Rab GTPases in Autophagy

Yuko Hirota, Keiko Fujimoto and Yoshitaka Tanaka

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/54578

1. Introduction

Rab proteins constitute a subfamily of small GTPases that play important roles in the spatio-temporal regulation of intracellular vesicle transport [1-3]. Rab GTPases represent a large family of small guanosine triphosphate (GTP)-binding proteins that comprise more than 60 known members. In mammalian cells, it is well established that different Rab proteins localize on distinct membrane-bound compartments, where they regulate multiple steps in membrane traffic, including vesicle budding, fusion, and movement, through cycling between an inactive guanosine diphosphate (GDP)-bound form and an active GTP-bound form [3]. Guanine nucleotide exchange factor (GEF) shifts GTPase from its inactive GDP-bound form to its active GTP-bound form, while GTPase activating domain protein (GAP) inactivates GTPase [Figure 1]. Many structural and biological studies have shown that specific amino acid mutation can make possible to keep Rab GTPase in its GDP-bound form or GTP-bound form; therefore, expression of GDP-bound form or GTP-bound form could imitate its function [3].

Autophagy is a degradation pathway that delivers cytoplasmic components and intracellular organelles at random and/or in a selective manner to lysosomes via doubled-membrane organelles called autophagosomes [4]. Although autophagy is induced by exposure of cells to nutrient- or growth factor-deprived medium, it also occurs at basal levels in most tissues and contributes to the routine turnover of cytoplasmic materials. So far, in the process of the formation of autophagosomes, many mammalian homologues of yeast ATG (autophagy-related) genes have been identified and extensively characterized, demonstrating that the molecular machinery of autophagy has been conserved from yeasts to mammals [5]. Analyses of the Atg proteins have identified two ubiquitin-like conjugation systems that are required for autophagosome formation [6]. Among these proteins, Atg5 and LC3 (a mammalian homolog of Atg8) have been analyzed in more detail. An Atg12-Atg5 conjugate is necessary...
for elongation of the isolation membrane on its outer side [7]. ProLC3 is processed by Atg4, a cystein protease, to a cytosolic form, LC3-I, exposing a carboxyl terminal glycine [8]. LC3-I is subsequently activated by the E1-like protein Atg7, and conjugates phosphatidylethanolamine to its C-terminal glycine via the E2-like enzyme Atg3, producing a membrane-bound form, LC3-II [9]. LC3-II localizes on the isolation membrane and the autophagosome membrane [10, 11]. Because the amount of LC3-II correlates with the number of autophagosomes, detection of LC3-II by Western blotting can be used to measure autophagic activity. In addition, dot-like or ring-like staining of LC3 in immunofluorescence is widely utilized as a specific marker of the formation of autophagosomes.

Akin to the involvement of Rab proteins in vesicle transport processes, there is a growing body of evidence that many Rab proteins, such as Rab7, Rab9, Rab11, Rab24, Rab32, and Rab33, function in the formation and/or maturation of autophagic vacuoles. Each of these Rab proteins localizes to distinct intracellular compartments and thereby appears to be involved in a distinct step of autophagic flux. In this chapter, we focus on the roles of these Rab proteins in the regulation of the autophagic process.

2. Rab7 GTPase and autophagy

2.1. Rab7 GTPase and autophagosome maturation

Rab7 is a member of the Rab family, which is involved in vesicle transport from early endosomes to late endosomes/lysosomes as well as lysosome biogenesis [12, 13]. Rab7 is also
implicated in the fusion between autophagosomes and lysosomes, i.e., autophagosome maturation [14-16]. Rab7 wild-type (WT) and active-forms of Rab7 (Rab7Q67L) are associated with ring-shaped vesicles labeled with the autofluorescent compound monodansylcadaverine (MDC), which is preferentially incorporated into mature autophagosomes and autolysosomes, and with LC3, which preferentially labels immature autophagosomes, indicating the association of Rab7 with autophagic vesicles [14]. On the other hand, overexpression of the inactive form of Rab7 (Rab7T22N) causes a marked increase in the size of MDC- and LC3-positive vesicles and the number of LC3-positive vesicles, but reduces the number of MDC-positive vesicles, indicating that the inactive-form of Rab7 impaired the fusion between autophagic vacuoles and lysosomes. Similar results were also obtained in cells depleted of Rab7 by RNAi [15]. Collectively, these results suggest that Rab7 is not essential for the initial step of autophagosome maturation, but is involved in the final step of the maturation of late autophagic vacuoles, possibly in the fusion with lysosomes [14, 15]. Interestingly, Rab7T22N that was diffusely localized in the cytoplasm under nutrient-rich conditions was redistributed to the membrane of MDC-positive vacuoles by amino acid starvation or by rapamycin treatment [14]. Thus, Rab7 is targeted to the autophagosomal membranes by a TOR (target of rapamycin)-kinase signal transduction mechanism in response to starvation.

SNARE proteins and the class C Vps (C-Vps) complex as well as Rab7 have been implicated in mammalian autophagy. In *Saccharomyces cerevisiae*, the fusion of autophagosomes and vacuoles is assumed to proceed in an identical manner to that of endocytic fusion, depending on SNARE proteins, Rab GTPase Ypt7, the yeast ortholog of mammalian Rab7, and its GEF, C-Vps tethering complex, all of which are known as regulators of the endocytic pathway [17-19]. Interestingly, while Rab7 and the C-Vps complex component Vps16 are essential for endocytic fusion with lysosomes, Rab7 but not Vps16 is required for complete autophagy flux in an autophagy induced by thapsigargin, an inhibitor of the sarco/ER Ca\(^{2+}\) ATPase [20]; therefore, autophagosomal-lysosomal fusion might be controlled by a molecular mechanism distinct from general endocytic fusion.

