Maturation of autophagosomes and endosomes: A key role for Rab7

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Abstract

Macroautophagy is an important route in cellular maintenance, in the breakdown and reuse of intracellular materials. It is closely related to endocytosis, the means by which the cell can absorb extracellular material, as both macroautophagy and endocytosis have converging steps and common participating molecules. The point where autophagosomes and endosomes fuse with lysosomes to permit for the final degradation of their contents is important. One of the most substantial molecules in the maturation of autophagosomes/endosomes is Rab7, a member of small GTPases. Rab7 designates the maturation of endosomes and also autophagosomes, directing the trafficking of cargos along microtubules, and finally, participating in the fusion step with lysosomes. Rab7 is an effective multifunctional regulator of autophagy and endocytosis. Since many aggregation-based diseases, e.g. age-related macular degeneration of the eye (AMD) and Alzheimer’s disease are due of malfunctioning in the autophagic process, the management of Rab7 activity might hold potential as a therapeutic target against these diseases.

1. Introduction

1.1. Autophagy

Macroautophagy, herein referred to as autophagy, literally meaning “self-eating”, is a cellular catabolic recycling process in which cytosolic components, especially protein aggregates, aged or even malfunctioning whole organelles, are degraded. Autophagy, which is important for maintaining cell homeostasis, is also induced by nutrient deficiency and during various stress conditions, when part of the material of the cell is digested to help in the cell’s own maintenance [1–4]. The components to be degraded are engulfed by a flattened membrane sack called the phagophore or the isolation membrane. The organelle becomes an autophagosome once the phagophore is sealed to form a closed, double-membrane organelle. Autophagosomes are formed with the aid of several Atg proteins (autophagy related gene), including LC3 protein (microtubule-associated protein 1 light-chain 3; Atg 8 in yeast), namely its lipidated form LC3-II. The autophagosomes become matured in specialized multi-step processes. Finally, autophagosomes are fused with lysosomes, specialized acidic organelles for degradation of macromolecules delivered by endocytosis, phagocytosis, and autophagocytic pathways [5,6], and their contents are thereafter digested by lysosomal proteases, and reused by the cell (Fig. 1). Autophagy is observed in all eukaryotes, and seems to be evolutionary well-preserved, with an ancient origin [7]. It can generally be divided into nonselective autophagy, e.g. occurring during starvation, when the cell may consume a portion of its cytoplasm for self-sustenance, whereas selective autophagy serves as an important form of quality control, discriminatingly removing damaged or aged proteins and organelles as well as harmful aggregated proteins. This pathway supplements the ubiquitin–proteasome degradation system, which is the major extralysosomal protein degradation system in the cell [8].

Autophagy is also a crucial process in programmed cell death, as well as in tumor suppression, antiaging, and regulation of immunity [9,10]. Weakening or failing autophagy makes a contribution to cell death, especially in neural cells, when metabolic stress, aggregation of mutant proteins, and normal aging can impair autophagic signaling, leading to decreased autophagic vesicle turnover and autophagy-associated cell death, also called autophagic stress. Abnormal autophagy has been found in several neurodegenerative diseases including Parkinson’s, Alzheimer’s, and Huntington’s disease [11], as well as to age-related macular degeneration (AMD) in the eye [12,13].

1.2. Endosomes

In the view of its parallels with autophagy in this review, the endosome system functions in importing nutrients and macromolecules into the cell from outside, and acts as a companion route as a complement
to autophagy (operating with intracellular material) to provide amino acids and other degraded basic components for use by the cell. An important feature of autophagy is its relationship with endocytosis, which effectively terminates signaling activities of receptors, for example those associated with normal or aberrant cell growth and proliferation [14]. In this sense, endocytosis provides nutrients and macromolecules for the use of the cell from internal and external resources, respectively. A close relationship exists between these two catabolic systems and they share lysosomal degradation as the common terminal end-point. In fact, autophagosomes are capable of fusing with early or late phase endosomes (MVBs) themselves, forming organelles called “amphisomes”, before fusion into lysosomes [22,23] (Fig. 1), proving the tight relationship between these two vesicular transport and degradation pathways. As will be discussed later, common molecular motors transport endosomes and autophagosomes along microtubules in these maturation processes, including the small Rab GTPase proteins [24], discussed closely in the following chapters. The overlap between endocytosis and autophagy can be also detected by the localisation of endocytosed material within autophagic organelles. It has been found that endocytosis markers appear in autophagosome pathway after the early endosome phase, but not before that stage [25]. Most of those numerous molecules participating in both metabolic routes mentioned in the following are discussed below in this review (Section 2 and forward).

