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Role of Rab family GTPases and their effectors in melanosomal logistics

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Rab GTPases constitute a family of small GTPases that regulate a variety of membrane trafficking events in all eukaryotic cells by recruiting their specific effector molecules. Recent accumulating evidence indicates that members of the mammalian Rab small GTPase family are involved in certain physiological and pathological processes. In particular, functional impairments of specific Rab proteins, *e.g.* Rab38 and Rab27A, their regulators or their effectors cause pigmentation disorders in humans and coat colour variations in mice because such impairments cause defects in melanosomal logistics, *i.e.* defects in melanosome biogenesis and transport. Genetic and biochemical analyses of the gene products responsible for mammalian pigmentation disorders in the past decade have revealed that Rab-mediated endosomal transport systems and melanosome transport systems play crucial roles in the efficient darkening of mammalian hair and skin. In this article, we review current knowledge regarding melanosomal logistics, with particular focus on the roles of Rab small GTPases and their effectors.

Keywords: Cellular logistics/melanocyte/membrane traffic/Rab effector/small GTPase.

Abbreviations: ABD, actin-binding domain; ANKR, ankyrin repeat; AP, adaptor protein complex; BLOC, biogenesis of lysosome-related organelles complex; *dsu*, *dilute suppressor*; GEF, guanine nucleotide exchange factor; HPS, Hermansky–Pudlak syndrome; MBD, myosin Va-binding domain; MC1R, melanocortin 1 receptor; Mreg, melanoregulin, MSH, melanocyte-stimulating hormone; REP-1, Rab escort protein-1; RGGT, Rab geranylgeranyl transferase; RILP, Rab7-interacting lysosomal protein; SHD, Slp homology domain; TGN, *trans*-Golgi network; Tyrp1, tyrosinase-related protein 1; VAMP, vesicle-associated membrane protein; Varp, VPS9-ankyrin-repeat protein; VID, VAMP7-interaction domain; VPS9, vacuolar protein sorting 9.

Mammalian epidermal melanocytes are specialized melanin pigment-producing cells that are responsible for protecting the human body from ultraviolet radiation, and their disorganized activity often causes hyperpigmentation or albinism. Melanosomes are tissue-specific lysosome-related organelles that synthesize and store melanin pigments in melanocytes (*1*). Melanosomes are produced around the nucleus mainly by the endosomal transport systems. Mature melanosomes are transported along two cytoskeletal components, *i.e.* microtubules and actin filaments, and eventually attached and tethered to the plasma membrane (*1*). Upon activation, melanocytes are thought to extend dendrites by reorganizing their cytoskeletons and membranes to efficiently transfer melanosomes to neighbouring keratinocytes and hair matrix cells (*2*). All of these intracellular and intercellular transport systems are required for efficient skin and hair pigmentation in mammals.

During the past few decades, a variety of key factors involved in the intracellular melanosome transport systems have been identified by genetic analyses of patients with albinism, *e.g.* patients with Hermansky–Pudlak syndrome (HPS), Griscelli syndrome and Chédiak–Higashi syndrome, and of coat colour mutant mice (*3*, *4*). It is noteworthy that the key factors include several Rab-type small GTPases and their regulators, indicating that they play crucial roles in melanosome biogenesis and transport (called 'melanosomal logistics'). Rab small GTPases are the key regulators of diverse membrane trafficking events, including cargo sorting, vesicle budding, vesicle formation, vesicle transport along the cytoskeleton and docking, tethering and fusion of vesicles with target membranes in all eukaryotic cells (*5*, *6*). The number of Rab proteins varies from species to species and approximately 60 distinct Rab proteins have been identified in mammals (*7*). Rab proteins are generally thought to function as molecular switches that shuttle between a GDP-bound inactive conformation (cytosolic localization) and a GTP-bound active conformation (membranous localization). The GTP-bound Rab proteins recruit specific effector molecules to specific membrane sites where they function. These effectors then execute diverse Rab-mediated membrane trafficking events described above (*5*, *6*). In this article, we review recent advances in knowledge of the functions of Rab proteins, their effectors and their regulators in mammalian epidermal melanocytes, with particular focus on the regulatory mechanisms of melanosomal logistics, including biogenesis, transport and transfer of melanosomes.

