Golgi-associated Rab GTPasesimplicated in autophagy

Qingchun Lu[†], Po-Shun Wang[†] and Ling Yang[*]

Abstract

Autophagy is a conserved cellular degradation process in eukaryotes that facilitates the recycling and reutilization of damaged organelles and compartments. It plays a pivotal role in cellular homeostasis, pathophysiological processes, and diverse diseases in humans. Autophagy involves dynamic crosstalk between different stages associated with intracellular vesicle trafficking. Golgi apparatus is the central organelle involved in intracellular vesicle trafficking where Golgi-associated Rab GTPases function as important mediators. This review focuses on the recent findings that highlight Golgi-associated Rab GTPases as master regulators of autophagic flux. The scope for future research in elucidating the role and mechanism of Golgi-associated Rab GTPases in autophagy and autophagy-related diseases is discussed further.

Keywords: Autophagy, Rab GTPase, Golgi, Vesicle trafficking

Background

Autophagy and its machinery

Autophagy is a cellular degradative pathway involving the delivery of cytoplasmic cargo to lysosomes and is highly conserved among eukaryotes ranging from yeast to mammalian cells [1–3]. To date, at least 30 different autophagy-related genes (Atg) have been identified to be associated with the regulation and execution of autophagy in yeast [4, 5]. The autophagic process comprises four major steps that are executed by specific *Atg* genes [6–8]. The first step of autophagy induction involves the formation (vesicle nucleation) and expansion (vesicle elongation and regulation) of an isolation membrane known as phagophore. Several distinct signaling pathways and proteins are involved in the execution of the initial step. However, it remains to be elucidated whether these signaling pathways work in concert or independently. The mammalian target of

rapamycin (mTOR) kinase, a major regulator of cap-dependent translation in cells, is known to strongly inhibit autophagy. Availability of nutrients stimulates the binding of mTOR and ULK1-Atg13-FIP200 to form complexes on autophagic isolation membranes, thereby suppressing the phagophore expansion. Nutrient deprivation or rapamycin inhibits the association of mTOR, ULK1, and mTOR kinase activity, leading to the upregulation of autophagy in both yeast and mammalian cells [9, 10]. In addition, several protein regulators such as p53, c-jun-N-terminal kinase 1 (JNK1), eukaryotic initiation factor 2α (eIF2α), GTPases, and intracellular calcium are known to play an important role in regulating this initial step of autophagy [11–16]. These molecules and/or signaling pathways promote autophagy by activating the class III phosphatidylinositol 3-kinase (PI3K) Vps34 that initiates the formation of phosphatidylinositol 3-phosphate (PIP3) on lipids. The activity of Vps34 in promoting autophagy depends on its interactions within a multi-protein complex including the essential mammalian autophagy protein beclin 1 (BECN1, homolog of yeast Atg6) [17]. Additional proteins, namely, Bcl-2, Ambra1, Bif-1, and Atg14L, interact with Vps34 and beclin 1 to form functionally distinct multi-protein complexes that regulate

*Correspondence: ling.yang@temple.edu
[†]Qingchun Lu and Po-ShunWang contributed equally to this work
Department of Medical Genetics and Molecular Biochemistry, Lewis Katz School of Medicine at Temple University, 3440 N Broad St, Kresge Hall, Rm. 624, Philadelphia PA19140, USA

Lu *et al. Cell Biosci* (2021) 11:35

Page 2 of 9

different stages of autophagosome development and vesicular trafficking [18, 19]. The drug 3-methyladenine (3-MA), an inhibitor of class III PI3K, is frequently used for the pharmacological inhibition of autophagy in cells [20]. Thus, both rapamycin and 3-MA exert a significant influence on autophagy and the associated cellular pathways and processes.

The second step of autophagy begins with elongation followed by closure of the phagophore to form an autophagosome. Autophagosome is a double-layered vesicle that sequesters the cytoplasmic material using two separated but evolutionarily conserved ubiquitin-like conjugation systems [7, 8, 21]. In the first system, the E1- and the E2-like enzymes, Atg7 and Atg10, respectively, promotes the covalent association between Atg12 and Atg5. Subsequently, Atg16 associates with this complex to form an Atg5-Atg12-Atg16 heterotrimeric complex that associates primarily on the outer membrane of the growing autophagosome, hypothesized to mediate the curvature of the growing membrane [22, 23]. The second ubiquitin-like conjugation system results in the cleavage of the C-terminal of the microtubule-associated protein light chain 3 (LC3, a homolog of yeast Atg8) by Atg7 and the cysteine protease Atg4. Following cleavage, the E2-like enzyme Atg3 adds phosphatidylethanolamine (PE) to a conserved glycine residue present at the C-terminus of the cleaved LC3 (designated LC3-I) to form the well-known LC3-II or LC3-PE. In general, LC3 is soluble and dispersed throughout the cytoplasm, but upon cleavage and lipidation with PE, it is recruited to the outer and inner membrane of the growing autophagosome. LC3 is the only known protein that stably associates with the completed autophagosomes and is commonly used as a marker for studying autophagy. Autophagosome formation can be assessed biochemically by the ratio of lipidated (LC3-II) and non-lipidated (LC3-I) forms of LC3 [24, 25]. Once the matured autophagosome is formed, Atg9 and Atg18 act to remove and recycle Atg16, Atg12, Atg5, and outer membrane LC3 [26].
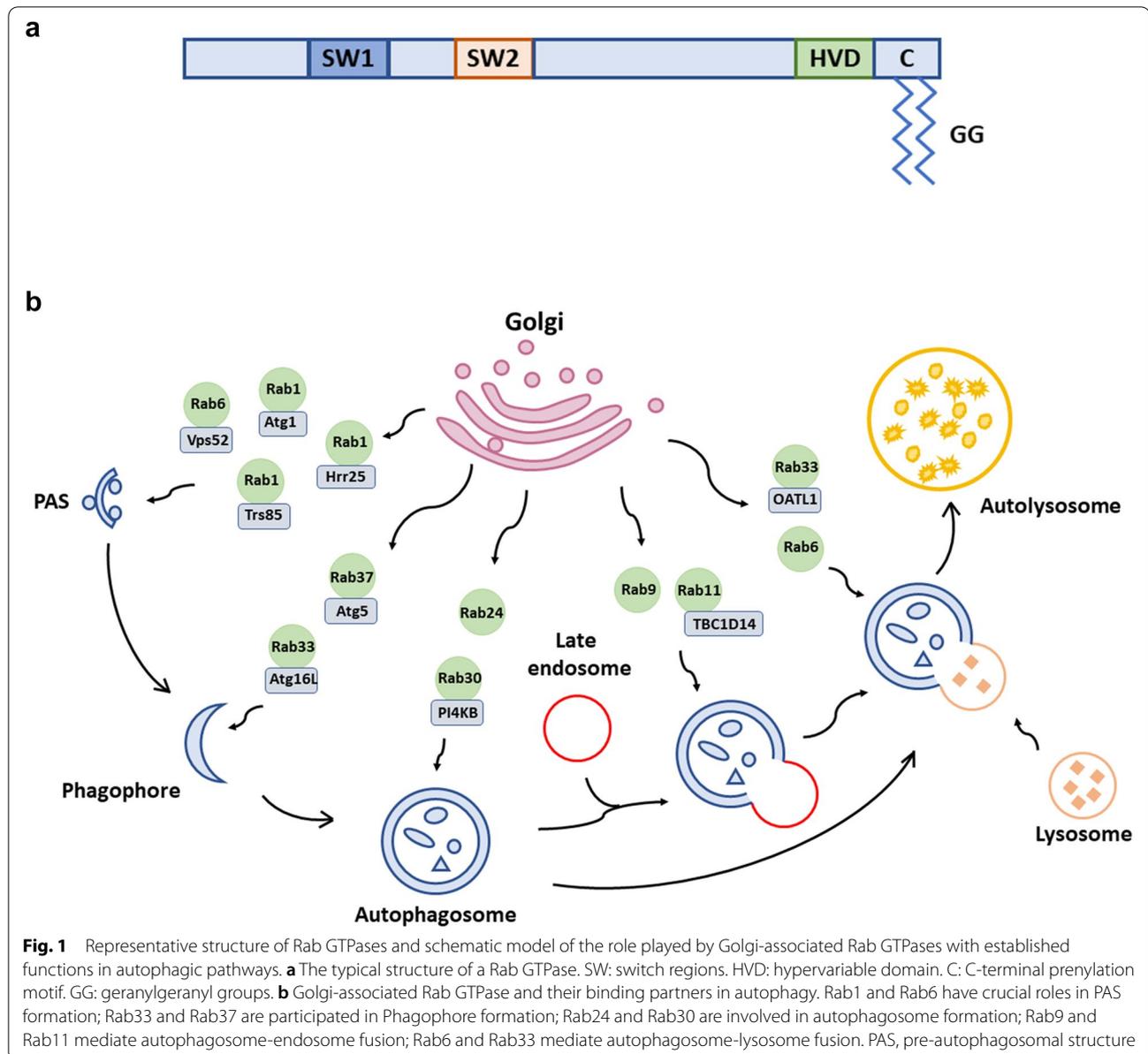
The third step of autophagy begins with the docking and fusion of mature autophagosomes with lysosomes to form autolysosomes. The engulfed material together with the inner membrane are degraded inside the autolysosome [7, 8, 27]. Lysosomal-associated membrane protein 1 and 2 (LAMP1 and LAMP2) and the GTP-binding protein Rab7 are involved in this process [28]. In addition, autophagosomes are capable of fusing with endosomes to form organelle amphisomes [29]. It remains to be elucidated whether amphisomes are distinct entities or are precursors for the formation of autolysosomes. The final step of autophagy is vesicular breakdown and degradation of cytoplasm-derived contents. Fusion of autophagosome and lysosome provide access to lysosomal proteases