During autophagy, autophagosomes fuse with lysosomes to degrade materials within them by lysosomal hydrolases. So far, little is known about the fate of autolysosomes. Recently, it has been shown that mTOR regulates the termination of autophagy and reformation of lysosomes [21]. When cells are exposed to starvation, mTOR is inhibited, leading to the autophagy induction; however, prolonged starvation causes reactivation of mTOR and this reactivation generates proto-lysosomal tubules and vesicles from autolysosomal membranes to reform into functional lysosomes. Interestingly, the dissociation of Rab7 from autolysosomes is required for the reformation of lysosomes, and overexpression of the active form of Rab7 results in the accumulation of enlarged autolysosomes [21]; therefore, mTOR might regulate the reformation of lysosomes from autolysosomes via Rab7.

### 2.2. Rab7 GTPase and pathogen-containing autophagosome

Many pathogens are sequestered in phagosomes and fated to be degraded, since these phagosomes undergo a process of maturation, fusing with lysosomes [22, 23]; however, some pathogens reside in vacuoles that interact with other organelles, such as mitochondria, ER and
Golgi, while others escape from phagosomes or remain in vacuoles that neither acidify nor fuse with lysosomes [24]. In contrast, *Coxiella burnetii* bacteria, the agent of Q fever in humans and of coxiellosis in other animals, live and replicate in acidified compartments with phagolysosomal characteristics. Lysosomal membrane proteins and enzymes are found in vacuoles containing *C. burnetii* [25]. In HeLa cells infected with *C. burnetii*, vacuoles containing these parasites and labeled with acidotropic probe LysoTracker were also labeled with MDC and LC3. Moreover, 3-methyladenine and wortmannin, known as reagents to inhibit the early stage of autophagosome formation, blocked the development of *Coxiella*-containing vacuoles [26]. These results suggest that *Coxiella*-containing vacuoles interact with the autophagic degradation pathway. Interestingly, exogenously expressed wild-type Rab7 and the active form of Rab7 colocalize with *Coxiella*-containing vacuoles, whereas the inactive form of Rab7 does not. This indicates that Rab7 associates with the biogenesis of *Coxiella*-containing vacuoles [26].

Also, the initial formation of Group A streptococcus-containing autophagosome-like vacuoles is prevented by expression of the inactive form of Rab7 or downregulation of Rab7 expression with RNAi, suggesting that Rab7 is required for the early stage of the formation of Group A streptococcus-containing autophagosome-like vacuoles [27].

### 2.3. Rab7 GTPase and interaction molecules

Rubicon (Run domain protein as Beclin 1 interacting and cysteine-rich containing) is a component of the class III phosphatidylinositol 3-kinase (PI3KC3) complex. PI3KC3 forms two protein subcomplexes that localize to autophagosomes or early endosomes and perform distinct functions. The autophagosomal subcomplex consists of the PI3KC3 core complex (hVps34, p150/Vps15, and Beclin 1) and Atg14L [28]. Atg14L is the targeting factor for this complex to the early stage of autophagosomes. The endosomal complex is composed of the PI3KC3 core complex, UV irradiation resistance-associated gene (UVRAG) and Rubicon. UVRAG activates PI3KC3 and is needed to mature autophagosomes and endosomes through its direct interaction [29]. UVRAG also interacts with C-Vps, and this interaction accelerates autophagosome recruitment and activation of Rab7, which facilitates autophagosome maturation [30]. On the other hand, Rubicon specifically interacts with Rab7 through the common C-terminal domain, called a regulator of G-protein signaling homology (RH) domain but not RUN domain (for RPIP8, UNC-14, and NESCA) to inhibit autophagosome maturation [31, 32]. The overexpressed active form of Rab7 competed with UVRAG for Rubicon binding much more efficiently than the inactive form of Rab7 [31]. Thus, Rubicon is a negative regulator of autophagosome maturation. Interestingly, Rubicon homologue, PLEKHM1, which contains an RH domain, specifically interacted with Rab7, and this interaction is important for their function [32]. In contrast to Rubicon, PLEKHM1 does not directly suppress autophagosome maturation. Rubicon, but not PLEKHM1, also interacted with the Beclin 1-PI3-kinase complex [32]. Rubicon functions to regulate the endocytic and autophagic pathways under the control of the association with Beclin 1-PI3-kinase complex or Rab7.

Phosphatidylinositol-3-phosphate (PI3P) is essential for autophagosome formation. Although the PI3P function in autophagy is unknown, it is already considered that effector proteins containing the FYVE (Fab1/YOTB/Vac1/EEA1) domain or PX (phox) domain are recruited to
and activated on PI3P-enriched membranes. Recently, FYCO1 was identified as a novel protein interacting with LC3, Rab7, and PI3P [33]. FYCO1 interacts with Rab7 and PI3P via part of the coiled-coil domain and FYVE domain, respectively. Overexpression of FYCO1 redistributes LC3, Rab7, and ORP1L, a Rab7 effector protein, to the cell periphery in a microtubule-dependent manner [33]. In contrast, FYCO1 depletion leads to the accumulation of perinuclear clustering autophagosomes, indicating that FYCO1 binds to PI3P via its FYVE domain and functions as a Rab7 and LC3 effector molecule with microtubules plus end-directed transport.

3. Rab9 GTPase and autophagy

Rab9 GTPase resides in late endosomes, in which Rab7 localizes in a distinct microdomain, and plays a role in vesicle transport from late endosomes to the TGN [34]. Rab9 depletion using siRNA decreased the size of late endosomes and reduced the number of late endosomes/lysosomes, which were clustered in the perinuclear region [35], implying that Rab9 is associated with the maintenance of late endosomes/lysosomes.

