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Chapter 1

Introductory Chapter: Overview on Autophagy in Burden of Functions

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> "The Nobel Assembly at Karolinska Institutet has today decided to award the 2016 Nobel Prize in Physiology or Medicine to Yoshinori Ohsumi for his discoveries of mechanisms for autophagy. 2016-10-03".
> [q]:www.nobelprize.org/nobel_prizes/medicine/laureates/2016/press.pdf
> This Nobel Assembly announcement culminates enormous efforts devoted over past decades to elucidate the molecular network of autophagy – fundamental "self-eating" machinery for decomposition and remodeling of cellular components - and thus, closes another chapter in the History of Cell Physiology.

1. Introduction to Autophagy: "Self-Eating" in the Pursuit of Cell Health and "Happiness"

Multicellular organisms evolved, adopted and conserved numerous mechanisms and pathways, which allow them to sustain essential metabolism and morphogenesis over life period and to proceed through aging. Remarkably, often the same mechanisms orchestrate response to impacts of environmental and oxidative stressogens, toxins and adverse conditions caused by a variety of infections, and degenerative and malignant transformations. Among these mechanisms, a crucial role is borne by autophagy-lysosomal catabolicmachinery or autophagy, which is constituted by a large interactome of autophagy-related genes and organelle networks, that is integrated within a distinct singularity along with newly introduced "omes" such as "exposome" and "stressome" [1–5]. The normal "housekeeping task burden" of the autophagy system (i.e., chaperone-mediated autophagy, microautophagy and the nonselective and selective

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macroautophagy) is to sustain, adjust, reconstitute and to remodel the cellular contents in order to maintain cell metabolism, phenotypes and structural integrity, especially in the case of starvation when energy molecules are depleted, nutrition is limited, and thus death risk is high [2, 5]. In these events, autophagy biogenesis is regulated with precision by the autophagy-response receptors and signaling systems at metabolic and immune checkpoints that operate in conjunction with other cellular elements and machineries, such as cellular energy sensors, the proteotoxic stress sensors the pattern recognition receptors, the ubiquitin system, galectins, danger and redox signaling cascades, the endoplasmic reticulum-mitochondrial system, *etc*. [2, 5, 6, current book Chpt. 4]. Overall, that phenomena make the autophagy-lysosomal pathway to be a universal mechanism for decomposition of biological targets regardless their biochemical nature yet vital for response to stress and damage [1, 2]. The functional role of the autophagy machinery in immunochemical and structural cellular homeostasis can be further elaborated within a framework of "entity component system architecture" with a perspective on "the autophagy-mediated intrinsic barrier network".

Historically, invention of the term "autophagy" is credited to Nobel Laureate Christian de Duve. Since his mid-1950s pioneering work on compartmentalization of hydrolytic enzymes in subcellular structures (defined thereafter as "lysosomes"), and then his postulation that the end-point of all organelles subjected to destruction is sequestration in lysosomal apparatus, it took Christian de Duve another decade to evolve a concept of biodegradation of cell constituents via ubiquitous lysosomal pathway, which he named as "autophagy" (from the Greek for self-eating) [7]. Over that time period, the crucial evidence supporting de Duve's concept was provided by several other scientists (Novikoff AB, Essner E, Clark SL, Holt SJ, Hruban Z, Spargo B, Swift H, Ashford TP, Porter KR), who developed and implemented new cell fractional analysis, organelle contrasting techniques and ultrastructural analyses of lysosomal sequestration of organelles and proteins with electron microscopy—the only advanced cell research technique available at that time [8–9]. The research conducted globally by thousands scientists over the following fifty years lead to development of new autophagy techniques and brought enormous progress in clarification of many fundamental aspects of autophagy biology and the pathway details [7, 10, 11]. This research includes remarkable work of Drs, Mortimore GE, Dice JF, Ohsumi Y, Mizushima N, Klionsky DJ, Levine B, Johansen T, Yoshimori T, and others who built up milestones toward development of the autophagy paradigm. That progress was culminated by the 2016 Nobel Prize Award to Ohsumi Yoshinori for his achievements in the molecular dissection of key autophagy pathways leading to discovery of two ubiquitin-like conjugation system (see below) essential for development of autophagic vesicles (i,e., autophagosomes) [7, 10]. But still a lot needs to be done, especially when it comes to understanding autophagy signaling, the biogenesis of phagophores (another name - "isolation membranes"), mechanisms of selective autophagy and autophagosome trafficking.

