SORTING AND TRANSPORT OF MELANOSOMAL ENZYMES BETWEEN POST-GOLGI COMPARTMENTS

VIORICA IVAN1*, PETER VAN DER SLUIJS2

1Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy, Splaiul Independenţei 296, 060031 Bucharest 17, Romania
2Department of Cell Biology, University Medical Center Utrecht, 3584 CX Utrecht, the Netherlands

(Received September 22, 2010)

Melanosomes are tissue-specific lysosome-related organelles (LROs) that synthesize and store melanins. They contain specific resident proteins, amongst which the melanogenic enzymes tyrosinase and tyrosinase-related proteins (TYRPs). Recent progress revealed that the early endosomal system serves as an intermediate station in the itinerary of melanosomal proteins from the trans-Golgi network to melanosomes. The occurrence of genetic disorders in humans and model organisms, in which the biogenesis of melanosomes and other LROs is impaired, has provided new insights into the molecular machinery that controls the endosomal sorting and transport events. In this review we summarize recent advances towards elucidating the intracellular pathways of melanosomal enzymes.

Key words: Lysosome-related organelles, tyrosinase, Hermansky-Pudlak syndrome, AP-3, BLOCs.

INTRODUCTION

In eukaryotic cells, transport between different intracellular compartments of the secretory and the endocytic pathway occurs via vesiculo-tubular carriers that bud from a donor organelle and are subsequently transported to- and fused with a specific acceptor organelle (1). Transmembrane proteins destined for transport are sorted away from the resident ones and concentrated on patches on the donor membrane before being incorporated into transport carriers. The sorting process is facilitated by a group of cytoplasmic coat proteins, called adaptors, through recognition and binding to sorting signals located in the cytoplasmic tail of transmembrane cargo molecules. Most common motifs consist of short peptides and are referred to as tyrosine- or dileucine-based signals or involve covalent modification by ubiquitination (2). Further interactions of the adaptors with phosphorylated phosphatidylinositol derivatives aid in cargo binding (3, 4). Adaptor

*Corresponding author (E-mail: viorica.ivan@biochim.ro)

ROM. J. BIOCHEM., 47, 2, 179–191 (2010)
proteins may recruit clathrin to the membrane and interact with accessory molecules that regulate assembly and disassembly of the coat, vesicle formation, vesicle targeting and interactions with the cytoskeleton (5).

Two main classes of cargo-binding adaptors are known: tetrameric adaptor complexes and monomeric adaptors. Mammalian cells contain four adaptor protein complexes (AP-1, AP-2, AP-3 and AP-4) and three monomeric adaptors, known as Golgi-localized, γ-ear-containing, ARF-binding proteins (GGA1, GGA2 and GGA3). Each of these adaptors mediates membrane cargo sorting at distinct intracellular locations. AP-2 is restricted to the plasma membrane (6) and is involved in the formation of endocytic clathrin-coated vesicles. AP-1 and the GGAs mediate protein sorting at the trans-Golgi network (TGN) and endosomes (7, 8). An AP-1 variant that is present in epithelial cells and the AP-4 complex are thought to mediate polarized sorting of cargo to the basolateral membrane (9, 10). Recent data revealed that the AP-4 complex mediates sorting of the Alzheimer’s disease amyloid precursor protein at the TGN (11). The AP-3 complex functions in trafficking pathways towards the lysosomes and LROs, such as melanosomes and platelet dense granules (12). A neuronal specific form of AP-3 regulates the formation and function of at least a subset of synaptic vesicles from endosomes (13–15).

Certain cell types employ components of the ubiquitous sorting machinery to create novel pathways for protein targeting to specialized LROs. LROs share features with lysosomes and other late endocytic organelles, including intraluminal acidic pH and resident lysosomal hydrolases and membrane proteins. Some LROs, such as melanosomes of melanocytes and retinal pigment epithelial cells coexist with classical lysosomes (16, 17).

