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Mini-Review

Autophagy and the endo/exosomal pathways in health and disease

Margarita-Elena Papandreou[1,2] *and Nektarios Tavernarakis*[1,2]

[1] Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology – Hellas, Heraklion, Greece
[2] Department of Basic Sciences, Faculty of Medicine, University of Crete, Heraklion, Greece

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Cell homeostasis requires the concerted action of cellular pathways involved in degradation, trafficking and intercellular communication, which are interlinked to satisfy the cell's needs upon demand. Defects in these pathways instigate the development of several age-related pathologies, such as neurodegenerative and chronic inflammatory diseases. Autophagy is an evolutionarily conserved and tightly regulated process of degrading cellular constituents. The endosomal and vesicular trafficking pathways contribute to this regulation and share common features with the autophagic process. Recently, autophagy has been implicated in the endosome/exosome secretory pathway. Importantly, current technological advances allow the manipulation of exosomes as drug nanocarriers in pharmaceutical intervention strategies. Here, we survey emerging findings relevant to the crosstalk between autophagy and the endo/exosomal vesicular trafficking pathways. In addition, we discuss novel methodologies that have recently been developed, which allow the utilization of these pathways for targeted drug delivery in disease.

Keywords: Endocytosis · Endosome · Neurodegenerative disorders · Polyglutamine repeat protein · Secretion · Trafficking

Correspondence: Professor Nektarios Tavernarakis, Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology – Hellas, Nikolaou Plastira 100, 70013 Heraklion, Greece
E-mail: tavernarakis@imbb.forth.gr

Abbreviations: Acbp1, acyl coenzyme A binding protein 1; **acyl-coA,** acyl coenzyme A; **Alix,** ALG-2-interacting protein X; **AMBRA1,** autophagy and Beclin 1 regulator 1; **ALS,** amyotrophic lateral sclerosis; **AP2,** adaptor protein 2; **ATG,** autophagy-related protein; **BCL-2,** B-cell lymphoma 2; **CHMP2B,** Chromatic modifying protein 2B; **COPI,** coat protein I; **CORVET,** Class C core vacuole/endosome tethering; **CUPS,** Compartment for unconventional protein secretion; **DFCP1,** Double FYVE-containing protein 1; **EEA1,** Early endosome antigen 1; **ER,** endoplasmic reticulum; **ESCRT,** endosomal sorting complexes required for transport; **EXO8,** exocyst 8; **Fab1,** formation of haploid and binucleate cells; **FIP200,** Focal adhesion kinase family interacting protein of 200-kDa; **FYVE,** Fab1, YOTB, Vac1, EEA1; **GABARAP,** Gamma-Aminobutyric acid type a (GABAA) receptor-associated protein; **GD3,** disialoganglioside synthase; **GRASP,** Golgi reassembly stacking protein; **Grh1,** GRASP65 homolog protein 1; **GTPase,** GTP-binding protein; **HOPS,** Hsp70-Hsp90 organizing protein; **IDE,** insulin-degrading enzyme; **ILV,** intraluminal vesicle; **IL-1β,** Interleukin-1β; **LAMP,** lysosomal-associated membrane protein; **LC3B-I,** light chain 3B isoform I; **MAM,** mitochondria-associated membranes; **MAP-LC3,** microtubule associated protein light chain 3; **MIF,** migration inhibitory factor; **miR-101,** miroRNA-101; **miRNA,** microRNA; **mTORC1,** mammalian target of rapamycin complex 1; **NBR-1,** neighbor of BRCA1 gene 1; **ORP1L,** oxysterol binding protein-related protein 1L; **PAS,** pre-autophagosomal structure; **PLEKHM1,** Pleckstrin homology domain-containing family M member 1; **PtdIns,** phosphatidylinositols; **RAB5,** Ras-related protein 5; **RabGAP,** Rab guanosine triphosphate-activating protein; **Ralb,** Ras-related protein Ral-B; **RILP,** Rab-interacting lysosomal protein; **ROS,** reactive oxygen species; **Sab1,** suppressor of Abl protein 1; **SNAP29,** synaptosome associated protein; **SNARE,** soluble NSF attachment protein receptor; **Snf-7,** vacuolar-sorting protein; **TBC1D,** TBC1 domain family member; **TDP-43,** TAR DNA-binding protein 43; **TFEB,** transcription factor EB; **ULK-1,** Unc-51 Like Autophagy; **VAMP,** vesicle associated membrane protein; **VAP-A,** VAMP associated protein A; **V-SNARE,** vesicle SNARE; **VPS34,** vacuolar protein sorting 34; **WIPI1,** WD repeat domain, phosphoinositide interacting 1

