Melanosomes on the move: a model to understand organelle dynamics

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Abstract

Advances in live-cell microscopy have revealed the extraordinarily dynamic nature of intracellular organelles. Moreover, movement appears to be critical in establishing and maintaining intracellular organization and organellar and cellular function. Motility is regulated by the activity of organelle-associated motor proteins, kinesins, dyneins and myosins, which move cargo along polar MT (microtubule) and actin tracks. However, in most instances, the motors that move specific organelles remain mysterious. Over recent years, pigment granules, or melanosomes, within pigment cells have provided an excellent model for understanding the molecular mechanisms by which motor proteins associate with and move intracellular organelles. In the present paper, we discuss recent discoveries that shed light on the mechanisms of melanosome transport and highlight future prospects for the use of pigment cells in unravelling general molecular mechanisms of intracellular transport.

Introduction

Mammalian skin melanocytes are specialized cells that reside on the superficial side of the epidermal basement membrane and within the bulb of the hair follicle. They produce melanin/pigment as a protection against, and in response to, environmental UV radiation (reviewed in [1]). In melanocytes, melanin is produced and stored in specialized organelles known as melanosomes [2,3]. Melanosomes then deliver mature melanin-packed melanosomes to a large number (~40) of nearby keratinocytes via an extensive and elaborate network of cytoplasmic extensions, or dendrites, which spread throughout the intercellular spaces of the lower epidermis (Figure 1). The mechanism of intracellular melanosome transfer in situ in skin remains little characterized [1]. Within keratinocytes, melanosomes are trafficked along MTs (microtubules) to the perinuclear region where they form an apically oriented cluster or ‘microparasol’ that protects the nuclear genetic material from UV-induced damage [4,5].

Although melanosomes are melanocyte-specific organelles, genetic studies of Hermansky–Pudlak, Chediak–Higashi and Griscelli syndromes and corresponding mutant mouse models, diseases that combine albinism with bleeding and immunological disorders, revealed that the molecular mechanism controlling melanosome biogenesis and transport are shared with other LROs (lysosome-related secretary organelles) in other cell types [6,7]. These include T-cell lytic granules, mast cell inflammatory mediator granules, neutrophil azurophilic granules, alveolar epithelium type II cell lamellar bodies, platelet dense granules, pancreatic β-cell insulin storage granules and others. Thus insight into melanosome biology may give broader insight into processes such as thrombosis, inflammation, immune response and glucose metabolism, which are linked to the function of these LROs.

To date, most of the studies using the melanosome as a model for organelle movement and cytoskeleton interaction have been performed in melanophores, a type of pigment cell in cold-blooded vertebrates. In these cells, rapid (~30 min) and reversible redistribution of pigment from perinuclear clustered to peripherally dispersed and vice versa is triggered by agents that alter intracellular cAMP levels [8]. Most mammalian melanocytes do not carry out such striking redistribution of melanosomes; nevertheless, several features make them an appealing cellular model for the study of organelle dynamics. First, they are large (dendrites may extend >100 μm) flat (peripheral cytoplasm ~1 μm thick) adherent cells that contain many melanosomes that are large (0.5 μm in diameter) high-contrast organelle that are easily visible using bright-field or phase-contrast microscopy (Figure 1A). Secondly, they are easily amenable to genetic modification such as siRNA (short interfering RNA)/shRNA (short hairpin RNA) knockdown and transgene expression. Thirdly, a large number of immortal melanocyte cell lines have been established from coat colour mutant mice and these contain melanosomes that are altered in size, distribution and shape (Figure 1). This means that fundamental biophysical issues such as the...
Figure 1 | Phase-contrast micrographs showing immortal mouse melanocytes
Melanocytes were derived from wild-type (C57Bl/6) mouse (A), Rab27a-null ashen mutant mouse showing pigment clustered around the perinuclear MTOC (B) and CHS/LYST (Chediak-Higashi syndrome/lysosomal trafficking regulator protein)-null beige mouse which contains enlarged melanosomes (up to 2 μm diameter compared with 0.5 μm in wild-type) (C). The black line in (B) indicates the extent of the peripheral cytoplasm of the cells pictured. All melanocytes pictured were obtained from the Wellcome Trust Functional Genomics Cell Bank managed by Elena Sviderskaya and Dorothy Bennett (St George’s, University of London, London, U.K.)

The effect of organelle size and motor number on movement may potentially be addressed using these cells (see http://www.sgul.ac.uk/depts/anatomy/pages/WTFGCB.htm).