Generally, it has been believed that Atg5 and Atg7 are essential for mammalian autophagy [36, 37]. In contrast, mouse embryonic fibroblasts deficient in Atg5 or Atg7 can still form autophagosomes and autophagic flux can function when exposed to autophagy-inducible stress conditions, and the lipidation of LC3 (autophagosome membrane-bound form) is also dispensable for this Atg5-/Atg7-independent autophagy [38]. Interestingly, in this alternative process of autophagy, but not in Atg5/Atg7-dependent conventional autophagy, the formation of autophagosomes seemed to be regulated in a Rab9-dependent manner by the fusion of isolation membranes with the TGN- and late endosome-derived vesicles [38]. In fact, the localization of Rab9 to autolysosomes was slightly increased with the active form of Rab9 (Rab9Q66L), but decreased with the dominant-negative form of Rab9 (Rab9S21N). Additionally, Rab9 silencing by siRNA decreased the number of autophagosomes but induced the accumulation of isolation membranes [38]. Thus, Rab9 plays a significant role in Atg5-/Atg7-independent autophagy.

4. Rab11 GTPase and autophagy

Rab11 has been shown to associate with perinuclear recycling endosomes and regulate transferrin recycling in CHO or BHK cells [39]; however, in K562, an erythroleukemic cell line, Rab11 localizes at MVBs, which are equivalent to late endosomes and are released into the extracellular space as so-called exosomes [40]. Overexpression of wild-type Rab11 and its active-form mutant produced large MVBs. Induction of autophagy by starvation or mTOR inhibitor rapamycin significantly increased the fusion between MVBs and autophagosomes [41]. This fusion was disturbed by the Ca\(^{2+}\) chelator BAPTA-AM and by the expression of the inactive form of Rab11 [41], indicating that the fusion of MVB with autophagosomes is a calcium- and Rab11 activity-dependent event.
Rab GTPase activity is controlled by GEF and GAP. Thirty-eight putative RabGAPs with a Tre-2/Bub2/Cdc16 (TBC) domain have been identified [43]. Recently, it was thought that RabGAP might be associated not only with the cellular endomembrane system but also with autophagy. In fact, TBC1D5 is identified as an interacting partner of LC3 and retromer complex and regulates the autophagy pathway and retrograde transport of cation-independent mannose 6-phosphate receptor from endosomes to the TGN [44]. Another RabGAP, TBC1D14, can bind a mammalian homologue of Atg1p ULK1, as can Rab11, and disrupts recycling endosome traffic [45]. Furthermore, under starvation conditions, TBC1D14 and Rab11 modulate the membrane transport from recycling endosomes to generate autophagosomes. TBC1D14 overexpression caused the tubulation of ULK1- and Rab11-positive recycling endosomes irrespective of nutrition conditions, impairing their function and preventing autophagosome formation [45]. However, the tubulation of recycling endosomes induced by the expression of TBC1D14 was dependent on Rab11 expression, since Rab11 depletion using siRNA gave rise to a loss of tubules and a diffuse distribution of TBC1D14 throughout the cytosol [45]. Amino acid-deprived starvation caused TBC1D14 relocation from recycling endosomes to Golgi, while the ULK1- and LC3-positive recycling endosome membrane was incorporated into the forming autophagosomes [45]. Thus, TBC1D14- and Rab11-dependent membrane transport from recycling endosomes participates in and controls starvation-induced autophagy.

5. Rab24 GTPase and autophagy

Rab24 is localized to perinuclear reticular structures that partially colocalize with marker proteins for ER, cis-Golgi, and ER-Golgi intermediate compartments [46]. Under starvation conditions, Rab24 relocated to large vesicles, where LC3 was localized. The appearance of these vesicles was enhanced in the presence of vinblastin, an agent that disrupts microtubules and prevents fusion of autophagosomes with lysosomes [46]. Interestingly, since no such distribution change was observed in cells expressing the mutant Rab24S67L that introduced the mutation into the GTP-binding motif, normally functioning Rab24 protein appears to be required for the formation of autophagosomes in response to starvation. *Coxiella burnetii* survives and replicates in MDC- and LC3-positive phagolysosomal compartments and Rab7 participates in the formation of *Coxiella*-containing vacuoles [26]. Overexpression of Rab24 or LC3 also accelerated the occurrence of *Coxiella*-containing vacuoles early after infection [47]. The expression of the Rab24 mutant (Rab24S67L), which does not localize to autophagosomes, significantly reduced the number and size of the phagolysosomal structures, although the inhibitory effect was not enduring but mutant expression delayed the generation of phagolysosomes [47]. Taken together, these results suggest that overexpression of proteins involved in the autophagic pathway, such as Rab24, increases the development of phagolysosomes for *Coxiella* replication.

Rab24 is also supposed to contribute to the degradation of aggregated proteins in rat cardiac myocytes. Glucose deprivation induced the formation of aggregates and aggresomes of polyubiquitinated proteins, and then they colocalized with exogenously expressing green
fluorescent protein (GFP) tagged-LC3 and endogenous Rab24 [48]. Autophagy induced by glucose deprivation seemed to depend on the reactive oxygen species, because the treatment with N-acetylcysteine prevented aggresome formation and autophagy [48]. These results might imply that glucose deprivation induces oxidative stress, which is involved in aggresome formation and autophagy via Rab24 in cardiac myocytes.

6. Rab32 GTPase and autophagy

Mouse Rab32 and Rab38 operate in a functionally redundant manner in regulating skin melanocyte pigmentation and regulate post-Golgi trafficking of tyrosinase and tyrosinase-related protein 1, thereby suggesting their critical roles in melanosome maturation [49]. In Xenopus melanophores, Rab32 is involved in the regulation of melanosome transport by cAMP-dependent protein kinase A [50]. Although Rab32 is expressed in most human tissues [51], little is known about the physiological roles of Rab32 in tissues and cells other than melanocytes.