1 Introduction

1.1 Autophagy

Autophagy, from the Greek words 'auto', self, and 'phagy', eating, is a vital cellular process shared by all eukaryotic organisms. It involves the degradation of a cell's constituents such as proteins and organelles. Although the traditional notion was that autophagy is a bulk degradation pathway, pioneering studies in the past decade have revealed its highly selective nature which involves distinct steps and intricate relationships and a fine balance. Autophagy is a general term for a cellular lysosomal degradation process which can be subdivided to macroautophagy, chaperone-mediated autophagy and microautophagy.

Macroautophagy, from now on referred to as autophagy, initiates with a 'crescent-shaped' phagophore followed by the formation of a double-membrane vesicle, the autophagosome, which encloses the autophagic substrates for sequestration. Subsequent trafficking of the autophagosomes for fusion to the lysosomes, forms the autophagolysosome, where the autophagosome inner components are degraded by hydrolytic enzymes. This catabolic process, as previously mentioned, invariably occurs at basal levels (basal autophagy) depending on the cell type/tissue. However, it can be induced under nutrient scarcity or starvation or other autophagy triggering conditions (induced autophagy) [1]. The end products of degradation can be used for anabolism in the cell. During high autophagic flux, the measure of autophagic degradation activity, there is a large requirement for membrane recycling and endosome contribution.

1.2 The endocytic pathway

Endosomes modulate intracellular trafficking and provide material from outside the cell. Endocytosis regulates internalization of cell surface receptors and their ligands for cell growth, proliferation and cell-to-cell communication. Material could include fluid solutes, membrane compartments, large macromolecules which are enclosed by synaptic vesicles which later fuse with endosomes. It initiates after binding of ligands on plasma membrane receptors which then are internalized into early endosomes transformed to multi-vesicular bodies which are degraded in the lysosome. Synthesis of endosomal membrane and luminal compartments as well as continuous recycling of the membrane is required for endosome maintenance.

Proteins and complexes of the endocytic pathway involve Rab proteins, clathrin coats and ESCRTs (endosomal sorting complex required for transport). Endocytosis initiates at the plasma membrane, where Rab5 becomes localized. After plasma membrane invagination and vesicle formation, Rab5 is incorporated in vesicles and early endosomes together with EEA1 [2]. Rab5-containing early endosomes are converted to Rab7-containing endosomes which then (i) return back to the membrane as Rab11-recycling endosomes or (ii) are transported to the Golgi by the retromer and (iii) are transported and mature into late endosomes, multivesicular bodies with intraluminal vesicles [3]. Late endosomes and multivesicular bodies fuse with lysosomes or mature themselves into lysosomes, incorporating lysosome associated membrane proteins (LAMPs), vacuolar H^+-ATPases for acidification, and hydrolases for degradation. Exocytosis of proteins destined for conventional secretion are synthesized in the ER and are usually acetylated in the ER, are delivered via secretory vesicles budding off the Golgi to the plasma membrane for fusion and secretion. Extracellular vesicles are called exosomes and are key players in intercellular communication.

2 Autophagy and the endosomal pathway

2.1 Autophagy initiation and endosomes

Regulation of autophagy and its interplay with the endosomal and exosomal pathways can occur at different stages of this multifaceted pathway, thus a detailed analysis of this intricate communication is required. Autophagy initiation occurs by the ULK-1-ATG13-FIP200 induction complex. Core autophagic proteins which participate in autophagosome assembly and elongation machinery are mainly divided into two conjugation systems. The first conjugation system involves ATG3, ATG4, ATG7 and the ubiquitin-like modifiers MAP-LC3A,B,C (microtubule-associated protein light chain) along with GABARAP and GATE-16 and the second conjugation system, ATG5, ATG12, ATG16, all of which colocalize with early autophagic structures.

