

1 Roles for RAB24 in autophagy and disease

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3 Päivi Ylä-Anttila\* and Eeva-Liisa Eskelinen

4 Department of Biosciences, University of Helsinki, Helsinki, Finland

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6 \*Current address: Department of Cell and Molecular Biology, Karolinska Institutet, Solna,

7 Stockholm, Sweden

8

9 Correspondence:

10 Eeva-Liisa Eskelinen

11 Department of Biosciences

12 University of Helsinki

13 PO Box 56, Viikinkaari 4, Cultivator II

14 00014-Helsinki, Finland

15 Tel +358 50 3199504

16 E-mail [eeva-liisa.eskelinen@helsinki.fi](mailto:eeva-liisa.eskelinen@helsinki.fi)

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18 Key words: RAB proteins, autophagy, RAB24, ataxia, hepatocellular carcinoma

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20 Abbreviations

21 GABARAP, gamma-aminobutyric acid receptor-associated protein

22 GAP, GTPase activating protein

23 GDF, GDI displacement factor

24 GDI, GDP dissociation inhibitor

25 GEF, GDP-GTP exchange factor

26 GOSR1, SNARE protein Golgi SNAP receptor complex member 1  
27 HCC, hepatocellular carcinoma  
28 Hsc70, heat shock cognate protein of 70 kDa  
29 LAMP2A, lysosomal associated membrane protein type 2A  
30 LC3, microtubule-associated protein light chain 3  
31 mTOR, mammalian target of rapamycin  
32 NSF, N-ethylmaleimide sensitive fusion protein  
33 RILP, RAB7 interacting lysosomal protein  
34 SNAP29, synaptosomal associated protein 29  
35 SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor

36

## 37 Abstract

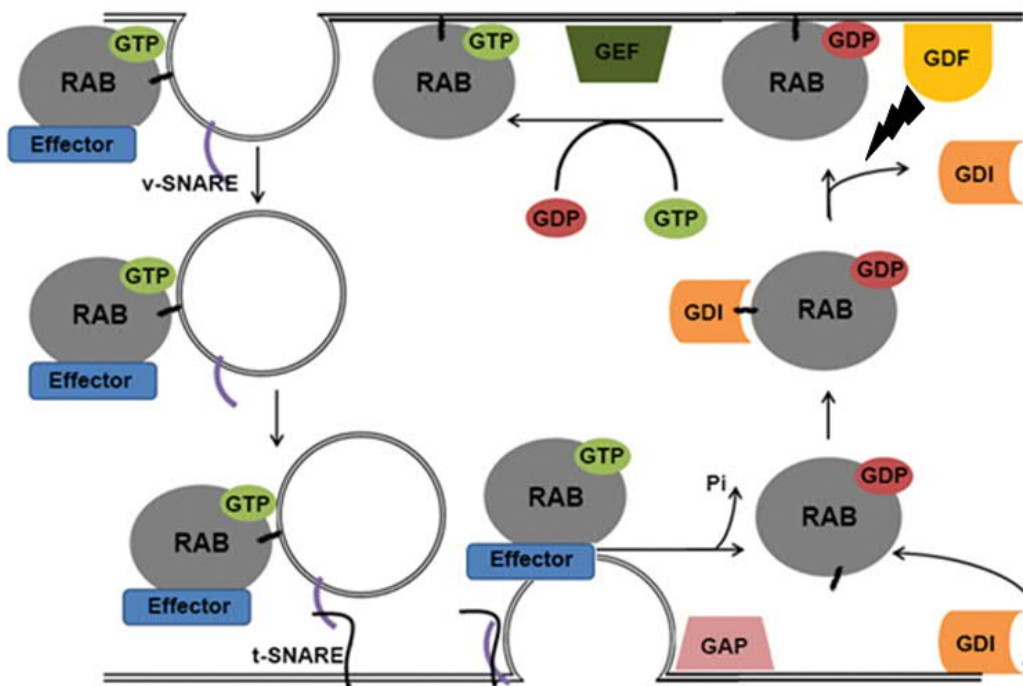
38 Autophagy is an evolutionarily conserved degradation pathway for cells to maintain homeostasis,  
39 produce energy, degrade misfolded proteins and damaged organelles, and fight against intracellular  
40 pathogens. The process of autophagy entails the isolation of cytoplasmic cargo into double  
41 membrane bound autophagosomes that undergo maturation by fusion with endosomes and  
42 lysosomes in order to obtain degradation capacity. RAB proteins regulate intracellular vesicle  
43 trafficking events including autophagy. RAB24 is an atypical RAB protein that is required for the  
44 clearance of late autophagic vacuoles under basal conditions. RAB24 has also been connected to  
45 several diseases including ataxia, cancer and tuberculosis. This review gives a short summary on  
46 autophagy and RAB proteins, and an overview on the current knowledge on the roles of RAB24 in  
47 autophagy and disease.

