

It takes two to tango to the melanosome

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The role of clathrin adaptor proteins in sorting cargo in the biosynthetic and recycling routes is an area of intense research. In this issue, Delevoye et al. (2009. *J. Cell Biol.* doi:10.1083/jcb.200907122) show that a close interaction between the clathrin adaptor AP-1 and a kinesin motor KIF13A is essential for delivering melanogenic enzymes from recycling endosomes to nascent melanosomes and for organelle biogenesis.

Melanosomes, along with platelet-dense granules and lung type II alveolar cell lamellar bodies, are lysosome-related organelles (LROs), compartments that originate from endosomes but are distinct from and usually coexist with lysosomes (Fig. 1). The most characteristic features of melanosomes are their ability to synthesize and store melanin and their presence in specialized pigmented cells such as skin melanocytes and iris and retinal pigment epithelial cells (Raposo and Marks, 2007; Wasmeier et al., 2008). In this issue, Delevoye et al. (see p. 247) report a melanogenic role for the clathrin adaptor AP-1 that involves interactions between the adaptor and the plus end kinesin motor KIF13A. An impressive set of data support a scenario in which the adaptor and the motor tightly interact, like in tango, to position donor recycling endosomes (REs) near nascent melanosomes at the cell periphery and to generate tubulovesicular intermediates that deliver newly synthesized pigmenting enzymes to melanosomes.

Extensive studies have shown that melanosome biogenesis occurs in two waves that correspond to four morphologically distinct stages (Fig. 1; Marks and Seabra, 2001; Raposo and Marks, 2007). The first wave (stages I and II) is the formation of immature, pigment-free ellipsoidal melanosomes from vacuolar domains of early sorting endosomes. This process requires Pmel17, an integral membrane protein that likely reaches sorting endosomes by clathrin-dependent endocytosis from the plasma membrane. Upon proteolysis in the sorting endosomes/stage I melanosomes, Pmel17 forms intraluminal proteinaceous fibrils with characteristics of amyloid. The second wave starts with the post-Golgi transport of enzymes involved in melanin synthesis such as tyrosinase and tyrosinase-related protein 1 (Tyrp1) to nascent melanosomes. Melanin deposition occurs on Pmel17 fibrils and leads to the biogenesis of mature (stages III

and IV) melanosomes. The clathrin adaptors AP-1 and -3 have partially redundant functions in sorting cargo proteins to melanosomes. Melanosomal cargo proteins have dileucine motifs that are recognized differentially by AP-1 and -3 in post-Golgi endosomes (Huizing et al., 2001; Theos et al., 2005). Nascent tyrosinase is found in distinct endosomal buds that contain either AP-3 or -1 in normal melanocytes and loss of AP-3 results only in a partial mislocalization of the enzyme. As these adaptors also mediate sorting from endosomes to other compartments, additional machinery, such as biogenesis of LRO complex 1 (BLOC-1), BLOC-2, and the tissue-specific small GTPases Rab32 and Rab38, regulate cargo delivery to melanosomes. Mutations in components of this melanosomal targeting machinery result in a variety of well-studied pigmentation defects in humans and animals such as Hermansky–Pudlak syndrome (Wei, 2006).