6.1. Rab32 GTPase and constitutive autophagy

Rab32 is supposed to localize to ER [52] and ER and mitochondria [53]. We showed previously that Rab32 participates in constitutive autophagy in HeLa cells derived from cervical cancer [52]. The expressed wild-type or GTP-bound active form of human Rab32 was primarily localized to the ER. Interestingly, overexpression of the wild-type or active form of Rab32 induced the formation of autophagic vacuoles containing LC3, the ER-resident protein calnexin and late endosomal/lysosomal membrane protein LAMP-2 even under nutrient-rich conditions. Moreover, the localization of Rab32 to ER was necessary for the formation of autophagosomes [52], because the expression of a mutant Rab32 deleted two cysteine residues that are essential for association with the membrane, impaired autophagy vacuole formation [50]. There is a long-standing debate concerning from where the autophagosomal membrane is derived. So far, two possibilities have been proposed: it arises from pre-existing organelles, such as the ER or Golgi, or from de novo formation [54]. Our findings mentioned above postulate, therefore, that Rab32 facilitates the formation of autophagic vacuoles whose membranes are derived from the ER. In addition, expression of the inactive form of Rab32 or depletion of Rab32 expression by siRNA caused the formation of p62 and ubiquitinated protein-accumulating aggresomes and prevented constitutive autophagy [52]. Thus, these results imply the physiological importance of Rab32 in the cellular clearance of aggregated proteins by basal constitutive autophagy.

As well as Rab7, Rab32 also seems to participate in phagosome maturation in pathogen-induced autophagic degradation by infection with Salmonella enterica serovar Typhimurium [55] or Mycobacterium tuberculosis [56], especially in the recruitment of lysosomal enzyme cathepsin D to phagosomes containing M. tuberculosis [56]. Rab32 and some other Rabs localized to M. tuberculosis-containing phagosomes transiently, and the expression of the inactive form of Rab32 showed the impairment of its recruitment to phagosomes [56], but had no effect on
phagosomal fusion with lysosomes [55]. Therefore, these results imply that Rab32 regulates the recruitment of cathepsin D to the phagosomes.

6.2. Rab32 GTPase and interaction molecules

Recently, it has been reported that, in *Drosophila*, Rab32 colocalized with LysoTracker labeling lysosomes and GFP-Atg8 (LC3 homologue), indicating that Rab32 is localized at lysosomes and/or autophagosomes during programmed autophagy for metamorphosis to differentiate the fat body, salivary gland, and midgut [57]. Previously, Ma et al. reported that the Claret encoded by *claret*, a member of the granule group eye color genes [58], coprecipitated not only with Rab-RP1, a Rab GTPase encoded by *Drosophila lightoid*, but also with its human homologues, Rab32 and Rab38 [59]. Furthermore, the autophagosome formation was impaired in Rab32/lighthoid mutants and Rab32 GEF/claret mutants, suggesting that Rab32 activity is required for the autophagic process of the fat body [57]. Previously, it has been suggested that autophagy impairment reduces lipid accumulation and impairs adipocytes differentiation in mice [60, 61]. In fact, downregulation of autophagy in *Drosophila* led to a decrease in the size of lipid droplets in *atg*-related genes in knocked down *Drosophila* fat body cells [57]; therefore, Rab32 appears to regulate lipid storage by controlling autophagy. Another report showed that Rab32 is upregulated in the epidermis and midgut during metamorphosis in *Helicoverpa armigera* [62], suggesting that Rab32 may participate in organogenesis in insects.

In addition to GEF, a GAP for Rab32, RUTBC1, was identified [63]. RUTBC1 is a TBC domain-containing protein that binds to Rab9A in a nucleotide binding-state-dependent manner both in vitro and in vivo but has no GAP activity for Rab9A [63]; however, RUTBC1 acts as a GAP for Rab32 and Rab33B, and its TBC domain stimulates GTP hydrolysis [63]. Therefore, RUTBC1 may function in the autophagy process, as both Rab32 and Rab33B are suggested to be regulatory factors of autophagy.

6.3. Rab32 GTPase and disease

Recently, *RAB32* and *IL23R* (*interleukin receptor 23*) were identified as susceptibility genes for leprosy in a genome-wide association study [64], although *IL23R* was previously reported to be a gene involved in Crohn’s disease [65]. Leprosy, also known as Hansen’s disease, is a chronic granulomatous infectious disease caused by *Mycobacterium leprae*, which affects both peripheral nerves and mucosa of the upper respiratory tract. As Rab32 participates in regulating the recruitment of cathepsin D to phagosomes containing *M. tuberculosis* [56], referred to above, Rab32 may have function in the pathogenesis of leprosy, such as host defense against *M. leprae* infection.