48

## 49 RAB proteins in membrane trafficking

50 RAB proteins regulate all steps in intracellular membrane dynamics such as cargo selection, vesicle  
51 budding and transport along cytoskeletal tracks, as well as vesicle docking and fusion.<sup>1, 2</sup> RABs are  
52 synthesized as soluble proteins that are post-translationally modified by the covalent attachment  
53 of a geranylgeranyl moiety, also called a prenyl group, to their C-terminal cysteines, which enables  
54 their association on the cytosolic side of intracellular membranes.<sup>3</sup> RAB proteins cycle between

55 active GTP- and membrane- bound state, and inactive GDP-bound cytosolic state. Inactive GDP-  
 56 bound RABs can be activated on membrane surfaces by the action of GDP-GTP exchange factors  
 57 (GEFs).<sup>4</sup> While in the active GTP-bound state, RAB proteins are able to recruit effectors that function  
 58 in the different vesicular trafficking steps. The GTPase activity of RABs is controlled by the GTPase  
 59 activating proteins (GAPs). GTP hydrolysis leads to the inactivation of the RAB and subsequently,  
 60 RAB-GDP re-associates with GDP dissociation inhibitors (GDIs) and is retrieved from the membrane.  
 61 GDIs hide the hydrophobic prenyl groups in their hydrophobic groove, making the RABs soluble in  
 62 the cytoplasm.<sup>5</sup> Dissociation of RAB-GDP from GDI, and subsequent membrane insertion, are  
 63 achieved by the action of a GDI displacement factor (GDF).<sup>6</sup> Unlike GDIs, GEFs and GAPs show more  
 64 specificity for their target RABs.<sup>4</sup> The RAB activation/inactivation cycle is schematically presented in  
 65 Figure 1.



66

67 Figure 1. The RAB activation/inactivation cycle. RAB proteins cycle between active membrane  
 68 bound state and inactive cytosolic state. RABs recruit effector proteins while in the active GTP-  
 69 bound state (left). See text for further details. GEF, guanine nucleotide exchange factor; GAP,  
 70 GTPase activating protein; GDI, GDP dissociation inhibitor; GDF, GDI displacement factor; Pi,  
 71 inorganic phosphate; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein  
 72 receptor; t-SNARE, SNARE on target membrane; v-SNARE, SNARE on vesicle membrane.

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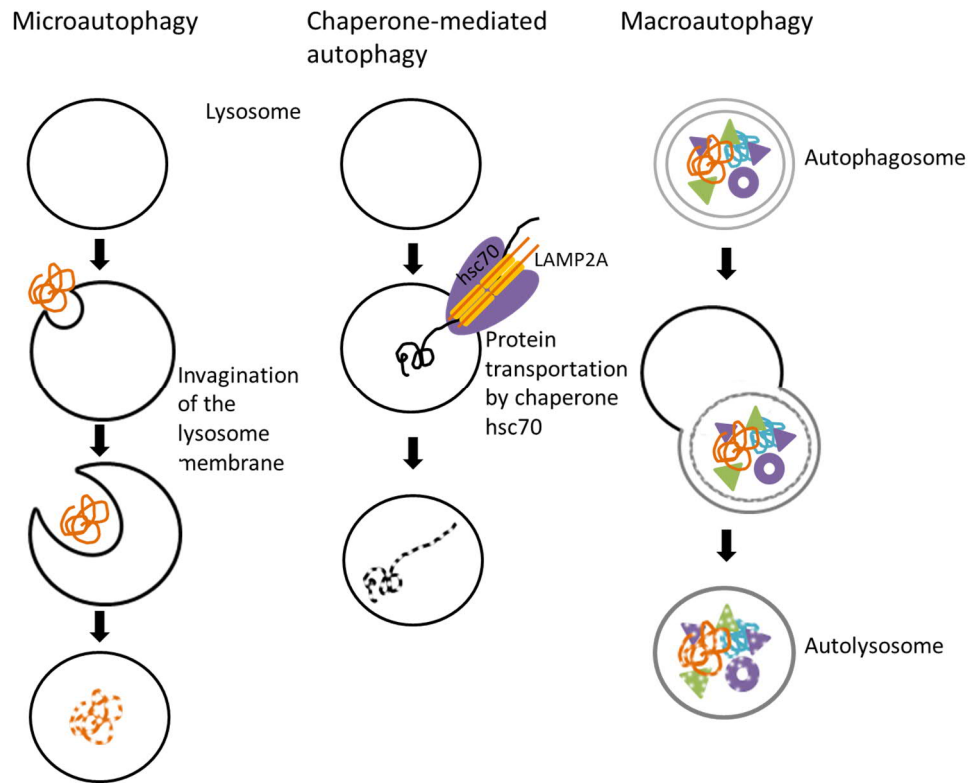
74 RAB effectors interact with the active, GTP-bound form of their partner RAB proteins and mediate  
75 at least one specific downstream effect. RAB effectors belong to many different protein families and  
76 mediate a wide selection of functions including the selection and concentration of vesicle cargo,  
77 vesicle formation, vesicle transport along actin filaments or microtubules, as well as vesicle  
78 recognition and fusion. Many RAB effectors serve a tethering function linking opposing membranes  
79 before SNARE pairing.<sup>7</sup> One RAB can interact with several different effector proteins.<sup>8</sup>

