PANCREAPEDIA
Exocrine Pancreas Knowledge Base

APA AMERICAN PANCREATIC ASSOCIATION

Rab8

Maria Gomez-Lazaro, Miguel Aroso, Michael Schrader

Centre for Cell Biology & Dept. of Biology, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

e-mail: mschrader@ua.pt

Version 1.0, January 4, 2011 [DOI: 10.3998/panc.2011.2]

Gene symbols: RAB8A, RAB8B

1. General Function

Rab8 (Ras-related proteins in brain 8) is a 24 kDa protein that belongs to the small Rab GTPase family (7). Rab proteins are known for their participation in and regulation of intracellular membrane trafficking pathways (17, 46, 48). In their active GTP-bound state different Rab proteins bind to different membrane compartments and recruit specific effector proteins, which are not only involved in docking and fusion with the target membrane but also in the formation of transport vesicles and in binding motor proteins for vesicle transport (17, 18, 28, 45, 48).

Rab8 displays high homology with the yeast *S. cerevisiae* protein SEC4 involved in post-Golgi traffic (16, 24) and with the yeast *S. pombe* protein Ypt2p that acts as well in the last stage of the secretory pathway (4). In humans there are two isoforms with 80% homology, Rab8a and Rab8b, which have a differential expression pattern (Figure 1). Rab8b is expressed in spleen, testis and brain (2, 8) while Rab8a is more ubiquitous expressed but shows low expression levels in the three organs (8). Nevertheless, Rab8a and Rab8b have been shown to play a similar role in vesicular traffic from the Golgi complex to the plasma membrane in regulated secretion in ATt20 cells (8). The Rab8 lipid motif differs from other Rabs resulting in only one geranylgeranylation (3, 27). A preliminary X-ray crystallographic analysis of mammalian Rab8 in complex with the nucleotide exchange factor MSS4 has been published (25).

Functionally, Rab8 has been proposed to act as a regulator of post-Golgi membrane traffic from the TGN to the plasma membrane. Rab8 has been localized to the Golgi region, to TGN-derived transport vesicles *en route* to the plasma membrane (22-24), and has been demonstrated to regulate biosynthetic trafficking pathways from the TGN to the cell surface (1, 24, 35). In *C. elegans*, depletion of ce-rab-8 inhibited the secretion of the growth factor EGL-17 *in vivo* (29). Rab8 depletion in developing neurons by antisense oligonucleotides inhibited the formation of exocytic vesicles at the Golgi apparatus and thus, neuronal process outgrowth (23). In addition, Rab8 is involved in the targeting of vesicles to the cilium, promoting the extension of the ciliary membrane (35). Rab8 can also modulate the transport of the adrenergic receptors α_{2B}- and β_2- by direct interaction (14).

