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ARTICLE ADDENDUM

The multiple roles of Rab9 in the endolysosomal system

Ana Kucera, Oddmund Bakke, and Cinzia Progida

Department of Biosciences, Centre for Immune Regulation, University of Oslo, Oslo, Norway

ABSTRACT

The small GTPase Rab9 has long been described as a protein that mediates endosome-to-trans-Golgi Network (TGN) transport, and specifically mannose-6-phospate receptor (MPR) recycling. However, studies have challenged this view by showing that Rab9 also is connected to sorting pathways toward the endolysosomal compartments. We recently characterized the spatio-temporal dynamics of Rab9 and, by using live cell imaging, we showed that it enters the endosomal pathway together with CI-MPR at the transition stage between early, Rab5-positive, and late, Rab7a-positive, endosomes. More so, the Rab9 constitutively active mutant, Rab9Q66L, accumulates on late endosomes and promotes carrier formation at the TGN. Here, we discuss our findings in light of previous reports on Rab9 in the retrograde transport pathway.

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Rab proteins belong to the Ras superfamily of small monomeric GTPases and are central regulators of intracellular trafficking events.[1] Rabs are associated with membranes in a dynamic on and off equilibrium which is regulated by the activities of guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). GTP-bound Rabs localize to specific organelle membranes where they are involved in regulation of defined intracellular events.

The process of endosome maturation is regulated by Rab5 and Rab7a. Here, endocytosed cargo accumulates in progressively more acidic compartments destined for degradation in the lysosomes. Rab5 binds to early endosomes and regulates homotypic early endosomal fusion events.[2] These endosomes undergo maturation to become Rab7a-positive late endosomal compartments targeted for lysosomes through the process of Rab conversion which consists of a coordinated loss of Rab5 and the concomitant acquisition of Rab7a.[3] These events are tightly regulated: in the initial steps, inactive GDP-bound Rab5 is activated and recruited to the early endosomal membrane through the actions of the GEF Rabex-5 and the effector Radaptin-5.[4] Among the downstream effectors of Rab5 is the SAND-1/Mon1-complex that binds Rab5-GTP but also initiates recruitment of Rab7a to maturing endosomes.[5,6] The SAND-1/Mon1-complex also promotes the dissociation of Rabex-5 from the endosome, thus leading to the detachment of Rab5 from the endosomal membrane.[7] Rab7a persists on late endosomes and is considered the key regulator of endosome maturation, lysosome biogenesis and endo-lysosomal transport.[8]

Late endosomes also carry other Rab proteins bound to their organelle membranes, such as Rab9 and Rab7b.[9-11] Rab7a and Rab9 use different machineries for recruitment to membranes,[12] and perhaps this explains why these 2 Rabs also locate to different domains while on the same endosome.[9] Rab9 has further been shown to act through specific downstream effectors not shared by Rab7a, such as GCC185, TIP47 and p40.[13-15] However, the greatest distinction between these 2 late endosomal Rab proteins is that Rab9 has been mainly reported to mediate the recycling pathway of sorting receptors as cation-independent mannose 6-phosphate receptors (CI-MPR)[16] and not to be directly involved in the process of endosome maturation.

Interestingly, in our recent work, we demonstrated through live confocal imaging that Rab9 entered the endosomal pathway at the Rab5-to-Rab7a transition.[17] Also, we showed for the first time that CI-MPR entered the endosomal pathway alongside Rab9 (Fig. 1).

We further characterized the constitutively active mutant of Rab9, Rab9Q66L, the live dynamics of which have been unknown. Upon mutation in the catalytically important glutamine in position 66, the intrinsic ability of

CONTACT Oddmund Bakke oddmund.bakke@ibv.uio.no; Cinzia Progida c.a.m.progida@ibv.uio.no Department of Biosciences, Centre for Immune Regulation, Blindernveien 31, University of Oslo, 0371 Oslo, Norway.

