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ORIGINAL ARTICLE

Rab27b is Involved in Lysosomal Exocytosis and Proteolipid Protein Trafficking in Oligodendrocytes

Yun-Tian Shen[1] · Yun Gu[1] · Wen-Feng Su[1] · Jing-fei Zhong[2] · Zi-Han Jin[1] · Xiao-Song Gu[1] · Gang Chen[1]

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Abstract Myelination by oligodendrocytes in the central nervous system requires coordinated exocytosis and endocytosis of the major myelin protein, proteolipid protein (PLP). Here, we demonstrated that a small GTPase, Rab27b, is involved in PLP trafficking in oligodendrocytes. We showed that PLP co-localized with Rab27b in late endosomes/lysosomes in oligodendrocytes. Short hairpin-mediated knockdown of Rab27b not only reduced lysosomal exocytosis but also greatly diminished the surface expression of PLP in oligodendrocytes. In addition, knockdown of Rab27b reduced the myelin-like membranes induced by co-culture of oligodendrocytes and neurons. Our data suggest that Rab27b is involved in myelin biogenesis by regulating PLP transport from late endosomes/lysosomes to the cell membrane in oligodendrocytes.

Keywords Myelination · Neuron-glial interaction · Oligodendrocyte · Lysosome · Rab27b

Introduction

In the central nervous system (CNS), the myelin sheath is made of a multilayered specialized oligodendrocyte plasma membrane with a specific protein composition, so that it functions as an insulator to increase the velocity of axonal impulse conduction [1, 2]. However, how myelin protein trafficking is regulated and myelin is established are still not well understood. Although mounting evidence suggests that distinct endocytic sorting and recycling associated with plasma membrane remodeling regulate the assembly of myelin proteins [3], for example, neuronal signals can induce exocytosis of the proteolipid protein (PLP), the major myelin protein in the CNS, from late endosome/lysosome membrane stores to the plasma membrane [4], our understanding of the cellular mechanisms by which PLP is transported from late endosomes/lysosomes is still limited.

The lysosome was thought to be a simple terminal degradation compartment. Recently, due to the finding that lysosomes in many cell types serve dual functions—degradation of proteins and storage of synthesized secretory products [5–7]—the concept of secretory lysosomes was proposed [8]. Our previous results showed that the major myelin protein in the peripheral nervous system, P0, is stored in late endosomes/lysosomes in Schwann cells and that the small GTPase Rab27a is involved in lysosomal exocytosis, P0 trafficking, and remyelination after nerve injury [9]. Previous studies have shown that members of the Rab27 subfamily, including Rab27a and Rab27b, and their multiple effectors play important roles in the regulation of lysosome-related organelle exocytosis [10–12]. A comprehensive analysis of Rab27b expression indicated notable differences in the expression patterns of these two isoforms; Rab27b is enriched in the CNS and Rab27a is primarily expressed outside the CNS [10]. Given the important role of Rab27b protein functions in the regulation of lysosome-related organelle exocytosis in the CNS, we hypothesized that Rab27b would also regulate PLP trafficking in oligodendrocytes. In this study, we set out to test this hypothesis.

✉ Gang Chen
chengang6626@ntu.edu.cn

[1] Jiangsu Key Laboratory of Neuroregeneration, Co-innovation Center of Neuroregeneration, Nantong University, Nantong 226001, China

[2] Xinglin College, Nantong University, Nantong 226007, China

Materials and Methods

Materials

All chemicals were from Sigma (St Louis, MO) unless otherwise noted. The three pSuper-siRNA Rab27b sequences were as follows: 1 forward, GGUACAUCUGCAGCU UUGGUU; 1 reverse, CCAAAGCUGCAGAUGUACCUU; 2 forward CUGCAGGCAAAUGCUUACUUU; 2 reverse, AGUAAGCAUUUGCCUGCAGUU; 3 forward, GGAGCA GAUGGAUCUUCAGUU; 3 reverse, CUGAAGAUCCAU CUGCUCCUU.

GFP-tagged ShRNA Rab27b constructs were generated by standard polymerase chain reaction techniques to introduce the desired restriction sites in the flanking sequences of the GFP and ShRNA Rab27b constructs. The mouse GFP wild-type Rab27b plasmid was from Sino Biological Inc. (Catalog: MG52671-ACG; Beijing, China).

Cell Culture, Transfection, and RNA Interference

Primary cultures of oligodendrocytes were prepared from the cerebral cortices of 1- to 2-day-old Sprague–Dawley rats. After 8–10 days, oligodendrocytes were isolated from mixed cultures using mechanical dissociation. Purified cells then were plated in Neurobasal medium with B27 supplement on poly-D-lysine-coated dishes. To promote survival and increase the proportion of precursor cells, 10 ng/mL basic fibroblast growth factor (Sigma) and 10 ng/mL platelet-derived growth factor (R&D, Minneapolis, MN) were added immediately after shaking. Mature cells were cultured for 4 days with 20 ng/mL triiodothyronine (Sigma). Short-hairpin RNA (shRNA) transfection of primary oligodendrocytes (immediately after shaking) was performed using a kit according to the manufacturer's instructions (Amaxa Nucleofector kit, Lonza, Cologne, Germany; program O-017).

Oligodendrocytes and neurons were co-cultured as described previously [13]. Rat cortices on embryonic day 15 were dissociated in 0.125% trypsin and cultured in Dulbecco's modified Eagle's medium/10% fetal bovine serum supplemented with penicillin/streptomycin. After 24 h, the medium was changed to feeding medium containing Neurobasal medium, B27 supplement, L-glutamine (0.5 mmol/L), and penicillin/streptomycin. One-half of the medium was changed every 2 days. After 7 days, shRNA-transfected primary oligodendrocytes were added on top of the neurons and then the co-cultures were grown in Neurobasal medium with B27. Fractionation of myelin-like membranes was assessed after 5 days of neuron–oligodendrocyte co-culture.