In the present short review, we focus on the processes that govern intracellular transport of melanosomes within melanocytes and highlight insights that have been obtained into the regulation of organelle dynamics using this model system and point the way to areas of future interest.

Myosin Va and peripheral melanosome capture
The first evidence of the importance of cytoskeleton proteins in the intracellular transport of melanosomes came with the finding that mutation of the MYO5A gene, which encodes the alternative myosin Va heavy chain, causes the dilute mutant phenotype in the mouse [9]. As the name suggests, dilute mice are characterized by partial albinism/pigment dilution and neurological disease characterized by muscle weakness and seizures, which results in premature death. Neurological deficit appears to result from defects in the distribution of ER (endoplasmic reticulum) into postsynaptic dendritic spines [10,11]. Studies of cultured mouse skin melanocytes revealed that myosin Va is a melanosome-associated protein whose loss-of-function results in perinuclear clustering of melanosomes around the MTOC (MT-organizing centre) (similar to ashen mutant shown in Figure 1B) [12–14]. These observations suggest that myosin Va-dependent movement of melanosomes along actin filaments drives their centrifugal transport into peripheral melanocyte dendrites.

However, seminal video microscopy studies revealed that melanosomes in dilute (and wild-type) melanocytes undertake episodes of bidirectional, MT-dependent fast (∼1.5 μm/s) transport that allow them to leave the central cluster of melanosomes, pass into the peripheral cytoplasm and then return [15]. These observations together with the earlier data, showing strong enrichment of myosin Va on melanosomes in actin-rich peripheral dendrites, suggest a capture model for myosin Va function in melanosome transport. In this model, melanosomes first undertake fast net plus-end-directed/centrifugal transport along MTs that takes them towards peripheral dendrites where they are retained or captured by myosin Va-dependent coupling to the dense cortical actin shell (Figures 2A and 2B). Thus MT and actin track and motor systems co-operate to promote melanosome transport and retention in peripheral dendrites. Several pieces of evidence support this. First, observation of reverse transport, i.e. induced perinuclear melanosomes clustering in wild-type cells, revealed this to be a two-step process: (i) an initial lag phase (several hours) during which melanosomes remain in the peripheral cytoplasm and presumably uncouple from the local actin cytoskeleton, and (ii) a 120 min phase of apparent MT-dependent net transport towards the perinuclear MTOC that is enriched in MT minus ends. Secondly, myosin Va/actin-dependent movements (∼140 nm/s) in MT-depleted melanocytes did not result in significant centrifugal transport of melanosomes over the time course measured (90 min). This suggests that myosin Va/actin-dependent transport alone is insufficient to allow the accumulation of melanosomes in peripheral dendrites. Thirdly, electron microscopy revealed that dendrites are packed with short randomly polarized actin filaments that are more likely to serve as a barrier to movement than tracks along which myosin Va might carry melanosomes [15].

Rab27a and Mlph (melanophilin) are a melanosomal myosin Va receptor
Leading from this, insight into the mechanism of myosin Va recruitment to melanosomes came from studies mapping the causative mutations in two other mouse mutants, ashen and leaen, to the Rab27a and Mlph genes [16,17]. In
Figure 2 | A summary of the molecular mechanisms thought to regulate melanosome transport in melanocytes

Newly synthesized melanosomes are transported to the dendrite tip by means of MT-based motors kinesin and dynein (A). Once at the periphery, track switching from MT- to actin-based transport occurs via melanosomal recruitment of active Rab27a-GTP and assembly of the capture complex of Rab27a effectors Mlph and myosin Va (A and B). Rab3GEP is a critical regulator Rab27 activation and membrane recruitment, and capture-complex assembly. However, the mechanisms regulating its activity are unknown. Myosin Va of the capture complex may then allow local melanosome transport to the membrane (B). A Rab27 effector cascade may then allow replacement of the capture complex with a docking effector Slp2-a before intercellular transfer of melanin to neighbouring keratinocytes (C).

these mice, similar to dilute, albinism results from defects in melanosome tethering in peripheral dendrites, suggesting that their gene products act in concert with myosin Va [12,18]. Consistent with this idea, albinism in dilute, ashen and leaden mice is partially suppressed in a dilute suppressor (dsu) mutant background [18]. dsu encodes melanoregulin, a vertebrate-specific protein proposed to regulate membrane fusion [19–21]. However, although crossing dsu with dilute restores pigment transfer to the hair shaft, it does not restore melanosome distribution in melanocytes, thus the mechanism of suppression remains unclear [19].

