

# The integration of autophagy and cellular trafficking pathways via RAB GAPs

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**Abbreviations:** ATG, autophagy related; BECN1, Beclin 1, autophagy related; CAL-COCO2, calcium binding and coiled-coil domain 2; ER, endoplasmic reticulum; GABARAP, GABA(A) receptor-associated protein; GDP, guanosine-5'-diphosphate; GTP, guanosine-5'-triphosphate; LRRK1, leucine-rich repeat kinase 1; MAP1LC3, microtubule-associated protein 1 light chain 3; NBR1, neighbor of BRCA1 gene 1; PAS, phagophore assembly site; PE, phosphatidylethanolamine; PIK3C3, phosphatidylinositol 3-kinase, catalytic subunit type 3; RAB GAP, RAB GTPase activating protein; RAB GEF, RAB GTPase guanine exchange factor; SQSTM1, sequestosome 1; TBC domain, TRE2-BUB2-CDC16 domain; TBCGAP, TBC domain-containing RAB GAP; ULK, unc-51 like autophagy activating kinase; WIPI, WD repeat domain, phosphoinositide interacting 1; ZFYVE1, zinc finger, FYVE domain containing 1

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**M**acroautophagy is a conserved degradative pathway in which a double-membrane compartment sequesters cytoplasmic cargo and delivers the contents to lysosomes for degradation. Efficient formation and maturation of autophagic vesicles, so-called phagophores that are precursors to autophagosomes, and their subsequent trafficking to lysosomes relies on the activity of small RAB GTPases, which are essential factors of cellular vesicle transport systems. The activity of RAB GTPases is coordinated by upstream factors, which include guanine nucleotide exchange factors (RAB GEFs) and RAB GTPase activating proteins (RAB GAPs). A role in macroautophagy regulation for different TRE2-BUB2-CDC16 (TBC) domain-containing RAB GAPs has been established. Recently, however, a positive modulation of macroautophagy has also been demonstrated for the TBC domain-free RAB3GAP1/2, adding to the family of RAB GAPs that coordinate macroautophagy and additional cellular trafficking pathways.

**Macroautophagy is a membrane mobilization and vesicle trafficking system**

Macroautophagy is an evolutionarily conserved eukaryotic process in which cytoplasmic contents are sequestered by phagophores, which mature into autophagosomes and deliver their cargo to lysosomes for degradation.<sup>1</sup> The pathway is induced under conditions of nutrient deprivation or stress and is an important functional component of the cellular homeostasis network. Deterioration of macroautophagy is associated with several disorders, including neurodegenerative diseases and cancer.<sup>2</sup>

One main characteristic of macroautophagy is the double-membrane autophagosome, which are generated at distinct cellular locations, the phagophore assembly sites (PAS). Upon macroautophagy induction, the activated ULK1/2 complex (including ATG13 and RB1CC1/FIP200) and phosphatidylinositol 3-kinase complex (including PIK3C3/Vps34, ATG14, and BECN1/Vps30/Atg6) are recruited to the PAS and initiate the formation of a phagophore by directing additional autophagic proteins to this site. These include WIPI1/Atg18, WIPI2/Atg18, ZFYVE1/DFCP1, ATG9, and the ATG12–ATG5–ATG16L1 complex.<sup>3</sup> The latter is part of a ubiquitin-like conjugation system and mediates the attachment of phosphatidylethanolamine to the C terminus of Atg8 family members. This protein family comprises the subfamilies of MAP1LC3 and GABARAP in mammals, and lipidation results in their binding to the growing phagophore membrane which is essential for phagophore expansion and maturation.<sup>4</sup>

Phagophore formation and autophagosome maturation are dependent on the adequate supply of membranes and appropriate cellular membrane dynamics. Recently, the plasma membrane, the Golgi, the ER,<sup>5,6</sup> and lipid droplets<sup>7</sup> have been recognized as lipid sources. In response to different regimens of macroautophagic activity they are considered to be selectively accessed to satisfy macroautophagic membrane requirements.<sup>8</sup> Interestingly, it is considered that the phagophore matures to an autophagosome by the addition of lipids via vesicular fusion rather than via lateral movement of membranes from existing cellular organelles.<sup>5,9</sup> Consequently, the resulting sophisticated and complex

membrane acquisition system needs to be carefully coordinated, and proteins that control vesicle transport systems are important factors for macroautophagy.