1.3. Convergence of autophagy and endocytosis

Both autophagy and endocytosis provide nutrients and macromolecules for the use of the cell from internal and external resources, respectively. A close relationship exists between these two catabolic systems and they share lysosomal degradation as the common terminal end-point. In fact, autophagosomes are capable of fusing with early or late phase endosomes (MVBs) themselves, forming organelles called “amphisomes”, before fusion into lysosomes [22,23] (Fig. 1), proving the tight relationship between these two vesicular transport and degradation pathways. As will be discussed later, common molecular motors transport endosomes and autophagosomes along microtubules in these maturation processes, including the small Rab GTPase proteins [24], discussed closely in the following chapters. The overlap between endocytosis and autophagy can be also detected by the localisation of endocytosed material within autophagic organelles. It has been found that endocytosis markers appear in autophagosome pathway after the early endosome phase, but not before that stage [25]. Most of those numerous molecules participating in both metabolic routes mentioned in the following are discussed below in this review (Section 2 and forward).

Fig. 1. Endosome maturation, autophagosome formation, and fusion with lysosome. Endocytosis is shown in the upper row. On the left, the early endosome is attached Rab5 on its surface, including the Mon1–Ccz1 complex. On the mature endosome (multivesicular body) Rab5 has been changed to Rab7, now itself with the Mon1–Ccz1 complex. The late endosome is fused with the lysosome (LY) and a hybrid organelle (on the right) is formed for the digestion of enclosed material. Autophagy is portrayed in the lower row. The phagophore membrane engulfs material to be enclosed in the autophagosome for degradation. Subsequently, a fully extended early autophagosome is formed. Thereafter, the mature autophagosome is labeled with the attachment of Rab7. The hybrid organelle (autolysosome) in the right is finally formed, when the autophagosome fuses with the lysosome. In the middle of the figure, in the fusion of the late autophagosome with the late endosome (or also with an early stage endosome) an amphisome is formed, and it is also fuses later with the lysosome and becomes degraded.

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paper we will discuss the role of Rab7, a protein belonging to the family of small GTPases, in these processes.

2. Rab 7 as a common modulator/participant in endocytosis and autophagy

2.1. Overview of Rab GTPases

GTP hydrolysis is a basic cellular reaction, which controls many vital processes. The GTPase proteins (guanosine-5’-triphosphatases) supply the catalytic apparatus which implements the conversion of GTP to GDP and vice versa. The superfamily of Ras GTPases comprise small sized proteins subdivided into Rab, Arf/Arl, Rho, Ras, and Ran families. It governs a broad range of cellular routes, like cellular trafficking, cytoskeletal regulation, proliferation, differentiation, sensory perception and signaling, and its members are interconnected together in diverse routes [26,27]. Changes in small GTPase functions are a sign of a number of diseases, for example due to alterations in the trafficking of cargo [28], and therefore members of this molecule group may serve as interesting targets for diagnostics as well as therapy. At present, the Rab family of GTPases consists of several dozens of members. These have been shown to be crucial or at least serve accessory roles in many of the steps regulating of several specialized processes of the cell connected to membrane trafficking: sorting, cytoskeletal transport of vesicles, and fusion with the target membranes. These processes take place in autophagy, endocytosis, phagocytosis, and pinocytosis, all of which are crucial cellular processes for taking up and autodigestion of material to be used by the cell [26,29].

Small Rab GTPases are regulated by various upstream regulators (Fig. 2). Lipid modification by Rab escort proteins (REPs) provides the lipid (geranyl) moieties to Rab for its stable and targeted attachment onto the membrane surfaces. Guanine nucleotide exchange factors (GEFs) change GDPs to GTPs. GTPase activation proteins (GAPs) change GTPs to GDPs. GDP dissociation inhibitors (GDIs) implement the attachment of Rab to the membrane, and GDI displacement factors (GDFs) [17,27,30]. A Rab effector protein is described as a protein or protein complex, which binds to a Rab in a GTP-dependent manner and is required for the downstream function determined by that particular Rab [31]. Consequently, the Rab effectors affect not directly that particular Rab, but indirectly through these effector types. Normally, Rab-GDP (inactive form) resides in the cytosol bound to its GDI. Rab is activated by GEF which converts the GDP bound form to the active GTP-form and it can associate onto membrane, and is inactivated by GAP, hydrolyzing GTP to GDP [31].

