Published August 1, 2016





# Coming or going? Un-BLOC-ing delivery and recycling pathways during melanosome maturation

Clare E. Futter[1] and Daniel F. Cutler[2]

[1]Univeristy College London Institute of Ophthalmology, London EC1V 9EL, England, UK
[2]Medical Research Council Laboratory for Molecular Cell Biology, University College London, London WC1E 6BT, England, UK

Melanosome biogenesis requires successive waves of cargo delivery from endosomes to immature melanosomes, coupled with recycling of the trafficking machinery. Dennis et al. (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201605090) report differential roles for BLOC-1 and BLOC-3 complexes in delivery and recycling of melanosomal biogenetic components, supplying directionality to melanosome maturation.

Melanosomes are the melanin-containing organelles that provide color to the skin, eyes, and hair, where they afford protection from solar ultraviolet radiation. Melanosomes are a core model for studying the formation and maturation of lysosome-related organelles (LROs). LROs include functionally diverse organelles such as platelet-dense granules and lytic granules of T cells, as well as the Weibel-Palade bodies of endothelial cells (Marks et al., 2013). The relationship between these functionally varied structures with their specific cargoes is defined by the common machinery involved in their formation. Although melanosome maturation progresses through sequential, morphologically identifiable and well-characterized stages, identifying the precise step at which individual molecular regulators operate has been challenging. Not only do melanosome biogenesis pathways involve pleiomorphic and complex early endosomes, which sort cargo to multiple destinations via multiple exit routes, but mature melanosomes are transferred from melanocytes to neighboring keratinocytes, so that the end-stage, mature organelle can have a limited lifetime in the cell. Furthermore, multiple nonredundant parallel pathways lead to melanosome biogenesis. As a result, it is very challenging to distinguish the direct effects of mutations on melanosome cargo delivery from the effects those mutations might have on the recycling pathways that retrieve key components from melanosomes to supply further rounds of cargo delivery. Despite these technical issues, dissecting the molecular pathways that lead to mature melanosome biogenesis is important, not only to understand the cell biology of pigmentation and pigmentation disorders but also to understand the effects of mutations that alter the biogenesis of other LROs and that are associated with immune dysfunction and bleeding disorders (Dell'Angelica, 2004; Marks et al., 2013).

One set of proteins whose functions in melanosome biogenesis have been particularly difficult to dissect is the biogenesis of lysosome-related organelles complexes (BLOCs). These proteins were first identified in patients with Hermansky–Pudlak syndrome (Dell'Angelica, 2004), in whom loss of BLOC proteins leads to dysfunctional LROs in a variety of tissues, including hair, skin, and lung, and in cells supporting the immune and hemostatic systems. Establishing the function of this trio of multiprotein machines has been hampered not only by a lack of conserved functional protein domains but also by a simple lack of immunogenicity. In melanosomes, depletion of the components of each of the BLOCs produces a variety of pigmentation phenotypes, including altered melanosome numbers, morphology, and size, that presumably reflect the different functions of each BLOC. For example, BLOC-1 is required for the exit of a subset of melanosomal cargos, including tyrosinase related protein TYRP1, from early endosomes into tubular carriers (Setty et al., 2007; Delevoye et al., 2016; Fig. 1), whereas BLOC-2 is needed for cargo delivery to melanosomes (Dennis et al., 2015). The function of BLOC-3 in melanosome biogenesis has been more elusive, but it was recently shown to be an exchange factor for Rab32/38 (Gerondopoulos et al., 2012), two tissue-restricted GTPases implicated in the delivery of cargo to melanosomes.

The final step in the delivery of cargo to melanosomes is the fusion of cargo-containing carriers with immature melanosomes. This process involves SNARE proteins, which mediate membrane fusion between transport vesicles and target membranes by interaction of a v-SNARE on the transport vesicle with t-SNAREs on the target membrane. In this issue, Dennis et al. focused on the trafficking of the v-SNARE VAMP7, which has previously been shown to be involved in pigmentation (Tamura et al., 2011; Jani et al., 2015). To operate as a v-SNARE in cargo delivery to melanosomes, VAMP7 must be present on the carriers that deliver melanosomal cargo and must also be retrieved from melanosomes to support further rounds of fusion. In an elegant series of experiments to assess its role, Dennis et al. (2016) took advantage of the following: (a) the obligate recycling of VAMP7; (b) the tubular nature of transport carriers on both forward and recycling arms of the pathway (which allow them to be reliably observed by live cell imaging); (c) the ability to inhibit exit from early endosomes or melanosomes by using BLOC mutant cells, followed by a pulse of traffic brought about by the release of inhibition through the transient transfection of the missing protein; and (d) the presence of markers that distinguish between anterograde and retrograde carriers.