The protein family of small RAB GTPases is specialized in the control of vesicle transport routes and ensures trafficking of vesicles to their appropriate target compartments.<sup>10</sup> RAB GTPases interact with effector proteins such as cargo sorting complexes, motor proteins, and tethering factors, which results in vesicle budding, transport, and fusion. The interactions with these effectors are precisely controlled by GDP/GTP exchange and hydrolysis of GTP. Since GDP is principally tightly bound by RAB GTPases and their intrinsic GTP hydrolysis rates are low, this cycle is regulated by guanine exchange factors (RAB GEFs) that catalyze the dissociation of GDP, and RAB GTPase activating proteins (RAB GAPs) that facilitate the hydrolysis of GTP.<sup>11</sup> Both regulators are required to coordinate the temporal-spatial activity of RAB GTPases. In recent years multiple RAB GTPases, RAB GEFs, and RAB GAPs have functionally been associated with macroautophagy.<sup>12</sup> This commentary will focus on RAB GAPs and briefly address their effects on this degradative pathway (schematically summarized in Fig. 1) and vesicle trafficking systems.

**TBCGAPs: TBC domain-containing RAB GAPs that function in macroautophagy**

In approaches aiming to identify RAB GAPs that affect macroautophagy, several

TBC domain-containing RAB GAPs have been characterized.<sup>13-15</sup> The TBC domain accelerates the hydrolysis of GTP by RAB GTPases and TBC domain-containing RAB GAPs (hereafter referred to as TBCGAPs) are linked to different trafficking routes, and are important factors that integrate diverse cellular pathways.<sup>16</sup>

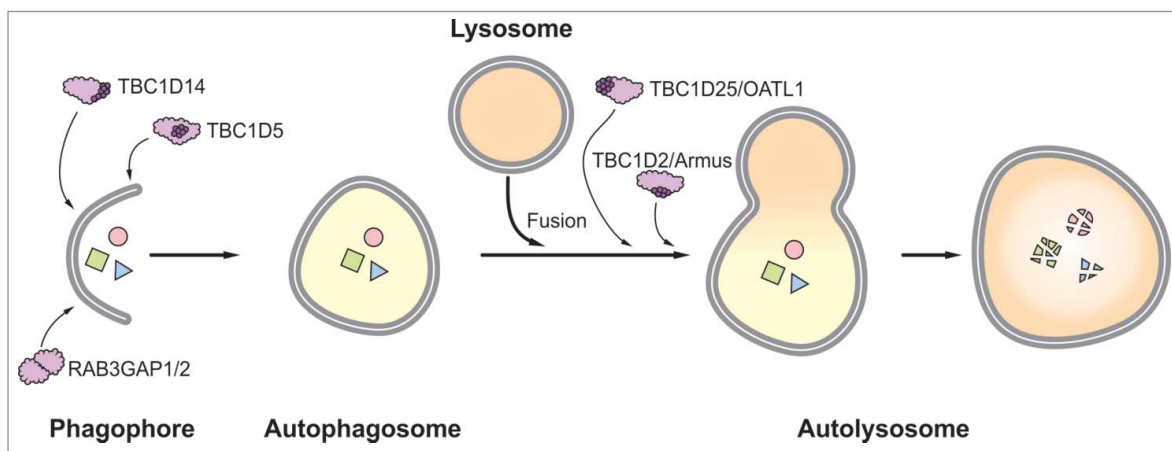
TBC1D25/OATL1 was identified in a study expressing 41 TBCGAPs in mouse embryonic fibroblasts and selecting proteins that colocalize with endogenous MAP1LC3.<sup>13</sup> TBC1D25/OATL1 targets the ATG16L1-interacting RAB GTPase RAB33B and is recruited to autophagosomes by direct binding to Atg8 family members. Increased levels of TBC1D25/OATL1 inhibit the fusion of autophagosomes with lysosomes and prevent autophagosomal maturation.