Featured Article

Rab proteins that regulate melanosome biogenesis

Melanosomes are thought to be produced from immature unpigmented endosome-derived organelles and to develop through a series of stages around the nucleus of melanocytes [see (*1*) for details]. Four morphologically distinct stages of melanosomes, referred to as stages I–IV, have been identified in the literature (Fig. 1). Stage I melanosomes are characterized by the presence of intraluminal vesicles and fibrils that emanate from the intraluminal membrane. The main component of the fibrils is the *silver* locus product Pmel17, an integral membrane protein (*8*). Stage II melanosomes have an oval shape and contain fibril-derived parallel sheet-like structures on which melanin is deposited at later stages. In stage III, melanogenic enzymes, such as tyrosinase and Tyrp1 (tyrosinase-related protein 1), are transported to premelanosomes and melanin pigments are synthesized and deposited until melanosome formation is completed in stage IV.

The results of proteomic analyses have indicated that a number of Rab proteins are associated with melanosomes (*9*, *10*). Two of them, Rab32 and Rab38, which are highly homologous Rab proteins in the phylogenetic tree (*6*), are localized on tyrosinase- and Tyrp1-containing vesicles and/or organelles around the *trans*-Golgi network (TGN) as well as on melanosomes (*11*). The weakly diluted mouse coat colour mutant *chocolate* has been shown to be caused by dysfunction of Rab38, and targeting of tyrosinase and Tyrp1 to melanosomes is mildly impaired in *chocolate* epidermal melanocytes (*11*, *12*). Rab38 and its close homologue Rab32 function redundantly in melanocytes, because additional depletion of Rab32 in cultured *chocolate* epidermal melanocytes severely impairs the transport of tyrosinase and Tyrp1 to melanosomes, resulting in severer hypopigmentation (*11*). In contrast to its role in epidermal melanocytes, Rab38 has a dominant role in the transport of tyrosinase to immature melanosomes in retinal pigment epithelial cells, because severe impairment of melanosome maturation is observed in the retinal pigment epithelial cells of *chocolate* mice (*13*). In other organisms, the *Rab38* gene is mutated in *ruby* rats (*14*), which also exhibit hypopigmentation in addition to the platelet storage pool deficiency related to HPS, a group of inherited disorders in humans (*4*, *15*), and thus *ruby* rats are considered a rat model of HPS. HPS is characterized by defective biogenesis of a group of specific organelles, including melanosomes, platelet dense granules and lysosomes. Subsets of the genes that encode components of adaptor protein complexes (AP-1/3), components of biogenesis of