Importantly, autophagosomes are highly dynamic structures, the origin and source of which should be considered at the level of the phagophore, a half-moon shaped membrane which surrounds the material to be degraded and then closes to form the autophagosome. Although in yeast the formation of the phagophore has been described at the phagophore assembly site (PAS) where autophagic machinery colocalizes, in mammals there are multiple sites of autophagosome assembly such as the ER, Golgi, endosomes, mitochondria, mitochondria-associated membranes and the plasma membrane [4]. Initially, early structures were observed named omegasomes derived from PtdIns(3)P, which interact with the ER and colocalize with autophagosomes. Autophagosomes have been shown to arise from omegasomes, however there is a possibility for different autophagosomes being generated from other sources as well [5]. There is more recent substantial evidence indicating that autophagosomes form at ER-mitochondria

contact sites where ATG14 is recruited by the ER SNARE protein syntaxin 17 and upon autophagy induction relocalizes together with ATG5 [6]. This has been further characterized, as ER subdomains which interact with mitochondria, the MAMs, mitochondria associated membranes, contain lipid raft components such as GD3 which associate with the core initiator proteins AMBRA1 and WIPI1 [7]. Moreover, origins of the pre-autophagosomal membranes have been identified at the plasma membrane as well. After endocytosis, clathrin adaptor AP2-coated vesicles, which are early endosomes containing ATG16L1 and ATG9, undergo homotypic fusion mediated by V-SNARE, VAMP7, which is usually contained in late endosomes, to generate autophagosome precursor membranes [8]. The differential origin of autophagosomes suggests that autophagosome subtypes with distinct enclosed material may be destined for different sub-cellular or even extracellular compartments.

Mechanistically, autophagy initiation is mainly induced under nutrient starvation by ULK-1 activation. VPS34 is activated after interaction with the early endosomal protein Rab5 followed by Beclin-1 interaction and ATG5-ATG12 conjugation. Moreover, activation of the class III phosphatidylinositol 3-kinase complex causes the production of PtdIns(3)P by Beclin-1 and VPS34 which is then recognized by PtdIns(3)P autophagic effector molecules DFCP1, double FYVE-containing protein, WIPI1/2 [5]. DFCP1 and ATG14L are both peripheral ER proteins which together with WIPI2 colocalize in distinct puncta after starvation. VAMP1 is a multispanning transmembrane protein of the ER and Golgi and interacts with Beclin-1 to dissociate it from BCL-2 indicating the importance of the ER in autophagy regulation [4]. Rab5 synergizes with VPS34 to induce autophagy upon virus infection [9]. The role of Rab5 in autophagy induction has been questioned due to the fact that in *Caenorhabditis elegans* genetic disruption of *Rab5* has actually been reported to induce autophagy [10, 11]. Nevertheless, starvation-induced upregulation of the miRNA miR-101 which reduces Rab5 expression, most probably serving as a negative feedback loop to keep autophagy induction under control [11]. These findings further demonstrate the modulatory effects of the endocytic pathway on autophagy.

ULK1 and Atg9 are found on recycling endosomes albeit on different subdomains. Upon starvation, transferrin and the transferrin receptor together with ULK1 are recruited to Rab-11 positive endosomes. This process is negatively modulated by Rab-11 effector TBC1D14, a RabGAP, which tubulates ULK1 and Atg9-positive recycling endosomes by directly binding to Rab11 [12]. Another RabGAP, TBC1D5, performs both retrograde transport from the endosomes to the trans-Golgi and regulates the autophagic rate or autophagic flux.

2.2 Autophagosome maturation, autophago-lysosome generation and endosomes

Autophagosome maturation includes autophagosome elongation, trafficking and autophagosome/lysosome fusion. Trafficking of autophagosomes is particularly important in large highly specialized cells such as neurons where the autophagosome needs to be transported to the soma for lysosomal degradation. Mature lysosomes are found at the perinuclear region and autophagosomes are generated at distal tips. Late endosomes loaded with dynein-snapin motor complexes fuse with autophagosomes and drive autophagic vacuole retrograde transport back to the soma. Dysregulation of this type of transport retains autophagosomes at neurites and synaptic terminals [13]. Syntaxin 17 genetic inhibition causes autophagosomal stalling in axons. Syntaxin 17 is also important at later steps of autophagy, that of autophagosome-endolysosome fusion when SNAREs syntaxin 17 and SNAP29 associate with ATG14 for stabilization on the autophagosomal membrane and concomitantly preparation for interaction with endosomal VAMP8 [14]. SNAP29 mutations cause autophagic degradation, endocytic and secretion deficits which could potentially contribute to neuropathies [15]. In ALS, equivalent trafficking defects have been detected where mitochondria-containing autophagosomes are stalled in motor neuron axons [16]. Moreover, the endosomal Rab7 GTPase is required for both autophagosomal and endosomal maturation and trafficking to lysosomes [17]. The Rab7 effector ORP1L, which is a cholesterol sensor localized on autophagosomes, orchestrates late trafficking of autophagosomes towards lysosomes. When cholesterol levels are low, ORPL1 associates with the ER protein VAP-A and forms contact sites between autophagosomes and the ER, preventing minus-end-transport by the Rab7-RILP-dynein complex [18]. Upon increase of cholesterol levels, this contact site is lost, and ORPL1 mediates the assembly of two Rab7 molecules with PLEKHM1 and RILP, which in turn recruit the HOPS complex (homotypic fusion and vacuole protein sorting). The HOPS complex, which associates with the autophagosomes via Syntaxin 17, SNAREs SNAP29, VAMP8 and ATG14, mediates the final fusion of autophagosomes with late endosomes to form amphisomes and lysosomes to form autophagolysosomes. Therefore, endosomes are actively involved in autophagosomal transport and maturation.

