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Lysosome-related organelles: Unusual compartments become mainstream

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Abstract

Lysosome-related organelles (LROs) comprise a group of cell type-specific subcellular compartments with unique composition, morphology and structure that share some features with endosomes and lysosomes and that function in varied processes such as pigmentation, hemostasis, lung plasticity and immunity. In recent years, studies of genetic diseases in which LRO functions are compromised have provided new insights into the mechanisms of LRO biogenesis and the regulated secretion of LRO contents. These insights have revealed previously unappreciated specialized endosomal sorting processes in all cell types, and are expanding our views of the plasticity of the endosomal and secretory systems in adapting to cell type-specific needs.

Introduction

The endocytic pathway fulfills many important functions in all cells, but additional adaptations of the endosomal system in specialized metazoan cell types underlie the formation of lysosome-related organelles (LROs). LROs comprise a group of functionally diverse compartments that share features with lysosomes but are distinct and harbor specific cargoes that confer their unique properties [1]. Consistent with their distinct functions, LROs vary in composition and morphology, ranging from pleiomorphic secretory granules in platelets, cytotoxic T lymphocytes (CTLs) and other hematopoietic cells to enormous fluid-filled vacuoles in the vertebrate notochord and complex subcompartmentalized structures such as pigment cell melanosomes, endothelial cell Weibel-Palade bodies, or platelet α granules. Maturing phagosomes in phagocytes such as dendritic cells, neutrophils and macrophages receive unique contents from endosomes, and thus can also be considered “inducible” LROs. LROs are diverse not only in morphology, but also in the origin of their membranes, the derivation of their contents from secretory and endosomal sources, and the

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complement of machineries exploited for their formation, maturation and secretion. Here we highlight recent advances in our understanding of these properties, focusing on a few areas that hold particular promise for future breakthroughs.

LRO biogenesis: Origin of LRO precursors

All LROs progressively mature from precursors by acquiring specialized cargoes and generating a lumenal environment conducive for their function (Figure 1). However, the origin of the precursor differs, as exemplified by four well-studied LROs – pigment cell melanosomes, endothelial cell Weibel Palade Bodies (WPBs), platelet α granules and CTL and natural killer (NK) cell lytic granules (LGs) (Figure 2). Non-pigmented melanosome precursors, or premelanosomes, develop from intermediate compartments with features of early endosomal vacuolar domains with few intraluminal vesicles (ILVs) and extended bilayered cytosolic coats [2]. The ILVs scaffold the polymerization of amyloid fibrils by the pigment cell-specific protein PMEL [3]; the fibrils assemble into sheets that distend the organelle into an ellipsoidal shape [2] and template melanin deposition [3]. Accordingly, pigment cells in *Pmel*$^{-/-}$ mice accumulate melanin in round melanosomes that lack characteristic striations, but that nevertheless remain segregated from the endolysosomal system [4]. Immature WPBs emerge from the *trans* Golgi Network (TGN) as von Willebrand Factor (vWF) assembles into tubules that shape the nascent compartment into a cigar-shaped organelle [5]. Platelet α granules derive from late endosomes/ multivesicular endosomes (MVEs) in megakaryocytes, and harbor both biosynthetic (e.g. vWF) and endocytic (e.g. fibrinogen) contents. Mature α granules retain CD63 on ILVs as they segregate from endosomes [6]. Whereas matured melanosomes, WPBs and α granules coexist with classical lysosomes, LGs are likely modified lysosomes with a ring of ILVs surrounding a dense core [7] that further mature upon T cell activation. The dense cores might derive like conventional secretory granules from the TGN and later fuse with MVEs to form a hybrid organelle (Figure 1).

Melanosomes and WPBs as model LROs

LROs such as LGs and platelet dense granules lack well-organized structural scaffolds, but WPBs and melanosomes develop around rigid matrices.