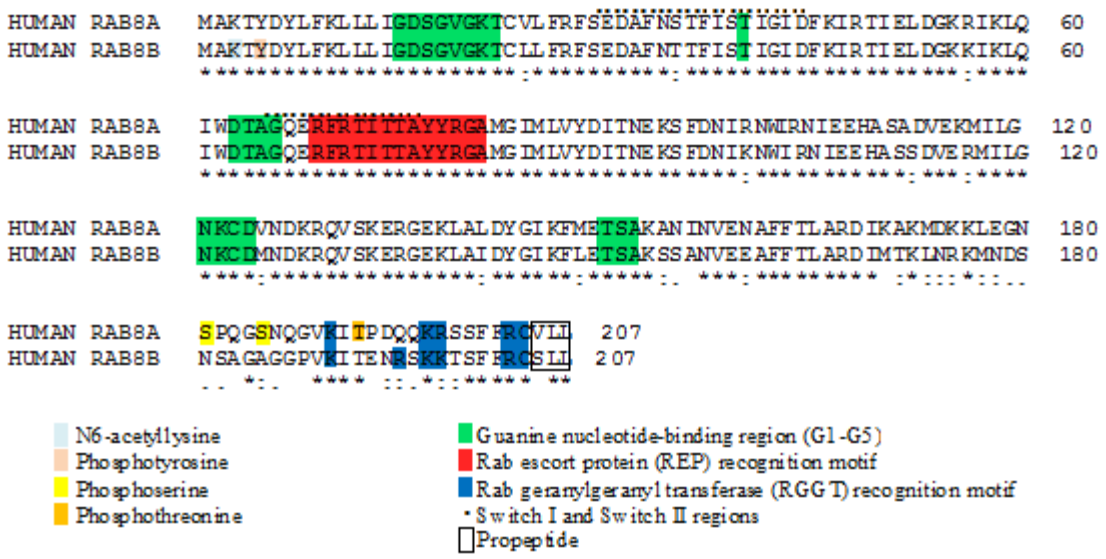

Figure 1. Alignment of the human Rab8A and Rab8B protein sequences. The online version of ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2/) was used to generate the alignment. The information of the Guanine nucleotide-binding (G1-G5) region and the Switch regions was taken from (26). The REP recognition motif and the RGGT recognition motif were obtained in the Prenylation Prediction Suite (http://mendel.imp.ac.at/sat/PrePS/index.html). All of the remaining information was extracted from the Protein Knowledgebase (UniprotKB) (http://www.uniprot.org/uniprot/P61006 for Rab8A and http://www.uniprot.org/uniprot/Q92930 for Rab8B). * — identical residues; : — conserved substitutions; . — semi-conserved substitutions.

Recently, it has been demonstrated that Rab8a regulates apical protein localization in intestinal epithelial cells by generating conditional Rab8 knockout mice (43). The mis-localization and degradation of apical peptidases and transporters in lysosomes was observed, thus leading to a marked reduction in the absorption rate of nutrients in the small intestine, and to death. In addition, we have reported on a role of Rab8 in the formation of zymogen granules in pancreatic acinar AR42J cells (15). These data provide evidence for a role of Rab8 in apical trafficking of digestive enzymes in acinar cells of the pancreas.

It has been shown that Rab8 promotes polarized membrane transport through reorganization of actin and microtubules. Rab8 is supposed to modify cellular shape by changing the organization of the plasma membrane and the underlying cytoskeleton (2, 37). Rab8 depletion promotes the formation of actin stress fibers, whereas activation of Rab8 has the opposite effect and causes the formation of cell protrusions (20, 37). Furthermore, Rab8 is supposed to interact via optineurin with myosin VI (42), and was reported to partially associate with mature melanosomes and to regulate actin-based movement of melanosomes (5, 6). Through its interaction with optineurin Rab8 is able to modify epithelial cell shape (21), and in response to apoptotic stimuli Rab8 is involved in the translocation of optineurin to the nucleus (11). It has also been described that the interaction of optineurin with Huntingtin links Huntingtin to Rab8 and promotes the relocalization of Huntingtin to vesicles (12, 49). Already in 1995, it was described that Rab8 may regulate some of the final steps in the post-Golgi transport of rhodopsin in retinal photoreceptor cells (13). Later it was observed that the expression of a Rab8 inactive mutant causes retinal degeneration in *Xenopus laevis* (33), and more recently that the disruption

of the interaction between optineurin and Rab8 leads to degeneration of the retina (10). Another Rab8 interacting protein is Mss4 that assists in nucleotide release (25).

Rab8 has also been linked to the organization of the endocytic compartment, (20). It has been localized to the endosomal recycling compartment (1, 47), where it colocalizes with EHD1 (41) and partially with Rab11 (20). Cells lacking Rab8 are defective in trafficking of the transferrin receptor (36) and display an inhibition of the retrograde transport of cholera toxin B to the Golgi compartment (20). Rab8 plays as well a role in the degradation of cholesterol (30, 31).