Immunocytochemistry and Immunohistochemistry

Oligodendrocytes were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS), and permeabilized with 0.01% Triton X-100 in PBS for 12 min before treatment with 10% bovine serum albumin for 1 h. Cultures were incubated overnight at 4 °C with the following primary antibodies: rabbit anti-galactocerebroside (Sigma); mouse anti-cathepsin D (Abcam, Cambridge, MA); rabbit anti-Rab27b (Synaptic Systems, Gottingen, Germany); goat anti-PLP (Santa Cruz Biotechnology, Inc., Paso Robles, CA); mouse anti-PLP, clone O10 recognizing a cell surface epitope of PLP (R&D); mouse anti-MBP (Abcam); or rabbit anti-NF-L (Millipore). After washing to remove excess primary antibodies, the cultures were incubated for 1 h at room temperature with the fluorescence-conjugated secondary antibodies (Jackson Immuno Research, West Grove, PA). After immunostaining, the nuclei were stained with Hoechst 33342 (5 μg/mL; Sigma) for 10 min at room temperature The stained sections were examined with a confocal microscope (TCS SP5; Leica Microsystems, Wetzlar, Germany).

For immunohistochemistry, adult rats were deeply anaesthetized with isoflurane and perfused with PBS, followed by 4% paraformaldehyde. The cerebral cortices were removed and post-fixed in the same fixative overnight. Sections (20 μm, free-floating) were cut on a cryostat and processed for immunohistochemistry as previously described [14].

Fluorescence Imaging

Oligodendrocytes were incubated with 200 nmol/L Lyso-Tracker Red DND-99 (Invitrogen-Molecular Probes, Carlsbad, CA) in culture medium for 10 min at 37 °C. The cells were then washed for 20 min in extracellular solution before transfer to a chamber for imaging under a microscope with a 40 × objective.

Real-Time Quantitative PCR Assay

Total RNA was extracted using an RNeasy Mini Kit (Qiagen, Hilden, Germany) and the cDNA was synthesized using a cDNA Reverse Transcription Kit (Applied Biosystems, Foster, CA). Real-time quantitative PCR was performed using the 7300 Real-Time PCR System (Applied Biosystems). The primers used were Rab27b F: CAACTGCAGG CAAATGCTTA, R: GTGTCCGGAACCTGTGTCTT and GAPDH F: AAGTTCAACGGCACAGTCAAG, R: CCAG TAGACTCCACGACATACTCA. Levels of mRNAs were normalized by GAPDH as a reference. All reactions were repeated in triplicate.

Isolation of Lysosomes from Oligodendrocytes

Enriched lysosomal fractions from pure cultures of oligodendrocytes were isolated as described previously [9]. In brief, cultured cells from 8 dishes (growth area 55 cm^2; Corning, NY, USA) were suspended in 250 mmol/L sucrose/10 mmol/L Tris/HCl buffer, pH 7.4 and disrupted by sonication on ice with two 15 s pulses 10 s apart, at 20 kHz and 90 W (Sonics and Materials VCX 600 Watt, Danbury, CT). The disrupted cells were centrifuged at 1,300 g for 15 min to remove nuclei and cell debris, and the supernatant was submitted to differential centrifugation at 26,000 g for 25 min to obtain an enriched lysosomal fraction.

Western Blotting and Enzymatic Activity Determination

Enriched lysosomal fractions or cultured oligodendrocytes were homogenized in a lysis buffer containing protease and phosphatase inhibitors, and 30 μg of proteins was loaded onto each lane and separated on 12% SDS–PAGE gels. After transfer, the blots were incubated overnight at 4 °C with antibody against PLP (1:1000, mouse, R&D), Rab27b (1:1200, rabbit, Synaptic Systems), and cathepsin D (1:1000, mouse, Abcam). For loading control, the blots were probed with GAPDH antibody (1:20000, mouse; Sigma). These blots were further incubated with HRP-conjugated secondary antibody and developed in ECL solution (Pierce Biotechnology, Rockford, lL). Specific bands were evaluated by apparent molecular size. The intensity of selected bands was analyzed using ImageJ (NIH, Bethesda, MD). β-Hexosaminidase and lactate dehydrogenase assays were carried out as described previously [15].

Statistical Analysis

All data are expressed as mean ± SEM. Differences between two groups were compared using Student's *t* test. One-way ANOVA was used for other analyses. The criterion for statistical significance was $P < 0.05$.

Results

Co-Localization of Rab27b with PLP in Late Endosomes/Lysosomes of Oligodendrocytes

To determine the role of Rab27b in oligodendrocyte signaling, we prepared primary oligodendrocyte cultures from the cerebral cortexes of neonatal rats (P0-2). To mimic mature oligodendrocytes that predominate in the adult brain, we differentiated the oligodendrocytes with triiodothyronine. Most of the cultured cells ($Hoechst^+$) were galactocerebroside-positive ($Galc^+$, a marker for mature oligodendrocytes) with fully-extended processes (Fig. 1A). To determine whether late endosomes/lysosomes in mature oligodendrocytes store the myelin protein PLP as in previous studies [13], we immunostained mature oligodendrocytes with anti-PLP and anti-cathepsin D (a secretory lysosomal marker [16]) antibodies and found that they showed good co-localization (85.81 ± 2.69%; Fig. 1B, D).

Several recent reports have highlighted the role of the small GTPase Rab27b in the exocytic mechanism of lysosome-related organelles in platelets [17], amylase-secreting cells [18], mast cells [19], and urothelial umbrella cells [20]. To define the cellular distribution of endogenous Rab27b in mature oligodendrocytes, we performed triple staining, which showed that Rab27b was co-localized with cathepsin D (82.42 ± 1.76%) and PLP (88.42 ± 2.16%) (Fig. 1B). Collectively, these results suggested that Rab27b is co-localized with PLP in late endosomes/lysosomes of mature oligodendrocytes.