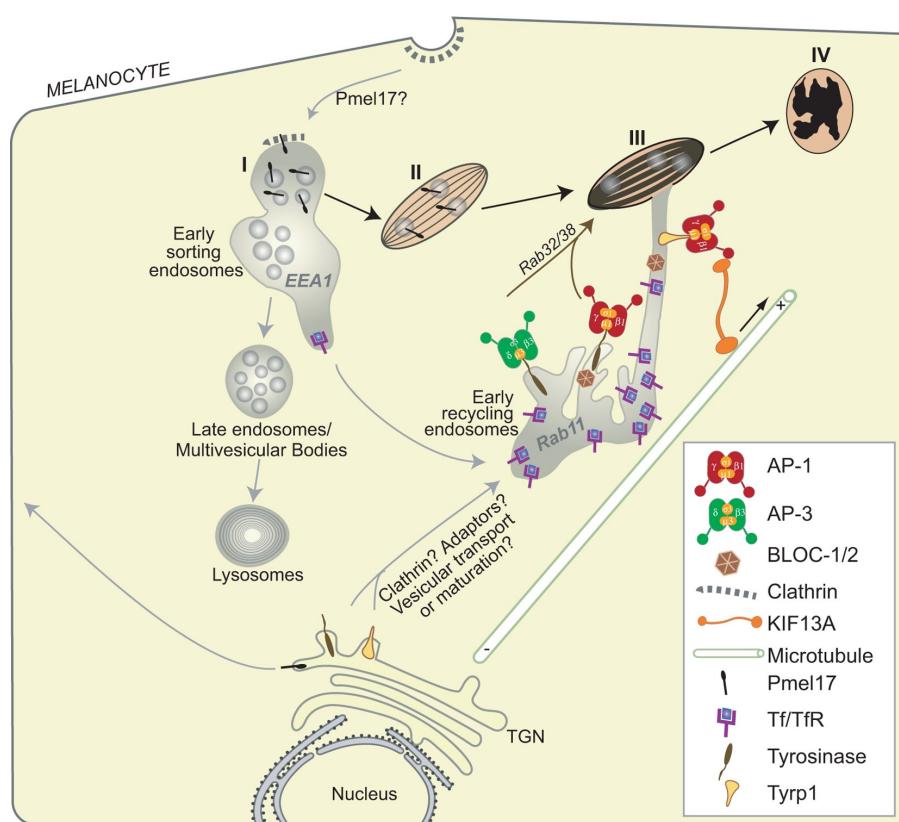
Delevoye et al. (2009) show that knockdown of AP-1 in melanocytic MNT-1 cells decreases melanin content, demonstrating that AP-1 has a role in melanogenesis. Only late-stage (III/IV) melanosomes are decreased in number; unpigmented (stage I/II) melanosomes are unaffected, indicating that AP-1 functions selectively in the second wave of melanosome biogenesis. In AP-1-depleted cells, the melanosome cargo protein Tyrp1 is retained in vacuolar endosomes in a manner similar to that seen in BLOC-1-deficient melanocytes (Setty et al., 2007). Using immunofluorescence to monitor markers of various endosomal compartments, Delevoye et al. (2009) show that AP-1 performs its melanogenic function in early REs. Interestingly, additional data show that AP-1-containing REs have a peripheral distribution in MNT-1 cells, which is strikingly different from the perinuclear localization observed in other cells. Furthermore, siRNA-mediated knockdown of AP-1, but not of AP-3, relocates RE to a pericentriolar location.

How might AP-1 influence endosome position? One possibility is by its association with the plus end-directed kinesin motor KIF13A (Fig. 1). Nakagawa et al. (2000) have previously shown that a subunit of AP-1 binds the C-terminal domain of KIF13A, mediating TGN to plasma membrane transport of the mannose 6-phosphate receptor. Indeed, Delevoye et al. (2009) show that KIF13A partially colocalizes with AP-1 in MNT-1 cells and coimmunoprecipitates with both AP-1 and Tyrp1.

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Figure 1. Role of clathrin adaptor proteins in melanosome biogenesis. Post-Golgi trafficking routes of three melanosome cargoes (Pmel17, tyrosinase, and Tyrp1) in melanocytes are shown. Newly synthesized Pmel17 is transported to the limiting membrane and intraluminal vesicles of stage I melanosomes/early sorting endosomes via the plasma membrane. This process (depicted by a question mark) might involve clathrin and AP-2. From these EEA1-positive vacuolar endosomes, Pmel17 is sorted away from the late endosome/multivesicular body pathway into stage II melanosomes. Little is known as to how the enzymes essential for melanin synthesis, tyrosinase and Tyrp1, are sorted from the TGN to early REs, and it is likely that clathrin and its adaptors are involved in this process. Tyrosinase, which binds both AP-1 and -3, is transported to stage III melanosomes from tubular regions of REs, containing Tf/TfR and Rab11, by two distinct routes: one regulated by AP-3 and the other regulated by BLOC-1, BLOC-2, and perhaps AP-1. However, Tyrp1 binds only AP-1 and not AP-3, indicating a divergence of sorting mechanisms between tyrosinase and Tyrp1. Delevoye et al. (2009) now show that AP-1 interacts with the kinesin motor KIF13A to transport recycling endosomal domains to the melanocytic cell periphery. The close apposition of Tyrp1-containing tubules with melanosomes allows cargo transfer and biogenesis of stage III and IV melanosomes. Although Tf is found in these peripheral endosomal tubules, there appears to be a filtering mechanism that sorts it out before the tubules fuse with melanosomes. It is likely, although not yet confirmed, that BLOC-1 and -2 act in concert with AP-1 to transport Tyrp1. The tissue-specific Rabs, Rab32 and Rab38, might function in any or all of these pathways.