80

## 81 Autophagy

82 Autophagy is an evolutionarily conserved cellular waste disposal and recycling mechanism, where  
83 cytoplasmic components are transported to lysosomes for degradation. Autophagy helps the cells  
84 to maintain homeostasis by producing energy and building blocks for vital biosynthetic reactions,  
85 degrading misfolded and aggregated proteins and unnecessary organelles, and fighting against  
86 intracellular pathogens.<sup>9, 10</sup> There are three ways to transport cytoplasmic material to lysosomes,  
87 called macroautophagy (or simply autophagy), microautophagy and chaperone-mediated  
88 autophagy (Figure 2). Macroautophagy involves the formation of an autophagosome, i.e., the  
89 enwrapping of the cytoplasmic cargo into a double membraned vacuole, and the subsequent  
90 delivery of the sequestered material for degradation by fusion with endosomes and lysosomes.  
91 Macroautophagy is able to degrade cytosolic proteins, ribosomes, protein aggregates and whole  
92 organelles. Microautophagy occurs by direct inward budding of the lysosomal limiting membrane  
93 with the engulfed cargo.<sup>11</sup> Chaperone-mediated autophagy is a specific transport route through the  
94 lysosomal membrane where the cargo protein must contain a recognition motif (KFERQ) that is  
95 recognized by a cytosolic chaperone, heat shock cognate protein of 70 kDa (Hsc70). This complex  
96 binds to the lysosomal receptor protein called lysosomal associated membrane protein type 2A  
97 (LAMP2A).<sup>12</sup> After unfolding, the cargo protein is transported across the lysosomal membrane with  
98 the help of the chaperone Hsc70. After degradation of autophagic substrates, the degradation  
99 products are transported back to the cytoplasm through several lysosomal permeases.

100



101

102 Figure 2. There are three types of autophagy: microautophagy, chaperone-mediated autophagy  
 103 and macroautophagy. See text for further details.

104 Autophagy is induced by different stimuli including stress and amino acid starvation, but autophagy  
 105 also exists when nutrients are available. The non-induced or basal autophagy enforces intracellular  
 106 quality control and is of particular importance for postmitotic cells like neurons and muscle cells.  
 107 Basal autophagy occurs continuously at a low level and degrades old organelles and aggregate-  
 108 prone proteins; common denominators in age-related disorders such as neurodegenerative  
 109 diseases. Starvation-induced autophagy and basal autophagy seem to differ in substrate selectivity  
 110 and in regulation. While starvation-induced autophagy is inhibited by the target of rapamycin  
 111 (mTOR) kinase, basal autophagy is less affected by mTOR.<sup>13</sup> Notably, when mTOR activation is  
 112 caused by overexpression of RHEB or activated RAGs (as opposed to activation by the presence of  
 113 nutrients), also basal autophagy is suppressed.<sup>14</sup> Further, basal autophagy is less dependent on  
 114 phosphatidylinositol 3-kinase activity than induced autophagy.<sup>14</sup> The maturation of basal and  
 115 starvation-induced autophagosomes also differs. One example is RAB7 that functions during the  
 116 maturation of starvation-induced autophagosomes but seems to be dispensable for basal  
 117 autophagy.<sup>15</sup>