Rab27a meanwhile encodes the small GTPase Rab27a and Mlph encodes the Rab effector (Rabphilin3A)-related protein Mlph [16,17]. The Rab GTPase family is a large protein group (>60 members in mammals) that regulate specific intracellular trafficking events in eukaryotic cells. They do this by acting as compartment-specific molecular switches that, in the GTP-bound/active conformation, recruit effector proteins to the membrane on which they reside. Effectors then mediate diverse vesicular transport functions, including membrane budding/vesicle formation, vesicle transport along cytoskeleton tracks, docking/tethering of vesicles with an acceptor compartment and membrane fusion [22–24]. Many studies over the intervening years indicate that Rab27 proteins are general regulators of movement and exocytosis of LROs and neuroendocrine secretory granules in a variety of cells [25,26]. Mlph was found to be a modular adaptor protein containing an N-terminal RBD (Rab27-GTP-binding domain), conserved among several Rab27a–b/3a–d effector proteins, and a C-terminal MBD (myosin Va-binding domain) [27–30], partially conserved with another Rab27 effector MyRIP (myosin Rab-interacting protein) that regulates melanosome transport in retinal pigment epithelium in complex with Rab27a and myosin VIIa [31–33] (Figure 2B). A number of studies found that active Rab27a-GTP sequentially recruits Mlph and myosin Va to the melanosome membrane and promotes dispersion of melanosome into peripheral dendrites, indicating that assembly of this tripartite capture complex on the membrane transfers melanosomes from MT- to actin-dependent travel [28–30,34–38] (Figure 2B). Consistent with this, we recently observed, using live-cell imaging and automated melanosome tracking, that capture-complex recruitment [reported using EGFP (enhanced green fluorescent protein)-tagged Rab27a and Mlph] correlated inversely with the frequency of MT-dependent melanosome movement (A.N. Hume, M.S. Wilson, D.S. Ushakov, M.A. Ferenczi and M.C. Seabra, unpublished work). In vitro studies reveal that the myosin...
Va-binding region of Mlp interacts with the cargo-binding tail of myosin Va and activates the motor activity, probably by disrupting the autoinhibitory motor/head–tail interaction of myosin Va [39,40].

The paradigm of MT-dependent fast long-range transport to the cell periphery coupled to actin-dependent local transport/tethering has found support from a number of studies of the transport of LROs [41–43]. Additionally, many studies have found that other Rabs link directly and indirectly to molecular motor effectors, suggesting this might be a general mechanism by which Rabs regulate intracellular transport [22,23,44,45].

### MT-dependent melanosome transport and track switching

In spite of the advances in understanding peripheral capture, less is understood about the motors that power upstream MT-dependent melanosome movement in mammalian melanocytes, how they are attached to their cargo and how their recruitment is integrated with melanogenesis. Immuno-electron microscopy studies revealed that conventional KHC (kinesin heavy chain) (Kif5b), kinecin, a peripheral membrane protein that links KHC to the ER, cytoplasmic DHC (dynein heavy chain) and components of the dynein-activating dynactin complex are localized to a proportion of melanosomes in human melanocytes [46–50]. Treatment of melanocytes with antisense oligonucleotides that specifically reduce the expression of Kif5b protein resulted in a decrease in the proportion of centrifugal melanosome movements [49]. However, no evidence was presented to show that the melanosome movements observed were MT-dependent, and it was not reported whether inhibition resulted in perinuclear melanosome clustering, as would be expected if this activity were essential for transport of melanosomes to dendrites [49] (Figure 2A). Interestingly, several studies of MT-dependent melanosome transport in melanophores implicate kinesin 2 (Kif3 family) rather than kinesin 1/Kif5b in plus-end-directed transport [8]. It should be interesting to resolve this issue. On the other hand, antisense-mediated knockdown of DHC resulted in centrifugal dispersion of melanosomes into the peripheral cytoplasm, consistent with inhibition of minus-end-directed transport [50]. However, observation of live cells revealed that bidirectional melanosome movement persisted in the knockdown cells, suggesting that another unidentified motor drives minus-end-directed movement (Figure 2A).

Leading from this, it is unclear precisely how MT-dependent melanosome transport is integrated with peripheral capture. The most likely mechanism is that recruitment of the capture complex and coupling to the actin meshwork is sufficient to arrest melanosomes in the periphery and separate MT motors from their tracks (Figures 2A and 2B). Thus recruitment and activation of Rab27a maybe a vital step in the regulation of melanosome transport. Insight into these processes was recently provided by the identification of Rab3GEP (previously identified as a GDP/GTP-exchange protein for Rab3 GTPases [also known as MADD/DENN for MAPK (mitogen-activated protein kinase)-activating death domain/differentially expressed in neoplastic versus normal] as a Rab27-specific GDP/GTP-exchange and also melanosome-targeting factor [51,52]. Consistent with this, siRNA-mediated knockdown of Rab3GEP in melanocytes resulted in perinuclear melanosome clustering and disassociation of capture complex from the melanosome membrane. Interestingly, Rab3GEP has also been found to interact with kinesin 3 motors (Kif1b/γ and Kif1α), suggesting a possible direction for future studies of the co-ordination of MT and actin-driven transport [53].

