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Loss of Rab27 function results in abnormal lung epithelium structure in mice

Giulia Bolasco,1 Dhani C. Tracey-White,1 Tanya Tolmachova,1 Andrew J. Thorley,2 Teresa D. Tetley,2 Miguel C. Seabra,1,3,4 and Alistair N. Hume1

1Molecular Medicine, 2Pharmacology and Toxicology, National Heart and Lung Institute, Imperial College London, London, United Kingdom; 3Instituto Gulbenkian de Ciência, Oeiras; and 4Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal

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Bolasco G, Tracey-White DC, Tolmachova T, Thorley AJ, Tetley TD, Seabra MC, Hume AN. Loss of Rab27 function results in abnormal lung epithelium structure in mice. Am J Physiol Cell Physiol 300: C466–C476, 2011. First published December 15, 2010; doi:10.1152/ajpcell.00446.2010.—Rab27 small GTPases regulate secretion and movement of lysosome-related organelles such as T cell cytolytic granules and platelet-dense granules. Previous studies indicated that Rab27a and Rab27b are expressed in the murine lung suggesting that they regulate secretory processes in the lung. Consistent with those studies, we found that Rab27a and Rab27b are expressed in cell types that contain secretory granules: alveolar epithelial type II (AEII) and Clara cells. We then used Rab27a/-/- and Rab27b/-/- double knockout (DKO) mice to examine the functional consequence of loss of Rab27 proteins in the murine lung. Light and electron microscopy revealed a number of morphological changes in lungs from DKO mice when compared with those in control animals. In aged DKO mice we observed atrophy of the bronchiolar and alveolar epithelium with reduction of cells numbers, thinning of the bronchiolar epithelium and alveolar walls, and enlargement of alveolar airspace. In these samples we also observed increased numbers of activated foamy alveolar macrophages and granulocyte containing infiltrates together with reduction in the numbers of Clara cells and AEII cells compared with control. At the ultrastructural level we observed accumulation of cytoplasmic membranes and vesicles in Clara cells. Meanwhile, AEII cells in DKO accumulated large mature lamellar bodies and lacked immature/precursor lamellar bodies. We hypothesize that the morphological changes observed at the ultrastructural level in DKO samples result from secretory defects in AEII and Clara cells and that over time these defects lead to atrophy of the epithelium.

Rab27 proteins; intracellular transport; alveolar epithelium type II cell; Clara cell

While a subset of “housekeeping” Rabs are expressed ubiquitously, Rab27 GTPases (Rab27a and Rab27b) are expressed in cell types with specialized secretory functions (6, 29). In these cells, Rab27 localizes to, and regulates, the motility and exocytosis of lysosome-related organelles (LROs) and secretory granules; e.g., melanosomes in melanocytes, lytic granules in cytotoxic T cells (CTL), and dense granules in platelets (1, 8, 11, 25, 28, 32). To facilitate these diverse functions Rab27 interacts with eleven known downstream effectors (5, 13). For instance, in melanocytes, we and others (9, 21) have shown that Rab27a-GTP sequentially recruits effector Melanophilin/Slac2-a and molecular motor myosin 5a to melanosomes thereby allowing their retention in actin-rich peripheral dendrites. Meanwhile, in CTLs, Rab27 mediates granule docking and exocytosis via effector Munc13–4 (17). Differential engagement of effectors is in part due to their cell type-specific pattern of expression (10). In some cell types, Rab27a and Rab27b isoforms are expressed together, and recent data suggest that they may perform sequential roles in granule docking and exocytosis via engagement of different effectors (14, 20).

Previous analysis revealed that Rab27 proteins are expressed in the murine lung, suggesting that they play an important role in lung cell types with specialized secretory functions (6, 29). Within the conducting airways (bronchioles), Clara cells perform an important role in production and release of lung lining fluid components; e.g., surfactant protein A, Clara cell-specific protein (CCSP). While in the gas exchange, (alveolar) epithelium type II (AEII) cells perform a similar function producing surfactant protein and phospholipid components (3, 15). Lining fluid proteins and lipids perform an essential role in the innate immune response to invading pathogens; e.g., defensins and collectins, and reduction of surface tension that maintains the alveolar structure; e.g., surfactant proteins and lipids. Interestingly, within AEII cells a subset of surfactant components are stored in lysosome-related organelles (LROs), known as lamellar bodies (LB), that release their cargo into the airspace upon stimulation (4).