Rab11 localizes both in early recycling endosomes, as described above, as well as in late endosomes or multivesicular bodies which carry LAMPs or lysosomal hydrolases. Upon starvation, autophagosomes can also fuse with Rab11-decorated multi-vesicular bodies to form enlarged hybrid organelles, the amphisomes [19]. Other Rab family proteins are also involved in autophagic regulation, such as Rab9A, Rab32, Rab33B for autophagosome formation as well as Rab8b and Rab24 for autophagosome

maturation. It is of particular interest how basal and induced autophagy are differentially regulated, perhaps through the involvement of different endosomal compartments. Under normal conditions, ATG12-ATG3 along with the ESCRT-associated protein PDCD6IP/Alix contribute to basal autophagy and endolysosomal trafficking as well as late endosome distribution, exosome secretion, and viral budding, intertwining autophagy, endocytosis and secretion [20].

Interestingly, whole exome analysis has revealed a causative mutation for leukoencephalopathy in Vps11, a core component of HOPS and CORVET (class C core vacuole/endosome tethering) protein complexes regulating autophagy as well [21]. Other v-SNAREs, such as VAMP7, are usually located to late endosomes and contribute to the formation of trans-Golgi-derived LAMP-1 vesicles called LAMP protein carriers which are essential for lysosomal stability and function [22]. Furthermore, endosome biogenesis itself regulates autophagy. In particular, intraluminal vesicles (ILV) are generated by the four complexes of the ESCRT machinery (0–III). Genetic inhibition of components of these complexes causes reduction in the endosome-autophagosome fusion significantly increasing the amount of immature autophagosomes. Moreover, late endosomes, the multivesicular bodies, are formed by the ESCRT machinery. Most neurons highly express Snf7-2, an ESCRT-III component, the absence of which causes dendritic retraction and cell death. In a similar fashion, a rare dominant negative mutation of CHMP2B, an ESCRT-III component, is linked to frontotemporal dementia and ALS [23]. Genetic ablation of one or the other ESCRT-III components causes autophagosome accumulation and neurodegeneration potentially because of defective trafficking and resulting inefficient clearance of protein aggregates and organelles. Other examples include Vps4 ATPase required for endosomal trafficking and autophagolysosome generation and PtdIns(3)P 5-kinase Fab1 which is critical for autophagosome maturation [24].

Polyglutamine disease symptoms are aggravated in a Huntington's disease model after ESCRT component genetic inhibition. Moreover, ESCRT-0 dysfunction has been recently shown to compromise autophagic degradation of the major autophagic receptor and substrate p62, α-synuclein, huntigtin or TDP-43 which ultimately causes ER stress-induced neurodegeneration [25]. Endosomes are also required for selective autophagy. HOPS is also involved in selective autophagy of mitochondria, namely mitophagy, as it is recruited by TBC1D15 which is responsible for late endosome trafficking and associates with mitochondria via fission protein Fis [26]. ESCRT-mediated selective microautophagy in yeast occurs without the involvement of core autophagic proteins, but instead with the autophagic receptor also found in mammals, Nbr1. The ESCRT machinery colocalizes with Nbr1-mediated vacuolar targeting components which are ubiquitinated. This molecular pathway is highly selective for degradation of two cytosolic hydrolases [27]. Selective degradation of plasma membrane proteins also occurs via their ubiquitination and ubiquitin-binding protein receptor recognition for both endolysosomal and autophagosomal degradation. An interesting case of endocytosis or phagocytosis is that of an LC3-positive phagosome containing bacteria entering the plasma membrane which later fuses with the lysosome for degradation [26].

