SECRETORY LYSOSOMES

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Regulated secretion of stored secretory products is important in many cell types. In contrast to professional secretory cells, which store their secretory products in specialized secretory granules, some secretory cells store their secretory proteins in a dual-function organelle, called a secretory lysosome. Functionally, secretory lysosomes are unusual in that they serve both as a degradative and as a secretory compartment. Recent work shows that cells with secretory lysosomes use new sorting and secretory pathways. The importance of these organelles is highlighted by several genetic diseases, in which immune function and pigmentation — two processes that normally involve secretory lysosomes — are impaired.

HAEMATOPOIETIC LINEAGE
The developmental series of cells that are derived from haematopoietic stem cells, which produce blood cells.

MELANOCYTE
A type of pigmented cell that can synthesize and store melanin in melanosomes.

LYSOSOMAL HYDROLASE
A soluble enzyme that is found in lysosomes that are involved in the hydrolytic breakdown of macromolecules. These include proteases, lipases and glycosidases.

CYTOTOXIC T CELL
(CTL). A T cell that can kill other cells. These are important in host defence against most viral pathogens.

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Most cells store their secretory products in specialized secretory granules, which can fuse with the plasma membrane when required. Conversely, components such as proteins, lipids and peptides are removed at the plasma membrane by the process of endocytosis. The endocytosed load is then delivered to the lysosome to be degraded (FIG. 1). Some cells, however, have modified their lysosomal compartment so that it also functions as their secretory compartment. This dual-function secretory–lysosomal organelle — termed a secretory lysosome — is unusual in that, in addition to the main function of conventional lysosomes (that is, the degradation of 'old' proteins), it is also used for storage of newly synthesized secretory proteins. Secretory lysosomes share many traits with conventional lysosomes — for example, they are acidic and contain the relevant degradative proteins. However, they are distinguished from conventional lysosomes by their ability to undergo regulated secretion.

Only a few cell types contain secretory lysosomes (TABLE 1). Most of these are derived from the HAEMATOPOIETIC LINEAGE but there are a few exceptions, the most notable being MELANOCYTES[1] (see below). Cells that are derived from the haematopoietic lineage secrete proteins (and other molecules, such as peptides) to carry out their specific effector functions. For example, osteoclasts — which are derived from the haematopoietic lineage — resorb bone by the fusion of their lysosomes with the plasma membrane; LYSOSOMAL HYDROLASES then degrade the bone[2]. CYTOTOXIC T CELLS (CTLs) and NATURAL KILLER CELLS recognize infected or tumorigenic cells and destroy them by secreting cytolytic proteins, which are stored in secretory lysosomes (also called lytic granules in these cells)[3,4]. MACROPHAGES, DENDRITIC CELLS and B CELLS present antigen to T CELLS in a complex with major histocompatibility complex (MHC) class II molecules. Until the MHC–peptide complex forms, both antigen and MHC class II molecules are stored in an MHC class II compartment, which has some, but not all, of the characteristics of a secretory lysosome[5]. Blood clotting is mediated by PLATELETS, which store clotting agents such as serotonin and P-selectin[6] in secretory lysosomes (also called dense granules in these cells). Stimulation of BASOPHILS and MAST CELLS through immunoglobulin E (IgE) or chemotactic agents[7] causes histamine and serotonin to be secreted from secretory lysosomes, and this elicits an inflammatory response. NEUTROPHILS engulf exogenous particles, such as invading bacteria, into PHAGOSOMES (also called azurophil granules in these cells), in which they store their antibacterial agents, including DEFENSINS and AZUROCIDIN[8]. After separation from the plasma membrane, the phagosome is transported through the endocytic pathway and eventually fuses with the lysosome to form a phagolysosome, an event which might be akin to lysosomal secretion[9]. Here, the phagocytosed material is degraded and presumably eventually secreted when the phagolysosome/secretory lysosome degranulates[10].