PMEL is an integral membrane protein that is proteolytically processed into (i) a luminally-exposed amyloidogenic domain that generates the melanosomal fibrils, and (ii) integral membrane fragments that are eventually degraded [3]. Association of the PMEL luminal domain with ILVs requires the tetraspanin (TSP) CD63, but – unlike for most known ILV cargoes – not ubiquitylation or ESCRT function [8,9••]. CD63 is also required for the sorting and proteolytic maturation of elastase – a component of primary granules in neutrophils [10] – suggesting that TSPs on endosomes might generally function to retain proteins that are destined for LROs and prevent them from ESCRT-dependent degradation. This might explain why CD63 associates with most LROs despite associating with late endosomes/ lysosomes in cells that lack LROs [11]. In melanocytes, the integral membrane PMEL fragments are degraded in an ubiquitin- and ESCRT-dependent manner [9••], thus illustrating independent sorting mechanisms for different topological domains of the same protein.

The tubular vWF structures that assemble at the TGN during WPB biogenesis differ substantially from the PMEL amyloid fibrils. Electron tomography identified the vWF tubules as regularly-spaced helical structures [12–14]. The vWF helices likely aggregate to form a paracrystalline core in the TGN [14], causing retention and ultimate segregation into distinct membrane structures that retain cargoes such as P-selectin or insulin growth factor binding protein-7 (IGFBP7) [15]. Interestingly, vWF in platelet α granules forms shorter

tubules that typically accumulate around a central core of heterogeneous, electron dense material (Figure 2), and are not responsible for the tubular shape of α granules [6].

LRO biogenesis: Precursor maturation

Whether they originate from endosomal or TGN membranes (Figure 1), LRO precursors mature by acquiring key transmembrane components – such as melanogenic enzymes in melanosomes or serotonin and calcium transporters in platelet dense granules – via membrane trafficking. Maturation also provides a means to acquire effectors that are required for LRO motility or secretion (Figure 1). LROs mature via an intimate dialogue between the immature organelle and MVEs and/or specialized domains of early sorting and recycling endosomes, ensuring that critical cargoes are diverted from classical endo-lysosomes and toward maturing LROs. Multiple non-redundant pathways generally deliver distinct transmembrane protein cargoes to the same maturing LRO, as exemplified by cargo acquisition from the TGN and early endosomes during WPB maturation, and from distinct early endosomal domains during melanosome maturation (Figure 1). The use of multiple pathways ensures that critical complex functions – such as the onset of melanin synthesis by convergence of the copper-dependent Tyrosinase enzyme with the copper transporter ATP7A [16] – are only observed in the mature organelle.

Hermansky-Pudlak syndrome and cargo delivery

Hermansky-Pudlak syndrome (HPS) and its mouse models comprise a group of genetic disorders characterized by malformation of melanosomes, platelet dense granules, and in some cases other LROs [17]. The affected genes encode subunits of five cytoplasmic multisubunit protein complexes – adaptor protein (AP)-3, vacuolar protein sorting (VPS)-C, and biogenesis of lysosome-related organelles complex (BLOC)-1, -2 and -3 [17] – that impact membrane trafficking and/or protein sorting to facilitate LRO maturation. The complexes are ubiquitously expressed, but altered expression levels, post-translational modifications, and/or unique interactions with trafficking machinery in LRO-containing cell types support their functions in critical non-redundant trafficking pathways. For these reasons, their loss of function preferentially impacts LRO maturation.

AP-3 and VPS-C

AP-3 is a heterotetrameric adaptor that engages transmembrane cargoes via cytoplasmic sorting signals in early endosomes, and packages them into clathrin-coated transport vesicles [18]. AP-3 is required for the biogenesis of eye pigment granules in *Drosophila melanogaster* and gut granules in *Caenorhabditis elegans*, and for delivery of multiple cargoes to vertebrate LROs [18] (Figure 1), including OCA2 to melanosomes [19,20], CD63 to WPBs [21], toll-like receptor 9 (TLR9) to an IRF7-signaling LRO in plasmacytoid dendritic cells [22], TLR4 to phagosomes in conventional dendritic cells [23•], and SLC35D3 likely to dense granules in platelets [24]. The heterotetrameric VPS-C core (containing VPS11, 16, 18 and 33) assembles into two larger complexes in the yeast *Saccharomyces cerevisiae*, HOPS and CORVET [25]. VPS-C/ HOPS in *S. cerevisiae*, *D. melanogaster* and vertebrates regulates tethering and SNARE-dependent fusion within the endosomal system [26,27], including of AP-3-coated vesicles with the lysosome-like vacuole [28]. Metazoans express distinct VPS-C complexes containing alternative VPS33 and VPS16-like isoforms. The mouse Vps33a *buff* mutation impacts melanosome and platelet dense granule maturation [29] and mutations in *D. melanogaster* VPS33A or VPS16A cause defects in eye pigment granules [30]. By contrast, mutations in *VPS33B* or the VPS16-like *VIPAS39* underlie Arthrogryposis-Renal dysfunction-Cholestasis (ARC) syndrome [31,32] characterized by a loss of platelet α granules among other system defects [33,34].