Melanosomes can be classified in four morphological stages (18). Stage I consists of vacuolar structures with a bilayered coat and internal vesicles. Stage II melanosomes have an ellipsoidal form with fibrous striations. These early stages lack melanin and are also called premelanosomes. As it is synthesized, melanin deposition begins on the fibers in stage III and fills the entire melanosome in stage IV. The main component of the fibers is the integral membrane protein Pmel17 (19,20), which in stage I melanosomes is enriched in the intralumenal vesicles (21). Sorting of Pmel17 to intralumenal vesicles and its subsequent processing by proteolytic cleavage, both mediated by luminal determinants, represent prerequisites in the formation of fibers (22–24). The fibers act as a matrix for melanosome formation (25).

Hermansky-Pudlak syndrome (HPS) is a genetic disorder in which the formation and function of LROs, including melanosomes and platelet dense granules, are impaired. This syndrome, characterized by partial albinism, excessive bleeding, lung fibrosis and sometimes immunodeficiency and granulomatous colitis, results from mutations in at least eight different genes in humans and their mouse orthologs (26, 27). Each of them causes a particular HPS subtype (Table 1). Another eight genes have been associated with HPS-like phenotypes in rodent models.
### Table 1

HPS protein complexes, human subtypes and mouse models

<table>
<thead>
<tr>
<th>Complex</th>
<th>subunits</th>
<th>human subtype</th>
<th>rodent model*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP-3</td>
<td>β3A δ μ3 σ3</td>
<td>HPS2</td>
<td>pearl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mocha</td>
</tr>
<tr>
<td>BLOC-1</td>
<td>Cappuccino</td>
<td>HPS7</td>
<td>cappuccino</td>
</tr>
<tr>
<td></td>
<td>Snapin</td>
<td>HPS8</td>
<td>sandy</td>
</tr>
<tr>
<td></td>
<td>Dysbindin</td>
<td></td>
<td>reduced pigmentation</td>
</tr>
<tr>
<td></td>
<td>Muted</td>
<td></td>
<td>muted</td>
</tr>
<tr>
<td></td>
<td>Palladin BLOS2</td>
<td></td>
<td>pallid</td>
</tr>
<tr>
<td>BLOC-2</td>
<td>HPS3 HPS5 HPS6</td>
<td>HPS3 HPS5 HPS6</td>
<td>cocoa</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ruby-eye2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ruby-eye</td>
</tr>
<tr>
<td>BLOC-3</td>
<td>HPS1 HPS4</td>
<td>HPS1 HPS4</td>
<td>pale ear</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>light ear</td>
</tr>
<tr>
<td>HOPS /VpsC</td>
<td>Vps33a Vps16a</td>
<td></td>
<td>buff</td>
</tr>
<tr>
<td></td>
<td>Vps11 Vps18 Vps41</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vps39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rab GGTase II</td>
<td>Rab GGTase β</td>
<td></td>
<td>gunmetal</td>
</tr>
<tr>
<td></td>
<td>Rab GGTase α</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monomeric protein</td>
<td>Rab38</td>
<td></td>
<td>ruby (rat)</td>
</tr>
<tr>
<td></td>
<td>xCT</td>
<td></td>
<td>subtle gray</td>
</tr>
</tbody>
</table>

*mouse, unless indicated otherwise

With perhaps one exception, all 16 genes encode proteins with role in cargo sorting and vesicular transport. Most of them are subunits of larger complexes such as the adaptor protein AP-3, the biogenesis of lysosome-related organelle (BLOC)-1, BLOC-2, BLOC-3, the homotypic fusion and vacuolar protein sorting (HOPS) and rab geranylgeranyl transferase II (28). Deficiency in AP-3 causes HPS2 in humans, for which two mouse models exist. BLOC-1 consists of eight subunits, two of which are encoded by genes that are mutated in patients with HPS7 and HPS8 and mouse models. Additional three subunits bear mutations in mouse models. The other two BLOC complexes, BLOC-2 and BLOC-3, consist of three and two subunits, respectively. All five proteins were found mutated in HPS patients and mouse models. Mutations in BLOC-2 components are associated with HPS3, HPS5...
and HPS6, whereas those of BLOC-3 with HPS1 and HPS4 (Table 1; see also (28–30)). Two other proteins that are known to form complexes – Vps33a (31) and RabGGTase II (32) – and the small GTPase rab38 are defective in rodent HPS, but no human disease has yet been associated with mutations in these proteins. The only HPS protein that has not been assigned a role in membrane transport is xCT, the product of $\text{Slc7a11}$ gene, which functions as a Cys-Glu exchanger required for the formation of pheomelanin in melanocytes (33). Several HPS proteins have orthologs in $\text{Drosophila melanogaster}$ and $\text{Caenorhabditis elegans}$ (34).