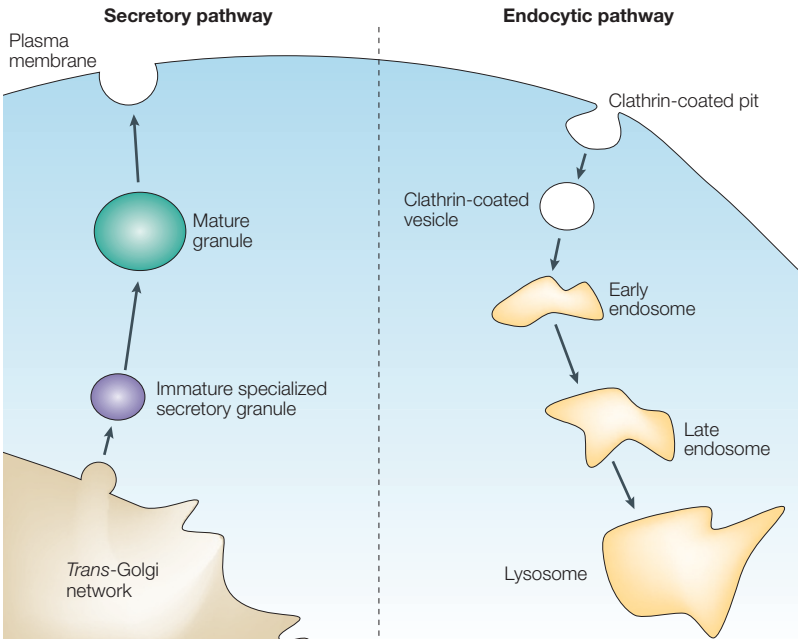

Figure 1 | **Secretory versus endocytic pathways.** Proteins that are destined for secretion exit the *trans*-Golgi network to be packaged into immature specialized secretory granules (which eventually form mature granules). When the mature granules fuse with the plasma membrane, soluble proteins are secreted from the cell, and membrane proteins are retained in the plasma membrane. Proteins at the plasma membrane that are destined for degradation are endocytosed by the formation of a clathrin-coated pit, which buds off from the plasma membrane to become a clathrin-coated vesicle. The contents of the clathrin-coated vesicle are sequentially delivered to the early endosome, which matures into the late endosome and subsequently into the lysosome by regulated fusion events. The contents are degraded once they reach the lysosome by a combination of the acidic environment and the action of the resident acid-dependent hydrolases.

NATURAL KILLER CELL
A class of lymphocyte that is crucial in the innate immune response. These exert a cytotoxic activity on target cells (such as virus-infected cells) that is enhanced by cytokines, such as interferons.

MACROPHAGE
Any cell of the mononuclear phagocyte system that is characterized by its ability to phagocytose foreign particulate and colloidal material.

DENDRITIC CELL
A professional antigen-presenting cell that is found in T-cell areas of lymphoid tissues, and also as a minor cellular component in most tissues. These have a branched or dendritic morphology and are the most potent stimulators of T-cell responses.

B CELL
A lymphocyte that develops in the bone marrow and produces antibody.

It is clear from the above discussion that secretion of a modified lysosome is crucial for many different cell types within the immune system to function properly, and illustrates the central role that secretory lysosomes have in the immune response. However, not all cells with secretory lysosomes are derived from haematopoietic lineage. Melanocytes are derived from the neural crest in vertebrates (reviewed in REF. 11) and produce a group of pigment proteins — the melanins — that are synthesized and stored in melanosomes. Melanosomes are lysosome-like organelles that are involved in the secretion of melanin and can be viewed as secretory lysosomes[12]. Perhaps the best evidence that melanocytes should be considered as a form of secretory lysosome comes from a series of diseases that affect secretory-lysosome function, in which melanosome release is also defective (BOX 1).

Secretory versus conventional lysosomes

So, what distinguishes conventional and secretory lysosomes? Secretory lysosomes differ from conventional lysosomes both morphologically and biochemically, with respect to the components that are packaged within them.

Structure. Morphologically, conventional and secretory lysosomes can appear similar. Both represent the end-point of the endocytic pathway at which endocytic tracers accumulate[13,14] (FIG. 1). In both cases, early endocytic compartments can fuse with the end-stage lysosomes and the formation of both conventional and secretory lysosomes probably involves a 'kiss-and-run' series of fusion and fission events[15,16]. A conventional lysosome is multi-vesicular in structure, as it contains internal vesicles that have budded off from the limiting membrane in a similar manner to a late endosome or multi-vesicular body (MVB). Secretory lysosomes have diverse types of structures — some have dense cores (for example, platelet dense granules), others have a multilaminar appearance (for example, MHC class II compartments), and others have unique structures (for example, melanosomes). Dense cores contain many of the secretory products of the cell, such as proteoglycans in T- and natural killer cells, which give the granules a characteristic electron density. However, not all secretory lysosomes contain dense cores — the number of these structures depends on the cell type (and hence the secretory product) and the activation or maturation state of the cell (discussed later).