One member of the family of GTPases, Rab7 (Rab7A, NCBI nucleotide sequence no. NM_004637; orthologue Ypt7p in yeast, Saccharomyces cerevisiae), a small 208-amino acid protein, has been found predominantly on endosomes in their later/matured stages (MVB). It is involved in the transport of endosomes from early to late endocytic compartments of the cell (Fig. 1) [32–37]. Similarly, Rab7 has been implicated in the late maturation of autophagosomes [36,37]. Another member of Rab family, Rab5, (orthologue in S. cerevisiae VPS2, vacuole protein sorting 2), another member of Rab family, is converted to Rab7 in the process of maturation of endosomes and autophagosomes, and it will be discussed in more detail below (Section 3).

Rab7 is also important in the biogenesis and maintenance of lysosomes. In circumstances when there are not enough functional lysosomes, bound cargos may remain trapped in late endosomes/MVBs by the action of Rab7 and its factors [38]. In addition to endocytosis and autophagy, Rab7 has so far been recognized to possess functions in cadherin degradation by epithelia and neurons, as well as in bone resorption by osteoclasts [27]. Autophagosome membrane marker protein LC3-positive autophagic vacuoles accumulate in large aggregates in the perinuclear area, and Rab7 is essential in this process [37].

2.2. Regulation of Rab7

Rab7 occupies a critical nexus in the endocytic pathway, since it governs the change of early into late endosomes, and then their trafficking, and fusion to lysosomes [39]. There have been conflicting opinions of the exact role of Rab7, since some researchers report that Rab7 regulates the trafficking as being from early to late endosomes [40], while others have reported its functions in trafficking between late endosomes and lysosomes [41]. It is unclear whether Rab7 is functional in both in early and late endosomes, or is it simply positioned in the early stages so that it can be activated and used immediately in the late phase endosomes. Vanlamingham and Ceresa [42] conducted RNA interference (RNAi) studies and reported that in the absence of Rab7, the trafficking did not change between early and late endosome stages, but exiting from the late endosomes/multivesicular bodies was blocked. Silencing of Rab7 by RNAi caused the accumulation of enlarged, densely packed late endosomes and MVBs, and decreased size and number of lysosomes. Consequently, Rab7 is required for the transfer of the cargo from the late endosomes and multivesicular bodies to lysosomes (Figs. 1 and 3). This engagement has been detected in both endosome and autophagosome trafficking (Section 4).

Rab7 has the general upstream effector types for small GTPase proteins (e.g. GEFs and GAPs) as discussed in Chapter 2.1 (Fig. 2) [43–45]. As an example, Homotypic fusion and vacuole protein sorting complex (HOPS), for example VPS39 and TBC1D15 (Gyp7p in yeast) are known as GEF and GAP factors for Rab7, respectively. The known upstream effectors regulating this balance include specific Rab7 REPs and GDIs [43–45].

