The small GTPase Rab27A is a crucial regulator of actin-based melanosome transport in melanocytes, and functionally defective Rab27A causes human Griscelli syndrome type 2, which is characterized by silvery hair. A GTPase-deficient, constitutively active Rab27A(Q78L) mutant has been shown to act as an inhibitor of melanosome transport and to induce perinuclear aggregation of melanosomes, but the molecular mechanism by which Rab27A(Q78L) inhibits melanosome transport remained to be determined. In this study, we attempted to identify the primary cause of the perinuclear melanosome aggregation induced by Rab27A(Q78L). The results showed that Rab27A(Q78L) is unable to localize on mature melanosomes and that its inhibitory activity on melanosome transport is completely dependent on its binding to the Rab27A effector Slac2-a/melanophilin. When we forcibly expressed Rab27A(Q78L) on mature melanosomes by using a novel melanosome-targeting tag that we developed in this study and named the MST tag, the MST-Rab27A(Q78L) fusion protein behaved in the same manner as wild-type Rab27A. It localized on mature melanosomes without inducing melanosome aggregation and restored normal peripheral melanosome distribution in Rab27A-deficient cells. These findings indicate that the GTPase activity of Rab27A is required for its melanosome localization but is not required for melanosome transport.

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3 The abbreviations used are: CA, constitutively active; CN, constitutively negative; MST, melanosome-targeting; Mreg, melanoregulin; EGFP, enhanced GFP; SHD, Slp homology domain; SR, siRNA-resistant; mStr, monomeric Strawberry.
Mechanism of Melanosomal Transport Inhibition by Rab27A(Q78L)

transport in melanocytes and secretory granule exocytosis in
secretory cells (reviewed in Ref. 8 and the references therein),
and it is well known that overexpression of the CA form of
Rab27A(Q78L) in melanocytes has been shown to cause inhibi-
tion of actin-based melanosomal transport (i.e. to cause induc-
tion of perinuclear melanosomal aggregation as a result)
rather than to promote melanosome transport (9). One hypoth-
esis to explain this inhibitory effect of Rab27A(Q78L) is that the
GT-Pase activity (or proper GDP-GTP cycling) of Rab27A is
essential to melanosomal transport in melanocytes. However,
this hypothesis has never been tested experimentally, and the
exact molecular mechanism by which Rab27A(Q78L) inhibits
Rab27A-dependent membrane traffic has remained unknown.

In this study, we analyzed the impact of Rab27A(Q78L)
expression on melanosomal transport in melanocytes, and the
results showed that Rab27A(Q78L) is not targeted to mature
melanosomes and that its inhibitory effect depends on binding
activity toward Slac2-a (also called melanophilin), a specific
Rab27A effector molecule (10–14). Our development of a novel
melanosome-targeting tag that we named the MST tag enabled
expression of Rab27A on melanosome transport in melanocytes, and the
basis of our findings, we discuss the possible molecular mech-
anism of the Rab27A(Q78L)-induced inhibition of melano-
some transport.

EXPERIMENTAL PROCEDURES

Materials—The following antibodies used in this study were
obtained commercially: anti-GFP rabbit polyclonal antibody
(MBL, Nagoya, Japan), anti-FLAG tag rabbit polyclonal anti-
body (Sigma-Aldrich), HRP-conjugated anti-T7 tag mouse
monoclonal antibody and anti-T7 tag antibody-conjugated
agarose beads (Merck Biosciences Novagen, Darmstadt, Ger-
many), and Alexa Fluor 488-conjugated anti-rabbit IgG goat
antibody (Invitrogen). Anti-Slac2-a rabbit polyclonal antibody
was prepared as described previously (15).

Plasmid Construction—pEGFP-C1-Rab27A and its mutant
eexpression plasmids (Y6F, Q78L, and C219A/C221A) used in
this study were prepared essentially as described previously
(25, 26). Plasmids were transfected into
black mouse-derived immortal melanocyte cell
line melan-a (a gift from Dorothy C. Bennett) was cultured as
described previously (25, 26). Plasmids were transfected into
melan-a cells by using Lipofectamine 2000 (Invitrogen) accord-
ing to the instructions of the manufacturer. Three days after
transfection, cells were fixed with 10% (w/v) TCA, permeab-
ilized with 0.3% Triton X-100, stained with anti-FLAG tag anti-
body (1/750 dilution) in 1% BSA/PBS or with anti-Slac2-a anti-
body (1/750 dilution, 5’-AAGAGATGTTGTCGACTCAGGAG
GAGG-3’) as a template and subcloned into the pBl-NotI site of the above pmStr-N1-MST-Gly-linker vector to express MST-That-aASSHD-FLAG (the mStr marker was replaced
by a FLAG tag). The Slac2-aASSHD-FLAG fragment was also
subcloned into the pEF-MyC expression vector (22). CDNA of enha-
ced GFP (EGFP) was amplified by PCR with pEGFP-C-1 as
a template and subcloned into the pEF-FLAG expression vector (23)
by conventional molecular biology techniques.