To exclude the possibility that the expression pattern of Rab27b in cultured oligodendrocytes is not exactly the same as in vivo, we also checked the co-localization of PLP, cathepsin D, and Rab27b in brain slices from adult rats. The co-localization ratio of PLP and Rab27b was 68.23 ± 2.31%, that of PLP and cathepsin D was 88.21 ± 3.11%, and that of Rab27b and cathepsin D was 71.28 ± 3.57% (Fig. 1C, E). Although the co-localization ratios were lower in the slices, perhaps due to the abundant myelin sheaths in brain, these results still supported our finding that Rab27b co-localized with PLP in late endosomes/lysosomes of oligodendrocytes.

Lysosomal Exocytosis Delivers PLP to the Plasma Membrane in Mature Oligodendrocytes

To investigate a potential mechanism for upregulation of PLP at the plasma membrane, we investigated the effects of the Ca^{2+} ionophore ionomycin on lysosomal exocytosis and the surface expression of PLP in mature oligodendrocytes. Similar to previous reports [9], ionomycin (10 μmol/L) induced the time-dependent release of β-hexosaminidase, which is an enzyme located specifically in lysosomes (Fig. 2A, left). The hexosaminidase release was not likely due to cell damage, because there was no significant release of lactate dehydrogenase, the usual indicator of cell lysis (Fig. 2A, right). Immunostaining of mature oligodendrocytes with anti-PLP that recognizes an extracellular epitope of PLP without permeabilization showed the surface expression of PLP, whereas second immunostaining of the cells after permeabilization showed the total expression, including the cytoplasmic and surface PLP. Significantly