Several studies provide evidence for an important role for Rab GTPases in the physiological function of the lung. For example, Rab3D has been found to localize to a population of LBs in AEII cells, although its precise function in this context remains unknown (30). Rab14, meanwhile, was found partially localized to LBs, and knockdown resulted in partial inhibition of their evoked release suggesting a role for Rab14 in this process (7). Most recently, analysis of the chocolate mutant mouse and the ruby mutant rat that contain Rab38 gene mutations revealed a role for this protein in surfactant exocytosis and LB structure (18, 19). In this study, we investigated the cell-type specific and intracellular localization of Rab27.
proteins in the murine lung as well as the effects of loss of Rab27 upon morphology of the pulmonary epithelium.

MATERIALS AND METHODS

Chemicals and antibodies. Unless otherwise stated all chemicals were obtained from Sigma-Aldrich (Poole, UK). The following antibodies were used for immunoblotting: rabbit polyclonals anti-Rab27a and anti-Rab27b raised against the COOH-terminus hypervariable region of each protein at 1:1,000 (Davids Biotechnologie, Regensburg, Germany) and rabbit anti-Calnexin Stresagen (SPA-860D, Bio-Whittaker; Wokingham, UK) at 1:10,000. For immunohistochemistry, rabbit anti-pro-surfactant protein C (SPC) antibody (Abcam ab28744, Cambridge, UK) was used at 1:1,000, rabbit anti-Clara cell-specific protein (CCSP) (UK cat no. 07-623, Upstate, Dundee) was at 1:1,000, goat anti-SPC (Research Diagnostics R7SURFCCabG) was at 1:100, and rabbit polyclonal anti-Rab27a and anti-Rab27b antibodies (as above) were each at 1:100.

Mouse strains. All mice were treated humanely and in accordance with the UK Home Office regulations under project license PPL 70/7078 at the Central Biomedical Services of Imperial College, London, UK. C57BL/6j wild-type mice were purchased from B&K Universal Limited (Hull, UK), Rab27a-deficient mice, ashen (Rab27aash/ash) mice were described previously (31) and the generation of Rab27b KO mice and double Rab27KO was described elsewhere (27). All strains were maintained on a C57Bl/6 background.

Immunoblotting. Immunoblotting was performed as described previously (12). Perfused lung lysates were prepared using a Polytron homogenizer and an appropriate volume (10× vol/wt) of lysis buffer (150 mM NaCl, 20 mM Tris-HCl, pH 7.5, 1 mM DTT, 1% CHAPS, and 1× PI cocktail) followed by incubation on ice for 15 min. Nuclei and debris were then harvested by centrifugation (3,000 g at 4°C for 10 min), and the protein content of the PNS was quantified using BCA protein assay kit (Pierce, UK).

Real-time PCR analysis. Total RNA from resected human lung tissue obtained from 19 transplant donors was reverse transcribed using a reaction mix of 1 tissue obtained from 19 transplant donors was reverse transcribed target genes (Rab27a, Rab27b) expression relative to the reference PCR products starts to be detected. Results, expressed as amount in which the increase in signal associated with exponential growth of MgCl2, 75 mM KCl, 50 mM Tris·HCl, pH 8.3), 20 units of RNasin and debris were then harvested by centrifugation (3,000 and 1

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Fig. 1. Rab27a and Rab27b proteins are expressed in the lungs of mice and humans. A: lung lysates were immunoblotted using Rab27a and Rab27b isoform-specific antibodies. Lysates from control animals (Rab27aash/ash and Rab27BKO) lacking Rab27a and Rab27b, respectively, confirm the specificity of the antibody staining. Additionally, lysates were blotted with calnexin-specific antibodies as a control for equal loading. B: real-time PCR was performed to measure the expression of Rab27a- and Rab27b-specific transcripts in total cDNA prepared from samples of resected human lung. Signal was normalized using 18s rRNA as control and expression level is displayed as ΔCt value (inversely related to expression level).
from each of 3 age-matched animals. P values were derived using the Student’s t-test.

