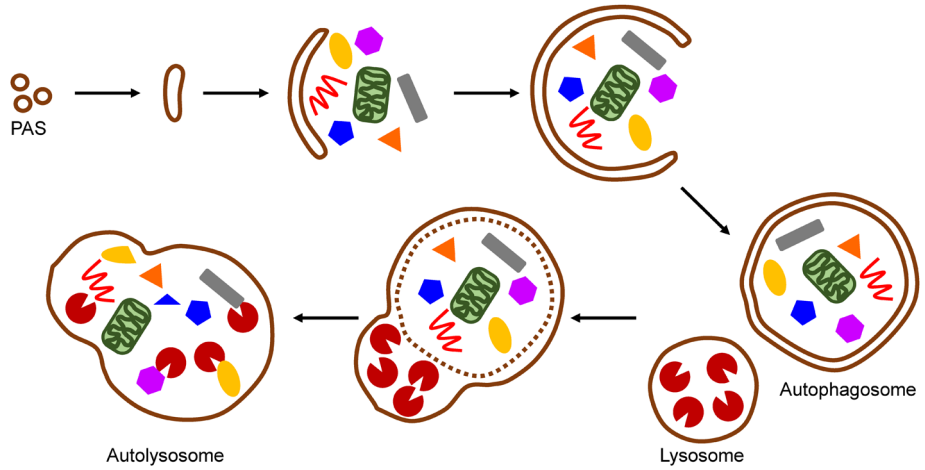

Fig. 1. Schematic diagram of autophagy. Autophagosome fuses with the lysosome containing various enzymes digesting contents in cytosol such as protein captured by autophagosome. Amino acids formed by hydrolysis of old proteins are recycled to produce new proteins.

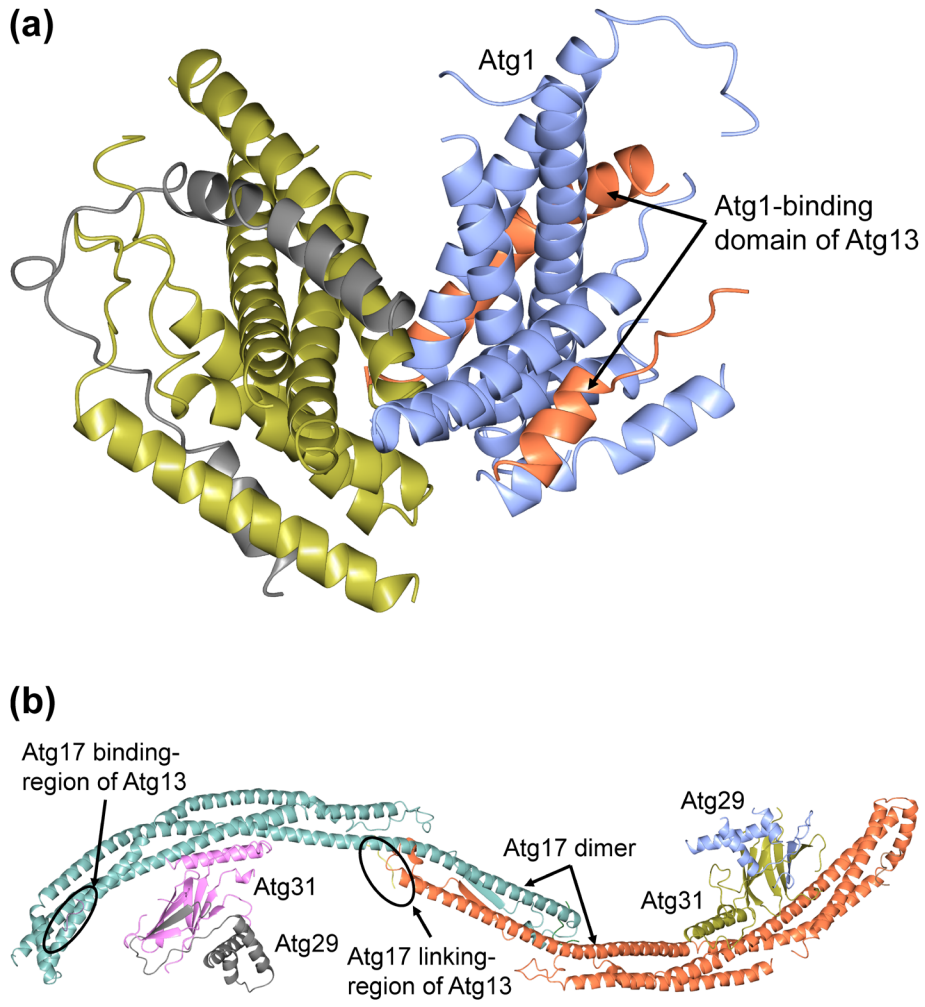

Fig. 2. (a) Structure of Atg1−Atg13 complex. (b) Structure of Atg−13−Atg17−Atg29−Atg31 complex. In both cases, X-ray structure analysis elucidated detailed interactions among Atg proteins at atomic level.

structure determined by single crystal X-ray structure analysis. From this point of view, comprehensive structure determination of Atg proteins having various functions and relevant shapes is ongoing. Once the mechanism is clarified at the atomic level, it will become possible to design a molecular targeting drugs to control autophagy. It may be a long way to completely elucidate the mechanism of autophagy at the atomic

level, the formation of the autophagosomal precursor (PAS: Pre-Autophagosomal Structure), which is the first stage of the formation of autophagosomes, has already been determined[6]. Dr. Noda of the Institute for Microbiological Chemistry and Prof. Inagaki of Hokkaido University, users of Rigaku instruments, have made significant contributions to understanding the mechanism[7]. It was a great loss for us and to the scientific community that Prof. Inagaki passed away last year in 2016.

18 Atg proteins are known to be involved in PAS formation, among which Atg1, 13, 17, 29 and 31 interact to constitute a protein complex triggering the PAS formation[8]. It is known that the target of rapamycin (TOR) is one of the kinases controlling the formation of this protein complex. TOR kinase is the prototype of trophic signaling and is also a regulator of major intracellular signaling by amino acids and insulin. This is the molecular level mechanism of activation of autophagy under conditions of starvation. Figure 2(a) and 2(b) denote crystal structures of Atg1−Atg13 complex[9] and Atg13−Atg17−Atg29−ATG31 complex[10], respectively. Starvation-induced dephosphorylation of Atg13 is required for the formation of the Atg1−Atg13−Atg17−Atg29−Atg31 complex (Atg1 complex) initiating the PAS assembly pathway.

Structural analysis of large protein complexes, which are extremely difficult to crystallize, is where cryo-TEM is gaining its popularity. However, the high resolution structure by single crystal structure analysis will continue to be used as an essential technique for elucidating the chemical reaction at the atomic level. Further contribution of Rigaku single crystal tools to the complete elucidation of the molecular mechanism of autophagy is expected.

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