A question which remains to be determined is the mechanism by which autophagosomes obtain proteins found in multivesicular bodies, late endosomes and lysosomes. On the one hand, one model supports that LAMPs and the V-ATPase are delivered by vesicles on the outer autophagosomal membrane shortly after autophagosomal formation. On the other hand, it has been proposed that autophagosomes become acidic prior to enzyme delivery causing rapid hydrolase activation and inner autophagosomal membrane degradation, transforming it into an amphisome. In order to become an autophagolysosome, the amphisome can either mature into one or fuse with another lysosome. There is strong evidence that it is the latter case, as disruption of trafficking of either early endosomes by COPI or trafficking and fusion of late endosomes to lysosomes by the ESCRT machinery causes autophagosome and amphisome accumulation [28].

Importantly, one process which is often overlooked is how the lysosomal pool is maintained especially under high autophagic flux. TFEB is the master regulator of lysosomal biogenesis, however, under high autophagic flux conditions reformation of the lysosome from the amphisome or the autophagolysosome is necessary. Specifically, under basal autophagy lysosome reformation is detected after 30 min, while under induced autophagy, where mTORC1 is required, reformation is first detected after 4 h [29]. This reformation necessitates removal of endosomal, autophagosomal membrane components and especially Rab7 after sustained starvation [29].

3 Autophagy and secretion

3.1 Secretory autophagy

The interplay between selective autophagy and the secretory pathway is quite intriguing as autophagy can mediate secretion while the secretory machinery can initiate autophagy. This intricate mechanistic relationship as well as the outcome of its dysregulation in multiple settings is discussed below.

An apparent paradox has recently emerged implicating autophagy-dependent secretion. How and why would a self-digestive process modulate secretion and how would it select between degradation of cellular constituents and secretion to the extracellular environment? In yeast, a novel structure has been uncovered which acts

as scaffold under starvation to promote secretion of proteins lacking the classic secretory signal sequence. This structure comprises of the orthologue of Golgi associated (stacking) proteins GRASP-65 and GRASP-66, Grh1 which is relocalized near ER exit sites where a compartment is assembled, that of unconventional protein secretion (CUPS). This structure is devoid of endosomal proteins but contains Vps23 which is a member of the ESCRT machinery, specifically ESCRT-I as well as the core autophagic components Atg8 and Atg9. CUPS is required for the subsequent secretion of Acyl-CoA binding protein, Acbp1, under starvation, which conversely requires the involvement of the endosomal pathway [30, 31]. Thus, CUPS is the site of biogenesis of secretory autophagosomes which is reminiscent of omegasome formation in mammals as it contains PtdIns(3)P, Atg8 and localizes near the ER [26]. These observations indicate that distinct autophagosome types arise depending on content and destination.

Conversely, Ral GTPases mainly regulate the exocyst complex for secretory vesicle trafficking and tethering. Active form of Ralb and Exo84 (EXO8) sub-complex has been shown to be essential for autophagosome formation under starvation or pathogen induced autophagy. Exo84 acts as a scaffold to assemble the ULK1-Beclin1-VPS34 complex [32]. Moreover, Sab1 and Rab1 are GTPases also involved in autophagosome formation. Sab1 is required for vesicle formation from the ER for secretion from the cell. Interestingly enough, Sab1 mutants also reduce autophagosome formation and exhibit a diffuse cytoplasmic and nuclear LC3B pattern instead of LC3B puncta as well as reduced LC3B-I conversion to LC3B-II [33]. Moreover, Rab1b controls anterograde transport from the ER to the Golgi, downstream of Sab1. Mutation or over-expression of Rab1b decreases or increases autophagosome formation respectively [33].

The coordinated action between the exosomal pathway and autophagic components regulates major developmental and homeostatic responses. Secretory autophagy is a pivotal homeostatic mechanism in the bone, contributing to bone resorption by osteoclasts. Secretory lysosomes generate the folds of the ruffled border, the site where bone is resorbed by osteoclasts. LC3B, ATG4B, ATG5, ATG7 are core autophagic players required for polarized lysosomal trafficking [34]. There is therefore speculation about autophagy negatively affecting postmenopausal osteoporosis during aging. Thus, potential therapeutic drugs for pathological bone loss could be autophagy inhibitors. Autophagy is necessary for otoconial protein secretion which occurs during ear development [35]. In the pancreas, β-cell specific deletion of *ATG7* decreased serum insulin levels as a result of defective insulin secretion [36]. More recently, there is additional evidence concerning insulin secretion and autophagy indicating that VAMP7, a SNARE protein responsible for membrane fusion in intracellular trafficking, regulates autophagosome formation to ultimately mediate insulin secretion in pancreatic β-cells [37].