**Immunohistochemistry.** Immunofluorescence was performed on either deparaffinized, formalin-fixed paraffin sections or frozen sections. Sections were deparaffinized using Histoclear and then taken through reducing alcohols to water, and rinsed in 1× PBS. For antigen retrieval, samples were boiled for 5 min in 10 mM sodium citrate buffer (pH 6.0) and cooled for 5–10 min in water. Slides were washed in 1× PBS and incubated with blocking buffer (1× PBS, 0.025% Triton X-100, 1% BSA, 10% donkey serum) for 1 h and then incubated overnight at 4°C with primary antibody diluted in blocking buffer. The following day slides were washed three times with washing buffer (1× PBS, 0.1% Triton X-100), incubated with Alexa488- or Alexa568-conjugated secondary antibodies, and finally washed as before and mounted using Immunofluor mountant (MP Biomedicals, Cambridge, UK). Nuclei were visualized using 4,6-diamidino-2-phenylindole (DAPI) stain. For immunofluorescence of frozen sections, perfused lungs were manually inflated via the trachea using a 1:1 mixture of OCT (Cellpath, Powys, UK) and PBS, and frozen sections, perfused lungs were manually inflated via the trachea.

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**Conventional electron microscopy.** Lungs of C57BL/6 mice were infused and fixed with a mixture of 2% (wt/vol) PFA, 2% (wt/vol) glutaraldehyde (TAAB) in 0.1 M sodium cacodylate buffer (Agar), pH 7.4, postfixed with 1% (wt/vol) OsO4 supplemented with 1.5% (wt/vol) potassium ferrocyanide, dehydrated in ethanol, and infiltrated with propylene oxide (Agar)/Epon (Leica), counter-stained with lead citrate, and observed with a transmission electron microscope (TEM) Jeol 1010. Images were obtained using a Gatan ORIUS CCD camera. For measurement of lamellar body area, electron microscope images were imported into Image J software, the perimeter of each organelle was defined manually, and the area was measured using the measure tool within the software.

**Ultracytotomy and immunogold labeling.** Lungs of C57BL/6 mice were infused and fixed with 2% (wt/vol) PFA and 0.1% (wt/vol) glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.1). Samples were cut in 0.5-mm³ blocks, embedded in 12% gelatin, and infused in 2.3 M sucrose (26). Mounted gelatin blocks were frozen in N2, and ultratrin (50 nm) cryosections were cut at −120°C with an Ultracytome (Leica). Sections were retrieved in 1.15 M (wt/vol) sucrose-2% (vol/vol) methylcellulose solution and processed for immunolabeling. After the sections were blocked with 0.5% (wt/vol) BSA, single immunolabeling was performed on the sections in a humid chamber with primary antibodies, and protein A was coupled to 10-nm diameter gold particles (PAG-10 nm).

**Fig. 2.** Immunohistochemistry of frozen sections of murine lung reveals that Rab27 proteins are expressed in the bronchiolar and alveolar epithelium. Frozen sections of C57BL/6 murine lungs were fixed and stained with antibodies specific for Rab27a (A and B) or Rab27b (C) (green in overlay images). In A and C, arrows indicate localization of Rab27a and Rab27b to alveolar epithelial type II (AEII) cells, whereas arrowhead indicates localization of Rab27a and Rab27b to the bronchiolar epithelium. In B, arrow shows localization of Rab27a to granules in the apical projections of Clara cells, whereas arrowhead highlights the expression of Rab27a in ciliated cells. Scale bars A and C = 100 μm and B = 5 μm.
RESULTS

Rab27A and Rab27B are expressed in the murine lung. As a first step to investigate the role of Rab27 proteins in the function of the pulmonary epithelium, we used isoform-specific Rab27 antibodies to test the expression of Rab27a and Rab27b proteins in murine lung lysates. In control (WT) lung lysate we observed that both isoforms are expressed (Fig. 1A). The specificity of Rab27a and Rab27b signal was confirmed by blotting of lysate derived from mutant mice lacking individually Rab27a (Rab27a<sup>ash/ash</sup>) or Rab27b (Rab27bKO), respectively. Moreover, quantitative real-time PCR analysis of Rab27a and Rab27b mRNA levels in resected human lung tissue samples showed that both genes are significantly expressed in the lung (Fig. 1B).

Rab27 proteins are expressed in Clara cells and AEII cells. We then used immunofluorescence microscopy to reveal the distribution of Rab27 isoforms in frozen sections of C57Bl/6 mouse lung. This showed that Rab27a is highly expressed in the bronchiolar epithelium (Fig. 2A, arrowhead) where it is present in both ciliated and Clara cells (Fig. 2B, arrowhead and arrow, respectively). In high-magnification images of bronchiolar epithelium, Rab27a was found to be particularly apparent in apical vesicles in Clara cells that may represent secretory vesicles (Fig. 2B, arrow). Within the alveolar epithelium, Rab27a was found to be highly expressed within a subset of cells (Fig. 2A, arrow). Double immunolabeling of surfactant protein C (SPC), which is specifically expressed in AEII cells, revealed that Rab27a-positive cells are AEII cells (Fig. 3A).