To the membrane . . . and beyond

Downstream events linking melanosome capture with melanocyte–keratinocyte transfer also remain poorly defined, largely due to the difficulties in modelling these processes in culture (Figure 2C). Given that capture requires myosin Va, it seems possible that this is an active process with motor activity driving melanosomes through the dendrite actin meshwork towards the plasma membrane by walking along actin filaments, as is observed in vitro [54]. However, the random polarity of actin filaments in melanocyte dendrites together with evidence from immunoelectron and immunofluorescence microscopy studies that melanosomes often contain multiple patches of myosin Va raise questions about how such transport might be organized ([15,35] and A.N. Hume, J.S. Ramalho and M.C. Seabra, unpublished work). For instance, how is the activity of different capture complexes at different positions on the same organelle co-ordinated? Alternatively, perhaps the collective activity of multiple disparate melanosome associated myosin Va molecules might locally remodel the actin cytoskeleton, thereby clearing a path towards the plasma membrane.

Once at the membrane, it is likely that melanosomes dock/attach to the membrane before transfer. Interestingly, aside from Mlp, melanocytes express significant quantities (estimated 5-fold in excess of Mlp) of one of the other ten currently known Rab27 effector proteins: Slp (synaptotagmin-like protein) 2-a. This implies an important function for Slp2-a in melanocytes. Slp2-a combines an N-terminal RBD, shared with Mlp and all but one other Rab27 effector (Munc13-4), with a C-terminal PS (phosphatidylerine)-binding tandem C2 domains. siRNA knockdown of Slp2-a in wild-type melanocytes results in modest perinuclear clustering of melanosomes (compared with Mlp-knockdown cells), referred to as ‘peripheral dilution’, and reduction in dendritic shape [55]. On the basis of these observations, it has been proposed that a Rab27a–Slp2-a complex acts downstream of the Rab27a–Mlp–myosin Va capture complex to dock melanosomes at the plasma membrane via simultaneous interaction of Slp2-a with Rab27a (on the melanosome) and PS (enriched in the plasma membrane) (Figure 2C). Interestingly, several studies reveal that other cell types, including pancreatic β-cells, PC12 cells,
neutrophils and CTLs (cytotoxic T-lymphocytes), express multiple Rab27 effectors suggesting that sequential effector engagement might be a general mechanism by which Rab27 could regulate different stages of transport [25]. Indirect support for this comes from surface plasmon resonance and pull-down binding studies showing differences in affinity for Rab27 between different effectors; for example, Mlph has a 10-fold lower affinity for Rab27a-GTP than does Slp2-a [56]. Also FRAP (fluorescence recovery after photobleaching) studies in PC12 cells show that, whereas granule-associated Rab27 and effectors, Slp4/granuphilin and Noc2, do not recover significantly 3 min after photobleaching, another effector, Rabphilin, completely recovered to pre-bleach levels within 2–3 min [57,58]. These observations suggest that lower-affinity/more dynamically associated effectors, e.g. Mlph, might be replaced over time by higher-affinity/less dynamically associated effectors, e.g. Slp2-a, and so could regulate early and late aspects of Rab27 function. However, caution must be maintained over these kinds of models of Rab27 function, as although mice deficient in Slp2-a have been generated, no differences in coat colour were reported [59]. Indeed, we found that expression of a Slp1–Mlph chimera in which the low-affinity RBD of Mlph was replaced with the high-affinity RBD from the effector Slp1 (that is expected to bind Rab27a more stably than Mlph and so compete the Rab27a–Slp2-a interaction more effectively) rescued melanosome transport defects in Mlph-null leaden melanocytes with similar efficiency to Mlph [60]. Finally, the mechanism regulating such a sequential, low- then high-affinity, effector interaction pathway remains obscure.

**Concluding remarks**

In summary, the study of melanosome transport over the last 10–15 years has provided unexpected insight into the global mechanisms regulating organelle transport and biogenesis. That said, it is clear that many important pieces of the puzzle of intracellular melanosome transport remain obscure. We anticipate that future clarification of these events will illuminate further the general mechanisms of membrane trafficking.

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**References**


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