118

119 Autophagic degradation requires several membrane fusion events, and not surprisingly, many RAB  
120 proteins and other small GTPases have been described to function in autophagy. Some GTP binding  
121 proteins function in autophagosome induction or formation (RAB1B, RAB4, RAB5, RAB11, RAB32,  
122 RAB33B, and SAR1)<sup>16-21</sup> and others later in the lysosomal fusion processes (RAB7, RAB8B, RAB11,  
123 RAB24 and RAB33B).<sup>15, 22-25</sup> RAB7 is also needed for the formation of autophagosomes induced by  
124 intracellular *Streptococcus* bacteria.<sup>26</sup> RAB9 is required for an unconventional form of  
125 macroautophagy that is independent of ATG5 and ATG7 autophagy proteins.<sup>27</sup> RAB8A plays a role  
126 in the unconventional autophagic secretory pathway for interleukin 1 $\beta$ .<sup>28</sup> RAB39A and RAB25 have  
127 been shown to negatively regulate autophagy.<sup>29, 30</sup> The roles of RAB GTPases and their regulators in  
128 autophagy have been summarized in several recent reviews.<sup>31-37</sup>

129

130 RAB24 is an atypical RAB protein that has been implicated in autophagy for a long time. Recent  
131 research has finally demonstrated that RAB24 is required in basal autophagy, and shown that RAB24  
132 may be connected to several diseases. In this review, we summarize what is currently known about  
133 the roles of RAB24 in macroautophagy and disease.

134

135 RAB24 is an unusual RAB protein

136 Elias et al. performed a genomics analysis on the evolutionary history of RAB proteins.<sup>38</sup> This analysis  
137 places RAB24 among the primordial RABs that were present in the last eukaryotic common ancestor.  
138 RAB24 was proposed to be one of the RABs that associate with the establishment of the endocytic  
139 pathway in eukaryotic cells. RAB24 is conserved in many species including *Dictyostelium discoideum*,  
140 zebrafish and mammals, but has been lost in others including *Saccharomyces cerevisiae*, *Drosophila*  
141 *melanogaster* and *Caenorhabditis elegans*.

142

143 RAB24 protein was first characterized by Olkkonen et al. as a perinuclear protein that colocalizes  
144 with Golgi markers, some late endosome markers, and with RAB2, an ER-Golgi intermediate  
145 compartment marker.<sup>39</sup> Later, RAB24 was reported to differ from a typical RAB protein in several  
146 aspects. Erdman et al. found that RAB24 has a low GTPase activity and thus predominantly occurs  
147 in the GTP-bound state. They also reported that RAB24 is inefficiently prenylated and that cytosolic

148 RAB24 only weakly associates with GDI.<sup>40</sup> Later studies have shown conflicting results on the GDI  
149 binding and prenylation. Using immunoprecipitation, Behrends et al. reported that RAB24 interacts  
150 with both GDI1 and GDI2.<sup>41</sup> Further, we showed that overexpressed RAB24 and RAB7 are prenylated  
151 at similar extents.<sup>42</sup> Ding et al. observed that the cytosolic pool of RAB24 is more phosphorylated  
152 than the membrane-associated pool.<sup>43</sup> Two tyrosines were found to be phosphorylated, Y17 within  
153 the GXXXGK(S/T) motif known as the P-loop, and Y172 in the YXXE motif in the hypervariable  
154 domain. The low GTPase activity of RAB24 is thought to be associated with the unusual amino acid  
155 at position 67 in the GTP-binding region: this amino acid is serine in RAB24, while in other RABs  
156 residue 67, or its equivalent, in the GTP-binding region is glutamine. The P-loop containing tyrosine  
157 Y17 may also influence GTP hydrolysis.<sup>43</sup> In other RAB proteins, Q67L or equivalent mutation causes  
158 a constitutively active RAB phenotype. However, RAB24-S67L mutant binds GTP less efficiently than  
159 wild type RAB24, does not localize to any membranous organelles, and acts as a dominant negative  
160 mutant when overexpressed in cells.<sup>42, 44, 45</sup>