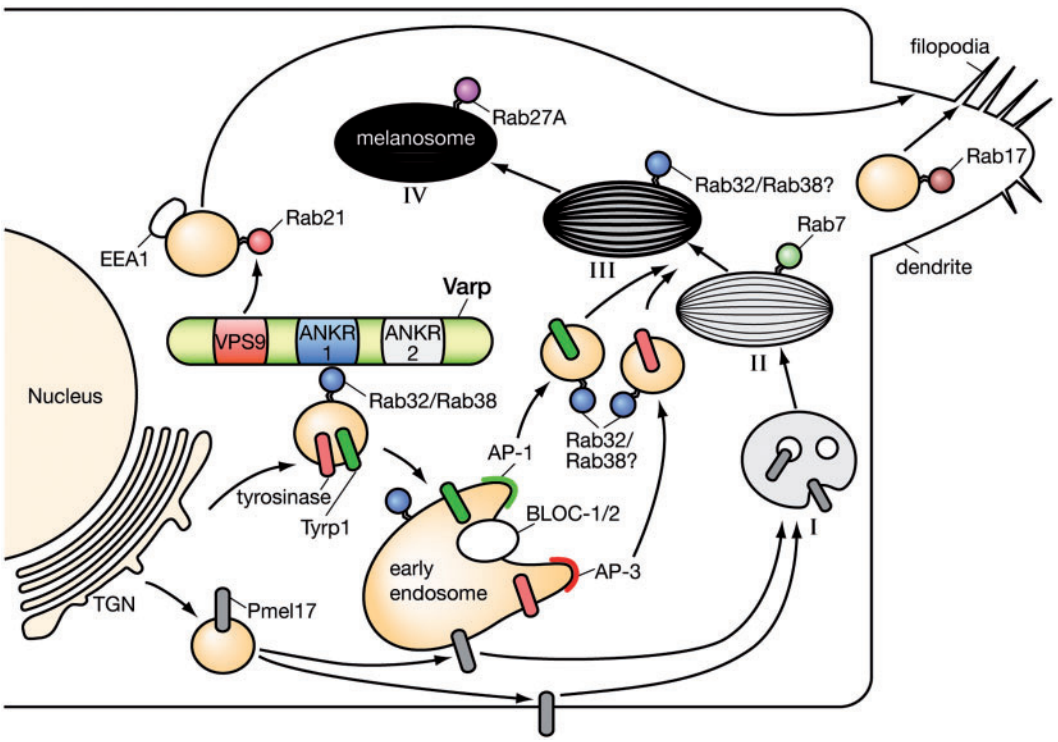

Fig. 1 Schematic diagram of endosomal transport systems that regulate melanosome biogenesis (from stage I to IV) and dendrite formation. The endosomal transport systems play important roles in the transport of tyrosinase and Tyrp1, because these melanogenic enzymes are transported from the TGN to stage III melanosomes via early endosomes. The BLOC and AP complexes are key players in the endosomal transport systems (*16*, *17*). AP-3 is required for tyrosinase transport from early endosomes to melanosomes, whereas BLOC-1/2 and AP-1 are required for Tyrp1 transport. Rab32/Rab38 and Varp are additional factors that are required for the transport of melanogenic enzymes (*22*), presumably for a step different from that regulated by BLOCs/APs. Varp also functions as a GEF for Rab21 to promote EEA1/Rab21-positive endosome trafficking for dendrite formation (*31*), which is essential for efficient melanosome transfer to neighbouring keratinocytes. Rab17 promotes the formation of dendritic filopodia by a largely unknown mechanism (*32*). The mechanism of Rab27A-dependent melanosome transport is illustrated in Fig. 2.

lysosome-related organelles complexes (BLOC-1, 2 and 3), or a component of the homotypic fusion and vacuole protein sorting (HOPS/VpsC) complex, are mutated in HPS patients (*16*, *17*). Furthermore, a *Drosophila melanogaster* Rab32/38 orthologue, Rab-RP1, is localized on pigment granules in the eye, and its mutation causes defects in eye pigmentation (*18*, *19*).