2. Specific Function in the Pancreas

Rab8a has been localized to zymogen granules in acinar cells of the exocrine rat pancreas and in pancreatic AR42J cells using immunocytochemistry (15) (Figure 2). It is present on isolated zymogen granules from rat pancreas and in the granule membrane fraction obtained after granule subfractionation (15). Furthermore, the presence of Rab8a and Rab8b in zymogen granule fractions was reported in recent proteomics studies (9, 39). Silencing of Rab8 (but not of Rab3) by siRNA inhibited granule formation and thus secretion of zymogens in AR42J cells, and resulted in an accumulation of granule marker proteins within the Golgi complex (15). By contrast, the trafficking of lysosomal and plasma membrane proteins was not affected. These data provide evidence for a role of Rab8 early on in zymogen granule formation at the Golgi complex and thus, apical trafficking of digestive enzymes.

Further evidence for a role of Rab8a in the regulation of apical protein localization has been obtained by the generation of conditional Rab8 knockout mice (43). These mice died 3–4 weeks after birth. In the intestinal epithelial cells of the wild type mice Rab8 localized to the Golgi complex and early endosomes. Interestingly, in the Rab8 knockout mice these cells displayed normal basolateral protein transport, but apical marker proteins accumulated intracellularly in late endosomes/lysosomes and were diminished in the apical membrane (43). The mislocalization and degradation of apical peptidases and transporters in lysosomes is supposed to lead to a marked reduction in the absorption rate of nutrients in the small intestine, and to death.

The small intestines of the knockouts were reported to be swollen and to contain undigested milk (43). Ultrastructural studies did, however, not reveal alterations in the number of zymogen granules at postnatal week 2 when controls and knockout mice were compared. Interestingly, a great reduction in the number of zymogen granules was observed at postnatal week 3, which might be explained by autophagy due to starvation conditions (32) (A. Harada, personal communication). Furthermore, the knockout mice still retain a functional Rab8b isoform, which could compensate for the absence of Rab8a in the pancreas. Rab8 knockout mice show a similar phenotype than patients with human microvillus inclusion disease but only one of the patients studied so far showed a decrease in Rab8 in the small intestine (43).

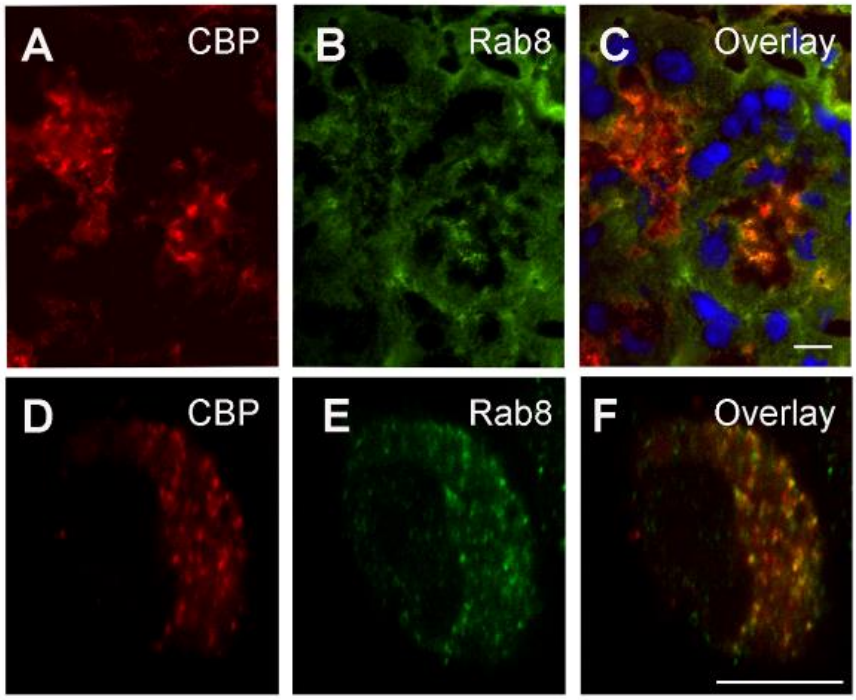

Figure 2. Immunofluorescence microscopy of pancreatic sections from rat pancreas and pancreatic AR42J cells. Cryosections of rat pancreas (A-C) and AR42J cells (D-F) were immunostained with antibodies specific for carboxypeptidase (CBP, A, D) and Rab8 (B, E). (C, F) Overlay of (A, B) and (D, E). Nuclei in (C) were stained with Hoechst 33258. Scale bars: 10 μm (C), 5 μm (F). Reproduced from (15) with permission from Traffic.