(such as cathepsin B, D, and L) and diverse hydrolytic enzymes to enter the autophagosome resulting in the degradation of the constituents of the inner autophagosomal membrane. Subsequent to degradation, the amino acids and lipids are exported from the autolysosome into the cytoplasm where they are recycled to generate new macromolecules [30].

Role of Rab proteins in vesicle transport

Rab proteins comprise a large family of small guanosine triphosphate (GTP)-binding proteins that play a crucial role in the regulation of intracellular vesicle trafficking [31–33]. The structure of Rab proteins is highly conserved across the family and consists of two switch regions, one hypervariable domain, and one C-terminal prenylation motif [34, 35]. The prenylation motif is responsible for modification of the geranylgeranyl group (GG) essential for the attachment of the Rab protein to the membrane (Fig. 1a). In mammalian cells, distinct Rab proteins localize at specific membrane-bound compartments or endocytic organelles where they modulate several cellular processes related to cytoplasmic cargo sorting, vesicle budding, docking, fusion, and membrane tabulation. In addition, they facilitate cytoskeletal translocation owing to their repeated alteration between an inactive guanosine diphosphate (GDP)-bound form and an active GTP-bound form [36]. The guanine nucleotide exchange factor (GEF) can essentially shift a Rab GTPase from the GDP-bound to the GTP-bound conformation, whereas the GTPase activating domain (GAP) exerts the opposite effect of inactivating GTPase [37, 38]. Importantly, several studies have shown that specific amino acid mutations could affect the switch from the GDP-bound to the GTP-bound conformation, and vice versa [39]. Therefore, an active GTP-bound form can further interact with its effectors or binding partners to fulfill the functions and mediate intracellular signals in response to physiological and metabolic demands.

Among the ~70 known Rab proteins, more than 20 are associated primarily with the Golgi apparatus and are considered Golgi-associated Rab GTPases [40]. Golgi is a highly dynamic organelle that plays a central role in the events related to vesicle transport. Vesicle organization and trafficking associated with Golgi relies on the Golgi-associated Rab GTPases [41]. Previous studies have demonstrated the role of Golgi-associated Rab proteins in regulating the formation of autophagosomes indicating its importance in autophagy [42–44]. A summative review covering the functions and mechanistic role of Golgi-associated Rab GTPases in autophagic flux would contribute to improved understanding of autophagy. Therefore, the present review is aimed at summarizing the existing knowledge related to the role of Golgi-related

Lu *et al. Cell Biosci* (2021) 11:35

Page 3 of 9

Fig. 1 Representative structure of Rab GTPases and schematic model of the role played by Golgi-associated Rab GTPases with established functions in autophagic pathways. **a** The typical structure of a Rab GTPase. SW: switch regions. HVD: hypervariable domain. C: C-terminal prenylation motif. GG: geranylgeranyl groups. **b** Golgi-associated Rab GTPase and their binding partners in autophagy. Rab1 and Rab6 have crucial roles in PAS formation; Rab33 and Rab37 are participated in Phagophore formation; Rab24 and Rab30 are involved in autophagosome formation; Rab9 and Rab11 mediate autophagosome-endosome fusion; Rab6 and Rab33 mediate autophagosome-lysosome fusion. PAS, pre-autophagosomal structure

Rab GTPases and their signaling partners in autophagy (Fig. 1b).

Main text

Rab1

Rab1 GTPase (22 kDa) localized in the ER-Golgi intermediate is involved in the regulation of membrane trafficking from the ER to the Golgi [45, 46]. Currently, two isoforms of Rab1 GTPase have been identified, namely, Rab1A and Rab1B. They are highly conserved proteins with similar biochemical properties and functions [30, 47, 48]. Rab1 is an important regulator of several signaling pathways, including the mTOR pathway [49].

Overexpression of Rab1A drives the mTORC1 signaling and mTORC1-dependent growth in tumor by modulating the direct interaction between mTORC1 and Rheb. The biological activity of Rab1 is dependent on its GTPase activity, whereas the dominant-negative mutant Rab1aS25N and the nucleotide-free mutant Rab1aN124I perturb transport processes leading to the disruption of the Golgi apparatus [50, 51]. Similar results were reported to be associated with the overexpression of the dominant-negative mutant Rab1bN121I, which contributes to the disarrangement of the Golgi structure followed by the release of β-COP into the cytosol [52]. In addition, Rab1 binds directly to Golgi-84, GM130, and

Lu *et al. Cell Biosci* (2021) 11:35

Page 4 of 9

p115, all of which are required for the generation and maintenance of the Golgi structure [53]. Interestingly, Rab1b knockdown or overexpression of nucleotide-free mutant Rab1bN121I in CHO cells robustly suppressed autophagosome formation, suggesting that Rab1b-dependent vesicular transport from the ER is crucial for the initiation of autophagy [54]. The results were supported by the finding of Meiling-Wesse K et al., who found that Rab1 GEF Trs85, a component of the TRAPP complex, is essential for the biogenesis of pre-autophagosomal structure (PAS) [55]. Activated Ypt1/Rab1 recruits Atg1 kinase to PAS, bringing it into close proximity with the binding partner, Atg17. In addition, Ypt1/Rab1 binds directly to and spatially activates HRR25/casein kinase 1 delta (CK1δ), which in turn regulates vesicle transport and autophagosome formation [56, 57]. These results indicate the role of Rab1 as a pivotal regulator controlling the crosstalk between ER-Golgi traffic and autophagy.