7. Rab33 GTPase and autophagy

Rab33 has two isoforms, Rab33A and Rab33B. Rab33B is expressed ubiquitously, although Rab33A is expressed exclusively in the brain and cells of the immune system [66]. Rab33B is localized at the Golgi apparatus [67], although Rab33A also targets dense-core vesicles in
neuroendocrine cells, not only in the Golgi [68]. It has been shown that both Rab33A and Rab33B interact with Atg16L, which associates with Atg5-12 complex on an isolation membrane for the duration of autophagosome formation and is essential for canonical autophagy [69]. These interactions occur in a GTP-dependent manner [70]. Expression of the active form of Rab33B induced the lipidation of LC3 even under nutrient-rich conditions, but this was not caused by the blockage of autophagic degradation [70]. Furthermore, this form of Rab33B inhibited constitutive autophagy, but not starvation-induced autophagy, as judged by the degradation of p62/SQSTM1, a substrate of autophagy [70]. The precise reason for this dysfunction is unknown, but it is possible to speculate that the active form of Rab33B recruits Atg5-12/16L complex to incorrect membranes because no obvious large LC3 dots were observed in cells expressing this mutant. Rab33B has another interaction partner, OATL1, a RabGAP, which can bind LC3 [71]. Rab33B seems to be a target substrate of OATL1 and to be involved in the fusion between autophagosomes and lysosomes. Overexpression of the wild-type or active form of Rab33B inhibits the fusion between autophagosomes and lysosomes, because OATL1 inactivates Rab33B as a GAP protein, and this inhibition leads to the blockade of autophagic flux. In fact, the expression of the active form of Rab33B increased the amount of LC3-II as previously reported [70], but did not show the colocalization of lysosomal membrane protein LAMP-1 with LC3-positive dots [71], suggesting that autophagosomes in the active form of Rab33B-expressing cells cannot efficiently fuse with lysosomes. Very recently, it was revealed that Atg16L interacts with Rab33A and that this interaction is required for the dense-core vesicle localization of Atg16L in neuroendocrine PC12 cells [72]. Knockdown of endogenous Atg16L in PC12 cells caused marked inhibition of hormone secretion independently of autophagic activity [72], indicating that Atg16L controls autophagy in all cell types as well as secretion from dense-core vesicles, presumably by acting as a Rab33A effector, in neuroendocrine cells.

8. Conclusion

Based on the results of a large body of research, regulation of autophagy by Rab proteins could be loosely classified into two types, (i) autophagosome formation or (ii) autophagosome maturation (i.e. the fusion of autophagosomes with lysosomes) [Figure 2]. We assumed that Rab7, Rab9, and Rab11 could be sorted into type (ii) profile, and Rab24, Rab32 and Rab33 into type (i). Autophagy indicates not only the sequestration of materials into autophagosomes but also the degradation by lysosomal enzymes. Therefore, Rab proteins associated with the fusion step of autophagosomes with lysosomes have a fatal role in autophagic flux, and Rabs also trigger autophagosome formation. Type (ii)-categorized Rab proteins are mostly localized at late endosomes and play a role in membrane traffic to lysosomes, recycling endosomes or TGN. It is conceivable that they are involved in autophagosome maturation, because late endosomes, TGN, recycling endosomes, or Golgi-derived vesicles fuse with lysosomes routinely [73]. So far, no clear result has shown that Rab24 and Rab33 directly associate with the formation of autophagosomes except for Rab32, which is associated with ER and/or mitochondria and is regarded as a key factor for the supply to autophagosome formation [52]. However, it could
be plausible that Rab33B is involved in autophagosome formation, since no obvious large LC3 dots were observed in the active form of Rab33B-expressing cells, but LC3 lipidation was induced [70]. Although the precise mechanism of autophagy by Rab24 is unknown, Rab24 might participate in the early stage of autophagy since overexpression of Rab24 stimulates the sequestration of *Coxiella* into vacuoles early after infection [47]. Thus, these Rab proteins, Rab24, Rab33, and Rab32, could be involved in autophagosome formation.

Although significant progress has been made in understanding the mechanisms of the formation and maturation of autophagic vacuoles through the function of Rab proteins, the precise molecular mechanism of how they regulate or interact with the core autophagic complex including Atg proteins is still unclear. Moreover, temporal ordering, such as a signal pathway to initiate the restricted molecules for autophagy, and spatial regulation including the source of isolation membranes are important in the overall understanding of autophagy involving Rab GTPases.

![Figure 2. Localization and function of Rab GTPases for autophagy](image-url)
Acknowledgements

This work was supported in part by a grant-in-aid from the Ministry of Education, Culture, Sports, Sciences and Technology of Japan.

Author details

Yuko Hirota¹, Keiko Fujimoto² and Yoshitaka Tanaka²*

*Address all correspondence to: ytanaka@phar.kyushu-u.ac.jp

¹ Department of Clinical Chemistry and Laboratory Medicine, Graduate School of Medical Sciences, Kyushu University, Maidashi, Fukuoka, Japan

² Division of Pharmaceutical Cell Biology, Graduate School of Pharmaceutical Sciences, Kyushu University, Maidashi, Fukuoka, Japan

References

[1] Klionsky DJ Emr SD. Autophagy as a regulated pathway of cellular degradation. Science 2000; 290(5497), 1717-1721.

[2] Mizushima N, Ohsumi Y, Yoshimori T. Autophagosome formation in mammalian cells. Cell Structure and Function 2002; 27(6), 421-429.

[3] Stenmark H. Rab GTPases as coordinators of vesicle traffic. Nature Reviews Molecular Cell Biology 2009; 10(8), 513-525.

[4] Weidberg H, Shvets E, Elazar Z. Biogenesis and cargo selectivity of autophagosomes. Annual Review of Biochemistry 2011; 80, 125-156.

[5] Nakatogawa H, Suzuki K, Kamada Y, Ohsumi Y. Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nature Reviews Molecular Cell Biology 2009; 10(7), 458-467.

[6] Shpilka T, Mizushima N, Elazar Z. Ubiquitin-like proteins and autophagy at a glance. Journal of Cell Science 2012; 125. 2343-2348.

[7] Mizushima N, Yamamoto A, Hatano M, Kobayashi Y, Kabeya Y, Suzuki K, Tokuhisa T, Ohsumi Y, Yoshimori T. Dissection of autophagosome formation using Apg5-deficient mouse embryonic stem cells. Journal of Cell Biology 2001; 152(4), 657-668.