2. Autophagy Molecular Paradigm: the Universal Mechanisms for Defining "to Be or not to Be"

According to the modern paradigm, autophagy represents a system of evolutionary conserved and strictly regulated "multitasking" mechanisms which is employed by eukaryotic cells (i) to

control quality of cellular constituents and to maintain AMP and ATP balance and cell metabolism—all by refurbishing unnecessary or compromised organelles and biomolecules; (ii) to compensate stress and danger and, thus to sustain the intrinsic resistance to harmful exposures; (iii) to decompose acquired xenobiotics (e.g., microorganisms and viruses) and, by these means, to mediate innate and adaptive immunity [1, 2, 5, 7, 10–16, current book Chpt. 18]. Detailed analyses of the autophagy interactive system revealed many aspects of the stress/damage-activated "eat-me" signaling mechanisms. Thus referring to macroautophagy the phagophore initiation, nucleation and elongation, the autophagic flux, the autophagy-regulated proteostasis, organelle biogenesis, pathogen sequestration, etc. are all borne by the highly conserved *ATG*-genes, Atg-proteins as well as by several signaling modules (e.g., mTOR, AMPK, the PI3K complexes, CREB transcriptional factor), sequestosome 1/p62-like receptors —adaptor proteins (e.g., p62/SQSTM1, NBR1, NDP52, T6BP, optineurin) and quality control modifiers (e.g., ubiquitins, galectins, STING) [1, 2, 5–7, 10, 11, 13, 16 and current book Chpts. 2, 3, 16, 17, 21]. In conjunction with this, interestingly that "membrane structural modules" arranged by mitochondria and endoplasmic reticulum (or the ER-mitochondrial axes), which are other key players in cell bioenergetics and proteostasis, are also involved in the emerging macroautophagy signaling mechanisms [5, 6, 12, 13]. Thus, numerous reports indicate that the ER-mitochondrial membrane modules along with their contact membranes (i.e., mitochondria-associated membranes – MAMs) can operate as a "fine stress-sensing interface", which either triggers prosurvival reconstitution of the damaged organelles or diverges the pathway to cell death [5, 12, 13]. Moreover, referring to the macroautophagy biogenesis, the ER-MAM-mitochondrial structures can originate omegasomes yet essentially contribute to formation, nucleation and elongation of the isolation membranes (phagophores), which further became sources of autophagosomes [5, 6, 10, 16]. Note the phagophore formation is mediated by the Atg12-Atg5-Atg16L and LC3/GABARAP/Atg8-phosphatidylethanolamine autophagy conjugates produced via activation of two ubiquitin-like enzymes E1 and E2 (i.e., Atg7 and Atg10—for Atg12 conjugation system and Atg7 and Atg3—for Atg8 conjugation system), and is assisted by an autophagosome-specific pool of phosphatidylinositol-3-phosphate and syntaxin-17 [1, 5, 13, current book Chpts. 2, 6, 12, 16, 21]. These observations are crucial for understanding efficacy of the macroautophagy target-sequestration from topological perspective. Indeed, according to the above model, potential sources of phagophores (i.e., the ER-MAM-mitochondrial structures) are ubiquitously present across cell volume (except other organelles), and thus the phagophore and autophagosome biogenesis can be specifically activated at the "target site". Further accomplishment of autophagic flux occurs with fusion of spatially separated autophagosomes, endosomes and lysosomes [5]. It should be denoted that spatiotemporal dynamics of autophagosome-to-lysosome trafficking through the cytoplasm still remains obscure.