The interplay between autophagy and secretion has recently been accentuated in the context of immunity. Interleukin-1 secretion is inhibited under basal autophagy and activated when autophagy is induced in macrophages [38]. Specifically, Beclin-1 and LC3B negatively regulate caspase-1-mediated immune responses under basal autophagic conditions. Under starvation, IL-1β secretion, which again lacks the signal peptide sequence to follow the conventional ER-regulated secretion pathway, dramatically increases by the coordinated action of ATG5, the inflammasome, GRASP and Rab8a and is referred to as autophagy-based unconventional secretion [39]. While conventional autophagy is implemented during viral infection to clear out the pathogen, recent evidence has revealed that non-lytic viruses can spread by potentially hijacking secretory autophagy [40].

In the context of autoimmunity, autophagy has been reported to cause exacerbated immune responses in several instances. Secretory granules in mast cells are decorated with LC3B-II under basal autophagic conditions and are required for mast degranulation. However, if autophagy is over-activated then this could cause major allergic reactions, that is why under autophagy hindering conditions, passive cutaneous anaphylaxis reactions are impaired [41]. In macrophages, a very recent report has highlighted that autophagic induction by starvation restricts mitochondrial ROS production thus inhibiting release of MIF (macrophage migration inhibitory factor) which has been linked to increased inflammation and tumorigenesis [42]. Moreover, ATG5, ATG7, ATG16L1 have been shown to regulate secretion of granule contents of Paneth cells. In complex heterogeneous inflammatory bowel diseases such as Crohn's disease, autophagy-dependent secretion appears to be dysregulated. This has been nicely demonstrated in an *ATG16L1* mutant mouse model in combination with virus infection as Crohn's disease is triggered by the combinatorial effects of a genetic and an environmental factor otherwise referred to as 'virus-plus-susceptibility gene' interaction [43]. One of the genetic factors is a variant of the *ATG16L1* gene which is apparently important in secretory functions for epithelial cell immunity in Paneth cells. Thus secretory autophagy may either protect against disease or contribute to disease via uncontrolled secretion.

Aberrant autophagy is a common denominator of several neurodegenerative diseases. In Alzheimer's disease autophagosomes accumulate in dystrophic neurites [44]. This indicates that autophagosomal maturation, transport or end-stage degradation is impaired. Beyond conventional degradation, autophagy has been shown to regulate amyloid beta secretion and plaque formation, two major hallmarks of the disease [45]. Although the exact mechanism has not been outlined, Alzheimer's patients' blood samples contain exosomes derived from the brain containing lysoso-

Table 1. Associations of autophagy and the endo/exosomal pathway with disease

Molecular pathway	Protein factors	Pathological condition	Reference
Autophagy induction	Rab5, Vps34	Virus infection	[9]
Autophagosome trafficking	SNAP29, syntaxin 17	Neurodegeneration	[15]
Endosome generation / Autophagosome trafficking	ESCRT-0	Neurodegeneration	[25]
Endosome generation / Autophagosome trafficking	Snf7-2 (ESCRT – III)	Neurodegeneration	[23]
Endosome generation / Autophagosome trafficking	CHMP2B (ESCRT – III)	Dementia, ALS	[23]
Autophagosome – endosome trafficking	Dynein	ALS	[13]
Autophagosome – endosome – lysosome tethering	Vps11	Leukoencephalopathy	[21]
Autophagic secretion	ATG7	Insulin secretion defects	[36]
Autophagic secretion	LC3B, ATG4B, ATG5, ATG7	Osteoporosis	[35]
Autophagic secretion	LC3	Allergies	[41]
Autophagic secretion	ATG16L1 + virus	Crohn's disease	[43]

mal proteins such as cathepsin-D, LAMP-1 [46]. Furthermore, astrocytes normally secrete an insulin-degrading enzyme (IDE) extracellularly in the brain, one of the major proteases of the amyloid-βpeptide which prevents its accumulation, a major hallmark of Alzheimer's disease. IDE is secreted into the cerebrospinal fluid by RAB8A and GORASP (Golgi reassembly stacking protein) while genetic inhibition of core autophagic components reduced IDE in the cerebrospinal fluid [47]. A-synuclein degradation is performed by autophagy, however recently α-synuclein secretion and propagation in a prion disease-like manner has been suggested in a Parkinson's disease model further accentuating the interplay between autophagy and secretion [48]. Moreover, in Parkinson's disease, mutations have been identified in components of the retromer, a protein assembly of the endosomal trafficking network, and other endosomal components, such as VPS35 [49].