The pigmentation defects observed in HPS are likely caused by miss sorting of melanogenic enzymes. In this review we discuss the molecular machinery that controls the post-Golgi sorting and transport of tyrosinase and TYRP-1 to melanosomes.

**SORTING OF MELANOSOMAL ENZYMES AT THE TGN**

While early events, such as biosynthesis and maturation of melanosomal enzymes have received considerable attention (35), little is known about how the mature enzymes exit the Golgi apparatus on their way to melanosomes. Early data proposed that melanogenic enzymes use a direct TGN-to-melanosome pathway that depends on clathrin-coated vesicles (21). Indeed, tyrosinase-a type I integral membrane protein contains a dileucin sorting signal in its cytoplasmic tail that is recognized and bound to by the coat complexes AP-1 and AP-3 (36, 37). A similar motif in TYRP1 serves to interact with AP-1 (36). Subsequent studies have implicated AP-3 and AP-1 in sorting of tyrosinase and TYRP1 from the endosomal compartment to melanosomes (36, 38). However, by isolating TGN-derived vesicles from melanocytes, Chapuy and colleagues have shown that incorporation of tyrosinase and TYRP1 in these vesicles was dependent on AP-3 and AP-1, respectively, in addition to other factors such as clathrin, ARF1, nucleotide, temperature and cytosol (39). The latter study suggests that AP-3 and AP-1 mediate sorting of melanosomal enzymes not only in endosomes, but to some extent also at the TGN. However, these exit pathways from the TGN are unlikely to be the major ones for tyrosinase and TYRP1, as both enzymes reach the endosomal compartment in cells lacking AP-3 or AP-1 (36). Interestingly, the TGN exit of TYRP2, another melanogenic enzyme that was reported initially to traffic in the secretory pathway via a TYRP1- distinct route (40), was independent of AP-3 or AP-1 (39).

The intracellular pathway(s) of TYRP1 and tyrosinase require glycosphingolipids, as they are routed through the plasma membrane in the glycosphingolipid-deficient cell line GM95 (41). The fact that in these cells tyrosinase accumulates in the TGN and multivesicular endosomes suggests that glycosphingolipids function in the sorting of melanosomal enzymes at the TGN. The authors propose that the luminal domain of melanosomal enzymes contains a sorting signal that directs them towards a glycosylceramide-dependent intracellular pathway which excludes lysosomal membrane proteins.
SORTING FROM ENDOSONES: CLUES FROM HPS

The pathways by which melanosomal enzymes are delivered from early endosomes towards melanosomes in melanocytes are incompletely understood. Studies on melanocytes isolated from HPS patients and model organisms have unraveled key components of the protein sorting machinery, such as the adaptor complex AP-3 and the BLOC complexes (28). Additional components were identified using melanoma cells or normal melanocytes. Amongst them, AP-1 (21, 36) and the ESCRT-1 complex (42).

AP-3 and AP-1

AP-3 and AP-1 are expressed in all cells, both complexes having also tissue specific isoforms. Whereas lack of ubiquitous AP-1 is embryonically lethal, AP-3-deficient animals are viable and show specific phenotype only in certain cell types (43). Mutations in the AP-3 complex are the cause of HPS type 2 in humans. In melanocytes from HPS2 patients and from pearl mice, tyrosinase is misslocalized to early and late endosomes, arguing for a role of AP-3 in tyrosinase sorting from early endosomes to melanosomes (36, 38). These cells show only partial pigmentation defects because a pool of tyrosinase still reaches the melanosomes. A partially redundant transport pathway of tyrosinase to melanosomes in melanocytes that is mediated by AP-1 has been described (36). Indeed, tyrosinase contains a sorting signal in its cytoplasmic tail that is recognized and bound to by AP-3, and also by AP-1 (36, 37). Consistently, tyrosinase colocalizes with both adaptor complexes on endosomes. Although both AP-3 and AP-1 localize in melanocytes mostly to clathrin-coated buds on tubular regions of early endosomes, they decorate distinct endosomal domains (36). An additional role for AP-1 in sorting of the melanosomal cargo TYRP1 has been inferred from their direct interaction and extensive colocalization in melanocytes (21, 36). The function of AP-1 in membrane transport is not restricted to cargo sorting, but rather to coupling the sorting process to organelle positioning through interaction with motor proteins. During melanosome biogenesis, AP-1 and the kinesin KIF13A create recycling endosomal subdomains and position them close to melanosomes to which they connect, thus ensuring efficient cargo delivery to melanosomes (44). An interaction between AP-1 and KIF13A has been previously reported to mediate transport of mannose-6-phosphate receptor from the TGN to the plasma membrane in non-melanocytic cells (45). Another complex formed by AP-1 with the kinesin motor KIF5 and the accessory protein Gadkin directs transport of TGN-derived endosomal vesicles in HeLa cells (46).
The BLOCs