In an approach overexpressing 38 TBCGAPs in HEK293 cells and analyzing their ability to inhibit autophagosome formation upon nutrient deprivation, 11 TBCGAPs were shown to negatively regulate macroautophagy.<sup>14</sup> The TBCGAP TBC1D14 was analyzed in detail and was shown to modify the trafficking of ULK1-containing recycling endosomes and to interfere with the activity of the RAB GTPase RAB11A/B. The function of RAB11 is required to transport recycling endosomes to the PAS and, thus, TBC1D14 and RAB11 regulate starvation-induced formation of autophagosomes.

In another study employing GST affinity isolation techniques, 14 TBCGAPs were

identified to interact with Atg8 family members.<sup>15</sup> Subsequently, the colocalization of these TBCGAPs with MAP1LC3 and SQSTM1 was analyzed, resulting in 4 promising candidates. The TBCGAP TBC1D5 was further characterized and was shown to have 2 binding motifs for Atg8 family members. During basal macroautophagy conditions TBC1D5 binds to the retromer complex and influences retrograde transport routes. Upon macroautophagy induction, TBC1D5 dissociates from the retromer, associates with MAP1LC3, and directs ATG9 and active ULK1 from the retromer to the PAS.<sup>17</sup> This rerouting of ATG9 is additionally regulated by the clathrin adaptor complex (AP2) and requires functional clathrin-mediated endocytosis. Thus, the dynamic translocation of TBC1D5 to autophagosomes is central for the trafficking of ATG9 from the retromer complex to the site of autophagosome biogenesis.

The protein TBC1D2/Armus is an additional TBCGAP that interacts with MAP1LC3 and integrates trafficking pathways and macroautophagy.<sup>18</sup> Overexpression of TBC1D2 results in the accumulation of enlarged autophagosomes, and its deficiency delays macroautophagic flux. Upon macroautophagy induction, TBC1D2 is recruited to autophagosomes by binding to Atg8 family members and regulates the activity of the RAB GTPase RAB7, which is essential for the fusion of autophagosomes and lysosomes.<sup>12</sup> Interestingly, TBC1D2 is also an effector of



**Figure 1.** Schematic representation of RAB GAPs established to function in macroautophagy. TBC1D5, TBC1D14, and RAB3GAP1/2 function during autophagosome formation, and TBC1D2 and TBC1D25 support autophagosome-lysosome fusion. The TBC domain is depicted by dark purple globules. Note that this domain is missing in the heterodimeric RAB3GAP complex.

the small GTPase RAC1, which is a negative regulator of macroautophagy. Nutrient deprivation inactivates RAC1, which allows the association of TBC1D2 with autophagosomes and results in regulation of RAB7. Thus, the interplay of TBC1D2, RAC1, and RAB7 underlines the coordinate character of macroautophagy and other cellular trafficking pathways mediated by RAB GTPases and RAB GAPs.

In these studies a multitude of TBCGAPs were linked to macroautophagy, which are summarized in **Table 1** with respect to their substrate RAB GTPases and their nonautophagic functions, if characterized. Although

the influence on macroautophagy of the majority of these RAB GAPs needs to be confirmed, the large number of potential candidates highlights the complexity of the coordination of membrane or vesicle trafficking and the macroautophagic pathway.

#### RAB3GAP1 and RAB3GAP2 as non-TBCGAPs and their function in macroautophagy and beyond

The introduced TBCGAPs function in macroautophagy and contribute to the reorganization of membrane trafficking routes according to the cellular requirements. This coordinate property has been well established for TBCGAPs that are ideally placed for

such a role, as one TBCGAP can act as an effector of different RAB GTPases. Interestingly, according to sequence homology the human TBCGAP family includes 44 proteins and is complemented by the RAB3-GAP complex, which is the only described RAB GAP without a TBC domain.<sup>16</sup> The heterodimeric complex consists of the catalytic subunit RAB3GAP1 and the noncatalytic subunit RAB3GAP2<sup>19</sup> and has been well established to regulate the name-giving RAB GTPase RAB3A-D and to modify neurotransmitter release at the neuronal synapse. In a RAB3GAP1 knockout mouse model, GTP-bound RAB3 accumulates in the brain and Ca<sup>2+</sup>-dependent glutamate release from