Correspondence to Clare E. Futter: c.futter@ucl.ac.uk



The Rockefeller University Press $30.00
J. Cell Biol. Vol. 214 No. 3 245–247
www.jcb.org/cgi/doi/10.1083/jcb.201607023

Published August 1, 2016

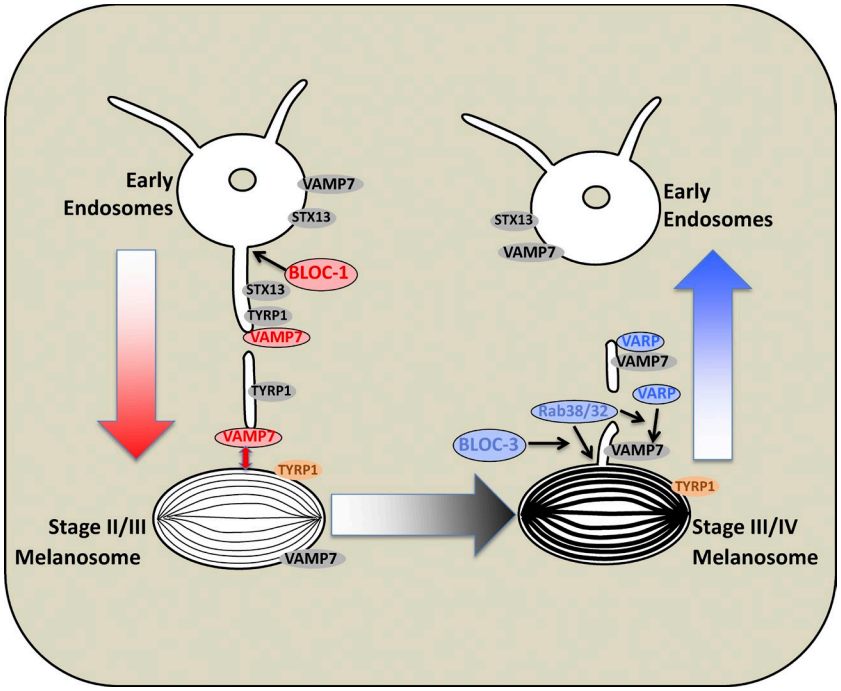


Figure 1. **Model of BLOC-dependent cycling of VAMP7 between endosomes and melanosomes.** BLOC-1 promotes the formation of STX13-positive tubules emanating from early endosomes. These tubular carriers carry TYRP1 and VAMP7 to immature melanosomes, where VAMP7 promotes membrane fusion. TYRP1 participates in the deposition of melanin in immature stage II and III melanosomes that accompanies the transition to mature stage IV melanosomes. BLOC-3 promotes the formation of VAMP7-positive tubules from mature melanosomes, recruiting Rab38/32, which in turn recruits VARP. VARP binds to SNARE and Longin domains of VAMP7, maintaining this v-SNARE in an inactive conformation. Anterograde machinery is shown in red, retrograde machinery in blue, and the melanin-synthesizing machinery in brown at points where they are active. Where proteins are likely to be passive cargo, they are shown in black.