Multiple downstream effectors of Rab7 have been characterized (Fig. 2). A liposome bioactive compound (LBC) was identified and characterized (Fig. 2). RILP (Rab7-interacting lysosomal protein) and FYCO1 (FYVE- and coiled-coil domain containing 1) act in the trafficking, Mon1/Ccz1 complex functions in the change of Rab (Section 3; Fig. 1). The HOPS complex acts as a Rab7 factor and participates in the trafficking and of organelle cargos and fusion with lysosome [46]. The Retromer trafficking complex (composed of sorting nexin subunits and a VPS26/29/35 trimer) regulates retrograde transport from endosomes to the trans-Golgi network, and also controls the late stages of endosome maturation [47,48]. Rab7-interacting ring-finger protein (Rabring 7), when overexpressed, can cause the perinuclear aggregation of lysosomes [49]. Rubicon protein controls the trafficking and maturation of endosomes by affecting Rab7 [50] (Section 6). This complex signaling system...
highlights the important role of Rab7 in the regulation of endo-lysosomal and autophagosomal membrane trafficking [30]. Recently, the knockout of COP9 signalosome protein complex (CSN, regulating ubiquitin–proteasome clearance system), in particular the member called CSN8 (one of its 8 subunits), was found to downregulate Rab7, causing impaired maturation of nonselective and selective autophagy, followed by autophagosome accumulation, and ultimately necrosis in cardiomyocytes [50]. Phafin1, a FYVE and PH (Pleckstrin homology) domain containing protein also participates in the activity of Rab7 both in endocytosis and autophagy. Binding of phafin1 to lysosomes seems to be mediated by Rab7 signaling. Furthermore, over-expression of phafin1 may induce autophagy by directly activating the signaling processes, bypassing the amino acid deprivation triggering mechanisms, and this is at least partly mediated by Rab7 [51]. Phafin1 also recruits p53 to lysosomes and triggers caspase-independent apoptosis [52]. Therefore, it is possible that phafin1 is involved in mediating the autophagy-related cell death [51].

Other molecules taking part in both autophagy and endocytosis, in connection with Rab7, include UVRAG (UV radiation resistance-associated gene) protein, a mediator of Rab7 activation and consequently endosome maturation, which is localized in both autophagosomes and early endosomes. UVRAG can also interact with Rab5, “the antecedent” of Rab7 on endosomes [53,54]. ESCRT (endosomal sorting complex required for transport) complexes (0, I, II, and III) perform the intricate task of endosomal sorting, and it involved in the maturation processes and promotes the fusion with other late endosomes and lysosomes. In yeast, Ypt7 is directly involved in the assembly of the fusion machinery at late endosomes and the yeast vacuole (corresponds to mammalian lysosome) membrane [60,61]. The depletion of Rab7 does not affect the maturation of late autophagic vacuoles developing from the fusion of MVBs and autophagic vacuoles, but Rab7 is essential for the final step in the fusion to lysosomes [37].

Only recently, the chemical modulation of Rab7 by synthetic molecules disturbing the change between GDP/GTP states has been illustrated by Agola and others [39]. A certain small molecule (a pyran compound derivative) proved to be a competitive inhibitor of GTP binding to Rab7. These types of compounds might hold potential as therapeutics in diseases where the modulation of the activity of small GTPases might represent a feasible strategy. Moreover, this approach may help to clarify the precise functions of individual GTPases, which are still largely unresolved.

3. Transition from early to late endosomes and the maturation of autophagosomes

Rab5 (Chapter 2.1) modulates the early endosome formation, e.g. by regulating PI(3)P synthesis on endosomal structures, and it participates the transition of endosomes from their early to late stage. Rab5 is activated by its GEF, Rabex5 protein. Subsequently, this activates effector molecules like VPS34, which produces PI(3)P, which in turn increases the binding of Rab5 and its effectors. There is obviously a strong positive feedback loop in the activation of Rab5 [62,63].

The crucial step in the maturation of endosomes is the change of Rab5 to Rab7 (Fig. 1). Mon1–Ccz1 is an evolutionary well preserved protein complex, physically interacting as a stable pair. It facilitates the Rab exchange by displacing Rabex5 (a GEF of Rab5) and activating a GEF of Rab7 [64–66]. According to Rink and others [67], this factor is VPS39, a HOPS member, as predicted from the studies conducted on the activity of the yeast orthologue VPS39p/Vam6p towards the Ypt7p (Rab7 orthologue in yeast). Mon1 (as also its orthologue Sand1 in Caenorhabditis elegans) is known to be an inhibitor of Rab5 activation, and it “prepares” for the exchange of Rab5 to Rab7 on endosomes with its interaction of endosomes, blockage of cargo trafficking to the lysosomes, and the deterioration of lysosome acidification and functionality [38]. Rab7/Ypt7p coordinates maturation processes and promotes the fusion with other late endosomes and lysosomes. In yeast, Ypt7 is directly involved in the assembly of the fusion machinery at late endosomes and the yeast vacuole (corresponds to mammalian lysosome) membrane [60,61]. The depletion of Rab7 does not affect the maturation of late autophagic vacuoles developing from the fusion of MVBs and autophagic vacuoles, but Rab7 is essential for the final step in the fusion to lysosomes [37].