Although the precise mechanism(s) underlying Rab32/38-mediated transport of melanogenic enzymes is not fully understood, recent identification of a Rab32/38-specific-binding protein Varp (VPS9-ankyrin-repeat protein; also called Ankrd27) has greatly improved our understanding of the function of Rab32/38 in melanocytes (*20*). As its name indicates, Varp protein contains an N-terminal VPS9 (vacuolar protein sorting 9) domain and C-terminal tandem ankyrin-repeat domains (named ANKR1 and ANKR2) (Fig. 1). The Varp VPS9 domain possesses Rab21-GEF (guanine nucleotide exchange factor) activity (*21*, *22*), and the ANKR1 domain functions as a specific GTP-Rab32/Rab38-binding site (*20*). Moreover, Varp interacts with VAMP7/TI-VAMP (vesicle-associated membrane protein) via the region between the ANKR1 domain and the ANKR2 domain (named VID, VAMP7-interaction domain) (*22*, *23*). Varp and Rab32/38 are well co-localized on Tyrp1-containing vesicles in melanocytes and knock-down of Varp or expression of the ANKR1 domain, namely the Rab32/38-binding site, in melanocytes causes a dramatic reduction in Tyrp1 signals on peripheral melanosomes (*20*, *22*). Since a Rab32/38-binding-deficient mutant of Varp is unable to support Tyrp1 transport, the Varp-Rab32/38 interaction is crucial to Tyrp1 transport in melanocytes (*22*). In contrast, the function of the VPS9 domain is not needed for Tyrp1 transport in melanocytes, because a Rab21-GEF-activity-deficient mutant of Varp fully supports this process (*22*) (a different function of the VPS9 domain in dendrite formation is described in a later section). Since the reduced expression of Tyrp1 in Varp-deficient melanocytes is recovered by treatment with a proteasome inhibitor, untransported or mis-targeted enzymes in Varp-deficient cells are selectively degraded by proteasomes (*20*). Consistent with this finding, melanogenic enzymes, especially tyrosinase, are known to be degraded by proteasomes via ER-associated protein degradation (ERAD), and it has been proposed that this process is a means of quality control of melanogenic enzymes (*24*). It is therefore possible that the Varp–Rab32/38 complex is also involved in the quality control of melanogenic enzymes in melanocytes.

Involvement of additional members of the Rab family in melanosome biogenesis has also been reported. For example, Rab7, which regulates the motility and fusion of late endosomes and lysosomes, has been reported to be involved in the maturation of the melanosomal matrix protein Pmel17 as well as in the transport of tyrosinase and Tyrp1 to melanosomes (*25*, *26*) (Fig. 1). Rab9, which regulates the retrograde transport from late endosomes to the TGN, has been reported to interact with HPS4, a component of the BLOC-3 complex, in a GTP-dependent manner, although the function of Rab9 in melanocytes remains to be elucidated (*27*). Rab11, a well-known regulator of recycling endosomes, has been reported to exhibit a genetic interaction with BLOC-1 in *Drosophila melanogaster*, suggesting that BLOC-1-containing vesicles and/or organelles may be transported in a Rab11-dependent manner (*28*). Actually, Delevoye *et al.* (*29*) demonstrated that direct contacts of stages III and IV melanosomes with tyrosinase-containing vesicles and/or organelles, for whose delivery Rab11 is responsible. More recently, RUTBC1, a specific GAP for Rab32 and Rab33B, has been shown to act as a Rab9A effector (*30*). Further research will be needed to determine the mechanisms that regulate the activation or inactivation of Rab32/38 and the functional relationships between Rab32/38 and other Rabs, *e.g.* Rab9 and Rab11, at the melanosome maturation step in melanocytes.

Rab proteins that regulate melanosome transport along microtubules

After melanosomes mature around the nucleus of melanocytes, they are transported from the perinucleus to the dendrite tips along microtubules and actin filaments by the coordinated actions of motor proteins. The rapid, bidirectional microtubule-based movements of melanosomes are regulated by two classes of motors, kinesins and dynein. Following the transfer of melanosomes from microtubules to actin filaments, an actin-based motor myosin Va regulates their slow and unidirectional movement in melanocytes (*33*). Kinesins regulate microtubule plus end-directed (outward) organelle transport (*34*), whereas dynein regulates minus end-directed (inward) transport (*35*). Several reports have described the existence of specific kinesins and dynein on melanosomes and their involvement in melanosome transport along microtubules (*36–40*). For example, the results of an antisense oligonucleotide-based experiment showed a correlation between reduced Kif5b (a conventional kinesin heavy chain) expression levels and lower rates of outward melanosome transport (*40*). However, the precise mechanism(s) underlying kinesin-mediated melanosome transport is still unclear, because there has been little conclusive evidence about the functional contribution of kinesin motors to melanosome transport in mammalian melanocytes.