BLOC-1

The eight subunits of BLOC-1 lack recognizable functional domains, and the molecular functions of the complex are not known. In melanocytes, BLOC-1 localizes to endosomal tubules [35] and is required to export melanosomal cargoes from vacuolar early endosomes [16,36,37] into recycling endosome-derived tubular carriers that fuse with melanosomes [38]. However, BLOC-1 unlikely functions as a sorting adaptor, as its architecture – a linear chain of eight globular domains [39•] – is unlike other adaptors, and direct interactions between BLOC-1 and cargoes have not been observed. BLOC-1 binds in vitro to endosomal Q SNAREs Syntaxin 13 and SNAP-25 [40], and might either sort them into transport carriers or regulate their interaction with a partner R-SNARE. A recent proteomics analysis identified tethering factors and two members of the peroxiredoxin family of oxidoreductases as additional binding partners [41].

AP-3 and BLOC-1 physically interact [35] and coordinately regulate cargo distribution in neurons [42,43]. However, in melanocytes BLOC-1 and AP-3 localize distinctly on early endosomes to tubular domains and buds, respectively, [35,36], and BLOC-1 functions in cargo transport to melanosomes either independently of AP-3 (as for ATP7A and the melanogenic enzyme TYRP1 [16,36]) or in conjunction with AP-3 (as for the transporter OCA2 [20]). Similarly, BLOC-1 regulates transport of cargoes that are both AP-3-dependent and independent to gut granules in *C. elegans* [44•]. This suggests that BLOC-1 acts in conjunction with sorting adaptors to effect cargo transport.

BLOC-2, BLOC-3, RAB32 and RAB38

The two-subunit BLOC-3 is a guanine nucleotide exchange factor (GEF) for two tissue-specific Rab GTPases, RAB38 and RAB32 [45••], that regulate cargo delivery to nascent melanosomes [46,47], platelet dense granules [48], lamellar bodies [49,50], and notochord vacuoles [51••]. How these two partially redundant small GTPases function is not clear. When bound to GTP, both interact with AP-3, the heterotetrameric BLOC-2 – which functions downstream of BLOC-1 in cargo transport [35,36,52] – and the heterotetrameric adaptor AP-1 [53]. They also both bind to VARP [54], a putative scaffold that engages and maintains the R-SNARE VAMP7/TI-VAMP in an inactive conformation [55,56••]. This suggests that RAB32 and RAB38, through multiple effectors, integrate cargo sorting into transport carriers and fusion of the carriers with target LROs. The latter would be consistent with the localization of a cohort of endogenous or overexpressed epitope tagged-RAB32 and/or -RAB38 to LROs [45••,46,48,50,51,53]. RAB32 and RAB38 activation is under complex regulation. VARP is a GEF for the early endosomal Rab GTPase, RAB21 [57], and BLOC-3 is an effector of RAB9 [58], implying that RAB32 and RAB38 participate in several Rab cascades during LRO maturation. Moreover, the existence of GEFs for RAB38 orthologues in *C. elegans* [59] and *D. melanogaster* [60] that are unrelated to BLOC-3 suggests that additional GEFs might exist in mammals, perhaps explaining why melanosome biogenesis is differentially affected by loss of BLOC-3 function in different pigment cell types [61].