3. Tools to Study Rab8

a. cDNA clones

Different plasmids codifying for the Rab8 wildtype and constitutively active and inactive forms have been generated by several laboratories. In 1993, Rab8 was cloned by Huber and coworkers by inserting full-length cDNA into pGEM1 (24). Rab8-pEGFP, pEGFP-Rab8-T22N, pEGFP-Rab8-Q67L, pEGFP-Rab8bwt, pEGFP-Rab8b-T22N, Rab8b-Q67L have been described (19, 21).

pBD-, pmCerulean, pmVenus, and pmCherry versions of wild-type and mutant Rab8a are as well available (41).

The open reading frames of Rab8-T22N and Rab8-Q67L were also cloned into pcDNA4/TO (Invitrogen) and pIRES (Clontech). GST-tagged fusions of Rab8, Rab8-T22N and Rab8-Q67L inserted into pEBG-SrfI have been generated (20). Rab8 and HA-Rab8 in pcDNA3.1 are available at www.cdna.org

For yeast two hybrid experiments Rab8 and its mutant forms (Rab8b-Q67L, Rab8b-T22N, Rab8b-C204S, and Rab8b-N121I) were cloned in the vector pAS2-1 (8). Hattula and coworkers also cloned the Rab8 lacking its carboxy-terminal codons CVLL (Rab8Δwt), Rab8, Rab8-67L (activated), Rab8-22N (dominant negative), Rab8Δ-67L, Rab8Δ-22N Rab8b, Rab8b-67L, Rab8b-22N into a pGilda vector (21).

For transfection in *X. laevis*, canine rab8 mutant and wild-type cDNAs (37) were cloned into the vector XOPeGFP-C1 (33, 34).

b. Antibodies

Purified mouse anti-Rab8 (BD biosciences, 610844) was used successfully for immunoblotting (15, 20, 31, 33, 38), immunohistochemistry of rat pancreas,

immunocytochemistry of AR42J cells and immunofluorescence on isolated zymogen granules (15) and other cell types (31, 40).

c. Viruses

Recombinant Semliki Forest (SFV) and Adeno (AdV) Viruses have been described (pRab8-SFV, (31), pAdEasy-1, (30)).

d. KO mice

A small-intestine-specific knockout mouse ($Rab8^{geo/geo}$) and a nullizygous mouse ($Rab8^{-/-}$) have been described (43).

e. Rab8 silencing

Rab8a expression has been silenced by small interfering RNA (siRNA) (15, 20, 30, 38, 44) or by using short hairpin RNA (shRNA) (14, 31). Rab8b-specific siRNA is also available (31, 44).

Acknowledgements

Supported by the German Research Foundation (DFG, SCHR 518/5-1, 2), the J. Manchot foundation (Düsseldorf, Germany), the Portuguese Foundation for Science and Technology (FCT) [SFRH/BPD/37725/2007 (to M. G. L), SFRH/BD/48722/2008 (to M. A.)], and the University of Aveiro.