Fig. 3. The motility of the late autophagosome/endosome on the microtubulus. The movement to proximal, perinuclear area (in the minus direction of the microtubulus) is directed by a dynein/dynactin (p150glued) motor. βIII spectrin acts as the dynactin receptor on the organelle to be moved. RILP mediates the action of Rab7. ORP1L inactivates dynein in the presence of low cholesterol. The movement to distal direction (in the plus end of the microtubulus) is driven by kinesin, mediated by FYCO1, which is attached to Rab7-GTP. Both apparatuses are represented here in simplified forms.
with the HOPS complex. Mon1/Ccz1 replaces Rabex5, Rab7 interacts with VPS39, and Mon1/Ccz1 complex binds to Rab7, and controls its localization on late endosome and subsequent activation. This is proved by the finding that Rab7 does not precede Sand1 (Mon1) on endosomes. Moreover, either Mon1 or Ccz1 alone cannot bind and activate Rab7. Consequently, the endosome becomes matured to the late phase and is ready to be transported and fused with lysosome [66,68]. However, it has also been proposed, that VPS39 does not have GEF activity in mammalian cells [69]. The GEF might be the Mon1-Ccz1 complex itself, as shown in yeast [70]. The timing of Mon1-Ccz1 association with early endosomes is crucial, because Rab conversion should not occur before the endosome has accumulated enough cargo for degradation. It has been suggested that the concentration of PI(3)P on endosomes is the determining factor in the timing of the Rab change [68,71]. Possibly this is sensed by Sand1 (or the Mon1 orthologue in mammals), which binds to endosomes enriched with PI(3)P [68]. Recently, a hypothetical mathematical model has been described for the change of Rab5 to Rab7 during endosome maturation. It has been proposed that the change of pH may be one crucial factor in the conformational change of endocytic cargo receptors [72].

Irrespective, whether or not Rab5 has direct role in autophagy, it was recently reported by Su et al. [73], that Rab5 inhibition could evoke a reduction of hepatitis C induced autophagy vesicle formation. In addition, Rab5 has been shown to act at the early stage of autophagosome formation in Huntington’s disease, accumulating a toxic protein [74]. Rab5 was found to form a macromolecular complex with Beclin 1, an essential autophagy-related protein (Section 6) and Vps34, and inhibition of Rab5 decreased the number of LC3-labeled autophagic vesicles, just as Rab5 overexpression increased their numbers. In addition, Ravikumar and others also [74] found that silencing of Rab5 caused a reduction in autophagic vesicle formation in hepatitis C induced autophagy. Although Rab5 has been previously thought to be exclusively involved in autophagocytosis, it might also possess a role also in autophagy, at least in the early stages.

Generally the Rab conversion allows the late endosomes to fuse only with other late endosomes, lysosomes and autophagosomes. In the late stages RILP (Rab7-interacting lysosomal protein)/ESCRT-II interacts with Rab7 [56]. These proteins might also be involved in the maturation of endosomes [30]. The endosome membrane-spanning cargo (excluding soluble endolytic consignments) is sequestered into intraluminal vesicles, coordinated by ESCRT complexes 0, I, II, and III [18,75]. Then the cargo reaches the CD63 (complex of differentiation)-positive compartments, fuses with lysosomes, and ultimate, the machinery achieves the proteolysis of the cargo [76].