Corroborating importance of crosstalk between autophagy and the ER-mitochondrial membrane modules in control of cell homeostasis, we can refer to the fact that suppression of *ATG*-genes and dysregulation of autophagic flux results in accumulation of damaged and therefore cytotoxic mitochondria and misfolded and oxidized proteins [11–14, 17, current book Chpts. 3, 14, 22]. These observations link impairment of autophagy machinery with pathogenesis of severe degenerative diseases and with promotion of aging, chronic viral infections and tumorigenesis, but also tumor cell death [11–14, 17], current book Chpts. 3,

14, 22]. Taking all the above into consideration, autophagy appears to be an efficient prosurvival adaptive and protective cellular mechanism [2, 5, 6, 12–16].

Evidently, autophagy-lysosomal pathway is adapted to recycle diverse intracellular components regardless of their size and biological nature, i.e., from polypeptide molecules (10^{-8} m) through microorganisms (10^{-6} m). That makes autophagy extremely efficient in barrier functions. From the evolutionary perspective it is interesting that host cells can eliminate bacteria and the "alleged" bacteria-derived endosymbionts, i.e., mitochondria, via selective autophagy, i.e., xenophagy and mitophagy, respectively [5, 6, 12–14, current book Chpt. 19]. Both xenophagy and mitophagy require implication of the "core" autophagy proteins [e.g., ULKs (Atg1), LC3/GABARAP, Atg5-Atg12, Atg9, Atg16L1], autophagy adaptors ("cargo" receptors) (e.g., p62, NBR1, NDP52), factor FIP200 and the poly-ubiquitin-modifiers—for the sequence of phagophore formation, cargo-selection and autophagosome enclosure and for autophagic flux. However, activation of these pathways and their spatial arrangements are regulated by distinct signaling mechanisms triggered by either invaded bacteria or damaged and depolarized mitochondria. Thus, in the first case the signaling cascade is comprised of pathogen recognition sensors (e.g., Toll and NOD-like receptors, antimicrobial GTPase proteins, STING, galectin-8), which can respond to entire microorganism or to the bacterium-containing vacuoles and their fragments [6, 15, 18]. While in the second case the signaling mechanism is initiated by mitochondrial expression of either Nix/Bnip3L and Bnip3 receptors of LC3/GABARAP or—PINK1 kinase followed by recruitment of cytosolic Parkin (E3 ubiquitin ligase), Mfn2, ubiquitin and p62 linking the mitochondrial cargo with LC3/GABARAP on isolation membrane [5, 6]. Seemingly, host cells endowed with these signaling mechanisms are capable of pursuing selective "multitargeting" autophagy, and thus sustaining resistance to invading pathogens, pathogenic factors as well as to the associated damage to mitochondria and ER [19].

Paradoxically, as many other crucial pathways, autophagy plays a dual role under normal and pathological conditions. In addition to the well-known role of autophagy in cell survival, autophagy-mediated type II programmed cell death has long been proposed [5, 12–14 and current book Chpt. 12]. The autophagic cell death was originally reported in tissues subjected to extensive development and remodeling. That effect seems to be analogous to apoptosis in similar metamorphoses [8, 14, current book Chpt. 13]. However, the autophagy implication in cell death is not restricted to the morphogenetic events; it can also drive cell death under various pathological conditions such as acute inflammation, ageing, malignancies and intracellular pathogens such as tuberculosis, borreliosis, etc., [5, 6, 8, 12–14, current book Chpt. 21]. Thus, many highly virulent intracellular pathogens can subvert and adapt different components of autophagy machinery to establish replicative niches eventually leading to host pathology and death [14, 16, 18, 20]. In conjunction with this, interestingly that upon invagination of hepatocytes, malaria sporozoites are able to adapt autophagy LC3-II protein to form host cell-derived parasitophorous vacuoles; and then to subvert amphisomes—originated from another autophagy pathway—as a nutritional source arranging "own feeding mechanisms", while avoiding degradation by lysosomes [20].

In addition, regulation of autophagic flux with pro- and anti-proteolytic enzymes can be a leading key for development of either "excessive autophagy" or "defective autophagy", such as described in the context of persistent chronic infections during sepsis [5, 11, 13–15, current book Chpt. 10]. These both autophagy conditions can affect the ER-mitochondrial network resulting in alterations in oxidative phosphorylation, energetic collapse, impairment of proteostasis, inflammation and the detrimental course of immune dysfunction [5, 12–15].