**Table 1.** Summary of macroautophagy-associated RAB GAPs.

RAB GAP	Substrate RAB GTPases	Nonautophagic function(s)	Association with Macroautophagy
TBC1D1	RAB2A, RAB8A, RAB8B, RAB10, RAB14	SLCA4/GLUT4 trafficking, <sup>31</sup> glucose metabolism <sup>32</sup>	binds to Atg8 family members <sup>15</sup>
TBC1D2 (Armus, PARIS1)	RAB7	CDH1/E-cadherin degradation <sup>33</sup>	binds to Atg8 family members; <sup>15</sup> modulates autophagosome-lysosome fusion <sup>18</sup>
TBC1D2B	RAB22A, RAB22B	ND	binds to Atg8 family members <sup>15</sup>
TBC1D4 (AS160)	RAB2A, RAB8A, RAB10, RAB14	SLC2A4/GLUT4 trafficking, insulin sensitivity <sup>34,35</sup>	OE inhibits autophagy <sup>14</sup>
TBC1D5	RAB7	endocytic trafficking <sup>17,36</sup>	binds to Atg8 family members; <sup>15</sup> regulates ATG9 trafficking and autophagosome formation <sup>15,17</sup>
TBC1D7	RAB17	primary cilium formation; <sup>37</sup> influences MTOR activity <sup>38</sup>	OE inhibits autophagy; <sup>14</sup> binds to Atg8 family members; <sup>15</sup> influences MTOR activity <sup>38</sup>
TBC1D9	ND	ND	OE inhibits autophagy; <sup>14</sup> binds to Atg8 family members <sup>15</sup>
TBC1D9B	RAB11A	basolateral-to-apical transcytosis <sup>39</sup>	OE inhibits autophagy <sup>14</sup>
TBC1D10A (EPI64)	RAB27A, RAB27B, RAB35, RAB8A	melanosome transport; <sup>40</sup> endocytic trafficking and microvillus structure; <sup>41</sup> exosome secretion <sup>42</sup>	OE inhibits autophagy; <sup>14</sup> binds to Atg8 family members <sup>15</sup>
TBC1D10B (EPI64B)	RAB3A, RAB22A, RAB27A, RAB27B, RAB31, RAB35	exosome secretion; <sup>42</sup> regulation of exocytosis <sup>43</sup>	OE inhibits autophagy; <sup>14</sup> binds to Atg8 family members <sup>15</sup>
TBC1D10C (EPI64C, Carabin)	RAB35	T cell receptor recycling, immunological synapse formation; <sup>44</sup> exosome secretion; <sup>42</sup> PPP3/calcineurin, NFAT, RAS, MAPK/ERK, and CAMK2 signaling <sup>45</sup>	OE inhibits autophagy <sup>14</sup>
TBC1D14	ND	endocytic trafficking <sup>14</sup>	OE inhibits autophagy; <sup>14</sup> regulates autophagosome formation <sup>14</sup>
TBC1D16	RAB4A, RAB5C	EGFR recycling from endosomes <sup>46</sup>	OE inhibits autophagy; <sup>14</sup> binds to Atg8 family members <sup>15</sup>
TBC1D17	RAB21, RAB8	endocytic trafficking, interacts with OPTN <sup>47</sup>	OE inhibits autophagy; <sup>14</sup> binds to Atg8 family members <sup>15</sup>
TBC1D25 (OATL1)	RAB2A, RAB13, RAB34, RAB33A, RAB33B	ND	binds to Atg8 family members; <sup>13,15</sup> regulates autophagosome formation <sup>13</sup>
RABGAP1 (TBC1D11, GAPCenA)	RAB2, RAB4, RAB6A, RAB6B, RAB11, RAB36	microtubule and Golgi dynamics, metaphase/anaphase transition <sup>48,49</sup>	binds to Atg8 family members <sup>15</sup>
RABGAP1L (TBC1D18, HHL)	RAB22A, RAB34, RAB39B	ND	binds to Atg8 family members <sup>15</sup>
SGSM2 (RUTBC1)	RAB9A, RAB32, RAB33B,	ND	OE inhibits autophagy <sup>14</sup>
RAB3GAP1/2	GAP: RAB3A-D, <sup>50</sup> GEF: RAB18 <sup>23</sup>	neurotransmitter release; <sup>20</sup> LMAN1 trafficking; <sup>25</sup> CLDN1 exocytosis; <sup>26</sup> maintenance of ER structure <sup>23</sup>	regulates autophagosome formation <sup>22</sup>