At least two pathways depending on either BLOC-1/BLOC-2 or the heterotetrameric clathrin adaptor complex AP3 are known to deliver cargo to melanosomes. It is not clear which of these two pathways depends on VAMP7 to deliver cargo to melanosomes. A major challenge is to follow the trafficking of the BLOC-1–dependent cargo protein TYRP1 to melanosomes. At steady state, most of TYRP1 is already on melanosomes, making it hard to track new deliveries. Dennis et al. (2016) overcame this problem by exploiting BLOC-1–deficient mouse melanocytes in which cargoes, including TYRP1, accumulate in Syntaxin13 (STX13)-positive early endosomes. The authors transiently transfected these cells with the missing BLOC-1 component to generate a pulse of cargo trafficking. They then followed this cargo as it left the early endosome. Live-cell imaging revealed tubular carriers that were positive for VAMP7 or TYRP1 and STX13 emanating from early endosomes. Depleting wild-type cells of VAMP7 caused the accumulation of TYRP1-positive vesicles and tubules surrounding the melanosomes. Together, these results provide strong evidence that VAMP7 is the v-SNARE for BLOC-1–dependent delivery of cargo to melanosomes (Fig. 1).

Dennis et al. (2016) used a similar strategy to analyze the subsequent recycling of VAMP7, using live-cell imaging to demonstrate the presence of VAMP7-containing tubular carriers forming from melanosomes (Fig. 1). These tubules are distinguished from those that deliver cargo to melanosomes because they are shorter, are less stable, do not contain STX13, and require BLOC-3 rather than BLOC-1 for their formation. These recycling tubules also contain the VAMP7- and Rab38/32-interacting protein (VARP), which binds both the SNARE domain and the longin domain of VAMP7 (Burgo et al., 2009), maintaining VAMP7 in an autoinhibited state (Schäfer et al., 2012). VARP additionally participates in retromer-mediated endocytic recycling of VAMP7 in nonpigmented cells (Hesketh et al., 2014). The study by Dennis et al. (2016) suggests that VARP maintains VAMP7 in an inactive state during its recycling from melanosomes, thereby preventing the promiscuous fusion of the recycling tubular carriers, helping to provide directionality to the whole cycle. VARP is an effector of Rab38 (Tamura et al., 2011), and Dennis et al. (2016) show that Rab38 recruits VARP to BLOC-3–dependent carriers. BLOC-3 is an exchange factor for Rab38, whose function in melanosome biogenesis has been difficult to pin down. A role for Rab38 in the delivery of melanin-synthesizing enzymes to the melanosome has been proposed previously (Wasmeier et al., 2006; Lopes et al., 2007; Bultema et al., 2014). However, a role for Rab38 in VAMP7 recycling, with its loss thus resulting in a depletion of the fusion machinery delivering melanin-synthesizing enzymes, would also be consistent with the phenotype of Rab38 depletion. It would also be consistent with the steady-state localization of Rab38, which is predominantly on melanosomes.

Overall, Dennis et al. (2016) reveal a BLOC-1–dependent delivery of VAMP7/TYRP1-positive carriers to melanosomes followed by a BLOC-3–dependent retrieval of VAMP7 (Fig. 1). This raises many further questions about the regulation of recycling pathways from melanosomes. The BLOC-3–dependent recycling carriers are not enriched in TYRP1, although many mature melanosomes appear depleted of this enzyme (see Fig. 1 of Dennis et al. [2016]). Indeed, the researchers point out that VAMP7 is a more faithful marker of peripheral melanosomes than TYRP1. This suggests that the melanin-synthesizing machinery is recycled when maturation is complete and would suggest that, as for melanosome delivery pathways, there is more than one route for recycling from melanosomes. If the absence of TYRP1 on melanosomes indicates melanosome maturity, then it raises the question: what signals that maturity to prevent further rounds of VAMP7-dependent fusion of BLOC-1 carriers to deliver more TYRP1? Interestingly, as Dennis et al. (2016) point out, lower levels of VAMP7 expression or higher levels of melanosome biogenesis could differentially affect the need for BLOC-3/Rab38-dependent VAMP7 recycling in different cell types/situations. For example, melanosome biogenesis occurs during a very short window in embryonic life in retinal pigment epithelial (RPE) cells, and melanosome biogenesis and thus

Published August 1, 2016

pigmentation are much more sensitive to the loss of Rab38 in RPE cells than in melanocytes (Lopes et al., 2007).