Other components of the biogenesis machinery

LYST and NBEAL2

Mutations in two members of a family of large proteins with a conserved BEACH domain cause LRO biogenesis disorders. Mutations in LYST/ CHS1 underlie Chediak-Higashi syndrome, in which many LROs and conventional lysosomes are grossly enlarged, but the molecular function of this 3801-residue protein is unclear. The orthologous lvsB controls cargo transport from lysosomes to an LRO in *Dictyostelium discoideum* [62], perhaps by antagonizing RAB14-dependent fusion between lysosomes and LROs [63]. Alternatively,

LYST might promote lysosome or LRO fission, as it does in macrophages [64]. Mutations in another BEACH protein, NBEAL2, underlie Gray Platelet Syndrome, characterized by an absence of platelet α granules [65–67•]. How NBEAL2 functions is unclear, but a role in fission of α granule contents from MVBs would parallel the proposed function of LYST in macrophages.

AP-1 and motors

A second ubiquitous heterotetrameric adaptor family member – AP-1 – plays an important but varied role in LRO generation. In melanocytes, AP-1 binds to targeting signals in several melanosome cargoes, [20,38,68,69] and is required for delivery of TYRP1 to mature melanosomes [38]. However, it is not clear whether the cargo sorting function of AP-1 is required. AP-1 on endosomal buds binds to the microtubule plus end-directed kinesin motor, KIF13A, promoting the extension of tubular Rab11-positive recycling endosomes to the cell periphery. This apposes the endosomes to maturing melanosomes, facilitating cargo delivery via the tubular transport carriers [38]. AP-1 also binds to TSG101, an ESCRT-I subunit involved in MVB formation [70] that is also required in TYRP1 delivery to melanosomes [71]. In endothelial cells, AP-1 and clathrin function as a scaffold to maintain the compacted, cigar-like structure of WPBs which is necessary for positioning vWF polymers to “unfurl” upon WPB secretion and for consequent platelet adhesion to the endothelial cell [5]. AP-1 plays a secondary role in facilitating WPB secretion through recruitment of its cofactors, amphiphilin and γ-synergin [72]. Platelet α granules are also bordered by clathrin lattices [6], but whether they contribute to α granule morphogenesis is not known.

Cytoskeletal motors have important roles in LRO motility and secretion (see below), but unconventional myosins function in LRO biogenesis likely by effecting actin rearrangements that facilitate membrane dynamics involved in budding and fusion. For example, in melanocytes, myosin VI – an actin-based motor involved in endocytic recycling – recruits actin to mature melanosomes and regulates melanosome size and melanization [73].

LRO secretion: polarization and docking

Most, if not all, LROs release their lumenal contents by secretion into the extracellular space or directly to neighbouring cells in response to signaling. This requires LRO transport to the cell periphery and stimulus-dependent fusion of the LRO and plasma membranes.

In endothelial cells, mature WPBs appear “ready” to release vWF – with the assistance of actin/ myosin II-based contractile forces – upon stimulation [74••]. Similarly, in resting platelets, fully matured α granules, dense granules and lysosomes are primed for stimulus-dependent fusion, but the kinetics of their release differ. Dense granules are likely docked at the plasma membrane and released immediately upon stimulus [75•], whereas content release from α granules is more heterogeneous and slightly delayed [75•,76]; the heterogeneity might reflect packaging into distinct α granule subsets [76,77] or spatial segregation within internally heterogeneous α granules [6]. Unlike platelet granules and WPBs, LGs in resting CTLs are functionally immature. Final maturation and fusion are triggered by target cell engagement, inducing the fusion of LGs with RAB11-containing compartments [78] (Figure 1) that deliver effectors required for positioning, fusion with the plasma membrane or both.

Genes mutated in Griscelli syndrome (GS) encode essential components of the molecular machinery that allow for LRO docking at the cell periphery. In melanocytes, RAB27A (GS1), myosin VA (GS2) and melanophilin (GS3) form a complex that tether melanosomes to cortical actin, permitting their ultimate transfer from dendritic tips to keratinocytes

(reviewed in [79]). WPB and LG secretion also require RAB27A at multiple steps, but the effectors are distinct from those on melanosomes. WPB secretion requires coordination of RAB27A, RAB15, and their effector MUNC13-4 with RAB3 [80], and is regulated by a balance between RAB27A availability, Slp4-a as a stimulating effector, and MyRIP as an inhibitory effector [81•]. In CTLs, a complex of the RAB27A effector Slp3 with kinesin-1 drives LGs to the plasma membrane for ultimate secretion [82•]. RAB27A and MUNC13-4 play independent roles in stimulus-induced LG maturation [78], but a Rab27A-MUNC13-4 complex is subsequently required to tether LGs to the plasma membrane for secretion [83•].