High-magnification images of AEII cells showed that while SPC is present in punctate structures (presumably LBs), Rab27a is present on small vesicles throughout the cytoplasm that do not significantly colocalize with LBs (Fig. 3B). Similar analysis using specific antibodies indicated that Rab27b is also expressed in ciliated and Clara cells of the bronchiolar epithelium (Fig. 2C, arrowhead), as well as a subset of cells in the alveolar epithelium (Fig. 2C, arrow). Double immunolabeling of frozen sections with SPC-specific antibodies confirmed that a subset of these Rab27b-positive cells are AEII cells (Fig. 3C).

![Image of immunofluorescence microscopy](image.png)

Fig. 3. Rab27 proteins are localized to cytoplasmic vesicles in AEII cells. Frozen sections of C57BL/6 murine lungs were fixed and stained with antibodies specific for Rab27a (A, B) or Rab27b (C, D) (green in overlay images), surfactant protein C (SPC, red in overlay images), and DAPI (blue in overlay images) and analyzed using confocal immunofluorescence microscopy. Low-magnification images show Rab27a (A) and Rab27b (C) expression in AEII cells (arrows). High-magnification images show the intracellular distribution of Rab27a (B) and Rab27b (D) in AEII. Scale bars A and C = 25 μm, B and D = 5 μm.
Fig. 4. Electron microscopy of ultrathin cryosections of murine lungs reveals Rab27 proteins localization to cytoplasmic vesicles, lamellar bodies, and multivesicular bodies in AEII cells. Ultrathin cryosections of C57Bl/6 murine lungs were labeled with antibodies specific for surfactant protein C (SPC; A and B), Rab27a (C and D) or Rab27b (E and F) and detected using second antibodies conjugated to 10-nm protein A gold (PAG). Electron microscopy analysis of labeled sections revealed strong expression of SPC (A and B) in the internal vesicular membranes of MVBs as well as LBs and cytoplasmic vesicles in AEII cells. Similarly Rab27a (C and D) and Rab27b (E and F) were localized to the membranes of cytoplasmic vesicles, LBs, and multivesicular bodies (MVBs) in AEII cells. Scale bars = 100 nm.
Limiting membrane of secretory CCSP granules in Clara cells (Fig. 3C). This analysis also showed that Rab27b is strongly expressed on cytoplasmic granules in an additional cell type present throughout the alveolar epithelium (Fig. 3C, arrowhead and see online supplementary Fig. S1A at AJP-Cell Physiol website). The specificity of antibody staining was confirmed by staining frozen sections of lung from double knockout (DKO) mice (see online supplementary Fig. S2).

Rab27 proteins associate with cytoplasmic vesicles, LBs, and multivesicular bodies in AETII cells and apical secretory granules in Clara cells. We then used cryoimmunoelectron microscopy to investigate further the identity of Rab27-positive structures within AEII cells in ultrathin cryosections of the lungs of C57BL/6 mice. Staining with SPC-specific antibodies (Fig. 4, A and B) showed that this protein is present in the internal membranes of multivesicular bodies (MVBs), LB precursor vesicles, and mature LBs. Similar analysis showed that Rab27a is associated with MVBs and vesicles present throughout the cytoplasm (Fig. 4, C and D). Furthermore, Rab27a did not show strong colocalization with LBs. Meanwhile, Rab27b was localized to cytoplasmic vesicles and in some cases to the limiting membrane of LBs (Fig. 4, E and F). Consistent with immunofluorescence, immunoelectron microscopy analysis confirmed the existence of a second class of Rab27b-expressing cell type within the capillary network of the alveolar wall. High-magnification images revealed that these cells are eosinophils, based on the presence of cytoplasmic crystalloid granules that were labeled with anti-Rab27b antibodies (see online supplementary Fig. S1, B–E). Meanwhile, in the bronchiolar epithelium, examination of immunogold-labeled cryosections revealed that Rab27a is present on the limiting membrane of secretory CCSP granules in Clara cells (Fig. 5, A and B). We also observed gold particles in Clara cells in cryosections labeled with Rab27b-specific antibodies (data not shown).