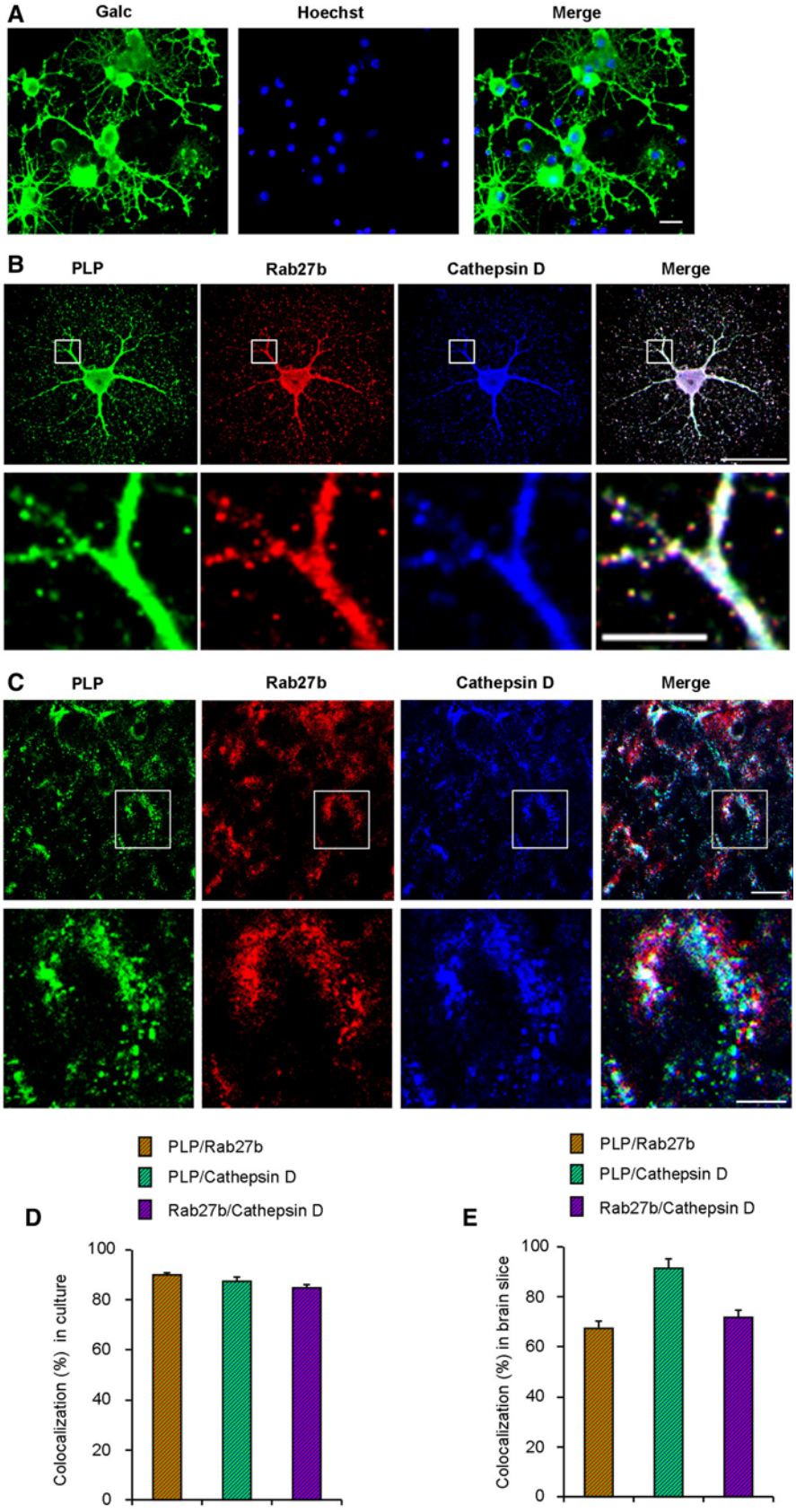

Fig. 1 Co-localization of Rab27b with PLP and Cathepsin D in Cultured Oligodendrocytes and in Brain Slices. **A** Double staining for galactocerebroside ($Galc^+$, mature oligodendrocyte marker, green) and Hoechst (nuclear marker, blue) shows that most $Hoechst^+$ cells also expressed Galc-IR. Scale bar, 25 μm. **B**, **C** Co-immunostaining for PLP, Rab27b, and cathepsin D reveals co-localization of Rab27b with PLP in late endosomes/lysosomes of cultured oligodendrocytes (**B**) and in rat brain slices (**C**). Confocal planes are shown; lower panels are enlarged images from upper panels. Scale bars, 25 μm (upper panels) and 5 μm (lower panels). **D**, **E** Summary of the co-localization of PLP with Rab27b or cathepsin D in cells ($n = 5$) (**D**) and slices ($n = 5$) (**E**). Error bars indicate SEM.

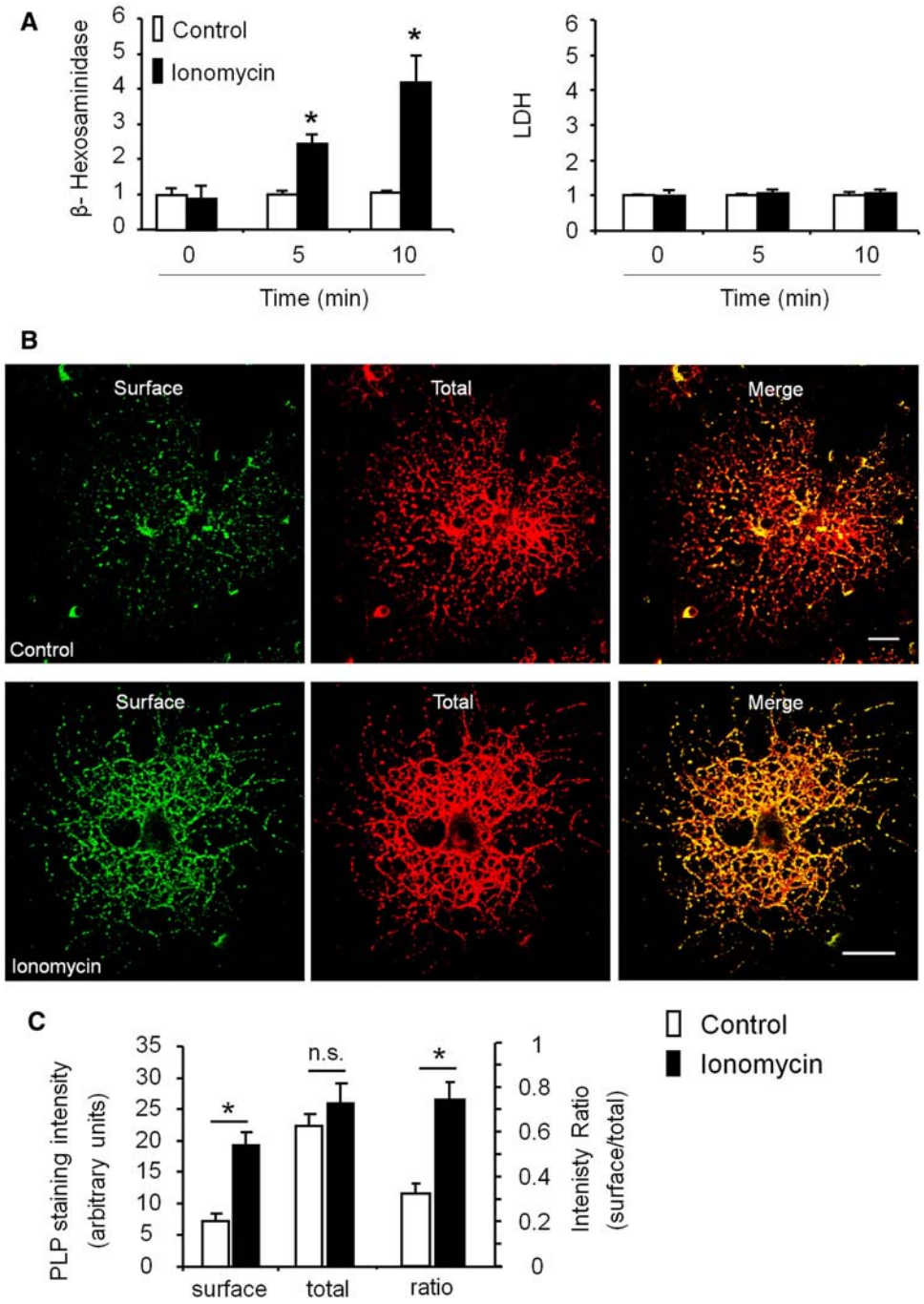

Fig. 2 Lysosomal exocytosis increases surface expression of PLP in mature oligodendrocytes. **A** Left panel: time course of the lysosomal enzyme β-hexosaminidase accumulation in the medium without (control, PBS) and with the Ca^{2+} ionophore ionomycin (10 μmol/L). Right panel: hexosaminidase release was not likely due to cell damage, because there was no significant release of lactate dehydrogenase (LDH), the usual indicator of cell lysis. $*P < 0.05$ compared with control. Data represent averages of at least 3 experiments for each group; error bars indicate SEM. **B** Immunostaining of oligodendrocytes with anti-PLP that recognizes an extracellular epitope of PLP without permeabilization shows the surface expression of PLP (surface, green), while the second immunostaining after permeabilization shows the total expression of PLP, including cytoplasmic and surface expression (total, red). The surface staining was significantly higher in the group treated with ionomycin (lower panels) than in controls (PBS, upper panels). Scale bars, 25 μm. **C** Quantification of surface and total PLP immunofluorescence intensity in control and ionomycin-treated groups. $*P < 0.05$, compared with control, Student's *t*-test, $n = 4$ cultures/group. All data are mean ± SEM.

increased surface staining was found in ionomycin-treated (10 μmol/L, 5 min) but not control cultures (Fig. 2B, C).