Stimulus-dependent LG secretion in CTLs and NK cells is under tight control to ensure that lumenal cytolytic contents are directed only toward a target cell at the immunological synapse. LGs polarize toward the synapse in association with the centrosome, which is repositioned toward the plasma membrane upon target cell contact [84,85••]. Centrosome motility is tightly controlled by the strength of T cell signaling [86], at least in part via activation of the Lck tyrosine kinase [86], and requires the minus end-directed microtubule motor dynein [84,87]. Apposition of LGs to the plasma membrane further requires actin rearrangements [84,88]. A similar mode of centrosome positioning and docking targets MHC class II compartments to the immunological synapse of B lymphocytes [89].

LRO secretion: FHL and the fusion apparatus

Familial hemophagocytic lymphohistiocytosis (FHL) is a genetic disorder characterized by unchecked lymphocyte expansion and inflammation due to impaired LG function in CTLs and NK cells [90]. FHL types 3–5, which are additionally associated with bleeding diathesis, reflect impaired LG and platelet granule release. FHL4 results from gene mutations in the Qa SNARE Syntaxin 11 (STX11). FHL4 CTLs and NK cells do not undergo target cell-induced LG degranulation [91,92], and STX11-deficient platelets fail to secrete α granule and dense granule contents in response to agonists [93]. This implies that a STX11-containing tSNARE mediates a fusion step required for LRO release. FHL5 results from mutations in STXBP2 encoding the Sec1-Munc18 family member, Munc18-2/ Munc18b [94]. Munc18b binds to and stabilizes STX11 and facilitates SNARE complex formation; accordingly, FHL4 and FHL5 CTLs and platelets show similar degranulation defects [93–95••]. In platelets STX11 and Munc18b complex with the Qbc SNARE SNAP-23 on the plasma membrane and the R-SNARE VAMP8 on granule membranes to mediate fusion and granule content release [93,95••]. A similar SNARE complex likely functions during LG degranulation in CTLs and NK cells, but where the complex forms in these cells is less clear. STX11 localizes in CTLs and NK cells primarily to intracellular structures that lack RAB27A [92,96] and that might be identical to the RAB11-containing compartments that fuse with immature LGs upon CTL stimulation [78]. This fusion step in CTLs requires the RAB27A effector MUNC13-4 [78]. MUNC13-4 is a SNARE interacting protein that is mutated in FHL3, is required for degranulation of LGs [97] and platelet granules [98], and forms a complex with STX11 and Munc18b in platelets [95••]. Together, the data suggest that whereas STX11/Munc18b/MUNC13-4-dependent SNARE complex formation directly mediates degranulation in platelets, it may mediate a preparatory maturation step in CTLs and NK cells.

Perspectives

While our understanding of LRO biogenesis and secretion has deepened considerably in recent years, many questions remain and will likely be the focus of study in coming years. Firstly, how do LRO precursors such as premelanosomes and α granules segregate from the endosomal system? In both cases, both LROs and lysosomes emerge from common MVEs, and segregation appears to be independent of structural rigidity imposed by vWF or PMEL.

Differential sorting of distinct cargo domains into separate ILVs might reflect a general feature of LRO precursors. Secondly, how is cargo assembly differentially regulated in distinct LRO-producing cells? For example, vWF polymerizes into elongated tubules in endothelial cell WPBs but into shorter tubules in α granules. Third, while we have amassed a reasonable "parts" list for both LRO maturation and the docking and fusion apparatus for secretion, the mechanisms by which these parts are integrated to effect their function is not yet understood. Understanding these mechanisms will require a combination of systems analyses, biochemical analyses of binding interactions among machinery components, and functional analyses of model cell types expressing targeted mutations that disrupt component interactions. Finally, comparative analyses between LRO-generating cell types will likely provide novel insights into common and distinctive features of LROs. For example, FHL is not characterized by hypopigmentation, and thus melanin transfer to keratinocytes is not likely mediated by the same fusion complex employed by platelets or CTLs to secrete their granules; indeed, a recent study suggests that melanocytes release melanosome clusters in dendritic tips by abscission of the plasma membrane in regions of adherence to keratinocytes [99]. Validation and molecular dissection of this mechanism might reveal novel ways in which LRO contents are released.