Similar to APs, the BLOCs are also ubiquitously expressed, but their defects are restricted to certain specific cell types. BLOC-1-deficient melanocytes show accumulation of TYRP1 in early endosomal vacuoles and at the cell surface, whereas localization of tyrosinase is only mildly affected (47,48). Immunoelectron microscopy analysis of AP-3 and BLOC-1 in human melanoma cells revealed that the two complexes localize to distinct endosomal domains, with AP-3 enriched in buds as opposed to BLOC-1 on tubules (47), which is consistent with their different effects on TYRP1 trafficking. These data argue that AP-3 and BLOC-1 define distinct sorting pathways from endosomes to melanosomes, in spite of their physical interaction, which may regulate each other’s association to the endosomal membrane (47). BLOC-2 localizes to early endosome-associated tubules (47) and seems to function in the same pathway as BLOC-1. Evidence for this comes from the analysis of melanosomal protein distribution in BLOC-2-deficient melanocytes. Whereas tyrosinase localization is not significantly affected, TYRP1 again accumulates in endosomes, but in a vesiculo-tubular compartment which seems to be downstream of that in BLOC-1-deficient cells. In addition, TYRP1 undergoes lysosomal degradation (47). These phenotypes suggest that BLOC-2 functions downstream of BLOC-1.

The function of the BLOC-3 complex is less understood. Whereas in non-pigment cells BLOC-3 regulates the motility of late endosomes and lysosomes (49), in melanocytes it was proposed to mediate transport of TYRP1 to melanosomes (50). Bioinformatics analyses have revealed the HPS1/HPS4 complex is evolutionary conserved (51). HPS1 and HPS4 contain an N-terminal CHiPS or longin domain homologous to that found in the Mon1/Ccz1 complex, which serves as a GEF for ypt7 (52) and is required for the conversion of rab5- into rab7 endosomes (53, 54). We recently showed that the BLOC-3 complex interacts directly through HPS4 with the active form of the late endosomal GTPase rab9 (55), suggesting that the BLOC-3 complex might function as a rab9 effector in the biogenesis of LROs.

The ESCRT-I complex

A novel endosome-to-melanosome pathway for TYRP1 has been recently described in normal melanocytic cells (42). This pathway involves the ESCRT-I component Tsg101 (56, 57), but not the endosomal coat protein Hrs that functions in sorting of ubiquitinated cargo to lysosomes (58). siRNA depletion of Tsg101 results in accumulation of TYRP1 in aberrant endosomal membranes that are distinct from those containing the ESCRT-dependent melanosomal membrane protein MART-1 (42). Based on the lack of TYRP1 recycling to the plasma membrane, the authors propose that ESCRT-1 functions downstream of BLOC-1 in cargo sorting from endosomes to melanosomes.
RAB PROTEINS IN BIOGENESIS OF MELANOSOMES

Rab GTPases are key regulators of vesicular transport. They are expressed ubiquitously, with a few members having restricted localization to certain tissues where they fulfill more specialized roles. Amongst those, the highly homologous rab38 and rab32 proteins are expressed in melanocytes, although expression in other cell types has also been documented (59). The rab38 gene is mutated in ruby rats (60), which suffer from HPS, and in the chocolate mutant mice (61). The chocolate mouse is not considered a bona fide HPS model because it lacks the platelet dense granule defects. In melanocytes from chocolate mice pigmentation is not severely impaired, likely due to the existence of a rab38-redundant pathway mediated by rab32. Depletion of rab32 by RNAi in melanocytes of chocolate mice causes a much reduced pigmentation associated with alteration of tyrosinase and TYRP1 localization and melanosome morphology. In melanocytes, both rab38 and rab32 localize to melanosomes and vesiculo-tubular structures carrying tyrosinase and TYRP1 (59), arguing for a role in cargo transport to melanosomes.

