GPHR is a Novel Anion Channel Critical for Acidification and Functions of the Golgi Apparatus

MAEDA Yusuke and KINOSHITA Taroh
(Research Institute for Microbial Diseases)
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Organelles, cells, organs and organisms elaborate a complex and fundamental system, so called homeostasis, that regulates its internal environment such as ion balance, pH, osmolarity and blood sugar and tends to maintain a stable, constant condition suitable for their activities. Organelles within cells are compartmented by lipid bilayer that enables cells to perform many tasks through fine-tuning of homeostasis. The organelles within secretory and endocytotic pathways such as Golgi apparatus, endosome and lysosome have acidified lumens, and maintaining their proper acidic pHs has been considered to be critical for the many functions of the organelles, based on evidence that these functions are disturbed by compounds that alkalinize acidic organelles. Therefore, regulation of pH in intracellular organelles is a fundamental component of homeostasis, but the issues of how organelle lumen acidification is regulated and how luminal pH elevation disturbs these fundamental cellular processes are largely unknown.

Recently, we established new mutant cells defective in Golgi acidification and identified a novel Golgi-resident multi-transmembrane protein by expression cloning, termed GPHR (Golgi pH Regulator), that is crucial for facilitating sufficient luminal acidification for normal Golgi functions, since the elevated pH in the mutant cells caused impaired trafficking and glycosylation of cargo proteins and lipids as well as disrupted morphological integrity of the Golgi. The luminal pH of the Golgi but not of the endosome and lysosome was selectively elevated by the defect of GPHR. After reconstitution in planar lipid bilayers, GPHR exhibited a voltage-dependent anion channel activity, indicating that GPHR functions as a molecular mechanism of counterion conductance that decreases the membrane potential formed by proton influx to allow proton pump to transfer more protons into the Golgi lumen. Thus, our results established the crucial role of counterion channel on physiological acidification of the Golgi, which had been under debates for a long time.

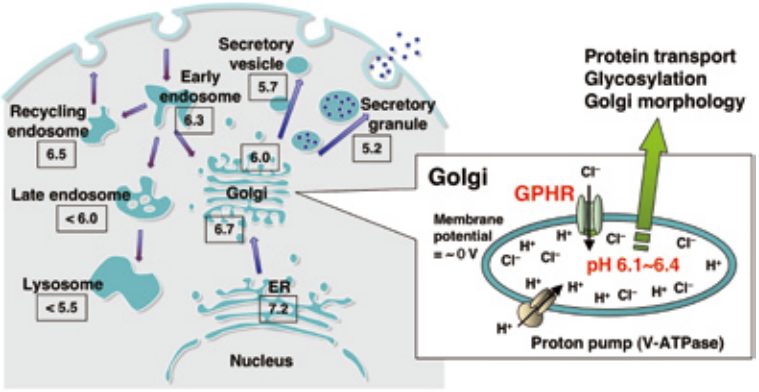

Fig. The organelles within secretory and endocytotic pathways possess proper acidic pHs (numbers in left figure). Golgi pH is regulated by balance between proton pump (V-ATPase), which is a sole proton delivery source, and GPHR, which is an anion channel and dissipates the membrane potential generated by the electrogenic proton pump to allow it to transfer more protons into the Golgi lumen. The regulated acidic pH plays crucial roles for trafficking and glycosylation of cargo proteins and lipids as well as maintaining normal Golgi morphology.

Two Beclin 1-binding Proteins, Atg14L and Rubicon, Reciprocally Regulate Autophagy at Different Stages

MATSUNAGA Kohichi and YOSHIMORI Tamotsu
(Research Institute for Microbial Deseases)
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Autophagy is an intracellular process in which cytoplasmic materials are sequestered and delivered by autophagosome to the lysosomes for degradation. Autophagy contributes to survival against starvation, cytoplasmic renewal, elimination of intracellular aggregate-prone proteins and pathogens, innate and acquired immunity, and context-dependent programmed cell death. Beclin 1, a protein essential for autophagy, binds to hVps34/Class III phosphatidylinositol 3-kinase producing phosphatidylinositol 3-phosphate, which is involved in membrane dynamics. We identified two novel Beclin 1 associated proteins and named them Atg14L and Rubicon, respectively. Atg14L and Rubicon bind to Beclin-1 in a mutually exclusive manner. While Atg14L-Beclin 1 complex positively regulated autophagy at an early step, Rubicon-Beclin 1 complex negatively regulated autophagy at a later step. The latter also suppresses endocytic traffic. It has been reported that Beclin $1^{+/-}$ mice increases the frequency of spontaneous tumor. Regulation of autophagic and endocytic pathways by the two Beclin 1 complexes may be critical for suppression of carcinogenesis.

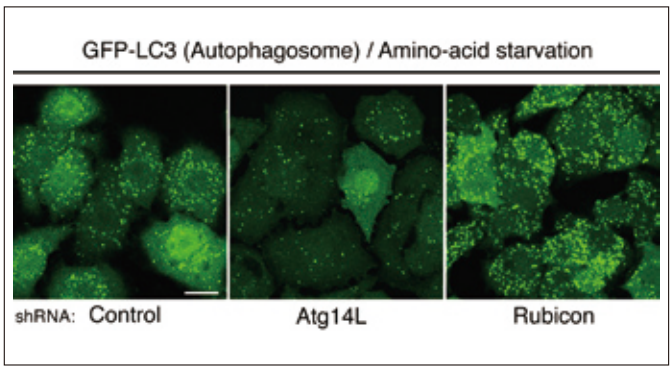

Fig. 1 The effects of Atg14L and Rubicon depletion by RNA interference (RNAi) on autophagosome formation
A549 cell line stably expressing GFP-LC3 (autophagosome marker) was infected with adenovirus harboring shRNA targeting Atg14L, Rubicon or control (luciferase). The cells were cultured in amino acid starvation conditions. GFP was observed by confocal laser microscopy. The bar indicates 10 μm.

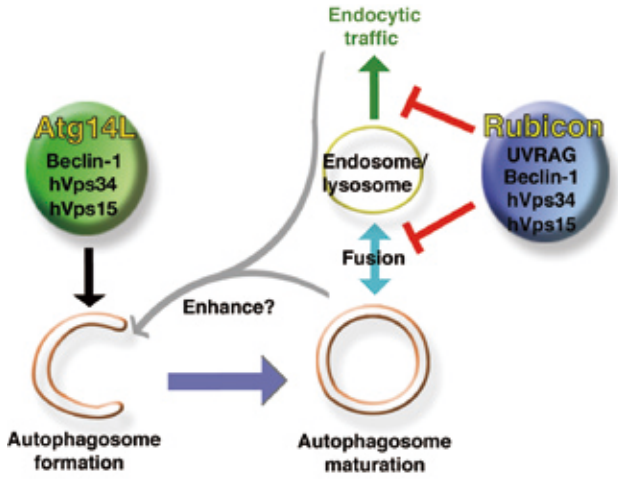

Fig. 2 Model of the two distinct Beclin 1-Vps34 complexes.
The Atg14L complex positively function in autophagosome formation. The Rubicon complex negatively function in autophagosome and endosome.