Rab27A siRNA was prepared as described previously (24)
(19-base target site, 5’-AAGAGATGTTGTCGACTCAGGAG
GAGG-3’). An siRNA-resistant (SR) form of Rab27A (wild-type and mut-
ants) was constructed by conventional PCR techniques using
the following oligonucleotides (substituted nucleotides are
shown in italics): 5’-AGGGAAAACCGGTATGATATAGG
GCCAAT-3’ (Rab27A-SR sense primer) and 5’-ATTGGCCCC
TATATACACTCCGTCTTTTCCCT-3’ (Rab27A-SR antisense primer).

Immunofluorescence Analysis and Melanosomal Distribution Assay—The black mouse-derived immortal melanocyte cell
line melan-a (a gift from Dorothy C. Bennett) was cultured as
described previously (25, 26). Plasmids were transfected into
melan-a cells by using Lipofectamine 2000 (Invitrogen) accord-
ing to the instructions of the manufacturer. Three days after
transfection, cells were fixed with 10% (w/v) TCA, permeab-
ilized with 0.3% Triton X-100, stained with anti-FLAG tag anti-
body (1/750 dilution) in 1% BSA/PBS or with anti-Slac2-a anti-
body (0.04 μg/μl) in Can Get Signal immunostain solution B
(Toyobo, Osaka, Japan), and then visualized with Alexa Fluor
488-conjugated anti-rabbit IgG secondary antibody. Cells were
examined for immunostaining signals with a confocal laser-
scanning microscope (Fluoview, Olympus, Tokyo, Japan) as
described previously (26). The images were processed with
Adobe Photoshop software (CS5). Melanosomal distribution
assays (normal peripheral distribution versus perinuclear
aggregation) were performed as described previously (n > 50
from three independent dishes) (26), and the data are expressed
as means ± S.E. Statistical analyses were performed by
unpaired Student’s t test. p < 0.05 was considered statistically
significant.
**Mechanism of Melanosome Transport Inhibition by Rab27A(Q78L)**

**RESULTS**

**Rab27A(Q78L) Is Not Targeted to Mature Melanosomes in Melanocytes**—When EGFP-tagged Rab27A(Q78L) was expressed in melan-a cells, it strongly induced perinuclear melanosome aggregation (Fig. 1B, third row, and D), consistent with a previous report (9). However, in contrast to the melanosome localization of wild-type Rab27A (Fig. 1B, second row), Rab27A(Q78L) did not localize on mature melanosomes and appeared to be dispersed throughout the cytosol, the same as EGFP alone (Fig. 1B, first and third rows). A similar cytosolic localization pattern of Rab27A(C219A/C221A) (Fig. 1B, fifth row, and D), despite not containing a Q78L mutation.

**Slac2-a Binding Ability of Rab27A(Q78L) Is Required for Induction of Perinuclear Melanosome Aggregation**—The inhibitory effect of Rab27A(C219A/C221A) on melanosome transport led us to hypothesize that cytosolic localization of