Downregulation of Rab27b in Mature Oligodendrocytes Reduces Lysosomal Exocytosis

To elucidate the function of Rab27b in oligodendrocytes, we first designed 3 different siRNA-Rab27b sequences and compared their gene-silencing efficacy using real-time quantitative PCR and downregulation of Rab27b protein expression by Western blot in Hepa (mouse hepatoma) cells. Hepa cells transfected with siRNA-Rab27b #1 or #2 dramatically reduced both the Rab27b gene and protein expression (Fig. 3A, B). Next, EGFP-tagged shRNA Rab27b constructs were generated using the sequence of siRNA-Rab27b #1 and mature oligodendrocytes were transfected with EGFP-Rab27b shRNA or non-target control EGFP-shRNA. Forty-eight hours after transfection, oligodendrocytes were immunostained with anti-Rab27b antibody to check the protein level of Rab27b.

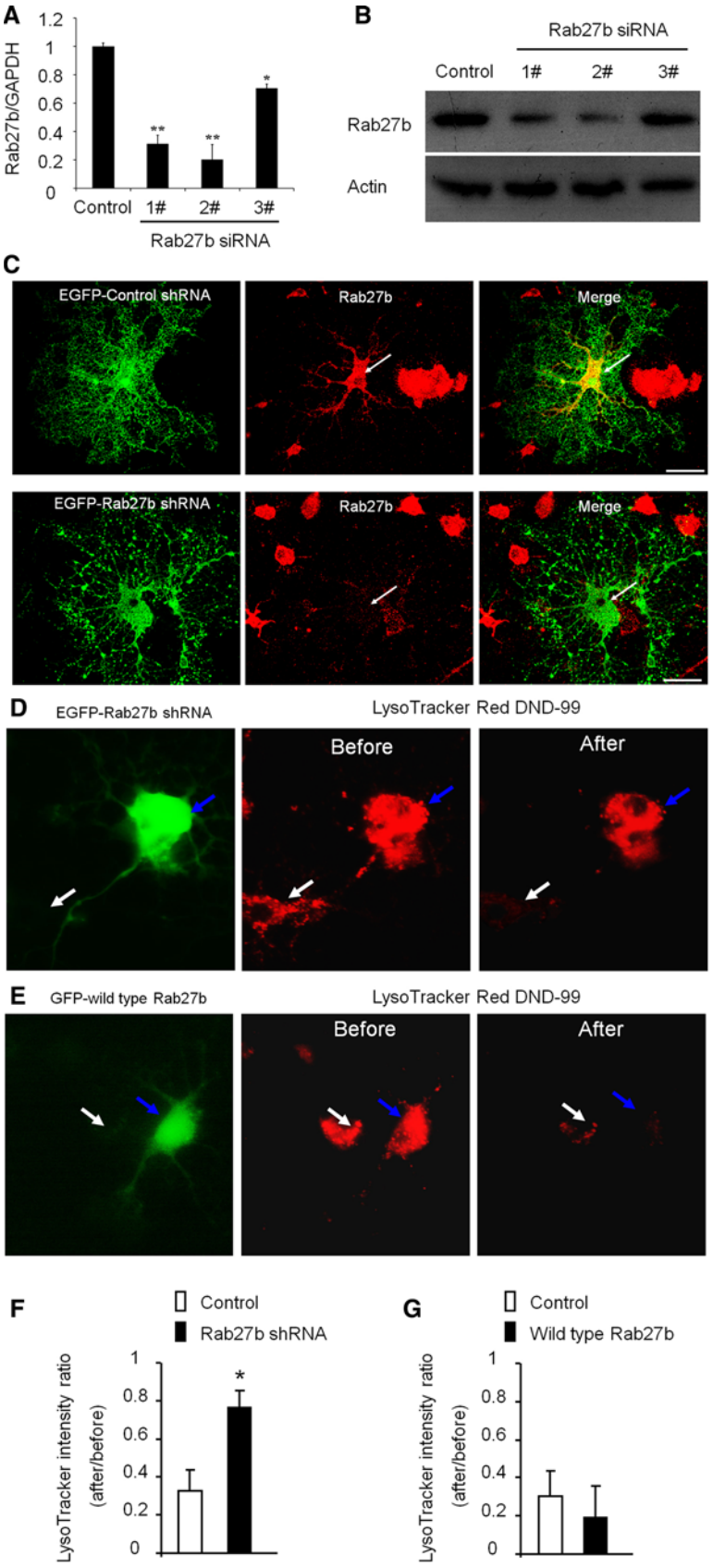

Fig. 3 Downregulation of Rab27b in mature oligodendrocytes reduces lysosomal exocytosis. **A**, **B** The #1 or #2 sequences of siRNA-Rab27b both dramatically reduced Rab27b gene and protein expression in Hepa cells. Real-time quantitative PCR (A, mean ± SEM; $n = 3$ cultures per group) and western blots (**B**) showing the Rab27b gene-silencing efficacy and Rab27b protein expression in transfected Hepa cells. **C** Oligodendrocytes transfected with EGFP-Rab27b shRNA plasmid had dramatically decreased Rab27b expression, whereas non-target control shRNA had no effect. Scale bars, 25 μm. **D**, **E** De-staining of the LysoTracker Red DND 99-labeled puncta induced by ionomycin (10 μmol/L, 10 min) was significantly lower in oligodendrocytes transfected with EGFP-Rab27b shRNA (**D**) [but not with GFP-wild-type Rab27b (**E**) (blue arrows)] than in non-transfected oligodendrocytes (white arrows). **F**, **G** Ratios of LysoTracker intensity before and after ionomycin treatment in non-transfected oligodendrocytes and in oligodendrocytes transfected with EGFP-Rab27b shRNA (**F**) or GFP-wild-type Rab27b (**G**). $^*P < 0.05$ compared with control, Student's *t*-test, $n = 4$ cultures/group. All data are mean ± SEM.