4. Mechanisms of transport of late endosomes and autophagosomes

Molecular motors transport endosomes along microtubules during maturation and finally they are moved to late endocytic/lysosome compartments. The movement occurs either to the minus-end (oriented towards the cell center, containing the microtubule organizing centre) or to peripheral, that is plus-end of the microtubulus [17,35,77]. Simplified molecular assemblies of both movement vehicles are illustrated in Fig. 3. The transport of late endosomes in the former direction is coordinated by a complex interaction system including a dynein-dynactin motor, the actual “machine” needed for the movement. ORP1L (oxysterol-binding protein-related protein 1 L) is colocalized with Rab7, Rab9, and LAMP1 (lysosome-associated membrane protein 1, a late endosome/lysosome marker protein) on endosome membrane [78]. Endosome-bound active GTP-Rab7 binds to ORP1L and Rab7 effector protein RILP simultaneously. Cholesterol also regulates the endosomal positioning and its level on late endosomes is screened by ORP1L [79]. The C-terminal ORD (oxysterol-binding protein-related ligand binding domain) of ORP1L interacts with membrane-bound cholesterol, creating a conformation state, which allows the Rab7-RILP complex to interact with the dynactin projecting arm p150Glued, which is required for dynein motor recruitment to late endocytic compartments. Next, the Rab7-RILP complex is transferred by ORP1L to the jill spectrin. Dynein can start the translocation of late endosomes to microtubules only after interacting with jill spectrin (the receptor for dynactin on the endosome), but these processes require the action of Rab7-RILP and ORP1L [80]. Dynein attaches to the cargo through its light chains. The other head, the heavy chain with its head moves along the microtubule and carries the cargo along in the minus-end direction to the late endocytic compartments [37,76].

Rab7 is also involved in microtubulus plus-end directed transport to the cell periphery, which is required during maturation [30]. In that movement FyCO1 (Chapter 2.2), a recently found large effector protein interacts with its C-terminal FYVE and GOLD (Golgi dynamic) domains with Rab7, LC3, and PI(3)P on endosomal or autophagosomal membrane, and with its N-terminal coiled-coil domain with the kinesin motor protein, which mediates the movement along the microtubulus in the plus-end direction (Fig. 3). If FyCO1 is overexpressed, it can redistribute Rab7-ORP1L containing vesicles to the periphery, suggesting that FyCO1 itself may regulate the microtubules plus-end directed movement of endosomes, by binding to kinesin motors [30,58].

As described earlier, Rab7 interacts with both the minus- and plus-end direction transport on microtubules, depending on the interacting effector(s) and motor protein (RILP/dynactin(p150Glued)/dynein), or FyCO1/kinesin, respectively, and both directions of movement, are controlled by several distinct effector-interacting cascades [30]. Interestingly, only one dynein protein is found so far, whereas a large family of kinesin motors is known to exist [81].

Similar to the endosomes, autophagosomes are moved with dynein and kinesin motors during maturation, as described recently in primary neurons by Maday and others [24]. Autophagosomes are formed in the distal parts of the cell, accumulated, and transported continuously bidirectionally in their maturing process, and finally they are switched into a unidirectional movement to the cell soma (proximal course) by a dynein/dynactin motor, when the kinesin motor which moves in the opposite (distal) direction, becomes downregulated. Furthermore, as autophagosomes move to proximal areas, they become more acidified. To sum up, autophagosome and endosome maturation to the late stage are both temporally and spatially regulated [24,82].

5. Fusion of endosomes/autophagosomes with lysosomes

The fusion of sealed membranes joins their enclosed contents while mixing the bilayers. The fusions of autophagosomes/endosomes with lysosomes have been studied morphologically in detail, but less progress has been made in the molecular elucidation [2]. A large superfamily of specific small proteins, SNAREs (SNAP (soluble NSF attachment protein) receptors) (NSF = N-ethyl-maleimide-sensitive fusion protein), increase the permeability of membranes and create the actual membrane opening leading to fusion of the contents of two adjacent organelles [31,83].

The dynamics of the yeast vacuole (resembling the lysosome in higher eukaryotes) fusion with late endosomes/MVBs has been used to model endosome and autophagosome fusion with lysosomes [61,84,85]. The reaction involves Rab7 (Ypt7 in yeast), HOPS effector and specific members of SNAREs, proteins, that become enriched on the structure surrounding the future fusion site. In the first phase, priming, the fusing organelles are prepared for the contact and fusion, Rab7/Ypt7 is converted into its GTP containing form by the action of HOPS, and the cis-SNARE complexes (neighbors on the same membrane) are disassembled to produce active, fusion competent single SNAREs. The tethering phase is dependent on the HOPS tethering complex and the Rab7/Ypt7-GTP proteins, present in both membranes to be fused. In the docking and fusion phases, unpaired single SNAREs, previously primed, assemble themselves into trans-complexes between opposite membranes in close proximity, this process being coordinated by Rab7/Ypt7 proteins. Consequently, the proteins form a “zipper”-complex which directly triggers the membrane fusion,
the “opening” of the bilayers and fusion of vacuoles occurs, either so-called kiss and run or direct fusions [61,84,85]. Neither the mechanism nor the various individual SNARE proteins participating in the endosome/autophagosome fusion with lysosomes will be discussed further in this review.