[8] Tanida I, Sou Y, Ezaki J, Minematsu-Ikeguchi N, Ueno T, Kominami E. HsAtg4B/HsApg4B/Autophagin-1 cleaves the carboxyl termini of three human Atg8 homologs. Nature 2005; 437(7060), 810-815.
logues and delipidates microtubule-associated protein light chain 3- and GABA<sub>A</sub> receptor-associated protein-phospholipid conjugates. The Journal of Biological Chemistry 2004; 279(35), 36268-36276.

[9] Tanida I, Tanida-Miyake E, Komatsu M, Ueno T, Kominami E. Human Apg3p/Aut1p homologue Is an authentic E2 enzyme for multiple substrates, GATE-16, GABARAP, and MAP-LC3, and facilitates the conjugation of hApg12p to hApg5p. The Journal of Biological Chemistry 2002; 277(16), 13739-13744.

[10] Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, Kominami E, Ohsumi Y, Yoshimori T. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. The EMBO Journal 2000; 19(21), 5720-5728.

[11] Kabeya Y, Mizushima N, Yamamoto A, Oshitani-Okamoto S, Ohsumi Y, Yoshimori T. LC3, GABARAP and GATE16 localize to autophagosomal membrane depending on form-II formation. Journal of Cell Science 2004; 117, 2805-2812.

[12] Méresse S, Gorvel JP, Chavrier P. The rab7 GTPase resides on a vesicular compartment connected to lysosomes. Journal of Cell Science 1995; 108, 3349-3358.

[13] Feng Y, Press B, Wandinger-Ness A. Rab7 an important regulator of late endocytic membrane traffic. Journal of Cell Biology 1995; 131(6), 1435-1452.

[14] Gutierrez MG, Munafó DB, Berón W, Colombo MI. Rab7 is required for the normal progression of the autophagic pathway in mammalian cells. Journal of Cell Science 2004; 117, 2687-2697.

[15] Jager S, Bucci C, Tanida I, Ueno T, Kominami E, Saftig P, Eskelinen E-L. Role for Rab7 in maturation of late autophagic vacuoles. Journal of Cell Science 2004; 117, 4837-4848.

[16] Liang C, Lee JS, Inn KS, Gack MU, Li Q, Roberts EA, Vergne I, Deretic V, Feng P, Akazawa C, Jung JU. Beclin1-binding UVRAG targets the class C Vps complex to coordinate autophagosome maturation and endocytic trafficking. Nature Cell Biology 2008; 10(7), 776-787.

[17] Kirisako T, Baba M, Ishihara N, Miyazawa K, Ohsumi M, Yoshimori T, Noda T, Ohsumi Y. Formation process of autophagosome is traced with Apg8/Aut7p in yeast. Journal of Cell Biology 1999; 147(2), 435-446.

[18] Sato TK, Rehling P, Peterson MR, Emr SD. Class C Vps protein complex regulates vacuolar SNARE pairing and is required for vesicle docking/fusion. Molecular Cell 2000; 6(3), 661-671.

[19] Wurmsser AE, Sato TK, Emr SD. New component of the vacuolar class C-Vps complex couples nucleotide exchange on the Ypt7 GTPase to SNARE-dependent docking and fusion. Journal of Cell Biology 2000; 151(3), 551-562.
[20] Ganley IG, Wong PM, Gammoh N, Jiang X. Distinct autophagosomal-lysosomal fusion mechanism revealed by thapsigargin-induced autophagy arrest. Molecular Cell 2011; 42(6), 731-743.

[21] Yu L, McPhee CK, Zheng L, Mardones GA, Rong Y, Peng J, Mi N, Zhao Y, Liu Z, Wan F, Hailey DW, Oorschot V, Klumperman J, Baehrecke EH, Lenardo MJ. Termination of autophagy and reformation of lysosomes regulated by mTOR. Nature 2010; 465(7300), 942-946.

[22] Alix E, Mukherjee S, Roy CR. Subversion of membrane transport pathways by vacuolar pathogens. Journal of Cell Biology 2011; 195(6), 943-952.

[23] Münz C. Enhancing immunity through autophagy. Annual Review of Immunology 2009; 27, 423-449.

[24] Birmingham CL, Canadien V, Kaniuk NA, Steinberg BE, Higgins DE, Brumell JH. Listeriolysin O allows Listeria monocytogenes replication in macrophage vacuoles. Nature 2008; 451(7176), 350-354.

[25] Heinzen RA, Hackstadt T, Samuel JE. Developmental Biology of Coxiella burnetii. Trends in Microbiology 1999; 7(4), 149-154.

[26] Berón W, Gutierrez MG, Rabinovitch M, Colombo MI. Coxiella burnetii localizes in a Rab7-labeled compartment with autophagic characteristics. Infection and Immunity 2002; 70(10), 5816-5821.

[27] Yamaguchi H, Nakagawa I, Yamamoto A, Amano A, Noda T, Yoshimori T. An initial step of GAS-containing autophagosome-like vacuoles formation requires Rab7. PLoS Pathogen 2009; 5(11), e1000670.

[28] Zhong Y, Wang QJ, Li X, Yan Y, Backer JM, Chait BT, Heintz N, Yue Z. Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1-phosphatidylinositol-3-kinase complex. Nature Cell Biology 2009; 11(4), 468-476.

[29] Matsunaga K, Saitoh T, Tabata K, Omori H, Satoh T, Kurotori N, Maejima I, Shirahama-Noda K, Ichimura T, Isobe T, Akira S, Noda T, Yoshimori T. Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. Nature Cell Biology 2009; 11(4), 385-396.

[30] Liang C, Lee JS, Inn KS, Gack MU, Li Q, Roberts EA, Vergne I, Deretic V, Feng P, Akazawa C, Jung JU. Beclin 1-binding UVRAG targets the class C Vps complex to coordinate autophagosome maturation and endocytic trafficking. Nature Cell Biology 2008; 10(7), 776-787.