Another rab member involved in melanosomal transport and motility is the late endosomal protein rab7 (62). Depletion of rab7 by RNAi or expression of a dominant negative rab7 mutant interferes with tyrosinase and TYRP1 trafficking, but how rab7 functions in their transport is not yet clear. Rab7 has also been involved in the motility of premelanosomes through its complex with RILP (rab7-interacting lysosomal protein) and dynein (63). Given the conversion of rab5 to rab7 on the endosomal membrane in achieving progression from early to late endosomes (64), a similar mechanism involving rab7 could operate in the formation of melanosomes.

FUNCTION OF OTHER HPS-ASSOCIATED PROTEINS

The homotypic fusion and protein sorting (HOPS) complex has initially been implicated in homotypic vacuole fusion and vacuole protein sorting in yeast (65) or in fusion of endosomal organelles in mammalian cells (66). Subsequent work has shown that the HOPS complex mediates transport to and from early endosomes (67, 68). Mutations in the murine gene encoding Vps33a cause HPS-like phenotype in the buff strain (69). Vps33a is a member of the Sec1/Munc18 family of proteins that function as regulators of SNARE complex formation (70). As Vps33a interacts directly with the endosomal SNARES syntaxin 6, 7 and 13 (66), the HOPS complex may be involved in regulating the assembly of SNARE proteins that are required for transport from endosomes to melanosomes.

Rab geranylgeranyl transferase α (RabGGTase α) catalyzes the prenylation of rab GTPases, a prerequisite for the membrane association of the latter (71, 72).
RabGGTase α is mutated in the gunmetal mouse, which displays defects in platelet α and dense granules and hypopigmentation (32, 73). Both platelets and melanocytes show decreased prenylation and, as a consequence, misslocalization of certain rab proteins to the cytoplasm, which is likely to account for the observed phenotype.

CONCLUSIONS AND PERSPECTIVES

In spite of the great progress in the field over the past ten years, to which we owe the identification of the molecular machinery involved in sorting of melanosomal proteins to the melanosome and its site of activity, the characterization of the transport pathways is still in its infancy. A schematic representation of the biosynthetic pathways for tyrosinase and TYRP1 and their corresponding transport machinery to melanosomes is shown in Fig. 1. Of a great importance to us is to understand how distinct endosomal domains that mediate different transport pathways towards the melanosome are formed and maintained without intermixing and how the APs and the BLOCs

![Fig. 1. – Schematic representation of the intracellular pathways of melanosomal enzymes. Tyrosinase (continuous line) and TYRP1 (dashed line) exit the TGN via two distinct pathways that require, at least in part, AP-3 and AP-1, respectively and depend on glycosylceramide. From endosomes, a pool of tyrosinase is transported to melanosomes in an AP-3-dependent manner, whereas another pool of tyrosinase and TYRP1 are transported through a second pathway that is regulated by AP-1 and KIF13A. BLOC-1 and BLOC-2 act in the same pathway for TYRP1. Rab32/38 could also function in endosome-to-melanosome transport routes of tyrosinase and TYRP1, but the exact site is not known.

A different pathway from endosomes directs endocytic cargo to lysosomes (dotted line).]
direct melanosomal cargo specifically to melanosomes and prevents it from entering the lysosomal degradation pathway. The fact that the same sorting machinery specifically operates in cargo transport to melanosomes in pigment cells, as opposed to late endosomes and lysosomes in generic cells, suggests that additional factors, including cell type-specific, contribute to the sorting of melanosomal proteins. The identification of these factors will shed light onto the mechanism of cargo sorting to melanosomes, with impact on melanosome biogenesis.

REFERENCES