4. References

1. **Ang AL, Folsch H, Koivisto UM, Pypaert M, Mellman I.** The Rab8 GTPase selectively regulates AP-1B-dependent basolateral transport in polarized Madin-Darby canine kidney cells. J Cell Biol 163: 339-350, 2003. PMID:14581456
2. **Armstrong J, Thompson N, Squire JH, Smith J, Hayes B, Solari R.** Identification of a novel member of the Rab8 family from the rat basophilic leukaemia cell line, RBL.2H3. J Cell Sci 109 (Pt 6): 1265-1274, 1996. PMID:8799816
3. **Casey PJ, Seabra MC.** Protein prenyltransferases. J Biol Chem 271: 5289-5292, 1996. PMID:8621375
4. **Craighead MW, Bowden S, Watson R, Armstrong J.** Function of the ypt2 gene in the exocytic pathway of Schizosaccharomyces pombe. Mol Biol Cell 4: 1069-1076, 1993. PMID:8298192
5. **Chabrillat ML, Wilhelm C, Wasmeier C, Sviderskaya EV, Louvard D, Coudrier E.** Rab8 regulates the actin-based movement of melanosomes. Mol Biol Cell 16: 1640-1650, 2005. PMID:15673612
6. **Chakraborty AK, Funasaka Y, Araki K, Horikawa T, Ichihashi M.** Evidence that the small GTPase Rab8 is involved in melanosome traffic and dendrite extension in B16 melanoma cells. Cell Tissue Res 314: 381-388, 2003. PMID:12942363
7. **Chavrier P, Vingron M, Sander C, Simons K, Zerial M.** Molecular cloning of YPT1/SEC4-related cDNAs from an epithelial cell line. Mol Cell Biol 10: 6578-6585, 1990. PMID:2123294
8. **Chen S, Liang MC, Chia JN, Ngsee JK, Ting AE.** Rab8b and its interacting partner TRIP8b are involved in regulated secretion in AtT20 cells. J Biol Chem 276: 13209-13216, 2001. PMID:11278749
9. **Chen X, Walker AK, Strahler JR, Simon ES, Tomanicek-Volk SL, Nelson BB, Hurley MC, Ernst SA, Williams JA, Andrews PC.** Organellar proteomics: analysis of pancreatic zymogen granule membranes. Mol Cell Proteomics 5: 306-312, 2006. PMID:16278343
10. **Chi ZL, Akahori M, Obazawa M, Minami M, Noda T, Nakaya N, Tomarev S, Kawase K, Yamamoto T, Noda S, Sasaoka M, Shimazaki A, Takada Y, Iwata T.** Overexpression of optineurin E50K disrupts Rab8 interaction and leads to a progressive retinal degeneration in mice. Hum Mol Genet 19: 2606-2615, 2010. PMID:20388642
11. **De Marco N, Buono M, Troise F, Diez-Roux G.** Optineurin increases cell survival and translocates to the nucleus in a Rab8-dependent manner upon an apoptotic stimulus. J Biol Chem 281: 16147-16156, 2006. PMID:16569640