Oligodendrocytes transfected with EGFP-Rab27b shRNA plasmids demonstrated a marked decrease in the expression level of Rab27b, while control shRNA had no effect (Fig. 3C).

To investigate the role of Rab27b in lysosomal exocytosis, both loss-of-function (EGFP-Rab27b shRNA transfection) and gain-of-function (GFP-wild-type Rab27b transfection) strategies were used. Transfected oligodendrocytes were incubated with LysoTracker DND99 for 10 min to label the lysosomes [9], then the de-staining of the Labeled lysosomes induced by ionomycin (10 μmol/L) was observed. The intensity of LysoTracker in EGFP-Rab27b shRNA-transfected oligodendrocytes was largely maintained compared with non-transfected cells (Fig. 3D, F). However, overexpression of Rab27b in oligodendrocytes did not increase lysosomal exocytosis (Fig. 3E, G). Together, these results suggest that Rab27b is necessary but not sufficient to regulate lysosomal exocytosis in oligodendrocytes.

Downregulation of Rab27b Reduces Surface Transport and Synthesis of PLP in Mature Oligodendrocytes

Next, we analyzed the functional role of Rab27b in the surface transport of PLP in mature oligodendrocytes in oligodendrocytes transfected with non-target control shRNA, EGFP-Rab27b shRNA, or GFP-wild type Rab27b. Indeed, compared with the control shRNA group, the surface expression of PLP was drastically reduced in EGFP-Rab27b shRNA-transfected cells, but there was no difference in GFP-wild-type Rab27b transfected cells (Fig. 4A, B). The total expression of PLP was similar in all three transfected groups (Fig. 4A, B).

To exclude the possibility that the decrease in surface expression of PLP might be due to impaired transport or membrane insertion of PLP, we checked the PLP protein level in lysosomes from oligodendrocytes with siRNA Rab27b or non-target siRNA treatment. We used a well-characterized method [9] for the separate enrichment of lysosomes and did western blot to analyze the expression of PLP in lysosomes. Western blotting showed PLP upregulation and Rab27b downregulation in lysosomes after siRNA-Rab27b treatment (Fig. 4C). The expression of cathepsin D was used as a lysosome loading control. The results indicated that knockdown of Rab27b reduces PLP release from lysosomes.

We also analyzed the expression of myelin basic protein (MBP) as a control, and found that it was unaffected (Fig. 4D), consistent with reports that lysosomal exocytosis is not involved in MBP transport in oligodendrocytes [13, 21].

Downregulation of Rab27b Impairs Myelin-Like Membrane (MLM) Formation in Oligodendrocyte–Neuron Co-cultures

To assess the impact of Rab27b-dependent PLP trafficking on myelin formation in vitro, we used the model of oligodendrocytes co-cultured with cortical neurons as described previously [13]. In this model, a few days after seeding purified oligodendrocytes on top of cortical neurons, oligodendrocytes have enwrapped the axons, forming many MLMs, which share the biophysical and biochemical properties of myelin in vivo [22]. Here, primary oligodendrocytes were transfected with EGFP-control shRNA, EGFP-Rab27b shRNA, or GFP-wild-type Rab27b before seeding onto cortical neurons. After 5 days of co-culture, we analyzed the myelin formation by double staining with anti-NF to mark axons and anti-MBP to mark MLMs. Compared with the control shRNA-treated group, when Rab27b was silenced, although oligodendrocytes were still able to establish contact with axons, the number of puncta of co-localization of MBP and NF in transfected cells (GFP^+) was drastically reduced, indicating that downregulation of Rab27b impairs MLM formation. However, compared with the control shRNA-treated group, overexpression of Rab27b had no effect on MLM formation (Fig. 5).

Discussion

The results of our study demonstrate that exocytosis of late endosomes/lysosomes in oligodendrocytes controlled by Rab27b contributes to myelin formation by delivering PLP to the myelin membrane. We have made the following findings. First, Rab27b is co-localized with PLP in late endosomes/lysosomes of mature oligodendrocytes. Second, lysosomal exocytosis delivers PLP to the plasma membrane in mature oligodendrocytes. Third, downregulation of Rab27b not only reduces lysosomal exocytosis but also inhibits PLP surface transport in mature oligodendrocytes. Finally, in vitro analysis using oligodendrocyte–neuron co-cultures provided evidence that downregulation of Rab27b affects the formation of MLMs.

Myelin plays an important role in efficient and complex brain processes. Interesting, abnormalities of myelin are common in lysosomal storage diseases [22–24] including multiple sulfatase deficiency, globoid cell leukodystrophy, and metachromatic leukodystrophy [25]. Recently, the role and mechanism of action of the endosomal/lysosomal system in myelin formation have been emphasized [5, 13]. For example, the degradation and ultimate recycling of molecules can regulate signal transduction pathways through receptor–ligand interactions and the generation of