Jordens *et al.* (*41*, *42*) investigated the inward immature melanosome transport and showed that Rab7 and its effector molecule, RILP (Rab7-interacting lysosomal protein), are localized on the membranes of not only late endosomes and lysosomes (*43*) but also Pmel17-positive immature melanosomes. Since RILP associates with the dynein–dynactin motor complex via direct binding with $p150^{Glued}$, a component of the dynactin complex, and regulates the microtubule minus end-directed transport of late endosomes and lysosomes, Rab7 is likely to regulate the inward transport of immature melanosomes as well as the biogenesis of melanosomes in human epidermal melanocytes (*42*). However, Rab7 is not involved in the inward transport of mature melanosomes, and instead,

melanoregulin has recently been shown to function as a cargo receptor for the dynein–dynactin motor complex during the microtubule minus end-directed transport of mature melanosomes (*44*) (see the later section on melanoregulin for details).

Rab proteins that regulate melanosome transport along actin filaments

In contrast to the microtubule-based melanosome transport, in the past decade, the molecular mechanism of actin-based melanosome transport has been well documented by genetic and biochemical analyses of three gene products responsible for the diluted coat colour in mutant mice. The first key regulator of actin-based melanosome transport discovered was myosin Va, and the other two were Rab27A and Slac2-a (also called melanophilin). Mutations of the genes that encode these three proteins are responsible for *dilute*, *ashen* and *leaden*, respectively (*45–47*). These molecules form a tripartite protein complex that regulates actin-based melanosome transport [see (*33*) and references therein], and loss of any one of the components of the complex causes perinuclear aggregation of melanosomes because of the defect in melanosome transfer from microtubules to actin filaments. The importance of this complex has also been demonstrated by the existence of mutations of the human *MYO5A*, *RAB27A* and *SLAC2-A*/*MLPH* genes, which are responsible for human hereditary diseases characterized by silvery hair that are called Griscelli syndrome types I, II and III, respectively (*48*).

The GTP-bound activated form of Rab27A first binds to the surface of mature melanosomes via the C-terminal geranylgeranylation site and recruits the melanocyte-specific Rab27A effector Slac2-a via the switch II region (*49*). The resulting Rab27–Slac2-a complex functions as a cargo (or melanosome) receptor for exon F-containing myosin-Va, which is abundantly expressed in melanocytes (*50–52*). Slac2-a is an important linker molecule in this tripartite complex, and the N-terminal SHD (Slp homology domain) and the middle domain (MBD, myosin Va-binding domain) are responsible for Rab27A-binding and myosin Va-binding, respectively (Fig. 2). Furthermore, the C-terminal domain of Slac2-a is able to bind actin (ABD, actin-binding domain) (*53*) and a microtubule plus end-tracking protein EB1 (*54*), whose interactions have been suggested to be involved in the efficient melanosome transfer from microtubules to actin filaments. Moreover, zebrafish Slac2-a/melanophilin has the ability to suppress dynein activity by an unknown mechanism in melanophores (*55*) and thereby facilitates melanosome transfer from microtubules to actin filaments. In contrast to the formation of the tripartite protein complex, very little is known about the mechanism by which the complex is disassembled after the transfer of melanosomes to actin filaments. Since Slac2-a contains multiple PEST-like sequences (potential signals for rapid protein degradation), degradation of the linker protein Slac2-a by certain proteases may be involved in the disassembly of the complex (*56*). Slac2-a functions as an epidermal melanocyte-specific Rab27A effector and it is not expressed in retinal pigment epithelial cells. In retinal pigment epithelial cells, Slac2-c/MYRIP, a homologue of Slac2-a, functions as an alternate linker protein between Rab27A on melanosomes and an actin-based motor myosin VIIa (*57–60*).

Slp2-a is a second Rab27A effector in melanocytes and consists of an N-terminal SHD and C-terminal tandem C2 domains that are essential for binding to phosphatidylserine, a major component of the cytosolic leaflet of the plasma membrane. Slp2-a regulates anchoring of melanosomes to the plasma membrane of melanocytes following actin-based melanosome transport regulated by the Rab27A–Slac2-a–myosin Va tripartite complex (*61*) (Fig. 2). These sequential effector interactions may be achieved by the higher Rab27A-binding activity of Slp2-a than of Slac2-a (*62*).