OE, overexpression

cerebrocortical synaptosomes is inhibited.<sup>20</sup> Indeed, by regulating the activity of RAB3, the RAB3GAPs are essential for maintenance of synaptic homeostasis.<sup>21</sup> Recently, we showed that the TBC domain-free RAB3-GAP1/2 also modulate macroautophagy and are essential factors of autophagosome formation.<sup>22</sup> Deficiency of both proteins in human primary fibroblasts deteriorates autophagosomal biogenesis and reduces macroautophagic activity at basal and induced macroautophagy conditions, whereas their overexpression enhances this process. The positive modulation of macroautophagy is dependent on the GAP activity of RAB3-GAP1 but independent of RAB3, suggesting that RAB3GAP1/2 access an alternative RAB GTPase, which has not been identified yet. Interestingly, the RAB3GAP complex was recently shown to be a RAB GEF for the RAB GTPase RAB18 and provokes localization of RAB18 to the ER, which is necessary for maintenance of ER structure.<sup>23</sup> Excitingly, mutations in RAB3GAP1/2 and RAB18 cause the Warburg Micro syndrome, a devastating developmental disorder.<sup>24</sup> The molecular mechanisms of this disease are not clarified yet but a functional association of RAB3GAP1/2 and RAB18 might support the identification of responsible pathogenetic pathways. Next to RAB3 regulation and its involvement in macroautophagy, RAB3-GAP1 interacts with LMAN1/ERGIC53<sup>25</sup> and mediates the exocytosis of CLDN1,<sup>26</sup> which highlights the coordinative character of this TBC domain-free RAB GAP in cellular trafficking systems.

As indicated above, several macroautophagy-modifying TBCGAPs were identified by their interaction with Atg8 family members and this interaction is counteracted by other interacting proteins that compete for binding sites. The ability of Atg8 family members to direct RAB GAPs to phagophores indicates that they might act as scaffolding molecules and, thus, are central partners for the activity of RAB GAPs in macroautophagy. This mechanism is comparable to the interaction of Atg8 family members with cargo receptors involved in selective macroautophagy, such as SQSTM1, NBR1, or CALCOCO2.<sup>27</sup> MAP1LC3 serves as a binding partner and recruits cargo receptors to phagophores, which mediates substrate-specificity to macroautophagy.

Interestingly, an interaction with Atg8 family members has also been indicated for RAB3GAP1/2 based on a proteomic approach,<sup>28</sup> although a direct physical interaction awaits confirmation.<sup>22</sup>

### Relevance of RAB GAPs in macroautophagy and compensatory mechanisms for membrane mobilization

The formation and transport of autophagosomes is one of the major challenges for the entire macroautophagy process and needs to be carefully controlled to reduce interference with other cellular trafficking pathways. The activity of RAB GTPases, RAB GEFs, and RAB GAPs positions these proteins as central factors for this coordination and their relevance for macroautophagy has been shown in multiple studies.<sup>12</sup> However, the selection of macroautophagy-deficient yeast strains resulted in the characterization of at least 40 Atg proteins, most of which do not appear to be involved in membrane mobilization or vesicle transport. An exception (although not an “Atg” protein) is the ortholog of RAB1, Ypt1,<sup>29</sup> and its RABGEF, the TRAPPIII complex,<sup>9</sup> which have been defined as important factors for autophagosome formation in yeast and possess a likewise important role for macroautophagy also in mammalian cell lines.<sup>12</sup> Interestingly, several RAB GAPs modulate macroautophagy particularly under induced conditions when macroautophagic membrane requirements are increased, which underlines the need for a stringent control, and some RAB GAPs seem to function in overlapping pathways. For example, TBC1D14 and TBC1D5 appear to be important both for the coordination of endosomal trafficking and autophagosome biogenesis.<sup>14,15,17</sup> Recently, TBC1D2, which effects the RAB GTPase RAB7 and modulates autophagosome-lysosome fusion, was shown to be activated by LRRK1 upon macroautophagy induction.<sup>30</sup> Therefore, the characterization of upstream factors that modulate the activity of RAB GAPs and the identification of target RAB GTPases will help to dissect the precise pathways that are modulated by these proteins and allow the identification of possible compensatory mechanisms. This will increase our understanding of the reorganization and the condition-dependent plasticity of cellular

trafficking systems that are necessary to keep macroautophagy going.

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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