Rab6

Rab6 GTPase (24 kDa) with its intra-Golgi localization, serves as a trans-Golgi marker with a well-established role in retrograde transport within the Golgi and between the Golgi and ER or endosome membrane [58–60]. Rab6 binds to Golgi resident proteins such as the coiled-coil homodimer Bicaudal-D (BicD), which is necessary for tethering vesicles and Golgi membranes to microtubules [61, 62]. In addition, Rab6 is involved in cell cycle regulation and apical-basal sorting [63]. The yeast Rab6 ortholog (Ypt6) plays a crucial role in sorting vacuolar hydrolases by regulating endosome-to-Golgi traffic and is essential for autophagy initiation. Rab6 binds directly to Vps52, a subunit of the Golgi-associated retrograde protein (GARP) complex. GARP facilitates the delivery of Atg9 to PAS under stress conditions [64, 65]. In addition, Ypt6 facilitates the recruitment of the GARP tethering complex to the Golgi ensuring the retrieval of lysosomal sorting receptors. Importantly, knockdown of Ypt6 or its GEF Ric1/Rgp1 leads to autophagy defects [66]. Recently, Ayala et al. reported a novel function of Rab6 as an important regulator of balance between mTOR signaling and autolysosome function [67]. Rab6 deficiency enhances the number of enlarged autophagic vesicles and suppresses lysosomal function, resulting in a partial inability to deliver lysosomal hydrolases to autolysosomes. Furthermore, loss of Rab6 expression leads to a significant reduction of cell size and inactivation of insulin-mTOR signaling because of mis-sorting and internalization of the insulin receptor. These findings suggest the importance of Rab6 in controlling the dynamic balance between the formation and turnover of autophagosomes under specific nutrient conditions to avoid autophagic stress.

Rab9

Rab9 GTPase (23 kDa) localized in the late endosome plays an important role in vesicle transport from the late endosome to the trans-Golgi network (TGN) [68]. Rab9 interacts with RhoBTB3, which is required for Golgi apparatus morphology as well as vesicle trafficking processes [69]. Knockdown of Rab9 attenuates the formation and decreases the number of late endosomes found in the perinuclear region, suggesting its necessity for the maintenance of late endosomes [70]. In addition, Rab9 is required for Atg5- and Atg7-independent autophagic pathways. Mouse embryonic fibroblasts lacking Atg5 or Atg7 expression could trigger autophagic flux and induce LC3 lipidation under specific metabolic stress conditions. The formation of autophagosomes, followed by fusion with late endosomes or TGN-derived vesicles, appears to be modulated in a Rab9-dependent manner [71, 72]. Intriguingly, increased Rab9 localization to autolysosomes was found with the active form Rab9Q66L, but not with the dominant-negative mutant Rab9S21 [70]. Thus, Rab9-dependent autophagy provides an alternative pathway to respond to metabolic stress in the absence of essential autophagy-related genes.

Rab11

Rab11 GTPase (25 kDa) localized in the TGN/post-Golgi vesicles interact with perinuclear recycling endosomes to regulate transferrin recycling in CHO or BHK cells [73–75]. Rab11 also localizes on multivesicular bodies (MVBs) in K562 cells, and its overexpression enhances the formation of large MVBs. Autophagy induction by starvation or rapamycin treatment robustly promotes the fusion of MVBs and autophagosomes. This is perturbed by calcium chelators or by the overexpression of the dominant-negative mutant Rab11S25N [76], suggesting the involvement of a calcium- and Rab11-dependent regulation. Rab GTPase activating proteins (RabGAPs) with a Tre-2/Bub2/Cdc16 (TBC) domain is associated with membrane-bound compartments and autophagy [77]. RabGAP TBC1D14 binds to ULK1 and Rab11 and is followed by the disruption of the recycling endosome traffic. TBC1D14 and Rab11 cooperate to regulate membrane transport from recycling endosomes to autophagosomes under starvation. Overexpression of TBC1D14 enhances tubulation of ULK1- and Rab11-positive recycling endosomes in a Rab11-dependent manner, perturbing autophagosome formation [78, 79]. Therefore, TBC1D14- and Rab11-dependent membrane transport from recycling endosomes plays a pivotal role in the regulation of starvation-induced autophagy.

Lu *et al. Cell Biosci* (2021) 11:35

Page 5 of 9

Rab24

Rab24 GTPase (24 kDa) localized in the ER/cis-Golgi and late endosomes participate in autophagy-related processes [80]. Rab24 relocates to autophagic vacuoles and localizes with LC3 under starvation. This process is facilitated by vinblastine treatment [36], suggesting that Rab24 is essential for autophagosome formation in response to starvation stress. In addition, the bacterial pathogen *Coxiella burnetii* survives and replicates in LC3-positive phagolysosome compartments, and Rab24 overexpression accelerate the formation of *Coxiella*-containing vacuoles after bacterial infection. Overexpression of the dominant-negative mutant Rab24S67L significantly decreased the number and size of phagolysosomal structures [81, 82], suggesting that Rab24 promotes the maturation of phagolysosomes for *Coxiella* replication. Furthermore, Rab24 contributes to the degradation of aggregated proteins in rat cardiomyocytes. Glucose deprivation induces oxidative stress and enhances the formation of aggregates and aggresomes of polyubiquitinated proteins that localize with endogenous Rab24 and LC3 [83].

Rab30

Rab30 GTPase (23 kDa) localized in trans-Golgi and is commonly found in metazoans. Previous studies have revealed the interaction of Rab30 with a number of Golgi proteins in *Drosophila melanogaster*, including golgins dGCC88, dGolgin-97, dGolgin-245, dGM130, as well as the fly orthologs of the coiled-coil proteins p115 and BicD [84]. GCC88, GM130, and p115 modulate the Golgi architecture, while Golgin-97, Golgin-245, and BicD are crucial for vesicular trafficking [85–89]. In addition, Rab30 has been identified as a target of c-Jun N-terminal kinase (JNK) signaling and functions as a TGN-resident modulator of intracellular trafficking during Drosophila morphogenesis [90]. Importantly, Rab30 primarily localizes in the trans-Golgi region to facilitate the structural maintenance of its morphological integrity. However, inhibition of Rab30 does not affect the trafficking of anterograde or retrograde cargo through the secretory pathway [91]. Furthermore, Oda et al. identified Rab30 as a novel regulator of autophagy induction in Group A *Streptococcus* (GAS) infection. They found that Rab30 is recruited to GAS-containing autophagosome-like vacuoles (GcAVs) that are dependent on its GTPase activity. In addition, Rab30 is essential for the formation of GcAV, followed by GAS degradation in autolysosomes. Although starvation induces Rab30 localization in autophagosomes, it is dispensable for starvation-induced autophagosome formation [92]. Recently, Nakajima et al. revealed that Rab30 promotes the recruitment of phosphatidylinositol 4-kinase beta (PI4KB) to the Golgi

and GcAVs. They revealed a direct interaction between RAB30 and PI4KB, in which knockdown of RAB30 inhibited the localization of PI4KB to the TGN and GcAVs, providing evidence for the coordinative functions of RAB30 and PI4KB with regard to the xenophagic machinery [93].