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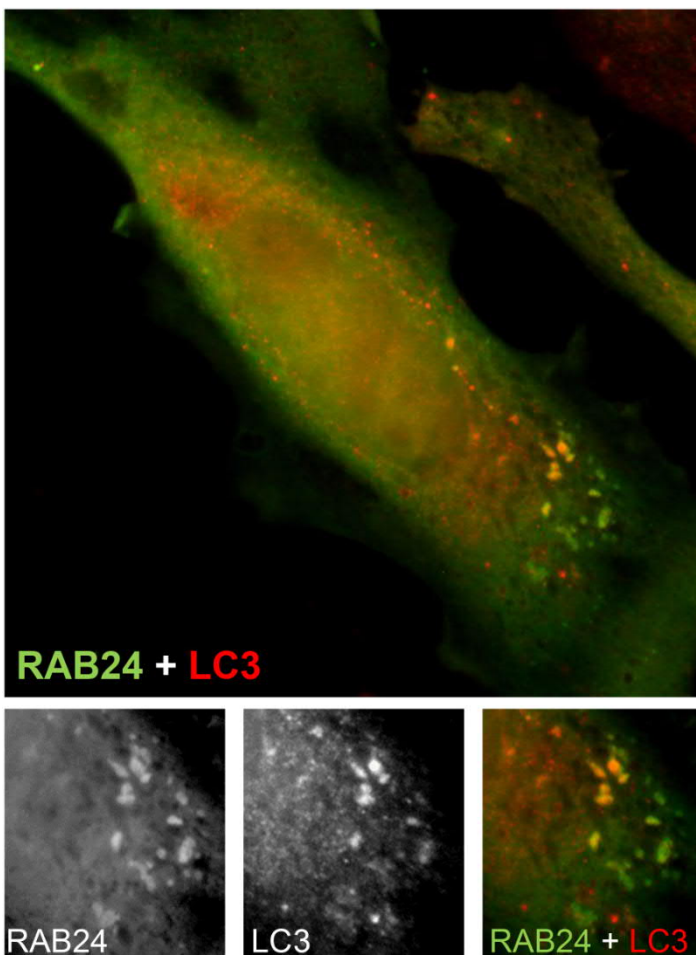
## 162 RAB24 and autophagy

163 Already Oikkonen et al. suggested that RAB24 may function in some sort of autophagy-related  
164 transport route between the ER-Golgi intermediate compartment and late endocytic  
165 compartments.<sup>39</sup> Later, several laboratories published reports supporting a role for RAB24 in  
166 autophagy. Munafo and Colombo showed that overexpressed RAB24 changes localization upon  
167 amino acid starvation.<sup>46</sup> In starved cells, RAB24 colocalized in vesicular structures with the  
168 autophagosome marker LC3 (microtubule-associated protein light chain 3) and with  
169 monodansylcadaverine, a marker for acidic compartments and putative autolysosomes. The same  
170 group observed that transit through the autophagic pathway increased the infection of CHO cells  
171 with Coxiella. Overexpression of wild type RAB24 accelerated, while expression of mutant RAB24-  
172 S67L inhibited the formation of Coxiella vacuoles.<sup>44</sup> Wu et al. observed that RAB24 colocalized with  
173 another autophagosome marker protein called GABARAP.<sup>47</sup> Tambe et al. reported that the tumor  
174 suppressor protein DRS is involved in autophagosome maturation.<sup>48</sup> They also showed  
175 colocalization of DRS and RAB24, and of DRS and the autophagosome marker LC3, in punctate  
176 structures that accumulated in low serum culture conditions. Marambio et al. studied aggresome  
177 formation in cultured cardiac myocytes exposed to glucose deprivation.<sup>49</sup> Aggresomes form when  
178 proteasomal degradation is overwhelmed with aggregate-prone proteins. Both LC3 and RAB24

179 colocalized with the aggresomes. Taken together, all these findings provided evidence for RAB24  
180 function in autophagy, but none of these studies addressed whether RAB24 was actually required  
181 for autophagy, and thus the exact step where RAB24 would function also remained unclear.