Finally, VAMP7 is required for the fusion of lysosomes with the plasma membrane during plasma membrane repair, and another VAMP7 longin domain interactor, Hrb, is required to maintain VAMP7 in its autoinhibited state during its recycling to lysosomes (Pryor et al., 2008). One mechanism by which melanosomes are transferred from melanocytes to keratinocytes is fusion with the plasma membrane, enabling the exocytosis of melanin, followed by its phagocytosis by keratinocytes (Tarafder et al., 2014). Does VAMP7 operate in melanosome fusion with the plasma membrane and then follow a similar recycling route, and does this involve a switch to Hrb? How is the need to recycle VAMP7 from melanosomes for further rounds of cargo delivery balanced against a possible need for VAMP7 in the fusion of melanosomes with the plasma membrane? It is worth pointing out that RPE cells and choroidal melanocytes, unlike skin melanocytes, do not transfer their melanosomes to other cells and so the fate of melanosomes may affect the need for VAMP7 after maturity is reached. This could be facilitated by regulating the BLOC-1– and BLOC-3–dependent anterograde and retrograde pathways identified in Dennis et al. (2016).

The study by Dennis et al. (2016) takes an important step in deciphering the pathways underlying SNARE cycling during melanosome maturation. They identify a new BLOC-3–dependent pathway for recycling of VAMP7 from melanosomes and provide a new hypothesis to explain the different pigmentation phenotypes associated with BLOC-3 deficiency. As multiple pathways that control the coming and going of cargo during melanosome formation are identified, attention will surely switch to the regulation and coordination of these pathways and the impact of their dysregulation on disease pathogenesis.

### Acknowledgments

The authors thank Louisa Cutler for drawing Fig. 1.

D.F. Cutler is funded by the UK Medical Research Council (MC-UU-12018/2) and C.E. Futter is funded by the Wellcome Trust (093445).

The authors declare no competing financial interests.

Submitted: 7 July 2016
Accepted: 8 July 2016

## References

Bultema, J.J., J.A. Boyle, P.B. Malenke, F.E. Martin, E.C. Dell'Angelica, R.E. Cheney, and S.M. Di Pietro. 2014. Myosin vc interacts with Rab32 and Rab38 proteins and works in the biogenesis and secretion of melanosomes. *J. Biol. Chem.* 289:33513–33528. http://dx.doi.org/10.1074/jbc.M114.578948

Burgo, A., E. Sotirakis, M.C. Simmler, A. Verraes, C. Chamot, J.C. Simpson, L. Lanzetti, V. Proux-Gillardeaux, and T. Galli. 2009. Role of Varp, a Rab21 exchange factor and TI-VAMP/VAMP7 partner, in neurite growth. *EMBO Rep.* 10:1117–1124. http://dx.doi.org/10.1038/embor.2009.186

Delevoye, C., X. Heiligenstein, L. Ripoll, F. Gilles-Marsens, M.K. Dennis, R.A. Linares, L. Derman, A. Gokhale, E. Morel, V. Faundez, et al. 2016. BLOC-1 brings together the actin and microtubule cytoskeletons to generate recycling endosomes. *Curr. Biol.* 26:1–13. http://dx.doi.org/10.1016/j.cub.2015.11.020

Dell'Angelica, E.C. 2004. The building BLOC(k)s of lysosomes and related organelles. *Curr. Opin. Cell Biol.* 16:458–464. http://dx.doi.org/10.1016/j.ceb.2004.05.001

Dennis, M.K., A.R. Mantegazza, O.L. Snir, D. Tenza, A. Acosta-Ruiz, C. Delevoye, R. Zorger, A. Sitaram, W. de Jesus-Rojas, K. Ravichandran, et al. 2015. BLOC-2 targets recycling endosomal tubules to melanosomes for cargo delivery. *J. Cell Biol.* 209:563–577. http://dx.doi.org/10.1083/jcb.201410026

Dennis, M.K., C. Delevoye, A. Acosta-Ruiz, I. Hurbain, M. Romao, G.G. Hesketh, P.S. Goff, E.V. Sviderskaya, D.C. Bennett, J.P. Luzio, et al. 2016. BLOC-1 and BLOC-3 regulate VAMP7 cycling to and from melanosomes via distinct tubular transport carriers. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201605090