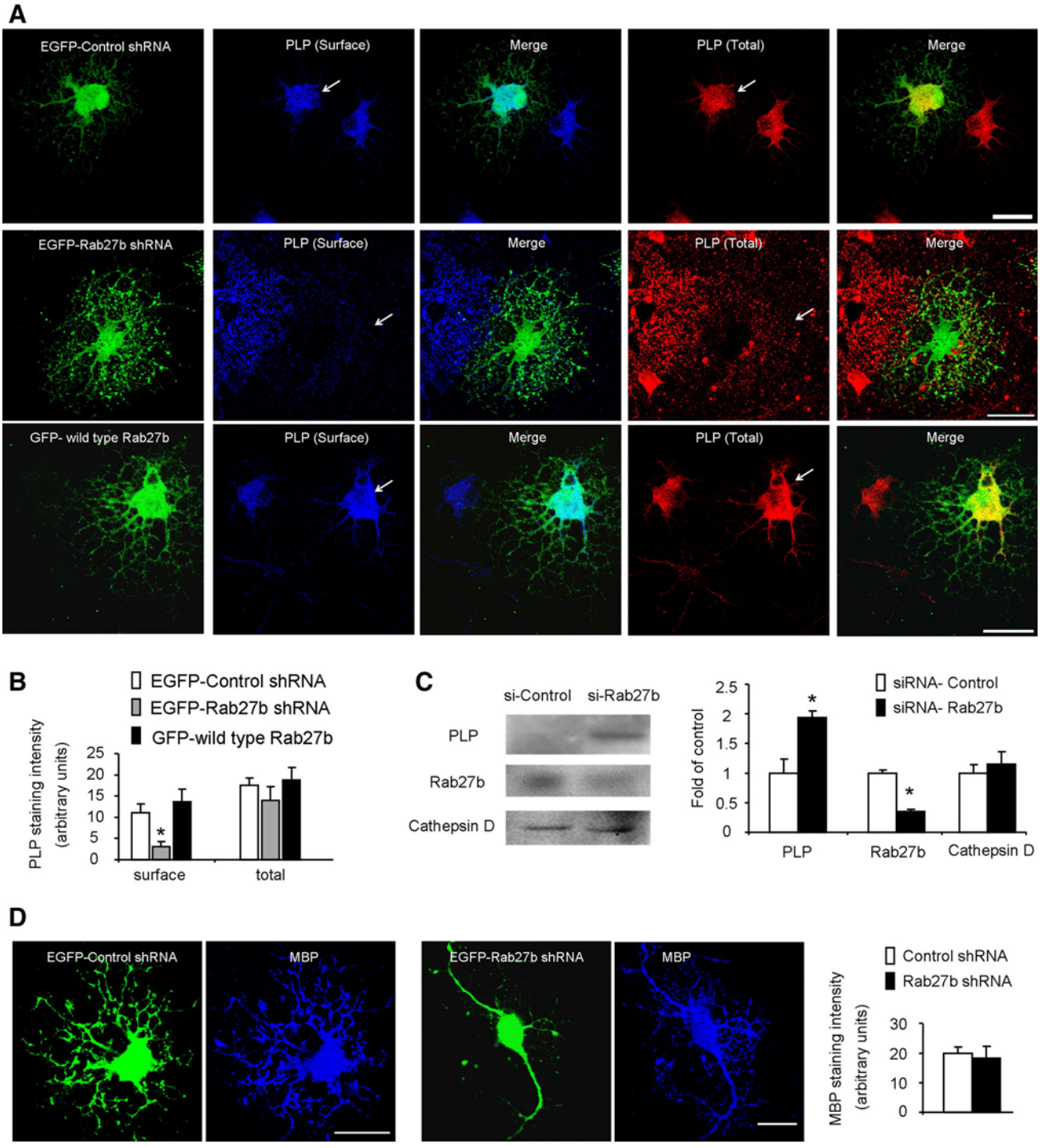

Fig. 4 Downregulation of Rab27b reduces PLP surface expression in mature oligodendrocytes. **A** Mature oligodendrocytes transfected with EGFP-Rab27b shRNA plasmid (middle panels) had significantly lower surface expression of PLP than those transfected with control shRNA (upper panels). Note that the total expression of Rab27b was also slightly reduced in cells transfected with Rab27b shRNA. Overexpression Rab27b by transfection with GFP-wild-type Rab27b had slightly higher surface expression of PLP (lower panels). Arrows indicate transfected oligodendrocytes. Scale bars, 25 μm. **B** Quantification of surface and total PLP immunofluorescence intensity in transfected oligodendrocytes (*$P < 0.05$ compared with control shRNA group, ANOVA followed by Newman–Keuls test; $n = 4$ cultures/group; mean ± SEM. **C** Western blotting showed PLP upregulation in lysosomes after siRNA-Rab27b treatment. Left panel, example of western blots with anti-PLP, anti-Rab27b, and anti-cathepsin D from enriched lysosomal fractions treated with siRNA-control or siRNA-Rab27b. Right panel, quantification of PLP, Rab27b, and cathepsin D levels in siRNA-transfected oligodendrocytes (fold of control; *$P < 0.05$ compared to control, Student's t-test, $n = 4$ mice/group, mean ± SEM). **D** The expression level of myelin basic protein (MBP) was unaffected in mature oligodendrocytes transfected with EGFP-Rab27b shRNA (scale bars, 25 μm; $n = 4$ cultures/group, mean ± SEM).