Even in the context of cancer, there is a notable example where exosomes derived from irradiated cancer cells induce autophagy in paracrine cells. During radiation therapy there is a phenomenon called radiation-induced bystander effect, which refers to effects on non-targeted cells caused by signals from irradiated cells [50]. Irradiated human bronchial cells secrete exosomes containing miR-7-5p, which in turn induces autophagy in non-targeted recipient cells. The paracrine effects of secretory autophagy have recently been highlighted in pancreatic stellate cells which contribute to tumor metabolism by secreting alanine. Indeed, cancer cells stimulate neighboring pancreatic stellate cells to secrete alanine via autophagy [51]. Disease-associated genes which regulate autophagy and the endosomal and exosomal pathway are reviewed in Table 1.

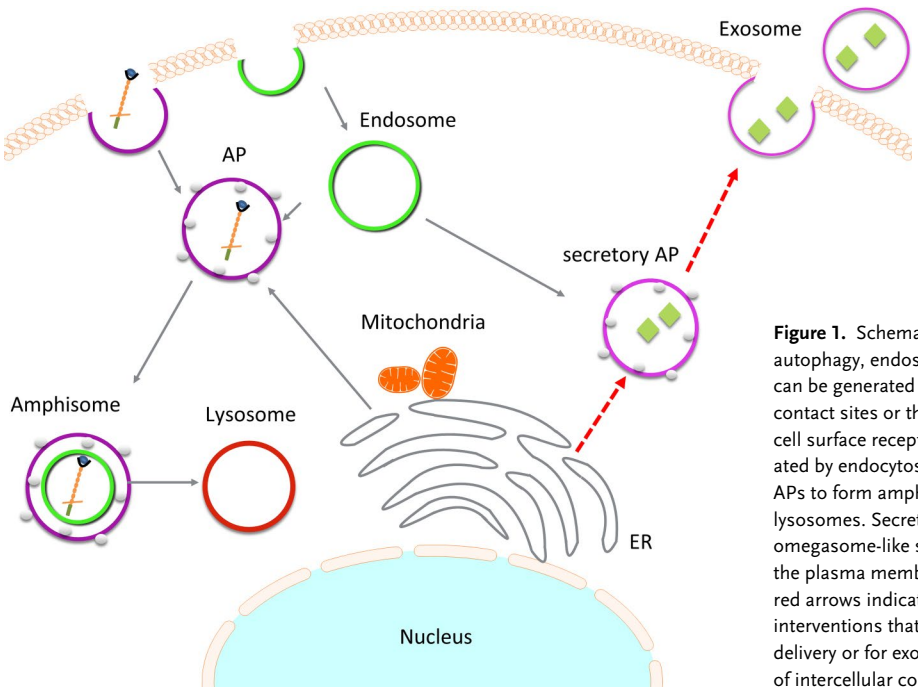


Figure 1. Schematic diagram of the interplay between autophagy, endosomes and secretion. Autophagosomes (AP) can be generated from endoplasmic reticulum-mitochondria contact sites or the plasma membrane where they internalize cell surface receptors and their ligands. Endosomes are generated by endocytosis of the plasma membrane and fuse with APs to form amphisomes, which are ultimately degraded into lysosomes. Secretory autophagosomes originated from omegasome-like structures, potentially from the ER, fuse with the plasma membrane, for secretion of their content. Dashed red arrows indicate processes amenable to bioengineering interventions that can be implemented for exosomal drug delivery or for exogenous interference with and manipulation of intercellular communication.

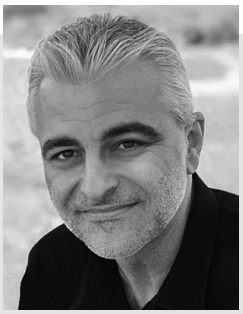
3.2 Exosomes as novel delivery vehicles

Cells which physiologically produce exosomes can be transfected to overexpress a specific gene such as an miRNA, water-soluble proteins or plasma membrane proteins which will then be contained in the exosome [52]. In a similar fashion, the exosome surface can itself be modified to be visualized and tracked in vivo after intravenous injection [53]. Moreover, cells can be engineered to package hydrophobic compounds into membrane vesicles by synthetic membrane fusogenic liposomes, which are efficiently incorporated into intracellular vesicles which are then secreted into the extracellular fluid [44]. This is of particular significance in the case of malignancy where chemotherapeutics cannot penetrate barriers in solid tumors. Recently, exosomes have been exploited for development of nanocarriers for drug delivery [52] . These exosomes are hybrids of exosomal membranes and liposomes. Genetically modified cells have been used to isolate exosomes embedded with specific membrane proteins which are then fused with liposome bionanotransporters. Whether specific autophagy inducing conditions could be implemented to trigger exosomal secretion for paracrine effects on neighboring cells remains to be tested.