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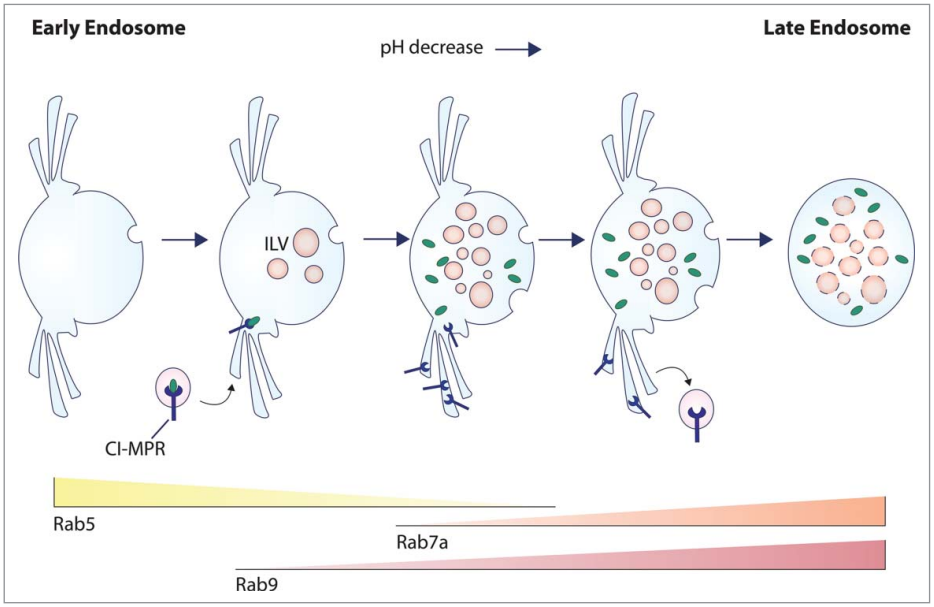

Figure 1. Rab9 in the endosomal pathway. During endosome maturation intraluminal vesicles (ILVs) are formed, the endosomes become more acidic and acquire lysosomal enzymes, which eventually degrade the luminal content. The maturation of endosomes is propelled through the process of Rab conversion, which consists of the coordinated loss of Rab5 and the concomitant acquisition of Rab7a. CI-MPR and Rab9 enter the endosomal pathway at the late stage of the early endosomes where CI-MPR delivers its ligands, newly synthesized lysosomal enzymes, to the maturing endosome. The drop in pH facilitates the release of the ligands from its sorting receptor and activated the enzymes. In order to avoid degradation, CI-MPR is sorted through retrograde transport pathways toward the TGN.

Rab9 to hydrolyze GTP is lowered, thus creating a constitutively active protein.[18] Rab proteins predominantly bind to their effectors and target membranes while in their active state. Therefore, these mutants facilitate the deciphering of the physiological functions of Rab proteins. In our report we showed that Rab9Q66L accumulated on late endosomes. Furthermore, the expression of Rab9Q66L spread CI-MPR from the TGN into the endosomal pathway, and increased tubulation and carrier formation originating from the TGN.[17] Together, these results show that Rab9 is involved in trafficking from the TGN to endosomes.

A body of evidence further supports a Rab9-dependent transport targeted toward the endosomal pathway, and several reports have shed light on the complexity of Rab9-mediated trafficking and its multiple functions in intracellular transport (Fig. 2). Rab9 mediates sorting of lysosomal enzymes into late endosomes[19,20] and is therefore involved in lysosome biogenesis.[20,21] Furthermore, formation of autophagosomes depends on Rab9, which mediates the fusion of isolation membranes with vesicles derived from the trans-Golgi and late endosomes.[22] Rab9 has additionally been linked to the release of viral particles[23] and shown to mediate intracellular transport of lipids.[24,25] Indeed, this small GTPase is involved in the Golgi targeting of glycosphingolipids and lipid transport from late endosomes.[24,25]

Nevertheless, in agreement with previous studies, silencing of Rab9 perturbed MPRs retrograde transport. Ganley and co-authors speculated that this is a consequence of a disrupted Rab9-dependent retrograde pathway.[19] However, silencing of Rab7a, which is involved in endosome maturation, also reduces the retrograde transport of MPRs.[26] This indicates that the perturbed retrograde transport of MPRs can also be a consequence of alterations in the endosomal maturation. Therefore, this could also explain the effects of Rab9 knock down on MPR retrograde transport, particularly in the light of the recent findings showing Rab9 recruitment to the endosomal pathway during the Rab5-to-Rab7a conversion.[17]