Rab33

Rab33 GTPase (27 kDa) localized in the Golgi apparatus is involved in intra-Golgi transport. Currently, it is known to have two isoforms: Rab33A and Rab33B. Rab33A is specifically expressed in the brain and immune system, while Rab33B is widely expressed in mammalian tissues [94–96]. Rab33B is known to bind with its effector, Golgi protein GM130, which is essential for Golgi-ER retrograde transport [97, 98]. Rab33A and Rab33B interact with Atg16L, regulating the stability of the Atg12-Atg5-Atg16 conjugation system essential for autophagosome formation. Overexpression of the constitutively active Rab33Q92L induces LC3 lipidation even under nutrient-rich conditions [99]. In addition, Rab33B interacts directly with RabGAP OATL1 to regulate autophagosomal maturation and participates in the fusion of autophagosomes and lysosomes. Overexpression of Rab33Q92L perturbs the fusion of the autophagic flux due to OATL1 inactivating Rab33B activity, thus increasing lipidation of LC3 but no colocalization with lysosomal membrane protein LAMP1 [100], indicating that Rab33B-overexpressed cells have limited capability to complete the autophagic flux. Recent studies prove the interaction of Rab33A with Atg16L and the necessity of this interaction for dense-core vesicle localization of Atg16L in neuroendocrine PC12 cells. Knockdown of endogenous Atg16L in cells results in perturbation of hormone secretion, independent of autophagic activity [99, 101, 102].Thus, Atg16L may serve as a Rab33A effector in neuroendocrine cells to control autophagy and secretion from dense-core vesicles.

Rab37

Rab37 GTPase (25 kDa) localized in the Golgi apparatus participates in autophagosome formation [103]. Rab37 is involved in various physiological and pathological processes, such as mast cell degranulation and insulin secretion by regulating exocytosis [104–106]. In addition, Rab37 functions as a tumor suppressor gene by suppressing cancer metastasis [107, 108]. Besides these functions, Rab37 is associated with autophagy. Previous studies have shown that upon starvation, Rab37 localizes to autophagosomes and interacts directly with ATG5, recruiting it to the isolation membrane. As a crucial protein required for the initiation of pre-autophagosome formation, ATG5 recruits ATG16L1 to promote

Lu *et al. Cell Biosci*   (2021) 11:35

Page 6 of 9

the assembly of the ATG5-ATG12-ATG16L1 complex, thus, facilitating autophagosome genesis. The constitutive negative mutant RAB37T43N, the mutated form of RAB37 that does not bind with GTP is scarcely associated with ATG5. Thus, RAB37 regulates autophagy in a GTP-dependent manner [109].

Conclusion and future perspectives

The involvement of Golgi-associated Rab GTPases in the initiation, formation, maturation, and fusion of autophagosome summarized in this review suggests the crucial role played by them in autophagy. Among the ~ 20 Golgi-associated Rab GTPases, eight have been shown to be associated with autophagy. The involvement of Golgi-associated Rab GTPases in autophagy has received increased attention. Rab1 and Rab6 are recruited to PAS and is necessary for autophagosome biogenesis [57, 64, 65]. Rab33 and Rab37 are participated in Phagophore formation [99, 109]. Rab24 and Rab30 are essential for autophagosome formation [81, 82, 92, 93]. Rab6 and Rab33 are crucial for autophagosome-lysosome fusion [100]. Rab9 and Rab11 play a pivotal role in the autophagosome-endosome fusion stage [71, 72, 78, 79]. However, a comprehensive understanding of Golgi-associated Rab GTPase-related membrane trafficking events regarding the optimization of autophagic flux remains limited.

Although studies elucidating the role and mechanism of Rab GTPases have greatly improved our understanding of them in the past years, the underlying mechanisms of these GTPases are complex and require further investigation. Previous studies have shown that Rab1 might regulate Atg19 by activating the kinase activity of Hrr25/CKIδ. In addition, the GEF TRAPPIII of Rab1 is essential for the trafficking of Atg9 vesicles in the Cvt pathway, implying diverse roles of Rab1 in autophagy [57]. Rab30 is essential for the morphological integrity of the Golgi complex. A previous proteomic screen revealed that Rab30 interacts with a large set of Golgi proteins (such as golgins and the GARP complex), implying its participation in autophagy and regulation of multiple stages of Golgi homeostasis [93]. Rab33 may regulate autophagy by forming a complex feedback loop with three other factors: the Atg12-5-16L1 complex, Atg8 homologs, and OATL1 [100]. In addition, Rab GTPases can fulfill their function through binding their Rab effectors, which are specific proteins that interact with the GTP-bound form of Rab GTPase [33, 110]. Some Rab effectors are involved in autophagy by interacting with autophagy proteins. For example, Rab1 effectors C9orf72 and SMCR8 regulate autophagy by facilitating ULK1 transport to phagophores [111–113], suggesting Rab GTPases may also control autophagy via their specific effectors. These findings

imply the diverse functions and mechanism of Golgi-associated Rab GTPases. Thus, future intensive studies on the precise molecular mechanism of Golgi-associated Rab GTPases are required to fully understand their importance in autophagy.

Autophagy is important for maintaining cellular homeostasis and is involved in the pathogenesis of a myriad of diseases, such as metabolic disorders, cancers, neurodegenerative diseases, and pathogen infections [114–117]. Interestingly, alteration in Golgi-associated Rab GTPase is also implicated in these diseases. Rab9 and Rab11 are important for energy metabolism: Rab9 overexpression reduces lipid storage [118]; Rab11, by affecting insulin sensitivity, is involved in diabetes pathogenesis [119]; [120–122] Rab1, Rab6, Rab9, and Rab11 participate in cancer progression: Rab1 regulates the development of hepatocellular carcinoma by modulating the mTOR pathway [120]; Rab6 interact with myosin II and acts as a negative regulator in multiple cancer cells, including lung cancer and osteosarcoma [121]; the progression of gastric cancer can be suppressed by inhibiting the Akt pathway via Rab9 silencing [122]; and the overexpression of Rab11 indicates poor survival time in patients with colorectal carcinoma [123]. In addition, Rab1 and Rab11 play crucial roles in the development of the nervous system[124, 125]: Rab1 protects against neuron loss in an animal model of Parkinson's disease [124]; decreased Rab11 is observed in multiple neurodegenerative diseases; and overexpression of Rab11 slows down the progress of these diseases [125]. Rab1, Rab6, Rab11, Rab30, and Rab33B are involved in pathogen infections: the *Legionella pneumophila* effector SetA modifies the GDP-bound form of Rab1, thus, regulating its activity [126]; Rab6 affects the proliferation of *Staphylococcus aureus* in macrophages [127]; Rab11 contributes to the assembly of the core of influenza A [128]; Rab30 is involved in the immune response to GAS infection [93]; and Rab33B participates in hepatitis B virus assembly by regulating its nucleocapsid formation and trafficking [129]. In summary, both Golgi-associated Rab GTPases and autophagy are involved in the pathogenesis of the same set of diseases. Therefore, it is important to determine whether the involvement of Golgi-associated Rab GTPases in the pathogenesis of these diseases is through regulation of autophagy. A comprehensive understanding of Golgi-associated Rab GTPases in autophagy provides novel scientific knowledge related to autophagy and the pathogenesis of autophagy-related diseases.