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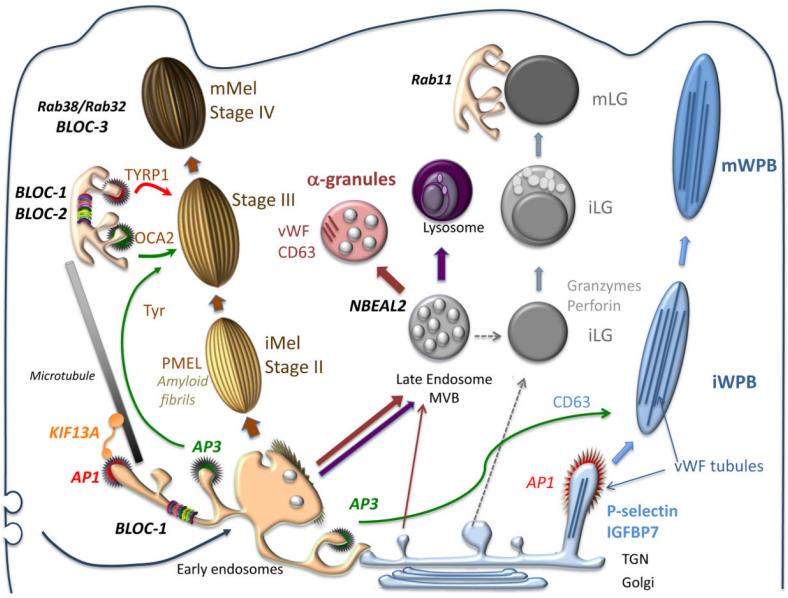

Figure 1. Model for biogenesis and docking of four vertebrate LROs

Shown are models for the biogenesis of immature (i) and mature (m) melanosomes (left, orange), platelet α granules (pink), lysosomes (violet), CTL LGs (gray) and WPBs (right, blue) relative to the endocytic and biosynthetic pathways (Golgi, TGN, early endosomes, late endosomes/ MVBs, and lysosomes). Key cargo molecules discussed in the text are noted in the same color as the LRO, and effectors involved in biogenetic steps are labeled in black text. Arrows indicate relevant trafficking pathways. Left, immature melanosomes (iMel) emerge from vacuolar domains of early endosomes, and mature by cargo delivery from tubulovesicular domains of early endosomes through AP-1- or AP-3-coated vesicles; recycling endosomal domains associated with KIF13A and AP-1 migrate along microtubules towards maturing melanosomes for delivery of some cargoes as indicated. BLOC-1 facilitates tubule-mediated transport; BLOC-2, BLOC-3, RAB32 and RAB38 likely function downstream. Platelet α granules derive in an NBEAL2-dependent process from late endosomes/ MVBs within megakaryocytes, and receive both biosynthetic and endocytic cargoes. MVBs in the same cells also fuse with lysosomes to deliver other cargoes. In CTLs and NK cells, immature LGs (iLGs) also derive by fusion of MVBs with dense core structures, and then fuse with recycling endosome-derived structures upon stimulation by target cells to form mature LGs (mLGs). The dense cores of iLGs contain perforin and granzymes that likely aggregate within the TGN. Likewise, vWF forms tubules in the TGN of endothelial cells, that then bud off perhaps together with cargoes such as P-selectin and IGFBP7 to form immature WPBs; other cargoes, such as CD63, are then delivered from early endosomes in an AP-3-dependent manner.