Gerondopoulos, A., L. Langemeyer, J.R. Liang, A. Linford, and F.A. Barr. 2012. BLOC-3 mutated in Hermansky-Pudlak syndrome is a Rab32/38 guanine nucleotide exchange factor. *Curr. Biol.* 22:2135–2139. http://dx.doi.org/10.1016/j.cub.2012.09.020

Hesketh, G.G., I. Pérez-Dorado, L.P. Jackson, L. Wartosch, I.B. Schäfer, S.R. Gray, A.J. McCoy, O.B. Zeldin, E.F. Garman, M.E. Harbour, et al. 2014. VARP is recruited on to endosomes by direct interaction with retromer, where together they function in export to the cell surface. *Dev. Cell.* 29:591–606. http://dx.doi.org/10.1016/j.devcel.2014.04.010

Jani, R.A., L.K. Purushothaman, S. Rani, P. Bergam, and S.R. Setty. 2015. STX13 regulates cargo delivery from recycling endosomes during melanosome biogenesis. *J. Cell Sci.* 128:3263–3276. http://dx.doi.org/10.1242/jcs.171165

Lopes, V.S., C. Wasmeier, M.C. Seabra, and C.E. Futter. 2007. Melanosome maturation defect in Rab38-deficient retinal pigment epithelium results in instability of immature melanosomes during transient melanogenesis. *Mol. Biol. Cell.* 18:3914–3927. http://dx.doi.org/10.1091/mbc.E07-03-0268

Marks, M.S., H.F. Heijnen, and G. Raposo. 2013. Lysosome-related organelles: unusual compartments become mainstream. *Curr. Opin. Cell Biol.* 25:495–505. http://dx.doi.org/10.1016/j.ceb.2013.04.008

Pryor, P.R., L. Jackson, S.R. Gray, M.A. Edeling, A. Thompson, C.M. Sanderson, P.R. Evans, D.J. Owen, and J.P. Luzio. 2008. Molecular basis for the sorting of the SNARE VAMP7 into endocytic clathrin-coated vesicles by the ArfGAP Hrb. *Cell.* 134:817–827. http://dx.doi.org/10.1016/j.cell.2008.07.023

Schäfer, I.B., G.G. Hesketh, N.A. Bright, S.R. Gray, P.R. Pryor, P.R. Evans, J.P. Luzio, and D.J. Owen. 2012. The binding of Varp to VAMP7 traps VAMP7 in a closed, fusogenically inactive conformation. *Nat. Struct. Mol. Biol.* 19:1300–1309. http://dx.doi.org/10.1038/nsmb.2414

Setty, S.R., D. Tenza, S.T. Truschel, E. Chou, E.V. Sviderskaya, A.C. Theos, M.L. Lamoreux, S.M. Di Pietro, M. Starcevic, D.C. Bennett, et al. 2007. BLOC-1 is required for cargo-specific sorting from vacuolar early endosomes toward lysosome-related organelles. *Mol. Biol. Cell.* 18:768–780. http://dx.doi.org/10.1091/mbc.E06-12-1066

Tamura, K., N. Ohbayashi, K. Ishibashi, and M. Fukuda. 2011. Structure-function analysis of VPS9-ankyrin-repeat protein (Varp) in the trafficking of tyrosinase-related protein 1 in melanocytes. *J. Biol. Chem.* 286:7507–7521. http://dx.doi.org/10.1074/jbc.M110.191205

Tarafder, A.K., G. Bolasco, M.S. Correia, F.J. Pereira, L. Iannone, A.N. Hume, N. Kirkpatrick, M. Picardo, M.R. Torrisi, I.P. Rodrigues, et al. 2014. Rab11b mediates melanin transfer between donor melanocytes and acceptor keratinocytes via coupled exo/endocytosis. *J. Invest. Dermatol.* 134:1056–1066. http://dx.doi.org/10.1038/jid.2013.432

Wasmeier, C., M. Romao, L. Plowright, D.C. Bennett, G. Raposo, and M.C. Seabra. 2006. Rab38 and Rab32 control post-Golgi trafficking of melanogenic enzymes. *J. Cell Biol.* 175:271–281. http://dx.doi.org/10.1083/jcb.200606050