182

183 RAB24 has also been observed to be upregulated during cellular stress, which is also known to  
184 induce autophagy. Egami et al. reported that RAB24 mRNA levels increased in nerve-injured  
185 hypoglossal motor neurons of rats.<sup>50</sup> Similar increase in RAB24 mRNA was observed in differentiated  
186 PC12 cells treated with a proteasomal inhibitor. Also mRNA levels of LC3 increased in both  
187 hypoglossal motor neurons and PC12 cells. Further, RAB24 showed partial colocalization with LC3  
188 in immunofluorescence staining. Similar increase in RAB24 protein level was reported by Seki et al.  
189 in trigeminal motor nucleus after denervation.<sup>51</sup>



190

191 Figure 3. RAB24 colocalizes with the autophagosome marker LC3. HeLa cells were transfected with  
192 RAB24 and immunolabeled with anti-RAB24 and anti-LC3. Before fixation, the cells were treated  
193 with 100 mM leupeptin and 10 mg/ml pepstatin for 6 h in full culture medium in order to accumulate



194 autophagic vacuoles (autophagosomes, amphisomes and autolysosomes) under basal conditions.  
195 Yellow color in the overlay images indicates colocalization.

196

197 RAB24 plays a role in basal autophagy and endosomal degradation

198 We studied the role of RAB24 in macroautophagy using HeLa and NRK cells.<sup>42</sup> We observed RAB24  
199 to colocalize with approximately 60% of LC3-positive autophagic structures both under basal and  
200 starvation conditions (Figure 3). Although the percentage of LC3-positive structures also positive for  
201 RAB24 did not increase during amino acid starvation, the amount of RAB24 per LC3 vesicle did  
202 increase. Immuno electron microscopy showed RAB24 to localize to both the inner and outer  
203 limiting membranes of autophagosomes. Using subcellular fractionation, we further showed that  
204 endogenous RAB24 localized to fractions positive for LC3-II, the membrane-associated form of LC3,  
205 and SQSTM1, an autophagic cargo protein. We also showed that targeting of RAB24 to  
206 autophagosomes requires prenylation and GTP binding, but not phosphorylation of tyrosines Y17  
207 or Y172. Our results further showed that RAB24 is dispensable for the formation, maturation and  
208 clearance of starvation-induced autophagosomes. However, under basal conditions, acidic  
209 autolysosomes accumulated in RAB24-depleted cells. Using bafilomycin to inhibit autophagic flux,  
210 we showed the accumulation to be due to decreased clearance of autolysosomes. We also showed  
211 that depletion of RAB24 retarded the clearance of a Huntingtin-polyglutamine probe, which has  
212 been shown to be a substrate for autophagic clearance.<sup>13</sup> Finally, we observed that the degradation  
213 of long-lived proteins was slightly decreased under basal conditions in RAB24 silenced cells.<sup>42</sup> We  
214 concluded that RAB24 functions in the clearance of autolysosomes under basal conditions, but is  
215 not needed for starvation-induced autophagy. Thus RAB24 is the first RAB protein shown to be  
216 required in the very late steps of basal autophagy. Two pathways have been described for  
217 autolysosome clearance: reformation of lysosomes from autolysosomes,<sup>52</sup> and fusion of  
218 autolysosomes with the plasma membrane.<sup>53</sup> It remains to be shown whether RAB24 plays a role in  
219 these processes.

220

221 A recent study by Amaya et al.<sup>54</sup> showed that RAB24 coprecipitated with the late  
222 endosomal/lysosomal RAB7 and its effector RILP (RAB7 interacting lysosomal protein). As  
223 mentioned earlier, RAB7 is also needed for the fusion of starvation-induced autophagosomes with

224 lysosomes.<sup>15, 22</sup> RAB24 was shown to colocalize with RAB7, and the localization of RAB7 to vesicular  
225 structures was shown to require RAB24.<sup>54</sup> Further, RAB24 was found to be needed for the  
226 degradation of endocytic cargo. The authors concluded that RAB24 forms a complex with RAB7 and  
227 RILP on the surface of late endosomal/lysosomal compartments and regulates endosomal  
228 degradation. Thus, two recent studies<sup>42, 54</sup> indicate that RAB24 functions in the late stages of  
229 autophagic and endocytic pathways.