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**FIGURE 1. Effect of expression of Rab27A mutants on melanosome distribution in melanocytes.** A, schematic of the Rab27A mutants used in this study. The melanosome localization activity, Slac2-a binding activity, and perinuclear aggregation activity of each mutant are shown in the right panel. The SF1 region is one of the conserved regions of a certain Rab subfamily (28), and the switch-2 region is thought to be sensitive to the nucleotide-binding state of Rab proteins. B, typical images of melanocytes expressing EGFP-tagged Rab27A mutants and EGFP alone (EGFP fluorescence images and their corresponding bright-field images). Cells exhibiting perinuclear melanosome aggregation are outlined with a dashed line. The melanosomes in the merged images (right column) are pseudocolored in red. The insets show magnified views of the boxed areas. Note that wild-type Rab27A alone, and not any of the Rab27A mutants, is present on mature melanosomes (second row, yellow signals in the inset). Scale bars = 20 μm. C, Slac2-a binding activity of the Rab27A mutants. Testing for associations between T7-tagged Slac2-a and EGFP-tagged Rab27A (wild-type and mutants) was performed by coimmunoprecipitation assays with anti-T7 tag antibody-conjugated agarose beads as described previously (23, 27). Coimmunoprecipitated EGFP-Rab27A (center panel) and immunoprecipitated T7-Slac2-a (IP) (bottom panel) were detected with the antibodies indicated. Input means 1/80 volume of the reaction mixture used for immunoprecipitation (top panel). The positions of the molecular mass markers (in kilodaltons) are shown at the left. IB, immunoblot. D, the number of melanocytes showing perinuclear melanosome aggregation is expressed as a percentage of the number of transfected melanocytes shown in B. **, p < 0.01, unpaired Student’s t test.
Rab27A(Q78L) is the primary cause of the inhibition of melanosome transport and that it traps the Rab27A effector Slac2-a, which functions as a linker protein between Rab27A on melanosomes and an actin-based motor myosin-Va (12–14), in the cytosol. Because the Y6F mutation in the SF1 region of Rab27A (17, 28) has been shown to specifically impair Slac2-a binding ability but to have no effect on binding to another Rab27 effector, Slp2-a, in melanocytes (17, 29, 30), we tested our hypothesis by introducing an additional mutation (Y6F) into Rab27A(Q78L). It should be noted that the resulting Rab27A(Y6F/Q78L) mutant exhibited cytosolic localization, the same as Rab27A(Q78L) did (Fig. 1B, fourth row), and that it did not induce perinuclear melanosome aggregation (Fig. 1D). Thus, there were good correlations between the Slac2-a binding activity of Rab27A mutants and their melanosome aggregation activity. Both Rab27A(Y6F/Q78L) and Rab27(C219A/C221A), but not Rab27A(Y6F/Q78L), were found to interact with Slac2-a in coimmunoprecipitation assays (Fig. 1C) and to induce perinuclear melanosome aggregation.

Melanoregulin Was Used to Develop a Novel Melanosome-targeting Tag—If the cytosolic localization of Rab27A(Q78L) is the primary cause of perinuclear melanosome aggregation, then forced targeting of Rab27A(Q78L) to mature melanosomes should restore peripheral melanosome distribution. Several signals that target certain organelles, including the nucleus, mitochondria, and peroxisomes, e.g. a nuclear localization signal of certain transcription factors (31), a mitochondrial targeting signal of mitochondrial outer membrane proteins (32), and a peroxisomal targeting signal 1 (SKL) of catalase (33), have been reported, and because their fusion with a target protein enables it to be targeted to a specific organelle, they are often used as specific organelle-targeting tags. Although the cytoplasmic region of several melanosomal membrane proteins, e.g. tyrosinase, Tyrp1 (tyrosinase-related protein 1), and OA1 (oculocutaneous albinism type 1), contains a dileucine-based motif that is necessary for melanosome targeting (34–37), no melanosome-targeting tag whose fusion with a target protein enables its melanosomal localization has ever been reported. To develop the novel mature melanosome-targeting tag that we named the MST tag, we searched the literature for proteins that are specifically localized on mature melanosomes. Among those that we found in our search, we focused our attention on Mreg, a dilute suppressor gene product (38, 39), because EGFP-tagged Mreg has been shown to target mature melanosomes via its N-terminal palmitoylation (19, 40). Because expression of the

FIGURE 2. The N-terminal portion of Mreg is necessary and sufficient for melanosome targeting in melanocytes. A, schematic of the Mreg mutants and MST tag used in this study. The N-terminal portion of Mreg is required for its melanosome localization (19, 40), whereas its C-terminal portion binds Rab-interacting lysosomal protein, which forms a retrograde melanosome transport complex (19). MST-Rab27A(Q78L)-mStr was produced by insertion of Gly-linker + Rab27A(Q78L/C219A/C221A) into the site between MST and mStr. BamHI, BgIII, and SalI are restriction enzyme sites that were used for the construction of MST-Rab27A(Q78L)+mStr (see “Experimental Procedures” for details). RILP, Rab-interacting lysosomal protein. B, typical images of melanocytes expressing mStr-tagged Mreg mutants and mStr alone (mStr fluorescence images and their corresponding bright-field images). Cells exhibiting perinuclear melanosome aggregation are outlined with a dashed line. The melanosomes in the merged images (right column) are pseudocolored green. The insets show magnified views of the boxed areas. Note that the full-length Mreg with mStr-tag was clearly targeted to mature melanosomes and that Mreg-mStr-expressing cells often exhibited perinuclear melanosome aggregation (center row). MregΔC/MST-mStr, on the other hand, was able to target mature melanosomes without altering peripheral melanosome distribution (bottom row). Scale bars = 20 μm. C, the number of melanocytes exhibiting perinuclear melanosome aggregation expressed as a percentage of the number of transfected melanocytes shown in B. **, p < 0.01; unpaired Student’s t test.
full-length Mreg in melanocytes induced perinuclear melanosome aggregation by activation of retrograde melanosome transport through its interaction with Rab-interacting lysosomal protein (RILP)–p150Glued (a dynactin subunit) via its C-terminal domain (19) (Fig. 2B, center row, and C), we decided to use the N-terminal domain of Mreg as the MST tag (Fig. 2A).