Content. Both conventional and secretory lysosomes contain the machinery — such as the mature forms of lysosomal acid hydrolases — that is required for their degradative function, and receptors — such as Lgp96 (a member of the lysosomal-associated membrane protein (LAMP) family[17,18]) and members of the heat shock 70 (Hsc70) family[19,20] — that are used for recognizing and delivering material (both cytosolic and membrane bound) to the lysosome. The Hsc70 family recognize a Lys–Phe–Glu–Arg–Gln motif in cytosolic proteins[21]. The function of other resident membrane proteins, such as LAMP-1, LAMP-2 and CD63, is largely unknown, but they might have a role in AUTOPHAGY. However, in addition to the standard set of lysosomal proteins, secretory lysosomes contain a specific, cell-type-dependent set of secreted components. These include soluble apoptotic granzymes in CTLs[4], serotonin and histamine in basophils and mast cells, or melanin in melanocytes (FIG. 2). In this way, secretory lysosomes differ from conventional lysosomes both in their structure and content.

Secretion. As the name indicates, the most obvious distinction should be that secretory, but not conventional, lysosomes can secrete their content. Historically, the principal role of a conventional lysosome has been considered to be intracellular digestion of macromolecules, and this organelle is not normally associated with secretion (FIG. 1). However, recent data indicate that conventional lysosomes could be important in membrane repair (reviewed in REF. 22). Andrews and colleagues[23] have shown that in response to plasma membrane damage, fibroblasts recruit conventional lysosomes, which can then fuse with the plasma membrane and repair it. This process requires synaptotagmin VII (SytVII; a calcium sensor) and can be inhibited by both recombinant SytVII C2A domain or anti-SytVII antibodies. So why would lysosomes, rather than another membranous organelle, be used for this process? There are at least three reasons for this: first, lysosomes contain excess membrane — both limiting and internal — which

Table 1 | **Cells that contain secretory lysosomes**

Cell type	Function	Soluble content	Specific membrane proteins	Stimulus for exocytosis	Cell-specific secretory lysosome
T cells	Target cell killing	Perforin granzymes	Fas ligand CTLA-4	T-cell receptor	Lytic granule
Mast cells	Parasite defence	Histamine serotonin	MHC class II	Fc receptor	
Eosinophils	Parasite defence	Major basic protein		Fc receptor	
Basophils	Inflammatory	Histamine		Fc receptor	Basophil cell granule
Neutrophils	Inflammatory phagocytosis	Chemoattractants		Fc receptor	Azurophil granule
Platelets	Clotting	Clotting factors	CD40 ligand	Fc receptor and collagen	Platelet dense granule
Macrophages	Phagocytosis antigen presentation		MHC class II		
Dendritic cells	Antigen presentation		MHC class II	MHC class II compartment	
B cells	Antigen presentation Ig secretion		MHC class II		
Melanocytes	Secretion of melanin for pigmentation	Melanin			
Osteoclasts	Bone resorption	Lysosomal hydrolyses			
Renal tubular cells	Kidney function				

Secretory lysosomes are generally found in cells that are derived from the haematopoietic lineage. Examples of both soluble and membrane lysosomal proteins are shown. The stimulus for exocytosis differs for each cell type. Cell-specific names for the secretory lysosome are also shown. MHC, major histocompatibility complex; CTLA-4, cytotoxic T-lymphocyte antigen 4.

might provide a large 'store' of membrane. Second, as lysosomes are the product of fusion and fission events from the endocytic pathway, they have the rudimentary complement of proteins that are needed for membrane fusion. Third, it has long been postulated that 'regurgitation' of lysosomes might be important in allowing the cell to rid itself of any build-up of cellular waste and membrane. These studies by Andrews and colleagues provide not only evidence, but also now functional significance, for lysosomal fusion with the plasma membrane. How damaging (if at all) the low pH of the lysosomes is to neighbouring cells has not been investigated. However, it is likely that the acidic content is dissipated fairly rapidly on exposure to the extracellular environment.