Lu *et al. Cell Biosci* (2021) 11:35

Page 7 of 9

protein; GAP: GTPase activating domain; GAS: Group A Streptococcus; GcAVs: GAS-containing autophagosome-like vacuoles; GDP: Guanosine diphosphate; GEF: Guanine nucleotide exchange factor; GG: Geranylgeranyl groups; GTP: Guanosine triphosphate; JNK: C-jun N-terminal kinase; LC3: Microtubule-associated protein light chain 3; LAMP1: Lysosomal-associated membrane protein 1; LAMP2: Lysosomal-associated membrane protein 2; MEFs: Mouse embryo fibroblasts; mTOR: Mammalian target of rapamycin; mTORC1: Mammalian target of rapamycin complex 1; MVB: Multivesicular bodies; PI3K: Phosphatidylinositol 3-kinase; PIP3: Phosphatidylinositol 3-phosphate; PE: Phosphatidylethanolamine; PAS: Pre-autophagosomal structure; PI4KB: Phosphatidylinositol 4-kinase beta; RabGAP: Rab GTPase activating protein; TGN: Trans-Golgi network; ULK1: Unc-51 Like Autophagy Activating Kinase 1.

References
1. Parzych KR, Klionsky DJ. An overview of autophagy: morphology, mechanism, and regulation. Antioxid Redox Signal. 2014;20(3):460–73.
2. Yang Z, Klionsky DJ. An overview of the molecular mechanism of autophagy. Curr Top Microbiol Immunol. 2009;335:1–32.
3. Wen X, Klionsky DJ. An overview of macroautophagy in yeast. J Mol Biol. 2016;428(9 Pt A):1681–99.
4. Klionsky DJ, Cregg JM, Dunn WA, Emr SD, Sakai Y, Sandoval IV, et al. A unified nomenclature for yeast autophagy-related genes. Dev Cell. 2003;5(4):539–45.
5. Weidberg H, Shvets E, Elazar Z. Biogenesis and cargo selectivity of autophagosomes. Annu Rev Biochem. 2011;80:125–56.
6. Hansen TE, Johansen T. Following autophagy step by step. BMC Biol. 2011;9:39.
7. Glick D, Barth S, Macleod KF. Autophagy: cellular and molecular mechanisms. J Pathol. 2010;221(1):3–12.
8. Yu L, Chen Y, Tooze SA. Autophagy pathway: Cellular and molecular mechanisms. Autophagy. 2018;14(2):207–15.
9. Hosokawa N, Hara T, Kaizuka T, Kishi C, Takamura A, Miura Y, et al. Nutrient-dependent mTORC1 Association with the ULK1-Atg13-FIP200 Complex Required for Autophagy. Mol Biol Cell. 2009;20(7):1981–91.
10. Jung CH, Jun CB, Ro SH, Kim YM, Otto NM, Cao J, et al. ULK-Atg13-FIP200 Complexes Mediate mTOR Signaling to the Autophagy Machinery. Mol Biol Cell. 2009;20(7):1992–2003.
11. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell. 2008;132(1):27–42.
12. Talloczy Z, Jiang WX, Virgin HW, Leib DA, Scheuner D, Kaufman RJ, et al. Regulation of starvation- and virus-induced autophagy by the eIF2 alpha kinase signaling pathway. Proc Natl Acad Sci USA. 2002;99(1):190–5.
13. Codogno P, Meijer AJ. Autophagy and signaling: their role in cell survival and cell death. Cell Death Differ. 2005;12:1509–18.
14. Wei YJ, Pattingre S, Sinha S, Bassik M, Levine B. JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. Mol Cell. 2008;30(6):678–88.
15. Tasdemir E, Maiuri MC, Galluzzi L, Vitale I, Djavaheri-Mergny M, D'Amelio M, et al. Regulation of autophagy by cytoplasmic p53. Nat Cell Biol. 2008;10(6):676–87.
16. Maiuri MC, Galluzzi L, Morselli E, Kepp O, Malik SA, Kroemer G. Autophagy regulation by p53. Curr Opin Cell Biol. 2010;22(2):181–5.
17. Cao Y, Klionsky DJ. Physiological functions of Atg6/Beclin 1: a unique autophagy-related protein. Cell Res. 2007;17(10):839–49.
18. Adi-Harel S, Erlich S, Schmukler E, Cohen-Kedar S, Segev O, Mizrachy L, et al. Beclin 1 self-association is independent of autophagy induction by amino acid deprivation and rapamycin treatment. J Cell Biochem. 2010;110(5):1262–71.
19. Kang R, Zeh HJ, Lotze MT, Tang D. The Beclin 1 network regulates autophagy and apoptosis. Cell Death Differ. 2011;18(4):571–80.
20. Wu YT, Tan HL, Shui GH, Bauvy C, Huang Q, Wenk MR, et al. Dual role of 3-methyladenine in modulation of autophagy via different temporal patterns of inhibition on class i and iii phosphoinositide 3-kinase. J Biol Chem. 2010;285(14):10850–61.
21. Nakatogawa H. Two ubiquitin-like conjugation systems that mediate membrane formation during autophagy. Essays Biochem. 2013;55:39–50.
22. Romanov J, Walczak M, Ibiricu I, Schuchner S, Ogris E, Kraft C, et al. Mechanism and functions of membrane binding by the Atg5-Atg12/Atg16 complex during autophagosome formation. Embo Journal. 2012;31(22):4304–17.
23. Klionsky DJ, Schulman BA. Dynamic regulation of macroautophagy by distinctive ubiquitin-like proteins. Nat Struct Mol Biol. 2014;21(4):336–45.
24. Mizushima N, Yoshimori T, Levine B. Methods in mammalian autophagy. Res Cell. 2010;140(3):313–26.
25. Yoshii SR, Mizushima N. Monitoring and measuring autophagy. Int J Mol Sci. 2017;18:9.
26. Feng YC, He D, Yao ZY, Klionsky DJ. The machinery of macroautophagy. Cell Res. 2014;24(1):24–41.
27. Nakamura S, Yoshimori T. New insights into autophagosome-lysosome fusion. J Cell Sci. 2017;130(7):1209–16.
28. Jager S, Bucci C, Tanida I, Ueno T, Kominami E, Saftig P, et al. Role for Rab7 in maturation of late autophagic vacuoles. J Cell Sci. 2004;117(20):4837–48.
29. Sanchez-Wandelmer J, Reggiori F. Amphisomes: out of the autophagosome shadow? Embo Journal. 2013;32(24):3116–8.
30. Kaminskyy V, Zhivotovsky B. Proteases in autophagy. Biochimica Et Biophysica Acta-Proteins Proteomics. 2012;1824(1):44–50.
31. Stenmark H. Rab GTPases as coordinators of vesicle traffic. Nat Rev Mol Cell Biol. 2009;10(8):513–25.
32. Wandinger-Ness A, Zerial M. Rab proteins and the compartmentalization of the endosomal system. Cold Spring Harb Perspect Biol. 2014;6(11):a022616.
33. Zhen Y, Stenmark H. Cellular functions of Rab GTPases at a glance. J Cell Sci. 2015;128(17):3171–6.
34. Ali BR, Seabra MC. Targeting of Rab GTPases to cellular membranes. Biochem Soc Trans. 2005;33(Pt 4):652–6.
35. Seabra MC, Wasmeier C. Controlling the location and activation of Rab GTPases. Curr Opin Cell Biol. 2004;16(4):451–7.
36. Egami Y, Kiryu-Seo S, Yoshimori T, Kiyama H. Induced expressions of Rab24 GTPase and LC3 in nerve-injured motor neurons. Biochem Biophys Res Commun. 2005;337(4):1206–13.
37. Cherfils J, Zeghouf M. Regulation of small GTPases by GEFs, GAPs, and GDIs. Physiol Rev. 2013;93(1):269–309.
38. Prieto-Dominguez N, Parnell C, Teng Y. Drugging the small GTPase pathways in cancer treatment: promises and challenges. Cells. 2019;8:3.
39. Grant BJ, McCammon JA, Gorfe AA. Conformational selection in G-proteins: lessons from Ras and Rho. Biophys J. 2010;99(11):L87-9.
40. Liu S, Storrie B. How Rab proteins determine Golgi structure. Int Rev Cell Mol Biol. 2015;315:1–22.
41. Liu S, Storrie B. Are Rab proteins the link between Golgi organization and membrane trafficking? Cell Mol Life Sci. 2012;69(24):4093–106.