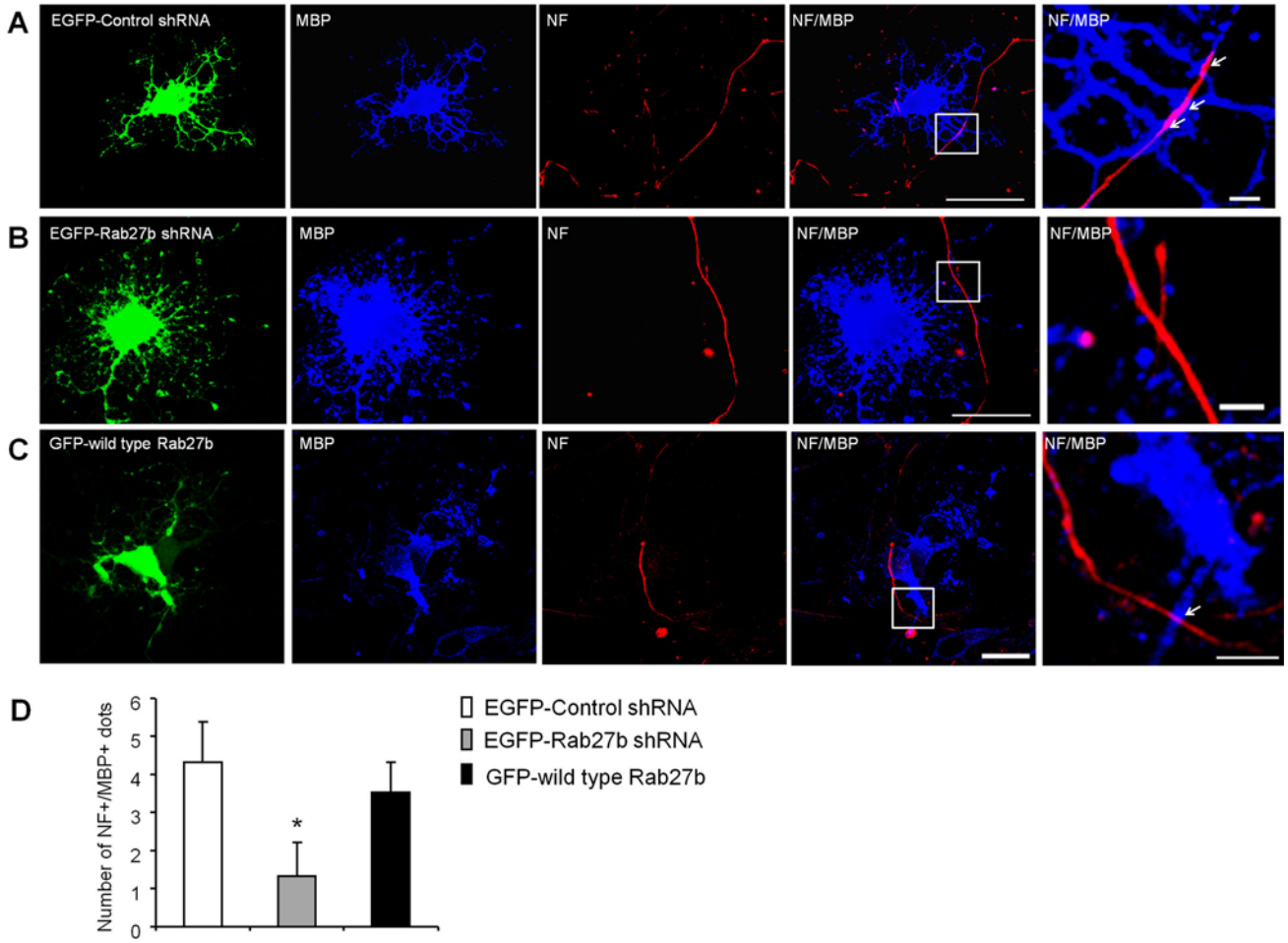

Fig. 5 Downregulation of Rab27b impairs myelin-like membrane formation in oligodendrocyte–neuron co-cultures. **A**–**C** Double immunostaining of oligodendrocyte–neuron co-cultures using antibodies recognizing NF in neurons (red) and MBP in mature oligodendrocytes (blue). On silencing of Rab27b (**B**), although oligodendrocytes still established axonal contacts, formation of myelin-like membranes (shown as co-localization of NF and MBP staining) was drastically reduced compared with the control shRNA-treated group (**A**). Over-expression of Rab27b by transfecting GFP-wild-type Rab27b (**C**) resulted in myelin formation similar to control shRNA-treated cells (**A**). Right panels are enlarged images from the left panels. Scale bars, 25 μm (left panels) and 5 μm (right panels). **D** Average numbers of co-localization puncta of MBP and NF in oligodendrocytes transfected with control shRNA, Rab27b shRNA, and wild-type Rab27b ($*P < 0.05$ compared with control shRNA group, ANOVA followed by Newman–Keuls test; $n = 4$ cultures/group, mean ± SEM).

signaling molecules [5, 14, 26]. Furthermore, in response to extracellular stimuli, secretory lysosomes fuse with the plasma membrane and then release their contents [8]. In this study, we found that PLP was stored in late endosomes/lysosomes of mature oligodendrocytes. In addition, the Ca^{2+} ionophore ionomycin induced lysosomal exocytosis and increased PLP surface expression in oligodendrocytes. Our results suggest that lysosomal exocytosis in oligodendrocytes regulates the surface expression of myelin protein in oligodendrocytes.

The physiologically important role of Rab27a protein is clear, because mutation of its gene causes defects in cytotoxic T-lymphocyte exocytosis in ashen mice [27] and human Griscelli syndrome [28]. Rab27a and Rab27b may play similar roles because they have highly-conserved primary sequences. In addition, Rab27b may be functionally redundant to Rab27a, as it can rescue Rab27a mutants. In this study, we found that downregulation of Rab27b not only reduced lysosomal exocytosis but also inhibited PLP surface transport in mature oligodendrocytes and reduced myelin formation in an oligodendrocyte–neuron co-culture system. However, the mechanisms by which Rab27b regulates PLP trafficking in oligodendrocytes remain unclear. Future studies are needed to establish how Rab27b regulates lysosomal exocytosis in oligodendrocytes and which Rab27a/b effectors are involved in this process.

Above all, to our knowledge, this is the first demonstration that Rab27b regulates myelin protein trafficking in oligodendrocytes through lysosomal exocytosis and contributes to myelin formation in vitro. These findings reveal

an interesting link between myelin biogenesis and lysosomal exocytosis. Now the challenge is to build an overall view of how myelination is regulated by integrating the signaling pathways and trafficking pathways.

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