To visualize the MST tag, we fused monomeric Strawberry (mStr) to the C terminus of MST rather than to its N terminus because the palmitoylation site was located near the N terminus of Mreg (40). As anticipated, in contrast to the cytosolic localization of mStr alone (Fig. 2B, top row), C-terminally mStr-tagged MST (MST-mStr) clearly targeted mature melanosomes without altering their normal peripheral distribution (Fig. 2B, bottom row, and C).

Next, we attempted to evaluate whether additional fusion to MST-mStr affects its melanosome-targeting activity. To do so, we turned our attention to Rab27A, an early endosome-resident Rab that is not normally associated with mature melanosomes (Fig. 3, center row; the red mStr-Rab27A signals and green melanosome signals were completely separate). When Rab27A was fused to MST-mStr (named MST-Rab27A-mStr) and expressed in melanocytes, it was clearly localized on mature melanosomes (Fig. 3, bottom row). These results indicate that the MST tag is likely to function as a melanosome-targeting tag.

**Forcible Expression of Rab27A(Q78L) on Mature Melanosomes by the MST Tag—**To forcibly target Rab27A(Q78L) to mature melanosomes, Rab27A(Q78L/C219A/C221A), which lacks C-terminal geranylgeranylation sites, was introduced into the above MST-mStr cassette (named MST-Rab27A(Q78L)-mStr, Fig. 2A). In contrast to the cytosolic localization of Rab27A(Q78L) (Fig. 4A, center row), MST-Rab27A(Q78L)-mStr was clearly targeted to mature melanosomes (Fig. 4A, bottom row). Most importantly, MST-Rab27A(Q78L)-mStr did not induce any perinuclear melanosome aggregation at all (Fig. 4B), indicating that the GTPase activity of Rab27A is not essential for peripheral melanosome distribution.

**MST-Rab27A(Q78L)-mStr Supports Normal Peripheral Melanosome Distribution in Rab27A-deficient Melanocytes—**Because MST-Rab27A(Q78L)-mStr was able to target mature melanosomes in Rab27A-deficient melanocytes, this approach might be used to rescue the function of Rab27A in these cells.

**Mechanism of Melanosome Transport Inhibition by Rab27A(Q78L)***

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**FIGURE 3.** Forcible targeting of Rab5A, an early endosome-resident Rab, to mature melanosomes in melanocytes by the MST tag. Typical images of melanocytes expressing mStr-tagged Rab5A and MST-Rab5A (mStr fluorescence images and their corresponding bright-field images). The melanosomes in the merged images (right column) are pseudocolored green. The insets show magnified views of the boxed areas. Note that MST-Rab5A was clearly targeted to mature melanosomes without altering peripheral melanosome distribution (bottom row). Scale bars = 20 μm.