3. Autophagy and Intrinsic Biological Barriers

In the light of the above considerations, we can say that autophagy mechanism is indispensable contributor to the interactive system of intrinsic biological barriers in living organisms, i.e., the barriers which are essential to maintain nonequilibrium dynamics of organic and inorganic metabolites, to control bioenergetics, antigen and redox status, to protect against thermal impacts and electromagnetic radiation, to interact with microbiota, etc. Based on biological nature of the elements which conduct sequestration, spatial isolation, shielding and target-processing, the barrier functions can be carried out at: (i) molecular level by e.g., ligands, carriers/transporters, proteasomes and redox "converters"; (ii) membrane subcellular level by e.g., mitochondria, endoplasmic reticulum, phagophores, the plasma membrane; (iii) extracellular level by e.g., mucus and other extracellular matrices. Moreover, at cellular level these barriers represent numerous interfaces of tissues and organs with ambient and internal environment to sustain structural, immune and metabolic integrity. From this perspective, while macroautophagy, microphagy and chaperone-mediated autophagy execute barrier functions at molecular and membrane levels [2, 5, 6, 13–17], it would be reasonable to assume that dynamics and efficacy of autophagy function can determine performance of the barrier-forming cells. Note, in the vertebrates the infection cellular barriers are constituted by multidimensional interactive networks of mesenchymal, epithelial, reticuloendothelial, endothelial and hematopoietic cells, where along with monocytes and polymorphonuclear granulocytes, a particular role in xenobiotic control and "cleaning function" is attributed to nonprofessional phagocytes, e.g., skin fibroblasts, bone marrow stromal cells, endothelial and epithelial cells [18, 19]. Evidently, nonprofessional phagocytes are very efficient in phagocytosis with "autophagy-to-pathogen" response mechanism and therefore, can compensate professional phagocyte function, when the last one declines [19]. Thus, a lack of the "canonical" phagocytic features (such as phagosome biogenesis, the oxidative burst, etc.) in nonprofessional phagocytes is presumably compensated by empowering xenophagy to control and execute all events from pathogen sequestration through degradation [5, 6, 18–21, current book Chpt. 15]. That infers increasing burden of autophagy function in nonprofessional phagocytes when professional phagocytes are depleted due to pathological conditions. Furthermore, considering that nonprofessional phagocytes can also orchestrate response to acute stress or trauma by expression and massive release of paracrine and endocrine factors, such as damage-associated molecular patterns (DAMPs), inflammatory cytokines, proteases, chemokines, defensins, nitric oxide, ROS, fragmented DNA, exosomes and microvesicles, which in turn can trigger and propagate autophagy stress response [6, 14–19].