Lu *et al. Cell Biosci*   (2021) 11:35

Page 8 of 9

42. Constantino-Jonapa LA, Hernandez-Ramirez VI, Osorio-Trujillo C, Talamas-Rohana P. EhRab21 associates with the Golgi apparatus in Entamoeba histolytica. Parasitol Res. 2020. https://doi.org/10.1007/s00436-020-06667-7.
43. Progida C. Multiple Roles of Rab GTPases at the Golgi. Results Probl Cell Differ. 2019;67:95–123.
44. Starr T, Sun Y, Wilkins N, Storrie B. Rab33b and Rab6 are functionally overlapping regulators of Golgi homeostasis and trafficking. Traffic. 2010;11(5):626–36.
45. Hutagalung AH, Novick PJ. Role of Rab GTPases in membrane traffic and cell physiology. Physiol Rev. 2011;91(1):119–49.
46. Sannerud R, Marie M, Nizak C, Dale HA, Pernet-Gallay K, Perez F, et al. Rab1 defines a novel pathway connecting the pre-Golgi intermediate compartment with the cell periphery. Mol Biol Cell. 2006;17(4):1514–26.
47. Touchot N, Zahraoui A, Vielh E, Tavitian A. Biochemical properties of the YPT-related rab1B protein. Comparison with rab1A. FEBS Lett. 1989;256(1–2):79–84.
48. Plutner H, Cox AD, Pind S, Khosravi-Far R, Bourne JR, Schwaninger R, et al. Rab1b regulates vesicular transport between the endoplasmic reticulum and successive Golgi compartments. J Cell Biol. 1991;115(1):31–43.
49. Thomas JD, Zhang YJ, Wei YH, Cho JH, Morris LE, Wang HY, et al. Rab1A Is an mTORC1 activator and a colorectal oncogene. Cancer Cell. 2014;26(5):754–69.
50. Tisdale EJ, Bourne JR, Khosravi-Far R, Der CJ, Balch WE. GTP-binding mutants of rab1 and rab2 are potent inhibitors of vesicular transport from the endoplasmic reticulum to the Golgi complex. J Cell Biol. 1992;119(4):749–61.
51. Nuoffer C, Davidson HW, Matteson J, Meinkoth J, Balch WE. A GDP-bound of rab1 inhibits protein export from the endoplasmic reticulum and transport between Golgi compartments. J Cell Biol. 1994;125(2):225–37.
52. Alvarez C, Garcia-Mata R, Brandon E, Sztul E. COPI recruitment is modulated by a Rab1b-dependent mechanism. Mol Biol Cell. 2003;14(5):2116–27.
53. Satoh A, Wang Y, Malsam J, Beard MB, Warren G. Golgin-84 is a rab1 binding partner involved in Golgi structure. Traffic. 2003;4(3):153–61.
54. Zoppino FC, Militello RD, Slavin I, Alvarez C, Colombo MI. Autophagosome formation depends on the small GTPase Rab1 and functional ER exit sites. Traffic. 2010;11(9):1246–61.
55. Meiling-Wesse K, Epple UD, Krick R, Barth H, Appelles A, Voss C, et al. Trs85 (Gsg1), a component of the TRAPP complexes, is required for the organization of the preautophagosomal structure during selective autophagy via the Cvt pathway. J Biol Chem. 2005;280(39):33669–78.
56. Wang J, Menon S, Yamasaki A, Chou HT, Walz T, Jiang Y, et al. Ypt1 recruits the Atg1 kinase to the preautophagosomal structure. Proc Natl Acad Sci U S A. 2013;110(24):9800–5.
57. Wang J, Davis S, Menon S, Zhang J, Ding J, Cervantes S, et al. Ypt1/Rab1 regulates Hrr25/CK1delta kinase activity in ER-Golgi traffic and macroautophagy. J Cell Biol. 2015;210(2):273–85.
58. Martinez O, Schmidt A, Salamero J, Hoflack B, Roa M, Goud B. The small GTP-binding protein Rab6 functions in intra-golgi transport. J Cell Biol. 1994;127(6):1575–88.
59. Martinez O, Antony C, PehauArnaudet G, Berger EG, Salamero J, Goud B. GTP-bound forms of rab6 induce the redistribution of Golgi proteins into the endoplasmic reticulum. Proc Natl Acad Sci USA. 1997;94(5):1828–33.
60. White J, Johannes L, Mallard F, Girod A, Grill S, Reinsch S, et al. Rab6 coordinates a novel Golgi to ER retrograde transport pathway in live cells. J Cell Biol. 1999;147(4):743–59.
61. Heffernan LF, Simpson JC. The trials and tubule-ations of Rab6 involvement in Golgi-to-ER retrograde transport. Biochem Soc Trans. 2014;42(5):1453–9.
62. Short B, Preisinger C, Schaletzky J, Kopajtich R, Barr FA. The Rab6 GTPase regulates recruitment of the dynactin complex to Golgi membranes. Curr Biol. 2002;12(20):1792–5.
63. Iwanami N, Nakamura Y, Satoh T, Liu ZG, Satoh AK. Rab6 is required for multiple apical transport pathways but not the basolateral transport pathway in drosophila photoreceptors. Plos Genetics. 2016;12(2):26.
64. Yang S, Rosenwald AG. Autophagy in Saccharomyces cerevisiae requires the monomeric GTP-binding proteins, Arl1 and Ypt6. Autophagy. 2016;12(10):1721–37.
65. Suda Y, Kurokawa K, Hirata R, Nakano A. Rab GAP cascade regulates dynamics of Ypt6 in the Golgi traffic. Proc Natl Acad Sci U S A. 2013;110(47):18976–81.
66. Ye M, Chen Y, Zou SS, Yu S, Liang YH. Ypt1 suppresses defects of vesicle trafficking and autophagy in Ypt6 related mutants. Cell Biol Int. 2014;38(5):663–74.
67. Ayala CI, Kim J, Neufeld TP. Rab6 promotes insulin receptor and cathepsin trafficking to regulate autophagy induction and activity in Drosophila. J Cell Sci. 2018;131:17.
68. Ganley IG, Carroll K, Bittova L, Pfeffer S. Rab9 GTPase regulates late endosome size and requires effector interaction for its stability. Mol Biol Cell. 2004;15(12):5420–30.
69. Ji W, Rivero F. Atypical Rho GTPases of the RhoBTB subfamily: roles in vesicle trafficking and tumorigenesis. Cells. 2016;5:2.
70. Kucera A, Bakke O, Progida C. The multiple roles of Rab9 in the endolysosomal system. Commun Integr Biol. 2016;9(4):e1204498.
71. Nishida Y, Arakawa S, Fujitani K, Yamaguchi H, Mizuta T, Kanaseki T, et al. Discovery of Atg5/Atg7-independent alternative macroautophagy. Nature. 2009;461(7264):654–8.
72. Arakawa S, Honda S, Yamaguchi H, Shimizu S. Molecular mechanisms and physiological roles of Atg5/Atg7-independent alternative autophagy. Proc Jpn Acad Ser B Phys Biol Sci. 2017;93(6):378–85.
73. Ullrich O, Reinsch S, Urbe S, Zerial M, Parton RG. Rab11 regulates recycling through the pericentriolar recycling endosome. J Cell Biol. 1996;135(4):913–24.
74. Wilcke M, Johannes L, Galli T, Mayau V, Goud B, Salamero J. Rab11 regulates the compartmentalization of early endosomes required for efficient transport from early endosomes to the trans-golgi network. J Cell Biol. 2000;151(6):1207–20.
75. Takahashi S, Kubo K, Waguri S, Yabashi A, Shin HW, Katoh Y, et al. Rab11 regulates exocytosis of recycling vesicles at the plasma membrane. J Cell Sci. 2012;125(17):4049–57.
76. Savina A, Fader CM, Damiani MT, Colombo MI. Rab11 promotes docking and fusion of multivesicular bodies in a calcium-dependent manner. Traffic. 2005;6(2):131–43.
77. Gabernet-Castello C, O'Reilly AJ, Dacks JB, Field MC. Evolution of Tre-2/Bub2/Cdc16 (TBC) Rab GTPase-activating proteins. Mol Biol Cell. 2013;24(10):1574–83.
78. Kern A, Dikic I, Behl C. The integration of autophagy and cellular trafficking pathways via RAB GAPs. Autophagy. 2015;11(12):2393–7.
79. Longatti A, Lamb CA, Razi M, Yoshimura S, Barr FA, Tooze SA. TBC1D14 regulates autophagosome formation via Rab11- and ULK1-positive recycling endosomes. J Cell Biol. 2012;197(5):659–75.
80. Munafo DB, Colombo MI. Induction of autophagy causes dramatic changes in the subcellular distribution of GFP-Rab24. Traffic. 2002;3(7):472–82.
81. Gutierrez MG, Vazquez CL, Munafo DB, Zoppino FC, Beron W, Rabinovitch M, et al. Autophagy induction favours the generation and maturation of the Coxiella-replicative vacuoles. Cell Microbiol. 2005;7(7):981–93.
82. Newton HJ, Kohler LJ, McDonough JA, Temoche-Diaz M, Crabill E, Hartland EL, et al. A screen of Coxiella burnetii mutants reveals important roles for Dot/Icm effectors and host autophagy in vacuole biogenesis. PLoS Pathog. 2014;10(7):e1004286.
83. Ylä-Anttila P, Mikkonen E, Happonen KE, Holland P, Ueno T, Simonsen A, et al. RAB24 facilitates clearance of autophagic compartments during basal conditions. Autophagy. 2015;11(10):1833–48.
84. Gillingham AK, Sinka R, Torres IL, Lilley KS, Munro S. Toward a comprehensive map of the effectors of rab GTPases. Dev Cell. 2014;31(3):358–73.
85. Makhoul C, Gosavi P, Duffield R, Delbridge B, Williamson NA, Gleeson PA. Intersectin-1 interacts with the golgin GCC88 to couple the actin network and Golgi architecture. Mol Biol Cell. 2019;30(3):370–86.
86. Hsu RM, Zhong CY, Wang CL, Liao WC, Yang C, Lin SY, et al. Golgi tethering factor golgin-97 suppresses breast cancer cell invasiveness by modulating NF-kappaB activity. Cell Commun Signal. 2018;16(1):19.