230

231 RAB24 associates with several diseases

232 Agler et al. reported that a mutation in RAB24 is associated with canine ataxia, a hereditary  
233 neurodegenerative disease.<sup>55</sup> The observed mutation results in glutamine to proline change in  
234 amino acid 38, located in the putative switch I region of RAB24. Glutamine 38 is well conserved in  
235 RAB24 in different species, and it is possible that the Q38P mutation has an effect on nucleotide  
236 binding. Affected dogs exhibit Purkinje neuron loss in the cerebellar cortex. Immunohistochemistry  
237 showed accumulation of ubiquitin-positive bodies in cells of the granular layer and at the junction  
238 of molecular and granular layers, and electron microscopy revealed axonal spheroids containing  
239 numerous late autophagic vacuoles in Purkinje cells of the granular layer. This study<sup>55</sup> is well in  
240 agreement with our findings, showing that nucleotide binding is important for the recruitment of  
241 RAB24 to autophagic compartments and that RAB24 is needed for autolysosome clearance.<sup>42</sup>

242

243 Altered expression of RAB24 has been reported in several human diseases, but further studies are  
244 needed to clarify whether the changes in RAB24 expression level are connected with alterations in  
245 autophagic activity. It is also unclear whether the altered RAB24 expression is a cause or  
246 consequence of the disease. Swaminathan et al. studied the mRNA levels of 59 selected genes  
247 between symptomatic patients (unstable plaques) and asymptomatic patients (stable plaques)  
248 suffering from carotid atherosclerosis.<sup>56</sup> LC3B showed the highest fold difference between the two  
249 groups: mRNA and proteins levels of LC3 were significantly decreased in the symptomatic samples.  
250 RAB24 mRNA was also significantly decreased in the symptomatic samples. Igci et al. studied gene  
251 expression profiles of autophagy-related genes in multiple sclerosis, an inflammatory disease of the  
252 central nervous system.<sup>57</sup> The expression of several genes, including RAB24, was observed to be  
253 altered (increased or decreased) in the patient samples. Jenum et al. aimed to find diagnostic  
254 biomarkers for pediatric tuberculosis.<sup>58</sup> They analyzed mRNA levels both direct ex-vivo, and using in

255 vitro whole blood stimulated with bacteria. They identified several biomarkers consistently  
256 associated with tuberculosis infections, one of them being RAB24. Elevated RAB24 mRNA levels  
257 were significantly associated with culture-positive tuberculosis.

258

259 Chen et al. investigated epigenetic silencing of micro RNA in hepatocellular carcinoma (HCC) and  
260 observed miR-615-5p to be downregulated in HCC.<sup>59</sup> Further, miR-615-5p was found to  
261 downregulate RAB24, while low levels of miR-615-5p increased the expression of RAB24 and  
262 facilitated the growth and metastasis of HCC both in vitro and in vivo. Downregulation of miR-615-  
263 5p and upregulation of RAB24 promoted the epithelial-mesenchymal transition, adhesion and  
264 vasculogenic mimicry of HCC cells. All these features enhance metastasis. Thus, RAB24 is a direct  
265 target of miR-615-5p, and RAB24 protein promotes the malignant phenotype of HCC cells. The  
266 authors concluded that miR-615-5p functions as a tumor suppressor by inhibiting RAB24 expression  
267 in HCC. These findings are in line with a report showing that RAB24 is required for normal cell  
268 division, modulating several mitotic events including chromosome segregation and cytokinesis.<sup>60</sup> It  
269 is currently unclear whether the roles of RAB24 in metastasis and cytokinesis are connected with its  
270 functions in autophagy or endocytosis.

271

272 Putative RAB24 effectors implicate a role in membrane fusion events

273 RAB24 effectors are at present unknown, but several studies support the hypothesis that RAB24  
274 may function in membrane fusion. As mentioned earlier, Amaya et al.<sup>54</sup> reported that RAB24  
275 coprecipitated with RAB7 and its effector RILP. Both RAB7 and RILP are known to function in  
276 endosome-lysosome fusion. Further, Schardt et al. found that RAB24 coprecipitated with  
277 synaptosomal associated protein 29 (SNAP29).<sup>61</sup> SNAP29 interacts with several syntaxins, SNARE  
278 proteins that participate in exocytosis.<sup>62</sup> The interaction of RAB24 with SNAP29 did not require the  
279 presence of GTP $\gamma$ S, unlike the interaction of SNAP29 with RAB3A.<sup>61</sup> Interestingly, SNAP29 was shown  
280 to play a role in autophagosome fusion with endosomes or lysosomes, acting in a SNARE complex  
281 with STX17/syntaxin 17.<sup>63-66</sup> Unlike RAB24, SNAP29 seems to be required for both basal and  
282 starvation-induced autophagy. Further, double-membrane autophagosomes accumulate in cells  
283 deficient in SNAP29<sup>65</sup> or STX17,<sup>66</sup> whereas we found that single-membrane bound, acidic and  
284 degradative autophagic vacuoles/autolysosomes accumulate in RAB24 deficient cells.<sup>42</sup>