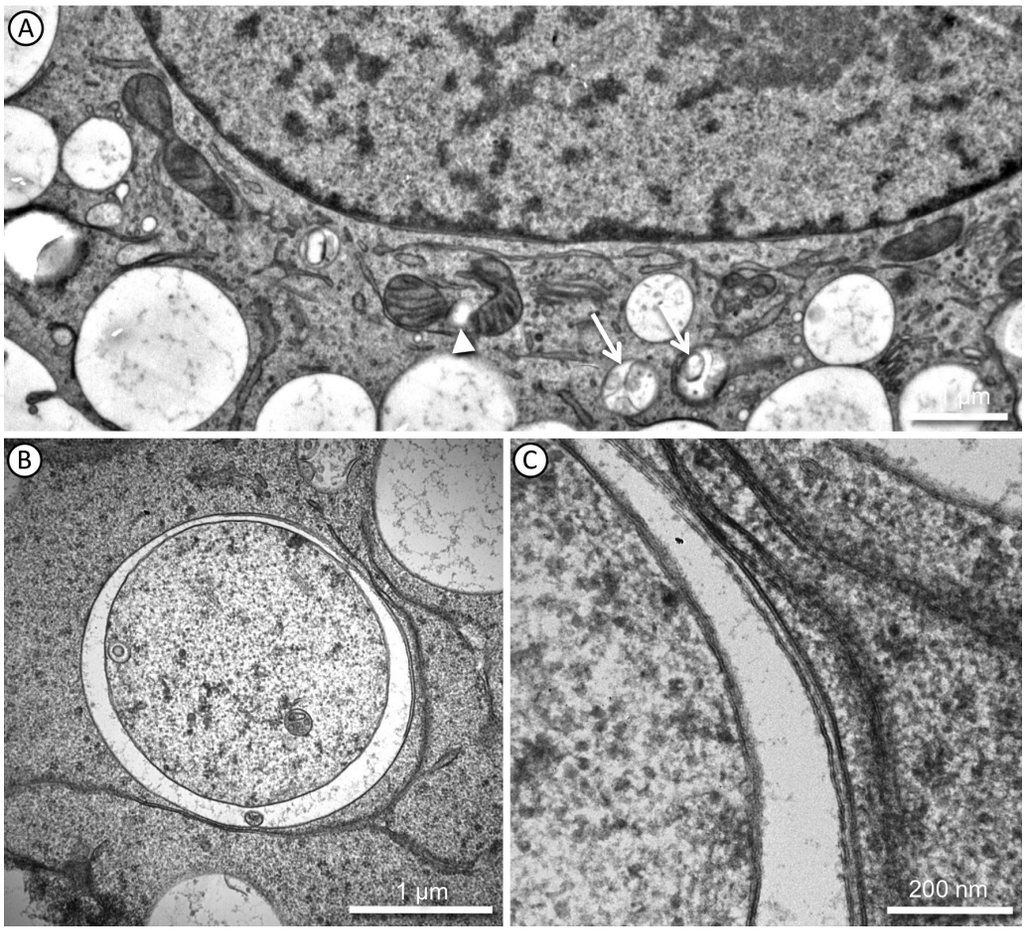

Figure 1. Various states of mitophagy in a glioblastoma cell and a clear contribution by endoplasmic reticulum forming the phagophore can be deduced; lysosomes are found adjacent to autophagosomes (arrow head) as well as following fusion (arrows) (A). Two lipid bilayers of a completed phagophore engulfing cytoplasm are surrounded by endoplasmic reticulum membranes (B). Lipid bilayers of the phagophore double membrane can be identified by higher magnification. The high resolution also shows the typical asymmetry of phagophore (and autophagosome membranes). Lower staining intensity is seen in the vesicle-faced bilayers whereas higher contrast is seen in the outer lipid bilayers (C) (trans electron microscopy performed with a JEOL 1400 at 120 keV).

These effects may suggest a presence of cross-talk in the "barrier network" assisted by autophagy mechanism.

4. Conclusion

Overall, it is hard to overestimate the vital role of autophagy in function of the intrinsic cellular barriers. Thus, autophagy machinery bears specific types of physical barriers emerging from activation and interaction of autophagy scaffolds, membrane-assembling proteins, ubiquitin-like modifiers and autophagy adaptors, which sustain autophagic flux. In this event, the hallmark of macroautophagy is formation of new organelles, i.e., double-membrane phagophores and then sequestration and compartmentalization of cellular constituents within autophagosomes (exemplified in **Figure 1** and **Supplement 1**).

Supplement 1

A tomogram depicting the glioblastoma cells (line #12537 GBM) with active autophagy and mitophagy. Different stages of mitophagy progression [e,g., advanced mitophagy (upper left) and just initiated mitophagy (lower left)] are detectable within numerous autophagosomes and newly formed intravesicular membranes. The residual mitochondrial constituents are observable at higher magnification (*see in the middle*). The autophagy tunneling system, shielded from the rest of the cell's cytoplasm can be best envisaged in a tomogram. Note numerous ribosomes and structural elements comprised of actin, intermediate filaments and microtubules.

The tomogram was taken by Paul Walther in the Central Facility for Electron Microscopy at Ulm University using Jeol 2100F TEM in STEM mode at 200 keV.

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