Loss of Rab27 protein results in atrophy of the pulmonary epithelium. Based on the previous observation that Rab27 proteins are expressed in the murine lung, we next aimed to investigate the function of Rab27 in this context. As a first step we examined the morphology of lungs from mice lacking both Rab27 isoforms (DKO). As shown in Fig. 6A, we observed thinning of the bronchiolar epithelium as well as marked enlargement of the alveolar air spaces in lungs from the DKO compared with control (WT) samples. These morphological alterations were clearly observed in high-magnification images of bronchioles (Fig. 6B), which show disorganization and shortening of bronchiolar cilia, while alveolar areas clearly show enlargement of alveolar spaces and thinning of the alveolar epithelium (Fig. 6C). Quantification using mean linear intercept measurement (Fig. 6D) indicated that airspace enlargement in the DKO reaches significance in the lungs of 12 wk- and 18-mo-old mice. This also indicated that airspace enlargement becomes more evident with increasing age. Meanwhile, measurement of the thickness of the epithelium in multiple bronchioles confirmed that there is significant thinning of the epithelium in DKO compared with age-matched, sex-matched control (WT) samples (Fig. 6E). In addition to these changes, we also observed increased incidence of activated foamy macrophages (see online supplementary Fig. S3A, arrows) and cellular infiltrates containing granulocytes (supplementary Fig. S3, B and C, arrows) in 12-mo-old DKO mice compared with those of age-matched, sex-matched controls (WT) (4/5 DKO vs. 2/9 control samples). Morphological analyses of the lungs of mice lacking individual Rab27 isoforms indicate that Rab27b plays a more significant role in maintenance of the integrity of the pulmonary epithelium than does Rab27a (data not shown).

The numbers of cells expressing AEII and Clara marker proteins are reduced in the lungs of DKO mice. Given that loss of Rab27 proteins resulted in generalized atrophy of the pulmonary epithelium, we investigated the effect of loss of Rab27...
specifically in Clara and AEII cells. To do this we stained deparaffinized sections of mutant and control lungs with pro-SPC and CCSP-specific antibodies that detect AEII and Clara cells, respectively, and then used immunofluorescence microscopy to investigate the effects of loss of Rab27 on the number and distribution of these cell types. By this approach we observed a significant reduction in the number of SP-C immune-positive cells in DKO compared with control samples from age-matched, sex-matched WT mice (mean AEII cells/20\texttimes H9262 field 32 vs. 49.3, respectively, Fig. 7A). Similar analysis of the number of Clara cells in both large and small bronchioles of DKO mice revealed a similar reduction in the number of CCSP immune-positive cells compared with control (WT) bronchioles (Fig. 7B). In addition, we frequently observed that, in contrast to the control, the coverage of Clara cells in DKO bronchioles was patchy with large areas entirely devoid of Clara cells. These observations suggest that the number of AEII and Clara cells is reduced in the lungs of DKO versus WT mice.

**DKO AEII cells accumulate large, mature LBs and contain few LB precursors.** Finally, we used electron microscopy to investigate the ultrastructural alterations in Clara and AEII cells resulting from loss of Rab27 protein. Consistent with immunofluorescence data, this indicated that the bronchiolar epithelium in DKO mice is thinner than that of controls (Fig. 8A). While in Clara cells we observed a dramatic accumulation of cytoplasmic vesicles that most likely represent CCSP-containing secretory granules, together with a reduction in the abundance of early secretory pathway organelles, such as endoplasmic reticulum, that was commonly observed in control samples (Fig. 8A, high magnification image). Similarly, using electron microscopy to study AEII cells, we observed a marked increase in both the number and size of mature LBs and a concomitant reduction in the number of LB precursor structures and mitochondria in DKO compared with control samples (Fig. 8B). This was confirmed by quantification (Fig. 8, C and D). This analysis also confirms that the changes in morphology are progressive and more apparent with aging (Fig. 8C). Parallel electron microscopy analysis of AEII cells in mice deficient in individual Rab27 isoforms revealed similar accumulation of mature LB in AEII cells of mice lacking Rab27b alone, indicating that Rab27b, and not Rab27a, plays a significant role in the regulation of LB homeostasis and AEII cell function.

![Image](image.png)

**Fig. 6.** Histological and morphometric analysis of the lungs of double knockout (DKO) mice reveals airspace enlargement and atrophy of the epithelium. The lungs of DKO and control mice were removed, inflation-fixed in formalin (as described in MATERIALS AND METHODS), and embedded in paraffin. Sections of 5 \textmu m were then cut and stained with hematoxylin and eosin. A–C show images of these stained sections from 12-mo-old DKO and age-matched, sex-matched control (WT) mice. A: low-power overview of lung structure (scale bar = 60 \textmu m); B and C: high-magnification images showing details of morphology of bronchiolar and alveolar epithelium (scale bars = 20 \textmu m). D: mean linear intercept analysis of the integrity of the alveolar epithelium in the lungs of age-matched, sex-matched DKO and control (WT) (*\textit{P} < 0.05; **\textit{P} < 0.002). E: analysis of the thickness of randomly selected areas of the bronchiolar epithelium of DKO and control (WT) (**\textit{P} < 0.0005).
Ultrastructural analysis of AEII cells confirmed that there is marked thinning of the alveolar epithelium in DKO compared with control lungs (Fig. 8B).