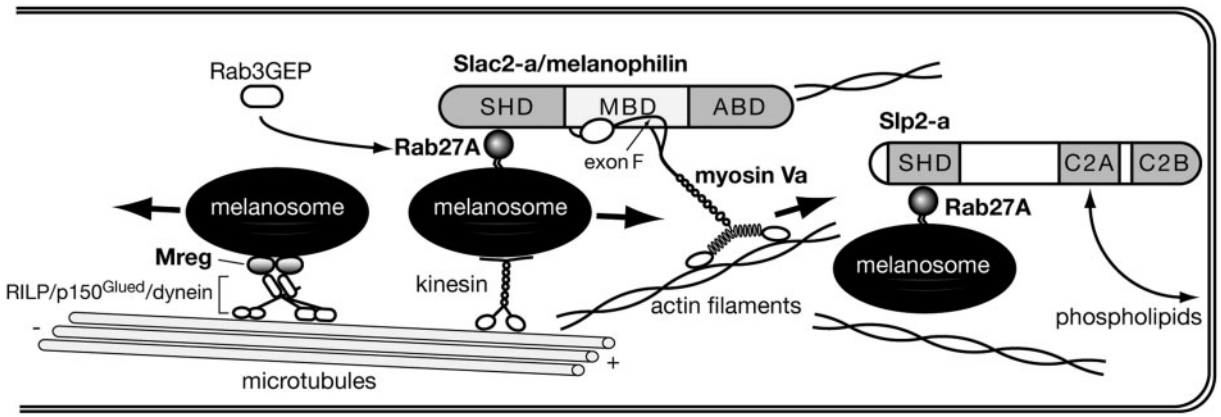

Fig. 2 Schematic diagram of melanosome transport along microtubules and actin filaments. Mature melanosomes are transported toward the peripheral region via microtubule-based motors (kinesin and dynein), but, except for the discovery that retrograde melanosome transport is regulated by the Mreg–RILP–p150^{Glued} complex in Rab27A-deficient melanocytes (*44*), the precise mechanisms underlying the microtubule-based melanosome transport remain to be elucidated. Mreg directly binds the C-terminal domain of RILP and functions as a melanosome receptor for the dynein–dynactin retrograde motor complex. After being transferred to actin filaments, melanosomes are transported by the tripartite complex consisting of Rab27A–Slac2-a–myosin Va (*33*) and then anchored to the plasma membrane by the Rab27A–Slp2-a complex (*61*). Activation of Rab27A by Rab3GEP is required for the recruitment of Rab27A to melanosomes before the actin-based melanosome transport (*63*).

The membrane targeting of Rab27A is the initial and crucial step in actin-based melanosome transport. Several regulatory factors have been reported to be involved in activation, inactivation and/or melanosomal targeting of Rab27A in melanocytes. Rab3GEP (also called DENN/MADD), originally described as a GEF for Rab3, is capable of activating Rab27A in melanocytes (*63*), whereas EPI64, which contains a conserved TBC/Rab-GAP domain (*64*), is capable of inactivating Rab27A (*65*, *66*). Consistent with their activities, both knockdown of Rab3GEP and overexpression of EPI64 in melanocytes induce the perinuclear melanosome aggregation phenotype (*63*, *65*), the same as in melanocytes from the coat colour mutants *ashen*, *dilute* and *leaden*. In addition to activation and inactivation of Rab proteins, C-terminal geranylgeranylation by Rab geranylgeranyl transferase (RGGT) is required for their membrane association and stability. Actually, *gunmetal* mice, which carry a mutation in the gene encoding the α subunit of RGGT II, exhibit diluted coat colour in addition to prolonged bleeding due to macrothrombocytopenia and a reduced platelet α- and dense-granule content. Consistent with these phenotypes of *gunmetal* mice, the geranylgeranylation level of several Rab proteins, especially Rab27A, is dramatically reduced (*67*). REP-1 (Rab escort protein-1) assists geranylgeranylation of Rab proteins and REP-1 deficiency causes choroideremia, an X-linked retinal degeneration in humans (*68*). Actually, deficient geranylgeranylation of Rab27A in retinal pigment epithelial cells is observed in choroideremia (*68*).