All the processed in endosome and autophagosome fusion and the endosomal-lysosomal pathway are NOT identical. Each route has its own distinctive characteristics. As shown, thapsigargin, an inhibitor of the endoplasmic reticulum calcium pump and also an autophagy inducer can block the recruitment of Rab7 to autophagosomes and this prevents the fusion between lysosomes, but this drug does not affect the fusion between endosomes and lysosomes [86]. This emphasizes again the vital role of Rab7 in the last phases of autophagy.

6. Regulation of endosome and autophagosome maturation by Rubicon

Rubicon (RUN domain protein as Beclin 1 interacting and cysteine-rich containing) (RUN: RPIPB–UNC-14–NESCA), a member of the PI3KC3 (phosphatidylinositol 3-kinase class 3) complex, inhibits both endosome and autophagosome maturation by preventing the activation of Rab7 [14,54]. Rubicon binds to Beclin 1, the mammalian homologue of yeast Apg6, an essential autophagy-related protein, and also a tumor suppressor and it also sequencers UVRAG (UV radiation resistance- associated gene) protein, and thus preventing it from activating class-C-VPS/HOPS, the GEF for Rab7 [87]. UVRAG protein activates the Beclin 1-Pi3(3)/Kc3 complex, thus promoting autophagy and suppressing tumorigenicity. Rubicon binds directly to Rab7 through its C-terminal RH (Rubicon homologue) domain, acting as the effector, and simultaneously to the Beclin 1-Pi3 kinase complex, which are required for the action of Rubicon and its localization to the endosome membrane. Overall, Rubicon mediates endocytic trafficking and endosome maturation by controlling the simultaneous action of beclin1, Rab7, and UVRAG [54,88].

PLEKH1M1 (pleckstrin homology domain containing, family M (with RUN domain) member 1), a Rubicon homologue, also binds to Rab7 with its C-terminal RH domain, but it does not bind to the Beclin 1–Pi3–kinase complex. This leads to the fact that PLEKH1M1 can regulate endocytic transport but not autophagosome maturation [88].

7. Rab GTPases and mTOR signaling

mTOR (mammalian target of rapamycin) protein kinase, in particular its functional complex 1 mTORC1, represents the link between nutrient and hormonal status with protein synthesis and cell growth [4]. mTORC1 operates also in endosomal trafficking and maturation in flies [89], and it has been indeed localized in endosomes in yeast and flies [90], as well as in mammalian cells [91].

The possibility that mTOR would be associated with endosomal trafficking in mammalian cells was recently proposed by Flinn and others [45]. The inactivation of Rab7 by knockdown of its GEF VPS39 evoked the inactivation of mTORC1/S6K1, and subsequently mTORC1 was localized to hybrid early/late endosomes. S6K1 is a S6 ribosomal kinase and it functions as a translational regulator [92]. The inhibition of other stages of endocytic trafficking did not change the activity of mTORC1. This suggests that intact late endosomes are crucial for the amino acid and insulin-stimulated mTORC1 signaling, and it is the endosome composition rather than its sorting functions that is important. In early endosomes/autophagosomes, Rab7 seems to regulate mTORC1 function, since nonfunctional Rab5 disrupts the activity and proper localization of mTORC1 [93].

8. Conclusions and future prospects

Disorders in autophagy clearance have been claimed to be involved in many neurodegenerative diseases including AMD. In post-mitotic cells, lysosomal function is usually impaired due to the accumulation of lipofuscin during the aging process. It has been speculated that this may secondarily disturb autophagy clearance and evoke cellular degeneration. However, the regulation of fusion processes between lysosomes and autophagosomes is poorly understood in neurodegenerative diseases. However, this may represent a critical property in these diseases since this fusion step determines the autophagy flux. The impaired function of a small GTPase protein, Rab7, which is crucial participant standing at the crossroads of the transition of both autophagosomes and endosomes where these structures move from early to late phase, trafficking of the cargos, and to fusion with lysosomes might be a potential point to target the pathology of neurodegenerative diseases. AMD is certainly a candidate disease in this respect.

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