DISCUSSION

In this study we used light and electron microscopy to investigate the localization and function of Rab27 proteins in the lung. Our main findings are that Rab27a and Rab27b are expressed in Clara and ciliated cells in bronchioles as well as AEII cells in the alveolar region. Rab27b is additionally strongly expressed in cells in the parenchyma that we have defined as eosinophils based on our finding that they contain eosinophil-specific crystalloid granules. In particular, we find that Rab27b is associated with cytoplasmic face of these granules. These observations are largely consistent with previous analysis of expression of GFP-Rab27a transgenic protein and Rab27b-driven lacZ expression in the lungs of mice (6, 29). However, our finding of high Rab27b expression in eosinophils is surprising given that previous studies indicated that Rab27a, not Rab27b, is expressed in circulating eosinophils in humans (2). Meanwhile, our analysis of the structure of the lungs of DKO mice revealed a generalized atrophy of both the alveolar and bronchiolar epithelium together with mild airspace enlargement that was particularly apparent in mice.
over 12 mo old. Moreover, we observed a reduction in the abundance of SP-C/CCSP-positive cells in DKO mice that was more apparent after 12 mo of age. This suggests that Rab27-expressing AEII and Clara cell numbers may be reduced in the DKO mutant compared with WT control. At the subcellular level, we observed that Rab27 was localized to apical granules in Clara cells that may represent secretory granules, whereas in AEII cells, Rab27 was located on cytoplasmic vesicles and MVBs. In DKO samples, we observed a striking accumulation of mature secretory organelles; i.e., Clara cell granules and LBs in AEII cells, and a reciprocal reduction in the numbers of mitochondria, granule precursors, and ER membranes.

Based on these findings, we hypothesize that the function of Rab27 proteins in the lung is to regulate the release of exocytic
granules in specialized secretory cell types such as Clara and AEII cells. This suggestion is consistent with the localization data, as well as the observed accumulation of end-stage secretory organelles in these cell types in mice lacking Rab27 proteins. Further support for this idea comes from the large number of other studies that have linked Rab27 function to regulated exocytosis in a variety of cell types, including CTLs, platelets, and neutrophils (5, 13). Leading from this, we hypothesize that chronic disruption of exocytosis in Clara and AEII cells is the cause of pulmonary epithelium atrophy in DKO. Consistent with this, the reduction in the number of granule precursor structures, early secretory pathway organelles, and mitochondria point to a mechanism whereby accumulation of end-stage organelles feeds back into a reduction in metabolic activity in these cells. Given that other cell types; e.g., bronchiolar ciliated cells and AEII cells, are thought to be derived from these Clara cells and AEII cells, reduced metabolic, and possibly proliferative, activity in these cells may go some way to explaining the widespread atrophy of the lung tissue observed in the DKO mice (16). Interestingly, the changes in lung architecture observed in DKO are similar to those that occur during natural aging, suggesting that the DKO may represent a useful model for the study of accelerated aging of the lung (23). The relatively mild phenotype observed in the DKO mutant lungs suggests that the function of Rab27 proteins is partially redundant. In line with this idea it has been reported that the closely protein Rab3d, whose function is also linked to exocytosis, is expressed in the pulmonary epithelium (30). Also consistent with the relatively mild nature of the DKO mutant phenotype, we did not observe significant differences in the levels of secreted SP-A, SP-D, SP-C, and CCSP in the bronchoalveolar lavage fluid of DKO and WT animals (data not shown).

Future studies should address the functional consequences of these changes in lung structure in the DKO mice; they should also identify the cargo whose exocytosis is regulated by Rab27 and the molecular mechanisms by which this occurs.

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The current address for A. N. Hume: School of Biomedical Sciences, Queen’s Medical Centre, Nottingham NG7 2UH, UK; and current address for G. Bolasco: EMBL Mouse Biology Unit, Campus A. Buzzati-Traverso, Via E. Pompadour 1, 50121 Florence, Italy.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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