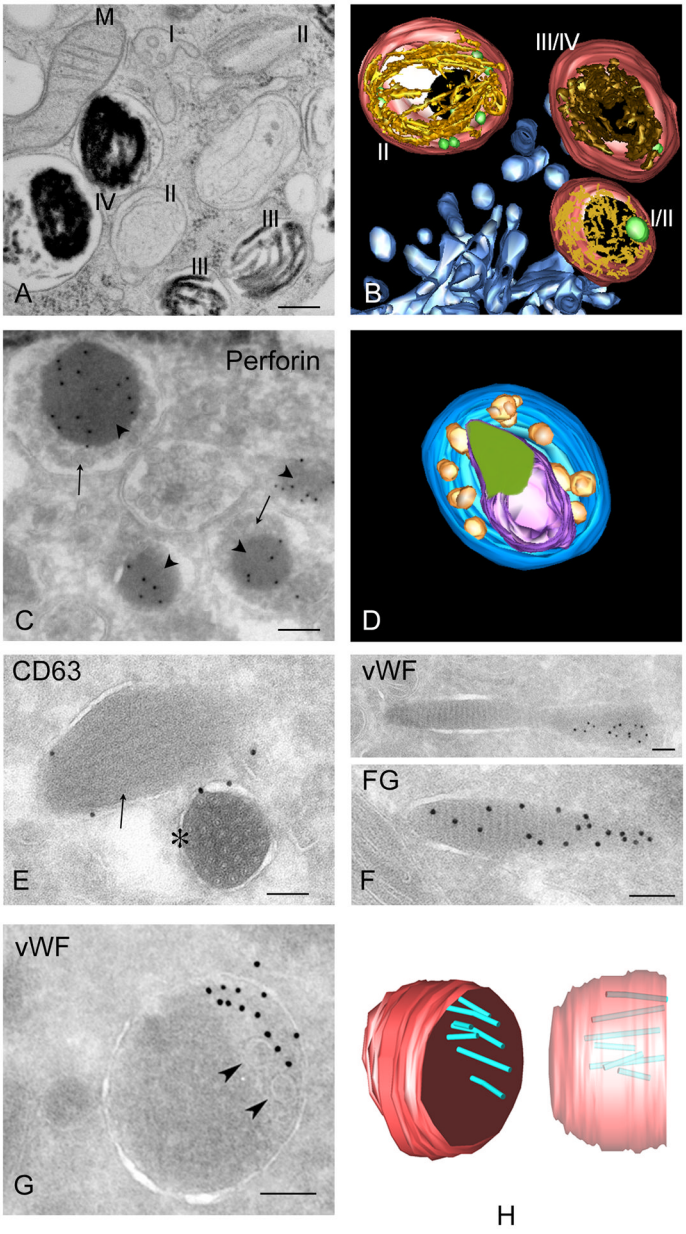

Figure 2. LRO ultrastructure

Shown are images from electron microscopy analyses or three-dimensional (3d) reconstructions of electron tomograms for four model LROs discussed in this review. A, B, melanosomes from MNT-1 human melanoma cells fixed by high pressure freezing and embedded in plastic by freeze substitution. A, a thin section emphasizing stage I and II premelanosomes and stage III and IV mature melanosomes, as indicated. Note the striated appearance in stages II and III. M, mitochondrion. B, 3d reconstruction emphasizing fibrillar structure (yellow) emanating from intralumenal vesicles (green) in stage I/ II melanosomes. Note the accumulation of melanin (brown) on the fibrils in a stage III melanosome. Melanosome limiting membranes are indicated in red; surrounding endosomal tubules are indicated in blue. C, D, cytolytic granules from primary human CTLs. C, an ultrathin cryosection was immunogold labeled for perforin. Note labeling over dense cores (arrowheads), with surrounding ILVs (arrows). D, 3d reconstruction emphasizing dense core

(green) surrounded by multilamellar membranes (violet) and ILVs (yellow). The limiting membrane is pseudocolored blue. E, WPBs within an ultrathin cryosection of a human umbilical vein endothelial cell are shown, immunogold labeled for CD63. Note the tubular vWF polymers captured in profile (arrow) or cross-section (*). F, G, H, α granules within human platelets. F, G, ultrathin cryosections were immunogold labeled for vWF or fibrinogen (FG). Note that fibrinogen is present throughout the tubular α granules but vWF is polarized to one side, adjacent to ILVs (arrows in G). H, 3d reconstruction emphasizing vWF tubules (blue) polarized to one side of a spherical α granule domain, shown in two orientations; the limiting membrane is pseudocolored red. Bars: A–D, 200 nm; E–H, 100 nm.