12. **del Toro D, Alberch J, Lazaro-Dieguez F, Martin-Ibanez R, Xifro X, Egea G, Canals JM.** Mutant huntingtin impairs post-Golgi trafficking to lysosomes by delocalizing optineurin/Rab8 complex from the Golgi apparatus. Mol Biol Cell 20: 1478-1492, 2009. PMID:19144827
13. **Deretic D, Huber LA, Ransom N, Mancini M, Simons K, Papermaster DS.** rab8 in retinal photoreceptors may participate in rhodopsin transport and in rod outer segment disk morphogenesis. J Cell Sci 108 (Pt 1): 215-224, 1995. PMID:7738098
14. **Dong C, Yang L, Zhang X, Gu H, Lam ML, Claycomb WC, Xia H, Wu G.** Rab8 interacts with distinct motifs in alpha2B- and beta2-adrenergic receptors and differentially modulates their transport. J Biol Chem 285: 20369-20380, 2010. PMID:20424170
15. **Faust F, Gomez-Lazaro M, Borta H, Agricola B, Schrader M.** Rab8 is involved in zymogen granule formation in pancreatic acinar AR42J cells. Traffic 9: 964-979, 2008. PMID:18363906
16. **Goud B, Salminen A, Walworth NC, Novick PJ.** A GTP-binding protein required for secretion rapidly associates with secretory vesicles and the plasma membrane in yeast. Cell 53: 753-768, 1988. PMID:3131018
17. **Grosshans BL, Ortiz D, Novick P.** Rabs and their effectors: achieving specificity in membrane traffic. Proc Natl Acad Sci U S A 103: 11821-11827, 2006. PMID:16882731
18. **Hammer JA, 3rd, Wu XS.** Rabs grab motors: defining the connections between Rab GTPases and motor proteins. Curr Opin Cell Biol 14: 69-75, 2002. PMID:11792547
19. **Hattula K, Furuhjelm J, Arffman A, Peranen J.** A Rab8-specific GDP/GTP exchange factor is involved in actin remodeling and polarized membrane transport. Mol Biol Cell 13: 3268-3280, 2002. PMID:12221131
20. **Hattula K, Furuhjelm J, Tikkanen J, Tanhuanpaa K, Laakkonen P, Peranen J.** Characterization of the Rab8-specific membrane traffic route linked to protrusion formation. J Cell Sci 119: 4866-4877, 2006. PMID:17105768
21. **Hattula K Peranen J.** FIP-2, a coiled-coil protein, links Huntingtin to Rab8 and modulates cellular morphogenesis. Curr Biol 10: 1603-1606, 2000. PMID:11137014
22. **Huber LA, de Hoop MJ, Dupree P, Zerial M, Simons K, Dotti C.** Protein transport to the dendritic plasma membrane of cultured neurons is regulated by rab8p. J Cell Biol 123: 47-55, 1993. PMID:8408204
23. **Huber LA, Dupree P, Dotti CG.** A deficiency of the small GTPase rab8 inhibits membrane traffic in developing neurons. Mol Cell Biol 15: 918-924, 1995. PMID:7823956
24. **Huber LA, Pimplikar S, Parton RG, Virta H, Zerial M, Simons K**. Rab8, a small GTPase involved in vesicular traffic between the TGN and the basolateral plasma membrane. J Cell Biol 123: 35-45, 1993. PMID:8408203
25. **Itzen A, Bleimling N, Ignatev A, Pylypenko O, Rak A.** Purification, crystallization and preliminary X-ray crystallographic analysis of mammalian MSS4-Rab8 GTPase protein complex. Acta Crystallogr Sect F Struct Biol Cryst Commun 62: 113-116, 2006. PMID:16511278
26. **Itzen A, Pylypenko O, Goody RS, Alexandrov K, Rak A.** Nucleotide exchange via local protein unfolding—structure of Rab8 in complex with MSS4. *EMBO J* 25: 1445-1455, 2006. PMID: 16541104
27. **Joberty G, Tavitian A, Zahraoui A.** Isoprenylation of Rab proteins possessing a C-terminal CaaX motif. FEBS Lett 330: 323-328, 1993. PMID:8375503
28. **Jordens I, Marsman M, Kuijl C, Neefjes J.** Rab proteins, connecting transport and vesicle fusion. Traffic 6: 1070-1077, 2005. PMID:16262719
29. **Kamikura DM, Cooper JA.** Clathrin interaction and subcellular localization of Ce-DAB-1, an adaptor for protein secretion in Caenorhabditis elegans. Traffic 7: 324-336, 2006. PMID:16497226
30. **Linder MD, Mayranpaa MI, Peranen J, Pietila TE, Pietiainen VM, Uronen RL, Olkkonen VM, Kovanen PT, Ikonen E.** Rab8 regulates ABCA1 cell surface expression and facilitates cholesterol efflux in primary human macrophages. Arterioscler Thromb Vasc Biol 29: 883-888, 2009. PMID:19304576
31. **Linder MD, Uronen RL, Holtta-Vuori M, van der Sluijs P, Peranen J, Ikonen E.** Rab8-dependent recycling promotes endosomal cholesterol removal in normal and sphingolipidosis cells. Mol Biol Cell 18: 47-56, 2007. PMID:17050734