285

286 Behrends et al. used HA-tagged RAB24 as one of the bait proteins in their proteomics study on  
287 interactions of autophagy proteins.<sup>41</sup> Their mass spectrometry primary data showed coprecipitation  
288 of RAB24 with GDP dissociation inhibitors 1 and 2 (GDI1 and GDI2), N-ethylmaleimide sensitive  
289 fusion protein (NSF), and plakophilin 1 (armadillo repeat protein implicated to function in  
290 desmosomes). Behrends et al. also found RAB24 among the proteins that coprecipitated with the  
291 SNARE protein Golgi SNAP receptor complex member 1 (GOSR1), but no coprecipitation was  
292 reported between RAB24 and SNAP29. In order to identify putative high-confidence interaction  
293 partners, Behrends et al. performed a comparative analysis of the proteomic results, and a  
294 subsequent analysis to validate and delineate the interaction network. This analysis placed RAB24  
295 in the NSF subnetwork together with GOSR1, SNAP29 and several other SNARE proteins. The  
296 analysis proposes that RAB24 has direct interactions with GDI1, GDI2, NSF and plakophilin 1, while  
297 NSF would mediate the interactions with the other proteins in the subnetwork. Taken together, the  
298 findings of Behrends et al. support the idea that RAB24 may function in membrane fusion together  
299 with NSF, SNAP29 and GOSR1. Interaction of RAB24 with GDIs is expected, while the significance of  
300 the interaction with plakophilin 1 remains unknown.

301

302 Several laboratories have reported single RAB24 interacting proteins, but the importance of these  
303 interactions is not known at present. Tambe et al. observed that RAB24 coprecipitated with the  
304 tumor suppressor protein DRS.<sup>48</sup> Schlager et al. performed a GST pulldown assay and observed that,  
305 similar to several other RABs, RAB24 weakly bound Bicaudal-D-related protein 2/BICDR2 (a putative  
306 RAB6 effector).<sup>67</sup> Fukuda et al. used yeast two hybrid assay and immunoprecipitation to show that  
307 two mutant versions of RAB24 (S67L and T21N) interacted with transcriptional corepressor C-  
308 terminal-binding protein 1, CtBP1.<sup>68</sup>

309

310 In summary, several of the putative indirect or direct RAB24 interacting proteins have been  
311 implicated in membrane fusion, including RAB7, SNAP29, GOSR1 and NSF. The candidate RAB24  
312 interactors are in agreement with the idea that RAB24 functions in membrane fusion events during  
313 the late steps of macroautophagic and endocytic pathways. However, further studies are required  
314 to elucidate the detailed molecular mechanisms of RAB24 functions.

315

316 Acknowledgments

317 The work in the E.-L.E. laboratory is supported by the Academy of Finland. P.Y.-A. was supported  
 318 by the Magnus Ehrnrooth Foundation, Finland.

319

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480 Figure legends

481

482 Figure 1. The RAB activation/inactivation cycle. RAB proteins cycle between active membrane  
483 bound state and inactive cytosolic state. RABs recruit effector proteins while in the active GTP-  
484 bound state (left). See text for further details. GEF, guanine nucleotide exchange factor; GAP,  
485 GTPase activating protein; GDI, GDP dissociation inhibitor; GDF, GDI displacement factor; Pi,  
486 inorganic phosphate; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein  
487 receptor; t-SNARE, SNARE on target membrane; v-SNARE, SNARE on vesicle membrane.

488

489 Figure 2. There are three types of autophagy: microautophagy, chaperone-mediated autophagy  
490 and macroautophagy. See text for further details.

491

492 Figure 3. RAB24 colocalizes with the autophagosome marker LC3. HeLa cells were transfected with  
493 RAB24 and immunolabeled with anti-RAB24 and anti-LC3. Before fixation, the cells were treated  
494 with 100  $\mu$ M leupeptin and 10  $\mu$ g/ml pepstatin for 6 h in full culture medium in order to accumulate  
495 autophagic vacuoles (autophagosomes, amphisomes and autolysosomes) under basal conditions.  
496 Yellow color in the overlay images indicates colocalization.