Melanoregulin functions as a cargo receptor for the dynein–dynactin motor complex

Melanoregulin (Mreg), a *dilute suppressor* (*dsu*) gene product, has been implicated in the regulation of melanosome transport in mammalian epidermal melanocytes, because in 1983 a spontaneous mutation in the *dsu* locus was reported to cause suppression of the diluted coat colour phenotype of *dilute*, *ashen* and *leaden* mice (*69–71*). Although Mreg was proposed to be involved in membrane fusion (*72*, *73*), the precise mechanism by which Mreg regulates melanosome transport long remained unknown. Recently, Mreg has been shown to function as a cargo receptor for the dynein–dynactin motor complex in microtubule minus end-directed melanosome transport (*44*). Mreg directly interacts with the C-terminal domain of RILP and forms a complex with RILP and $p150^{Glued}$ (Fig. 2), which is analogous to the retrograde lysosomal transport complex (Rab7–RILP–$p150^{Glued}$) (*41*, *43*). siRNA-mediated knockdown of each component of the Mreg–RILP–$p150^{Glued}$ complex restores the peripheral melanosome distribution in Rab27A-deficient melanocytes (*44*). However, similar knockdown in normal melanocytes appears to have no effect on melanosome distribution. Further work will be necessary to determine how the microtubule-based minus end-directed melanosome transport is regulated in normal melanocytes.

Rab proteins that regulate dendrite formation of melanocytes

Melanocytes are stimulated by paracrine factors, including α-MSH (melanocyte-stimulating hormone), endothelin-1 and prostaglandins that are secreted from surrounding keratinocytes (*74*), and the stimulation results in dendrite formation and the transfer of melanosomes from melanocytes to neighbouring keratinocytes. α-MSH, presumably the most important factor that stimulates melanocytes, activates the melanocortin 1 receptor (MC1R), a G-protein-coupled receptor on the plasma membrane of melanocytes, and thereby stimulates cAMP synthesis. Due to the morphology of its dendrites, each dendritic melanocyte is able to provide melanin to approximately 36 keratinocytes (*75*). The transferred melanin accumulates in the perinuclear region of the keratinocytes and protects them against UV injury. Several possible mechanisms of melanosome transfer have been proposed (see below). In any event, dendrite formation has been proposed to be crucial to the subsequent melanosome transfer step. The Rho family GTPases (Rac, Rho and Cdc42) play a pivotal role in cytoskeletal dynamics, including in actin polymerization, and regulate neurite formation (*76*). Consistent with the fact that the elevated cAMP level induces Rac activation and Rho inhibition in a variety of cells, expression of either a constitutive active mutant Rac (V12Rac) or Rho inhibitor (Rho GDP dissociation inhibitor) is capable of stimulating dendrite formation in melanocytes (*77*). In addition to cytoskeletal reorganization, a supply of new membrane proteins and lipids to the plasma membrane, *i.e.* membrane trafficking, must be required for neurite formation of neurons (*78*) and dendrite formation of melanocytes. It is therefore not surprising that certain Rab small GTPases are involved in the regulation of the dendrite formation.

The first evidence that Rab-mediated membrane trafficking is involved in dendrite formation of melanocytes was obtained in an analysis of the VPS9 domain of Varp (*31*). As mentioned above, Varp functions as a Rab32/38 effector through the ANKR1 domain that transports Tyrp1 to melanosomes, but the Rab21-GEF activity of the VPS9 domain of Varp is not required for this process (*22*). Surprisingly, the Rab21-GEF activity of Varp (or Rab21 itself), but not the Rab32/38 effector function, is required for forskolin-induced dendrite formation in cultured melanocytes (*31*). Thus, Varp regulates two distinct steps in the process of melanogenesis, transport of melanogenic enzymes and dendrite formation, through two different Rab-signalling domains, the ANKR1 domain (Rab32/Rab38-binding activity) and the VPS9 domain (Rab21-GEF activity), respectively (Fig. 1). In addition to these two Rab-signalling domains, Varp possesses the VAMP7/TI-VAMP interaction domain (VID) between the ANKR1 domain and the ANKR2 domain (*23*). Interestingly, the VID is required for both dendrite formation and the transport of melanogenic enzymes (*22*, *31*). Extensive research will be necessary to determine the precise mechanism(s) by which the interaction between VAMP7 and Varp regulates these two processes.