Lu *et al. Cell Biosci*   (2021) 11:35

Page 9 of 9

87. Sohda M, Misumi Y, Ogata S, Sakisaka S, Hirose S, Ikehara Y, et al. Trans-Golgi protein p230/golgin-245 is involved in phagophore formation. Biochem Biophys Res Commun. 2015;456(1):275–81.

88. Linders PT, Horst CV, Beest MT, van den Bogaart G. Stx5-mediated ER-golgi transport in mammals and yeast. Cells. 2019;8:8.

89. Claussen M, Suter B. BicD-dependent localization processes: from Drosophilia development to human cell biology. Ann Anat. 2005;187(5–6):539–53.

90. Thomas C, Rousset R, Noselli S. JNK signalling influences intracellular trafficking during Drosophila morphogenesis through regulation of the novel target gene Rab30. Dev Biol. 2009;331(2):250–60.

91. Kelly EE, Giordano F, Horgan CP, Jollivet F, Raposo G, McCaffrey MW. Rab30 is required for the morphological integrity of the Golgi apparatus. Biol Cell. 2012;104(2):84–101.

92. Oda S, Nozawa T, Nozawa-Minowa A, Tanaka M, Aikawa C, Harada H, et al. Golgi-resident GTPase Rab30 promotes the biogenesis of pathogen-containing autophagosomes. PLoS One. 2016;11(1):e0147061.

93. Nakajima K, Nozawa T, Minowa-Nozawa A, Toh H, Yamada S, Aikawa C, et al. RAB30 regulates PI4KB (phosphatidylinositol 4-kinase beta)-dependent autophagy against group A Streptococcus. Autophagy. 2019;15(3):466–77.

94. 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. J Cell Sci. 1998;111:1061–9.

95. Cheng E, Trombetta SE, Kovacs D, Beech RD, Ariyan S, Reyes-Mugica M, et al. Rab33A: Characterization, expression, and suppression by epigenetic modification. J Investig Dermatol. 2006;126(10):2257–71.

96. Huang LG, Urasaki A, Inagaki N. Rab33a and Rab33ba mediate the outgrowth of forebrain commissural axons in the zebrafish brain. Sci Rep. 2019;9:9.

97. Ortiz Sandoval C, Simmen T. Rab proteins of the endoplasmic reticulum: functions and interactors. Biochem Soc Trans. 2012;40(6):1426–32.