[31] Sun Q, Westphal W, Wong KN, Tan I, Zhong Q. Rubicon controls endosome maturation as a Rab7 effector. Proceedings of the National Academy of Sciences of the United States of America 2010; 107(45), 19338-19343.
Tabata K, Matsunaga K, Sakane A, Sasaki T, Noda T, Yoshimori T. Rubicon and PLEKHM1 negatively regulate the endocytic/autophagic pathway via a novel Rab7-binding domain. Molecular Biology of the Cell 2010; 21(23), 4167-4172.

Pankiv S, Alemu EA, Brech A, Bruun JA, Lamark T, Overvatn A, Bjørkøy G, Johannsen T. FYCO1 is a Rab7 effector that binds to LC3 and PI3P to mediate microtubule plus end-directed vesicle transport. Journal of Cell Biology 2010; 188(2), 253-269.

Barbero P, Bittova L, Pfeffer SR. Visualization of Rab9-mediated vesicle transport from endosomes to the trans-Golgi in living cells. Journal of Cell Biology 2002; 156(3), 511-518.

Ganley IG, Carroll K, Bittova L, Pfeffer S. Rab9 GTPase regulates late endosome size and requires effector interaction for its stability. Molecular Biology of the Cell 2004; 15(12), 5420-5430.

Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, Ohsumi Y, Tokuhisa T, Mizushima N. The role of autophagy during the early neonatal starvation period. Nature 2004; 432(7020), 1032-1036.

Komatsu M, Waguri S, Ueno T, Iwata J, Murata S, Tanida I, Ezaki J, Mizushima N, Ohsumi Y, Uchiyama Y, Kominami E, Tanaka K, Chiba T. Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. Journal of Cell Biology 2005; 169(3), 425-434.

Nishida Y, Arakawa S, Fujitani K, Yamaguchi H, Mizuta T, Kanaseki T, Komatsu M, Otsu K, Tsujimoto Y, Shimizu S. Discovery of Atg5/Atg7-independent alternative macroautophagy. Nature 2009; 461(7264), 654-658.

Ullrich O, Reinsch S, Urbé S, Zerial M, Parton RG. Rab11 regulates recycling through the pericentriolar recycling endosome. Journal of Cell Biology 1996; 135(4), 913-924.

Savina A, Vidal M, Colombo MI. The exosome pathway in K562 cells is regulated by Rab11. Journal of Cell Science 2002; 115, 2505-2515.

Fader CM, Sánchez D, Furlán M, Colombo MI. Induction of Autophagy promotes fusion of multivesicular bodies with autophagic vacuoles in K562 cells. Traffic 2008; 9(2), 230-250.

Berg TO, Fengsrud M, Strømhaug PE, Berg T, Seglen PO. Isolation and characterization of rat liver amphisomes. Evidence for fusion of autophagosomes with both early and late endosomes. The Journal of Biological Chemistry 1998; 273(34), 21883-21892.

Fuchs E, Haas AK, Spooner RA, Yoshimura S, Lord JM, Barr FA. Specific Rab GTPase-activating proteins define the Shiga toxin and epidermal growth factor uptake pathways. Journal of Cell Biology 2007; 177(6), 1133-1143.

Popovic D, Akutsu M, Novak I, Harper JW, Behrends C, Dikic I. Rab GTPase-activating proteins in autophagy: regulation of endocytic and autophagy pathways by di-
rect binding to human ATG8 modifiers. Molecular and Cellular Biology 2012; 32(9), 1733-1744.

[45] Longatti A, Lamb CA, Razi M, Yoshimura S, Barr FA, Tooze SA. TBC1D14 regulates autophagosome formation via Rab11- and ULK1-positive recycling endosomes. Journal of Cell Biology 2012; 197(5), 659-675.

[46] Munafó DB, Colombo MI. Induction of autophagy causes dramatic changes in the subcellular distribution of GFP-Rab24. Traffic 2002; 3(7), 472-482.

[47] Gutierrez MG, Vázquez CL, Munafó DB, Zoppino FC, Berón W, Rabinovitch M, Colombo MI. Autophagy induction favours the generation and maturation of the Cox-iella-replicative vacuoles. Cellular Microbiology 2005; 7(7), 981-993.

[48] Marambio P, Toro B, Sanhueza C, Troncoso R, Parra V, Verdejo H, García L, Quiroga C, Munáfo D, Díaz-Elizondo J, Bravo R, González MJ, Díaz-Araya G, Pedrozo Z, Chiong M, Colombo MI, Lavandero S. Glucose deprivation causes oxidative stress and stimulates aggresome formation and autophagy in cultured cardiac myocytes. Biochimica et Biophysica Acta 2010; 1802(6), 509-518.

[49] Wasmeier C, Romano M, Plowright L, Bennet DC, Raposo G, Seabra MC. Rab38 and Rab32 control post-Golgi trafficking of melanogenic enzymes. Journal of Cell Biology 2006; 175(2), 271-281.

[50] Park M, Serpinskiy A, Papalopulu N and Gelfand VI. (2007) Rab32 regulates melanosome transport in Xenopus melanophores by protein kinase A recruitment. Current Biology 2007; 17(23), 1-5.

[51] Bao, X., Faris, A.E., Jang, E.K. and Haslam, R.J. Molecular cloning, bacterial expression and properties of Rab31 and Rab32. European Journal of Biochemistry 2002; 269(1), 259-271.

[52] Hirota Y, Tanaka Y. A small GTPase, human Rab32, is required for the formation of autophagic vacuoles under basal conditions. Cellular and Molecular Life Sciences 2009; 66(17), 2913-2932.