32. **Mizushima N, Yamamoto A, Matsui M, Yoshimori T, Ohsumi Y.** In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. Mol Biol Cell 15: 1101-1111, 2004. PMID:14699058
33. **Moritz OL, Tam BM, Hurd LL, Peranen J, Deretic D, Papermaster DS.** Mutant rab8 Impairs docking and fusion of rhodopsin-bearing post-Golgi membranes and causes cell death of transgenic Xenopus rods. Mol Biol Cell 12: 2341-2351, 2001. PMID:11514620
34. **Moritz OL, Tam BM, Knox BE, Papermaster DS.** Fluorescent photoreceptors of transgenic Xenopus laevis imaged in vivo by two microscopy techniques. Invest Ophthalmol Vis Sci 40: 3276-3280, 1999. PMID:10586953
35. **Nachury MV, Loktev AV, Zhang Q, Westlake CJ, Peranen J, Merdes A, Slusarski DC, Scheller RH, Bazan JF, Sheffield VC, Jackson PK.** A Core Complex of BBS Proteins Cooperates with the GTPase Rab8 to Promote Ciliary Membrane Biogenesis. Cell 129: 1201-1213, 2007. PMID:17574030
36. **Nagabhushana A, Chalasani ML, Jain N, Radha V, Rangaraj N, Balasubramanian D, Swarup G.** Regulation of endocytic trafficking of transferrin receptor by optineurin and its impairment by a glaucoma-associated mutant. BMC Cell Biol 11: 4, 2010. PMID:20085643
37. **Peranen J, Auvinen P, Virta H, Wepf R, Simons K.** Rab8 promotes polarized membrane transport through reorganization of actin and microtubules in fibroblasts. J Cell Biol 135: 153-167, 1996. PMID:8858170
38. **Randhawa VK, Ishikura S, Talior-Volodarsky I, Cheng AW, Patel N, Hartwig JH, Klip A.** GLUT4 vesicle recruitment and fusion are differentially regulated by Rac, AS160, and Rab8A in muscle cells. J Biol Chem 283: 27208-27219, 2008. PMID:18650435
39. **Rindler MJ, Xu CF, Gumper I, Smith NN, Neubert TA.** Proteomic Analysis of Pancreatic Zymogen Granules: Identification of New Granule Proteins. J Proteome Res, 2007. PMID:17583932
40. **Rodriguez OC, Cheney RE.** Human myosin-Vc is a novel class V myosin expressed in epithelial cells. J Cell Sci 115: 991-1004, 2002. PMID:11870218
41. **Roland JT, Kenworthy AK, Peranen J, Caplan S, Goldenring JR.** Myosin Vb interacts with Rab8a on a tubular network containing EHD1 and EHD3. Mol Biol Cell 18: 2828-2837, 2007. PMID:17507647
42. **Sahlender DA, Roberts RC, Arden SD, Spudich G, Taylor MJ, Luzio JP, Kendrick-Jones J, Buss F.** Optineurin links myosin VI to the Golgi complex and is involved in Golgi organization and exocytosis. J Cell Biol 169: 285-295, 2005. PMID:15837803
43. **Sato T, Mushiake S, Kato Y, Sato K, Sato M, Takeda N, Ozono K, Miki K, Kubo Y, Tsuji A, Harada R, Harada A.** The Rab8 GTPase regulates apical protein localization in intestinal cells. Nature 448: 366-369, 2007. PMID:17597763
44. **Schuck S, Manninen A, Honsho M, Fullekrug J, Simons K.** Generation of single and double knockdowns in polarized epithelial cells by retrovirus-mediated RNA interference. Proc Natl Acad Sci U S A 101: 4912-4917, 2004. PMID:15051873
45. **Seabra MC, Coudrier E.** Rab GTPases and myosin motors in organelle motility. Traffic 5: 393-399, 2004. PMID:15117313
46. **Stenmark H, Olkkonen VM.** The Rab GTPase family. Genome Biol 2: REVIEWS3007, 2001. PMID:11387043
47. **Verma P, Ostermeyer-Fay AG, Brown DA.** Caveolin-1 induces formation of membrane tubules that sense actomyosin tension and are inhibited by polymerase I and transcript release factor/cavin-1. Mol Biol Cell 21: 2226-2240, 2010. PMID:20427576
48. **Zerial M, McBride H.** Rab proteins as membrane organizers. Nat Rev Mol Cell Biol 2: 107-117, 2001. PMID:11252952
49. **Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L, MacDonald ME, Friedlander RM, Silani V, Hayden MR, Timmusk T, Sipione S, Cattaneo E.** Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. Science 293: 493-498, 2001. PMID:11408619