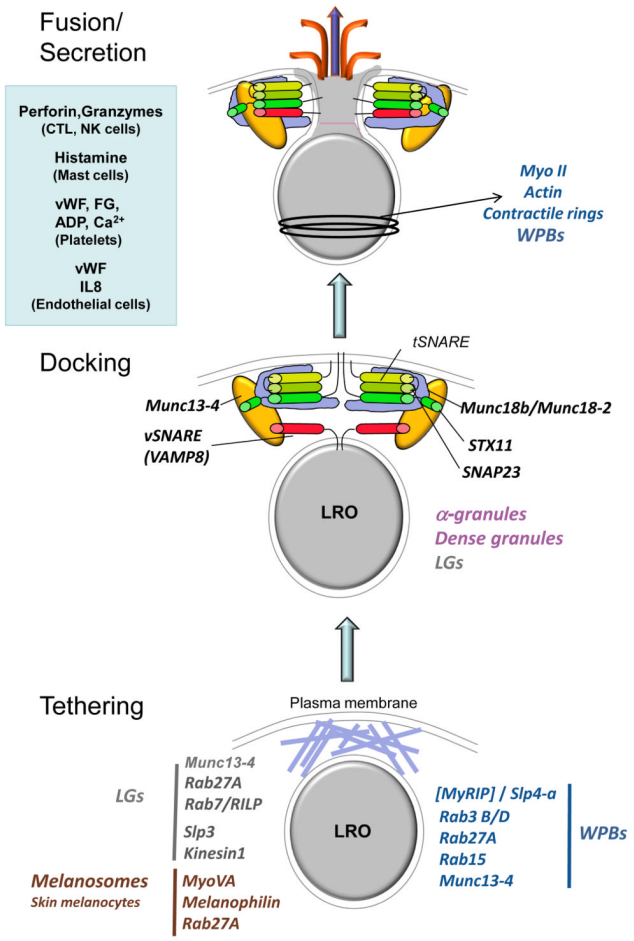

Figure 3. Model for molecular control of LRO secretion

Shown is a schematic diagram of discrete steps in LRO secretion. *Tethering* (bottom) refers to the attachment of LROs to the subplasmalemmal actin cytoskeleton (royal blue network). RAB27A facilitates tethering of LGs, melanosomes and WPBs, but by distinct effectors as shown, and in several kinetically distinct stages that have not yet been detailed. In CTLs and NK cells, LGs become tethered following centriole polarisation in a process requiring RAB7, its effector RILP, and dynein (not shown). RAB27A and Munc13-4 function independently to regulate fusion of immature LGs with RAB11-positive exocytic vesicles (see Figure 1), and then together at a later step of tethering; RAB27A also functions together with a Slp3/kinesin-1 complex at a yet undefined stage. In endothelial cells, RAB27A, RAB15 and their joint effector, Munc13-4, facilitate WPB tethering; the RAB27A effectors Slp4-a and MyRIP also function in a mutually antagonistic manner at a distinct stage. In melanocytes, a complex of RAB27A, melanophilin and Myosin VA (MYOVA) recruit melanosomes from microtubules to cortical actin. *Docking* (middle) refers to the engagement of SNAREs on the LRO and target membranes in a pre-fusion complex. In platelets, docking is mediated by VAMP8 on α granule and dense granule membranes, a syntaxin 11 (STX11)/ SNAP-23 complex on the plasma membrane, and accessory proteins Munc13-4 and Munc18b/MUNC18-2. The same components promote docking in CTLs and NK cells for LG secretion, but it is not yet clear whether this is at the plasma membrane or at a pre-secretory step. *Fusion/ secretion* involves the zippering of opposing SNAREs, applying force to fuse the LRO and plasma membranes. This permits release of the lumenal LRO contents; a few examples of some LRO contents are indicated in the blue box. In

endothelial cells, contractile forces from actin and myosin II are required to “squeeze” the elongated vWF tubules from fused WPBs. In skin melanocytes, it is not yet clear whether melanin is secreted like other LRO contents or whether melanin transfer is mediated by a non-secretory process.