Varp and Rab21 have also been reported to be involved in neurite outgrowth of mouse hippocampal neurons (*23*), indicating that Varp also regulates trafficking of Rab21-positive endosomes in cells other than melanocytes.

Although the precise mechanism of the melanosome transfer step from melanocytes to neighbouring keratinocytes remains to be elucidated, several groups have proposed possible mechanisms, including (i) phagocytosis of melanocyte dendrite tips or filopodia by keratinocytes, (ii) secretion of melanosomes or multi-melanosome-containing globules from melanocytes and their uptake by keratinocytes via endocytosis or phagocytosis and (iii) direct fusion of the plasma membranes of the two cells (*2*, *79*, *80*). Although we still do not know which of the proposed mechanisms is correct, we especially noted the recent findings by Beaumont *et al*. (*32*) that a recycling endosomal protein Rab17 regulates filopodia formation in melanocytes and that its absence results in melanosome accumulation within melanocytes, presumably because of impaired melanosome transfer to keratinocytes. Consistent with these findings, Singh *et al*. (*80*) showed by a melanocyte–keratinocyte co-culture system that melanosomes are transported along the filopodia and phagocytosed by neighbouring keratinocytes in a myosin X motor-dependent manner.

Concluding remarks and perspectives

In this article, we have reviewed current knowledge regarding the functions of Rab small GTPases, their effectors, and their regulators in melanosomal logistics. Rab32/38 and its effector Varp play a crucial role in the transport of melanogenic enzymes to melanosomes in the melanosome maturation step (*22*). The results of analyses of HPS factors, *e.g*. BLOCs and APs, in the past few decades have also pointed to the importance of the endosomal transport systems in melanosome biogenesis (*16*, *17*). Although these factors are ubiquitously expressed, their functional outputs are observed exclusively in specific cells, specifically in cells that contain lysosome-related organelles, such as melanocytes. One possible explanation is that the component(s) of the BLOC and/or AP complexes may regulate the biogenesis of melanosomes (or lysosome-related organelles) by interacting with a cell-specific factor. Since some Rab proteins, *e.g*. Rab32 and Rab38 (*11*), are expressed in a tissue/cell-specific manner, they are likely candidates for the functional interactors with BLOCs/APs (*27*). Therefore, it would be of great interest to test BLOC components for interactions with members of the Rab family by means of Rab panels (*81*). The Rab-mediated endosomal transport systems have also been implicated in both dendrite formation and filopodia formation of melanocytes (*31*, *32*) to efficiently transfer melanosomes to neighbouring keratinocytes and hair matrix cells. Elucidating the precise and consensus mechanism underlying melanin transfer from melanocytes to keratinocytes will be an important task in the next decade.

The mechanism of the actin-based melanosome transport regulated by the Rab27A–Slac2-a–myosin Va tripartite complex is well understood (*33*). Although Mreg-dependent microtubule-based minus end-directed (inward) transport in Rab27A-deficient melanocytes has just been discovered (*44*), the mechanism(s) of microtubule-based melanosome transport under normal conditions remains unknown. Since accumulating evidence indicates that there are direct or indirect interactions between kinesin motors and Rab proteins (*82*), a specific Rab protein may also be involved in the microtubule-based plus end-directed (outward) transport of melanosomes. A future genome-wide investigation of the Rab family (*83*) and the kinesin family will clarify their involvement in the microtubule-based melanosome transport.

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Conflict of interest

None declared.

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