The two main pathways involved in retrograde transport of CI-MPRs use Rab9[20] and the retromer complex.[27] For long time these 2 pathways have been considered independent, however, recent evidence suggests that they may be interconnected.[28] It is very likely that additional mechanisms exist, and in line with this, at least 2 other Rab proteins have been shown to mediate retrograde transport of CI-MPR: Rab7b and Rab29.[10,11,29] CI-MPRs are also subjected to complex regulation through AP-1, AP-2 and GGAs as their sorting adaptors for intracellular trafficking.[30] Each of these sorting complexes bind CI-MPR cytosolic tail by means of distinct sorting signals, and these variations imply that CI-MPR follows various sorting pathways. Interestingly, AP-1 mediates not only the transport of CI-MPRs from the TGN to endosomes, but also the retrograde transport of these sorting receptors to the TGN.[30-32]

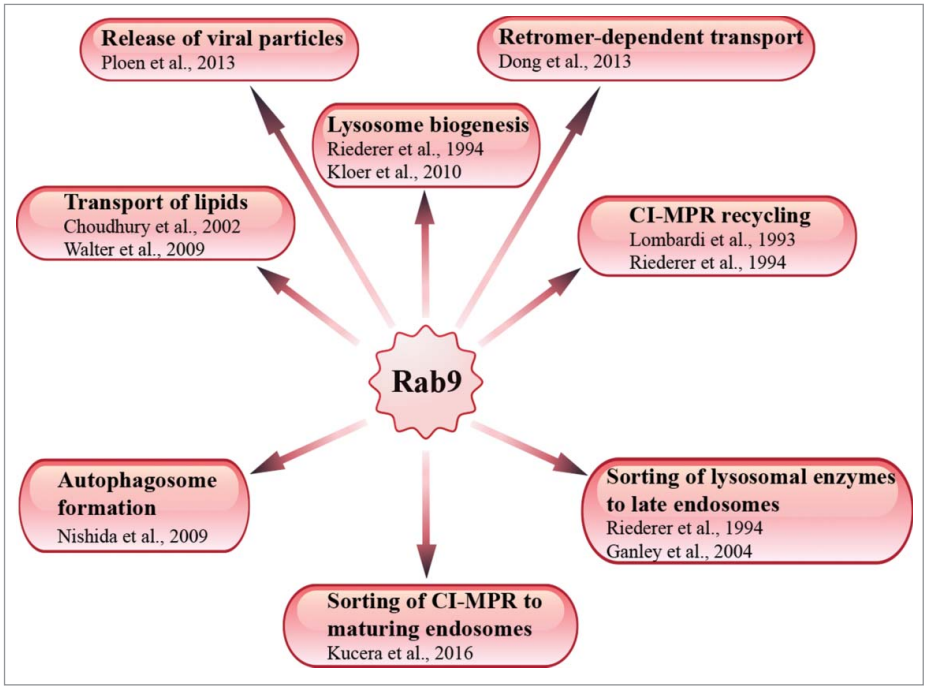

Figure 2. The multiple roles of Rab9 in the endolysosomal system.

Within the adaptor diversity, the tail-interacting protein of 47 kDa (TIP47) has long been considered the retrograde adaptor that connects Rab9 to CI-MPR on late endosomes.[14] However, a careful study by Bulankina et al.[33] challenged this view by demonstrating that knockdown of TIP47 had no consequence for CI-MPR recycling and distribution, nor did it affect lysosomal enzyme sorting. TIP47 is indeed involved in the biogenesis of lipid droplets in the cell.[33,34]

The retromer complex also mediates CI-MPR retrograde transport and consists of a conserved heterotrimer of the vacuolar protein sorting (Vps) proteins Vps26, Vps35 and Vps29 and a dimer of phosphoinositide-binding sorting nexins (SNXs).[35] It has been suggested that the choice of retrograde pathway (Rab9- or retromer-dependent) is regulated by both the cytoplasmic domain and the transmembrane region of the cargo.[36] Intriguingly, a recent publication connected Rab9 to retromer transport in Drosophila during embryonic development through an interaction with Vps35,[37] and while speculative for now, further investigations are required to unravel a possible role of Rab9 in retromer-dependent CI-MPR transport.

The retromer recruitment to endosomes is mediated by the sequential action of Rab5 and Rab7.[38] In our recent work, we found Rab9 to enter the endosomal pathway at the late stages of early endosomes,[17] which coincides with the point of retromer recruitment to the endosomal pathway.[38,39] Together, these findings suggest a connection between Rab9 and the retromer in the transport between endosomes and the TGN that will be important to elucidate in the future.

Disclosure of potential conflicts of interest

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