4 Concluding remarks

In essence, autophagy is a highly dynamic process which demands trafficking and membrane components, much of which is 'delivered' from the endocytic pathway. Thus, they are converging pathways with many common players and their interplay is essential for the continuous changing needs of the cell, which is illustrated in Fig. 1. Autophagy can regulate endosomal secretion to form extracellular vesicles, which can in turn also regulate autophagy in a paracrine manner.

The notion that autophagy, endosomes and secretion are three distinct pathways which share components should be reconsidered as they are intertwined in a highly intricate manner and there are no clear-cut borders between these processes. A more spherical comprehension of their coordinated modulation should be performed by new technological advances, high throughput methods, imaging techniques and genetic engineering. This is of particular significance especially in the field of neuroscience, where most emerging age-related multifactorial diseases of this century involve abnormal protein aggregation, trafficking and secretion defects, the source of which could be common. The interaction between autophagy and the endosomal pathway targeted for either degradation or secretion will potentially permit novel bioengineering tools for various biomedical applications combining membrane engineering methods with endogenous inducers or genetic modification techniques.



Nektarios Tavernarakis is a Research Director at the Institute of Molecular Biology and Biotechnology, and Professor of Molecular Systems Biology at the Medical School of the University of Crete in Heraklion, Greece. He earned his PhD at the University of Crete, studying gene expression in yeast, and trained in *Caenorhabditis elegans* biology at Rutgers University, New Jersey, USA. His research focuses on the molecular mechanisms of necrotic cell death and neurodegeneration, the mechanisms of sensory transduction and integration by the nervous system, the interplay between cellular metabolism and ageing, and the development of genetic tools for *C. elegans* research. He is a member of the ERC Scientific Council, EMBO, and Academia Europaea. He is the recipient of two ERC Advanced Investigator Grants, the EMBO Young Investigator award, the Alexander von Humboldt Foundation, Friedrich Wilhelm Bessel research award, and the BioMedical Research Award of the Academy of Athens.

Margarita Elena Papandreou is a Marie Curie PhD student in the laboratory of Nektarios Tavernarakis, at the Institute of Molecular Biology and Biotechnology in Crete, Greece. She obtained her BSc from Imperial College London in Biology with a Year in Research in 2012 and her MRes in Biomedical Research, personalized healthcare, with an MRC fellowship. Her main objectives are to combine *C. elegans* genetics and mouse studies to discern the conserved mechanisms of neurodegeneration.

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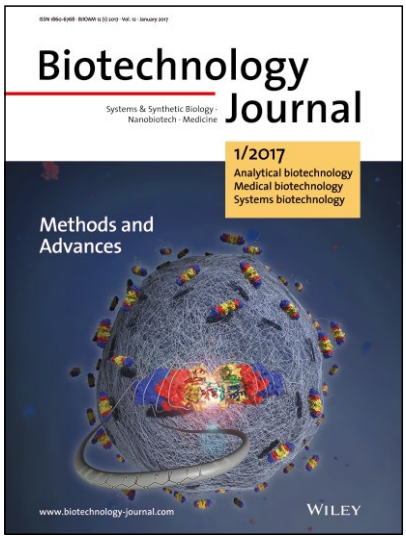

Cover illustration

Each year *Biotechnology Journal* kicks off with the special Methods and Advances issue which includes a series of Reviews and Methods on systems and synthetic biology, nanobiotech and medicine. The cover was provided by the authors of two back-to-back papers which unveil the 30-year mystery of polyhydroxyalkanoate (PHA) synthase from the groups of Professors Kyung-Jin Kim and Sang Yup Lee. The cover shows a cartoon of *Ralstonia eutropha* PHA synthase (shown in yellow, red and blue at the center and small ones around the granule) working on polymerization of hydroxyalkanoate substrates into PHA together with a PHA granule being formed (http://dx.doi.org/10.1002/biot.201600648; (http://dx.doi.org/10.1002/biot.201600649). The cover image was created by So Young Choi and Kyung-Jin Kim.

Biotechnology Journal – list of articles published in the January 2017 issue.