[53] Bui M, Gilady SY, Fitzsimmons RE, Benson MD, Lynes EM, Gesson K, Alto NM, Strack S, Scott JD, Simmen T. Rab32 modulates apoptosis onset and mitochondria-associated membrane (MAM) properties. The Journal of Biological Chemistry 2010; 285(41), 31590-31602.

[54] Juhasz G, Neufeld TP. Autophagy: a forty-year search for a missing membrane source. PLoS Biology 2006; 4(2), e36.

[55] Smith AC, Heo WD, Braun V, Jiang X, Macrae C, Casanova JE, Scidmore MA, Grinstein S, Meyer T, Brumell JH. A network of Rab GTPases controls phagosome maturation and is modulated by Salmonella enterica serovar Typhimurium. Journal of Cell Biology 2007; 176(3), 263-268.
[56] Seto S, Tsujimura K, Koide Y. Rab GTPases regulating phagosome maturation are differentially recruited to mycobacterial phagosomes. Traffic 2011; 12(4), 407-420.

[57] Wang C, Liu Z, Huang X. Rab32 is important for autophagy and lipid storage in Drosophila. PLoS One 2012; 7(2), e32086.

[58] Yamamoto AH, Komma DJ, Shaffer CD, Pirrotta V, Endow SA. The claret locus in Drosophila encodes products required for eyecolor and for meiotic chromosome segregation. The EMBO Journal 1989; 8(12), 3543-3552.

[59] Ma J, Plesken H, Treisman JE, Edelman-Novemsky I, Ren M. Lightoid and Claret: a rab GTPase and its putative guanine nucleotide exchange factor in biogenesis of Drosophila eye pigment granules. Proceedings of the National Academy of Sciences of the United States of America 2004; 101(32), 11652-11657.

[60] Baerga R, Zhang Y, Chen PH, Goldman S, Jin S. Targeted deletion of autophagy-related 5 (atg5) impairs adipogenesis in a cellular model and in mice. Autophagy 2009; 5(8), 1118-1130.

[61] Zhang Y, Goldman S, Baerga R, Zhao Y, Komatsu M, Jin S. Adipose-specific deletion of autophagy-related gene 7 (atg7) in mice reveals a role in adipogenesis. Proceedings of the National Academy of Sciences of the United States of America 2009; 106(47), 19860-19865.

[62] Hou L, Wang JX, Zhao XF. Rab32 and the remodeling of the imaginal midgut in Helicoverpa armigera. Amino Acids 2011; 40(3), 953-961.

[63] Nottingham RM, Ganley IG, Barr FA, Lambright DG, Pfeffer SR. RUTBC1 protein, a Rab9A effector that activates GTP hydrolysis by Rab32 and Rab33B proteins. The Journal of Biological Chemistry 2011; 286(38), 33213-33222.

[64] Zhang F, Liu H, Chen S, Low H, Sun L, Cui Y, Chu T, Li Y, Fu X, Yu Y, Yu G, Shi B, Tian H, Liu D, Yu X, Li J, Lu N, Bao F, Yuan C, Liu J, Liu H, Zhang L, Sun Y, Chen M, Yang Q, Yang H, Yang R, Zhang L, Wang Q, Liu H, Zuo F, Zhang H, Khor CC, Hibberd ML, Yang S, Liu J, Zhang X. Identification of two new loci at IL23R and RAB32 that influence susceptibility to leprosy. Nature Genetics 2011; 43(12), 1247-1251.

[65] Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, Abraham C, Regueiro M, Griffiths A, Dassopoulos T, Bitton A, Yang H, Targan S, Datta LW, Kistner EO, Schumm LP, Lee AT, Gregersen PK, Barmada MM, Rotter JI, Nicolae DL, Cho JH. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. Science 2006; 314(5804), 1461-1463.

[66] Zheng JY, Koda T, Arimura Y, Kishi M, Kakinuma M. Structure and expression of the mouse S10 gene. Biochimica et Biophysica Acta 1997; 1351(1-2), 47-50.

[67] Zheng JY, Koda T, Fujiwara T, Kishi M, Ikehara Y, Kakinuma M. A novel Rab GTPase, Rab33B, is ubiquitously expressed and localized to the medial Golgi cisternae. Journal of Cell Science 1998; 111, 1061-1069.
[68] Tsuboi T, Fukuda M. Rab3A and Rab27A cooperatively regulate the docking step of dense-core vesicle exocytosis in PC12 cells. Journal of Cell Science 2006; 119, 2196-2203.

[69] Mizushima N, Kuma A, Kobayashi Y, Yamamoto A, Matsubae M, Takao T, Natsume T, Ohsumi Y, Yoshimori T. Mouse Apg16L, a novel WD-repeat protein, targets to the autophagic isolation membrane with the Apg12-Apg5 conjugate. Journal of Cell Science 2003; 116, 1679-1688.

[70] Itoh T, Fujita N, Kanno E, Yamamoto A, Yoshimori T, Fukuda M. Golgi-resident small GTPase Rab33B interacts with Atg16L and modulates autophagosome formation. Molecular Biology of the Cell 2008; 19(7), 2916-2925.

[71] Itoh T, Kanno E, Uemura T, Waguri S, Fukuda M. OATL1, a novel autophagosome-resident Rab33B-GAP, regulates autophagosomal maturation. Journal of Cell Biology 2011; 192(5), 839-853.

[72] Ishibashi K, Uemura T, Waguri S, Fukuda M. Atg16L1, an essential factor for canonical autophagy, participates in hormone secretion from PC12 cells independently of autophagic activity. Molecular Biology of the Cell 2012; 23(16), 3193-3202.

[73] Maxfield FR, McGraw TE. Endocytic recycling. Nature Reviews Molecular Cell Biology 2004; 5(2), 121-132.