Furthermore, knockdown of KIF13A replicates the phenotype seen with AP-1 depletion: pericentriolar clustering of RE, accumulation of Tyrp1 in vacuolar endosomes, and reduction in mature melanosomes and melanin content. Delevoye et al. (2009) go on to show that the peripheral RE localization facilitates sorting of melanosomal proteins but decreases the efficiency of transferrin (Tf) receptor (TfR) recycling to the plasma membrane. They also show the converse; i.e., the pericentriolar localization of RE decreases the efficiency of melanosomal targeting and increases the efficiency of TfR recycling. Thus, the position of REs, determined by the interaction between a clathrin adaptor and a kinesin, is key for specific sorting functions of this organelle (like TfR recycling) and also regulates the biogenesis of another organelle (the melanosome). This is a novel and exciting finding and is an emerging theme in cell biology. It was recently reported that AP-1 interacts with another plus end-directed kinesin, KIF5, which helps transport endosomes to the cell periphery (Schmidt et al., 2009).

The next question that Delevoye et al. (2009) approach is what is the nature of the carriers that transport melanosomal proteins from peripheral REs to immediately adjacent stage III/IV melanosomes? Live imaging experiments showed a dynamic network of Tf-containing RE tubules that extend and retract, making contact with melanosomes for at least 30 s. Double-tilt 3D electron tomography of thick (350–400 nm) sections of cells

preserved by high pressure freezing and freeze substitution, a technique recently adapted to the study of melanosomes by Hurbain et al. (2008), revealed that some of these tubular elements are continuous with the melanosomal limiting membrane and that their lumens are often connected. Collectively, these results indicate that peripheral RE domains serve to deliver biosynthetic cargo to maturing melanosomes by the coordinated actions of AP-1 and KIF13A and that the mechanism involves tubular connections rather than vesicular transport (Fig. 1).

The study by Delevoye et al. (2009) beautifully demonstrates the power of carefully chosen morphological and live imaging techniques, in combination with siRNA-mediated knockdown of molecules under study, to elucidate important details of cellular sorting processes. As always, several questions emerge from their results. Does this type of mechanism also operate in perinuclear REs, which were recently shown to cooperate with adjacent TGN in biosynthetic trafficking to the plasma membrane (Cancino et al., 2007; Gravotta et al., 2007)? Do newly synthesized melanosomal enzymes move from the TGN to REs using vesicular trafficking and clathrin adaptors or, rather, result from “maturation” of REs from the TGN? What is the role of clathrin in melanosome maturation? Are AP-1 and KIF13A essential for tubulogenesis from REs as the authors speculate? How are RE proteins (e.g., TfR) prevented from incorporating into melanosomes through the tubular connections? What is the mechanism

that regulates docking and fusion of RE tubules with melanosomes? Likely, Rab32 and Rab38 participate in this process, as these proteins localize to tubulovesicular endosomal structures, and their loss causes mislocalization of tyrosinase and Tyrp1 (Wasmeier et al., 2006), but the SNAREs (if any) that participate in the mechanism are still unknown. Lastly, another intriguing aspect of this study is how adaptors sort proteins by differential recognition of dileucine motifs. Tyrp1 also has a dileucine motif that exclusively binds AP-1, but not AP-3, in melanocytic cells (Theos et al., 2005), whereas tyrosinase has dileucine motifs that bind AP-1 and -3, indicating that not all dileucine motifs are equal in the eyes of the adaptor.

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