**FIGURE 4.** Forcible targeting of Rab27A(Q78L) to mature melanosomes in melanocytes by the MST tag. A, typical images of melanocytes expressing mStr-tagged Rab27A(Q78L) and MST-Rab27A(Q78L) (mStr fluorescence images and their corresponding bright-field images). Cells exhibiting perinuclear melanosome aggregation are outlined with a dashed line. The melanosomes in the merged images (right column) are pseudocolored green. The insets show magnified views of the boxed areas. In contrast to Rab27A(Q78L), MST-Rab27A(Q78L) was clearly targeted to mature melanosomes without inducing perinuclear melanosome aggregation (bottom row). Scale bars = 20 μm. B, the number of melanocytes exhibiting perinuclear melanosome aggregation expressed as a percentage of the number of transfected melanocytes shown in A. **, p < 0.01, unpaired Student’s t test.
melanosomes (Fig. 4A, bottom row, insets), we next investigated whether MST-Rab27A(Q78L)-mStr was able to functionally replace endogenous Rab27A in actin-based melanosome transport. To do so, we coexpressed MST-Rab27A(Q78L)-mStr with an siRNA against Rab27A in melanocytes (24) and evaluated their effect on melanosome distribution. When Rab27A was depleted by the specific Rab27A siRNA, actin-based melanosome transport was impaired (41–43), and melanosomes that had not been transferred to actin filaments were returned to the center of the cell because of increased retrograde melanosome transport activity on microtubules, thereby resulting in melanosome aggregation around the nucleus (19, 44) (Fig. 5A and B, first row). This aggregation phenotype was clearly rescued by coexpression of an SR form of wild-type Rab27A (mStr-Rab27A\textsuperscript{SR}) with the Rab27A siRNA because the mStr-Rab27A\textsuperscript{SR} in the Rab27A-deficient cells localized to the melanosomes, and they exhibited normal peripheral melanosome distribution (Fig. 5B, second row, and C). By contrast, mStr-Rab27A(Q78L)\textsuperscript{SR} was mainly present in the cytosol, and its expression did not restore peripheral melanosome distribution in Rab27A-deficient cells (Fig. 5B, third row, and C). However, although MST-mStr alone did not restore peripheral melanosome distribution, when the MST tag was added to the N terminus of Rab27A(Q78L)\textsuperscript{SR}-mStr, MST-Rab27A(Q78L)\textsuperscript{SR}-mStr was clearly targeted to mature melanosomes and completely rescued Rab27A deficiency in relation to melanosome distribution (Fig. 5B, fourth and fifth rows, and C). Consistent with the normal peripheral melanosome distribution of MST-Rab27A-

**FIGURE 5.** MST-Rab27A(Q78L)-mStr can compensate for the function of endogenous Rab27A in melanosome transport in melanocytes. A and B, typical images of melanocytes expressing mStr together with control siRNA (A) and mStr, mStr-Rab27A\textsuperscript{SR}, mStr-Rab27A(Q78L)\textsuperscript{SR}, MST-mStr, or MST-Rab27A(Q78L)\textsuperscript{SR}-mStr together with Rab27A siRNA (B) (mStr fluorescence images and their corresponding bright-field images). Cells exhibiting perinuclear melanosome aggregation are outlined with a dashed line. The melanosomes in the merged images (right column) are pseudocolored green. The insets show magnified views of the boxed areas. Note that MST-Rab27A(Q78L)\textsuperscript{SR}-mStr was able to restore peripheral melanosome distribution in Rab27A-deficient cells (B, fifth row), the same as wild-type Rab27A\textsuperscript{SR} (B, second row). Scale bars = 20 μm. C, the number of melanocytes exhibiting perinuclear melanosome aggregation expressed as a percentage of the number of transfected melanocytes shown in B. **, p < 0.01, unpaired Student’s t test. NS, not significant. D, subcellular localization of endogenous Slac2-a molecules in melanocytes expressing mStr, mStr-Rab27A\textsuperscript{SR}, mStr-Rab27A(Q78L)\textsuperscript{SR}, or MST-Rab27A(Q78L)\textsuperscript{SR}-mStr together with Rab27A siRNA. Cells exhibiting perinuclear melanosome aggregation are outlined with a dashed line. The insets show magnified views of the boxed areas. Note that both wild-type mStr-Rab27A\textsuperscript{SR} and MST-Rab27A(Q78L)\textsuperscript{SR}-mStr recruited Slac2-a to mature melanosomes in Rab27A-deficient cells (second and fourth rows), whereas mStr-Rab27A(Q78L)\textsuperscript{SR} did not (third row). The lower insets in the right column are merged images of mStr fluorescence, Slac2-a, and melanosomes (pseudocolored in blue). Scale bars = 20 μm.
Forcible Expression of Slac2-aΔSHD on Mature Melanosomes by the MST Tag—To assess the MST tag as a general tool for melanosome targeting, we finally focused our attention on a Rab27A binding-deficient Slac2-a mutant. As described above, Slac2-a functions as a linker protein between Rab27A and the actin-based motor myosin Va, which is recruited to mature melanosomes in a Rab27A-dependent manner (12–14) (see also Fig. 5D), and impairment of its Rab27A-binding ability causes the perinuclear melanosome aggregation phenotype in human Griscelli syndrome type 3 (45) and in leaden, its corre-
Mechanism of Melanosome Transport Inhibition by Rab27A(Q78L)