98. Valsdottir R, Hashimoto H, Ashman K, Koda T, Storrie B, Nilsson T. Identification of rabaptin-5, rabex-5, and GM130 as putative effectors of rab33b, a regulator of retrograde traffic between the Golgi apparatus and ER. FEBS Lett. 2001;508(2):201–9.

99. Itoh T, Fujita N, Kanno E, Yamamoto A, Yoshimori T, Fukuda M. Golgi-resident small GTPase Rab33B interacts with Atg16L and modulates autophagosome formation. Mol Biol Cell. 2008;19(7):2916–25.

100. Itoh T, Kanno E, Uemura T, Waguri S, Fukuda M. OATL1, a novel autophagosome-resident Rab33B-GAP, regulates autophagosomal maturation. J Cell Biol. 2011;192(5):839–53.

101. Fukuda M, Itoh T. Direct link between Atg protein and small GTPase Rab: Atg16L functions as a potential Rab33 effector in mammals. Autophagy. 2008;4(6):824–6.

102. 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. Mol Biol Cell. 2012;23(16):3193–202.

103. Sundberg TB, Darricarrere N, Cirone P, Li X, McDonald L, Mei X, et al. Disruption of Wnt planar cell polarity signaling by aberrant accumulation of the MetAP-2 substrate Rab37. Chem Biol. 2011;18(10):1300–11.

104. Masuda ES, Luo Y, Young C, Shen M, Rossi AB, Huang BC, et al. Rab37 is a novel mast cell specific GTPase localized to secretory granules. FEBS Lett. 2000;470(1):61–4.

105. Tsuboi T, Fukuda M. Rab3A and Rab27A cooperatively regulate the docking step of dense-core vesicle exocytosis in PC12 cells. J Cell Sci. 2006;119(Pt 11):2196–203.

106. Zografou S, Basagiannis D, Papafotika A, Shirakawa R, Horiuchi H, Auerbach D, et al. A complete Rab screening reveals novel insights in Weibel-Palade body exocytosis. J Cell Sci. 2012;125(Pt 20):4780–90.

107. Tsai CH, Cheng HC, Wang YS, Lin H, Jen J, Kuo IY, et al. Small GTPase Rab37 targets tissue inhibitor of metalloproteinase 1 for exocytosis and thus suppresses tumour metastasis. Nat Commun. 2014;5:4804.

108. Wu CY, Tseng RC, Hsu HS, Wang YC, Hsu MT. Frequent down-regulation of hRAB37 in metastatic tumor by genetic and epigenetic mechanisms in lung cancer. Lung Cancer. 2009;63(3):360–7.

109. Sheng Y, Song Y, Li Z, Wang Y, Lin H, Cheng H, et al. RAB37 interacts directly with ATG5 and promotes autophagosome formation via regulating ATG5-12-16 complex assembly. Cell Death Differ. 2018;25(5):918–34.

110. Grosshans BL, Ortiz D, Novick P. Rabs and their effectors: achieving specificity in membrane traffic. Proc Natl Acad Sci U S A. 2006;103(32):11821–7.

111. Webster CP, Smith EF, Bauer CS, Moller A, Hautbergue GM, Ferraiuolo L, et al. The C9orf72 protein interacts with Rab1a and the ULK1 complex to regulate initiation of autophagy. EMBO J. 2016;35(15):1656–76.

112. Yang M, Liang C, Swaminathan K, Herrlinger S, Lai F, Shiekhattar R, et al. A C9ORF72/SMCR8-containing complex regulates ULK1 and plays a dual role in autophagy. Sci Adv. 2016;2(9):e1601167.

113. Jung J, Nayak A, Schaeffer V, Starzetz T, Kirsch AK, Muller S, et al. Multi-plex image-based autophagy RNAi screening identifies SMCR8 as ULK1 kinase activity and gene expression regulator. Elife. 2017;6:e23063.

114. Yang Y, Klionsky DJ. Autophagy and disease: unanswered questions. Cell Death Differ. 2020;27(3):858–71.

115. Marsh T, Debnath J. Autophagy suppresses breast cancer metastasis by degrading NBR1. Autophagy. 2020;9:1–2.

116. Heras-Sandoval D, Perez-Rojas JM, Pedraza-Chaverri J. Novel compounds for the modulation of mTOR and autophagy to treat neurodegenerative diseases. Cell Signal. 2020;65:109442.

117. Zhou J, Kang X, Luo Y, Yuan Y, Wu Y, Wang M, et al. Glibenclamide-induced autophagy inhibits its insulin secretion-improving function in beta cells. Int J Endocrinol. 2019;2019:1265175.

118. Narita K, Choudhury A, Dobrenis K, Sharma DK, Holicky EL, Marks DL, et al. Protein transduction of Rab9 in Niemann-Pick C cells reduces cholesterol storage. FASEB J. 2005;19(11):1558–60.

119. Du W, Zhao S, Gao F, Wei M, An J, Jia K, et al. IFN-gamma/mTORC1 decreased Rab11 in Schwann cells of diabetic peripheral neuropathy, inhibiting cell proliferation via GLUT1 downregulation. J Cell Physiol. 2020;235(7–8):5764–76.

120. Dong Z, Qi R, Guo X, Zhao X, Li Y, Zeng Z, et al. MiR-223 modulates hepatocellular carcinoma cell proliferation through promoting apoptosis via the Rab1-mediated mTOR activation. Biochem Biophys Res Commun. 2017;483(1):630–7.

121. Vestre K, Kjos I, Guadagno NA, Borg Distefano M, Kohler F, Fenaroli F, et al. Rab6 regulates cell migration and invasion by recruiting Cdc42 and modulating its activity. Cell Mol Life Sci. 2019;76(13):2593–614.

122. Zhu Y, Shi F, Wang M, Ding J. Knockdown of Rab9 suppresses the progression of gastric cancer through regulation of akt signaling pathway. Technol Cancer Res Treat. 2020;19:1533033820915958.

123. Chung YC, Wei WC, Hung CN, Kuo JF, Hsu CP, Chang KJ, et al. Rab11 collaborates E-cadherin to promote collective cell migration and indicates a poor prognosis in colorectal carcinoma. Eur J Clin Invest. 2016;46(12):1002–11.

124. Cooper AA, Gitler AD, Cashikar A, Haynes CM, Hill KJ, Bhullar B, et al. Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. Science. 2006;313(5785):324–8.

125. Zhang J, Su G, Wu Q, Liu J, Tian Y, Liu X, et al. Rab11-mediated recycling endosome role in nervous system development and neurodegenerative diseases. Int J Neurosci. 2020;8:1–12.

126. Wang Z, McCloskey A, Cheng S, Wu M, Xue C, Yu Z, et al. Regulation of the small GTPase Rab1 function by a bacterial glucosyltransferase. Cell Discov. 2018;4:53.

127. Chen Y, Jiang C, Jin M, Gong Y, Zhang X. The role of Rab6 GTPase in the maturation of phagosome against Staphylococcus aureus. Int J Biochem Cell Biol. 2015;61:35–44.

128. Vale-Costa S, Amorim MJ. Clustering of Rab11 vesicles in influenza A virus infected cells creates hotspots containing the 8 viral ribonucleo-proteins. Small GTPases. 2017;8(2):71–7.

129. Bartusch C, Doring T, Prange R. Rab33B controls hepatitis b virus assembly by regulating core membrane association and nucleocapsid processing. Viruses. 2017;9(6):e23.

Publisher's note