Corresponding mouse model (46). When wild-type Slac2-a (FLAG-Slac2-a) was expressed in Rab27A-deficient melanocytes, it failed to target mature melanosomes because of the absence of its binding partner, Rab27A, on mature melanosomes, and it was impossible to restore peripheral melanosome distribution in Rab27A-deficient melanocytes (13) (Fig. 6C, second row). Similarly, a Slac2-aSHD mutant (Slac2-aΔSHD-FLAG) lacking a Rab27A-binding SHD (12) mainly localized in the cytosol and had no effect on perinuclear melanosome aggregation (Fig. 6C, third row, and D). However, when the MST tag was added to the N terminus of Slac2-aΔSHD (Fig. 6A), the resulting MST-Slac2-aΔSHD-FLAG mutant localized on mature melanosomes and clearly restored peripheral melanosome distribution even in the absence of Rab27A (Fig. 6C, fourth row, and D). Thus, forcible targeting of Slac2-a to mature melanosomes can rescue the Griscelli syndrome phenotype (i.e. perinuclear melanosome aggregation) induced by Rab27A deficiency. These results indicate that the MST tag functions as a universal melanosome-targeting tag that is capable of recruiting a variety of proteins to melanosomes.

DISCUSSION

We previously showed that expression of the GTPase-deficient Rab27A(Q78L) mutant in melanocytes induced perinuclear melanosome aggregation (9). However, the molecular mechanism by which Rab27A(Q78L) inhibits melanosome transport has remained unknown throughout the past decade. The results of this study provide two lines of evidence that Rab27A(Q78L) is unable to target mature melanosomes and that it traps the Rab27A effector Slac2-a in the cytosol, thereby resulting in inhibition of actin-based melanosome transport. The first line of evidence is that the Rab27A(Y6F/Q78L) mutant, which specifically impairs Slac2-a binding activity (17, 29), was unable to induce perinuclear melanosome aggregation (Fig. 1). The second line of evidence is that forcible targeting of Rab27A(Q78L) to mature melanosomes by the novel MST tag both neutralized the inhibitory effect of the Q78L mutation in wild-type melanocytes (Fig. 4) and restored peripheral melanosome distribution in Rab27A-deficient melanocytes (Fig. 5). These lines of evidence allow us to conclude that the GTPase activity of Rab27A is not essential for melanosome transport. However, that does not mean that inactivation of Rab27A is not essential for melanocyte function. Because Rab27A(Q78L) was mainly localized in the cytosol (Fig. 1), proper GDP-GTP cycling of Rab27A must be necessary for melanosome targeting by Rab27A (29). Moreover, Rab27A GTPase activity must also be required for Rab27A dissociation from melanosomes after actin-based melanosome transport to enable the next round of melanosome transport from around the nucleus (48).

Because Rab27A is now widely thought to be a general regulator of secretion (8), in the future it will be very interesting to determine whether the GTPase activity of Rab27A is involved in the exocytosis of secretory vesicles. Consistent with our finding in melanocytes, it has recently been shown that induction of platelet secretion does not require the GTPase activity of Rab27 and that cytosolic expression of Rab27 in platelets strongly inhibits secretion by platelets (49). Furthermore, inactivation of Rab27 by Rab27A-GAP EPI64 in rat parotid acinar cells has been shown to occur only after amylase release from secretory granules (47). Therefore, we speculate that the GTPase activity of Rab27A is not essential for secretory activity in general, just as it is also not essential for melanosome transport.

We also developed a novel melanosome-targeting tag that we named the MST tag (Fig. 2) and succeeded in using it to target three different proteins, i.e. Rab5, Rab27A(Q78L), and Slac2-aΔSHD, to mature melanosomes (Figs. 3, 4, and 6). It should be noted that the MST tag did not seem to interfere with the function of the tagged proteins. That is, MST-Slac2-aΔSHD functioned as a linker protein between melanosomes and myosin-Va (Fig. 6). Thus, the MST tag should be a powerful tool for use in future investigations of the function of proteins involved in melanosome biogenesis and/or transport.

In conclusion, by developing the novel MST tag, we were able to demonstrate that the inhibitory effect of the GTPase activity-deficient Rab27A(Q78L) on melanosome transport is primarily attributable to its cytosolic localization of the Rab27A(Q78L) protein. In other words, proper GDP-GTP cycling of Rab27A is necessary for Rab27A targeting to mature melanosomes. Our findings should provide an important clue to understanding why some GTPase-deficient Rab mutants exhibit an inhibitory effect on membrane traffic.

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