There are other examples of endosomal–lysosomal compartments fusing with the plasma membrane. However, it is not clear whether these compartments are more closely related to conventional or secretory lysosomes. Wubbolts *et al.*[24] showed that, in the melanoma cell line Mel JuSo, an MHC-class-II-positive multivesicular compartment that contains MHC class II molecules was able to fuse directly with the plasma membrane. Although the MHC class II molecules were located on the inner vesicles of this compartment, they appeared on the plasma membrane and were not secreted as released vesicles, as has been seen in other cell types such as B cells[25] and dendritic cells[26]. These secreted vesicles are termed exosomes (BOX 2). Recent data however, have shown that when dendritic cells mature, MHC class II molecules that are resident on the inner vesicles on the MVB are transferred to the limiting membrane[27], thereby resulting in the appearance of MHC class II molecules on the plasma membrane when the MVB and the plasma membrane fuse. This membrane re-organization also caused tubular extensions from the MVB to extend towards the plasma membrane, thereby forming MHC-class-II-positive vesicles at their tips. However, it is not known which of these processes (exosome release or vesicle shuttling) occurs when conventional (and secretory) lysosomes fuse with the plasma membrane.

So, how similar are the processes that underlie the secretion of conventional and secretory lysosomes? The strongest evidence that they differ comes from the existence of genetic diseases that selectively affect secretory lysosome function (BOX 1 and discussed later) without apparently affecting membrane repair or the release of conventional secretory granules. This raises the question of whether cells can contain both conventional and secretory lysosomes. Conventional lysosomes can be identified in melanosomes[28] and platelets, which indicates that the two types of lysosomes can coexist in these cell types. However, in CTLs, all lysosomes seem to contain secretory proteins, which indicates that, at least in these cells, there is only one population of lysosomes.

Biogenesis of the secretory lysosome

Mature secretory lysosomes, like conventional lysosomes, receive both biosynthetic and degradative transport. This includes biosynthetic proteins that are required for the organelles to function; for example, degradative hydrolases and secretory proteins (such as perforin in CTLs or melanin in melanocytes). These

T CELL
A lymphocyte that develops primarily in the thymus and is important for the regulation and development of immune responses.

PLATELET
The smallest blood cell, which is important in haemostasis and blood coagulation.

BASOPHIL
A polymorphonuclear phagocytic leukocyte of the myeloid series.

MAST CELL
A type of leukocyte of the granulocyte subclass.

NEUTROPHIL
A phagocytic cell of the myeloid lineage that has an important role in the inflammatory response, and undergoes chemotaxis towards sites of infection or wounding.

PHAGOSOME
Large particles, such as bacteria, are engulfed by phagocytosis and are transported to phagosomes. Phagosomal and endosomal pathways undergo interconnected maturation and merge before fusion with lysosomes. *Mycobacterium tuberculosis* can modify this pathway and prevent phagosomal maturation.

DEFENSIN
An antimicrobial peptide that is secreted by Paneth cells in the villus crypt.

AZUROCIDIN
A glycoprotein that is produced in neutrophils, and has broad-spectrum antimicrobial activity and chemotactic activity towards monocytes.

AUTOPHAGY
A complete intracellular mechanism, which leads to bulk protein degradation, and involves the sequestering of cytosol into vesicles for delivery to a degradative organelle.

Box 1 | **Lessons from genetic diseases**

Several genetic diseases result in the selective impairment of the function of secretory lysosomes in a small number of cell types (see table below). This indicates that secretory lysosomes in these cell types might share a unique exocytic mechanism that is different from conventional secretion. This is most clearly shown by the autosomal recessive diseases Chediak–Higashi syndrome, Griscelli's syndrome and Hermansky–Pudlak syndrome (HPS), which are characterized by varying degrees of hypopigmentation (caused by impaired melanosome secretion), prolonged bleeding (due to reduced granule secretion from platelets) and severe immunological deficiency (a result of impaired secretion in cells of the immune system). Furthermore, more than 15 different mouse models of HPS (reviewed in REF. 94) are now available, mostly thanks to pioneering work from the laboratory of Richard Swank. By combining studies from these mutant mice and from cells that are derived from human patients, much progress has been made in understanding the mechanisms that control intracellular transport and, in particular, the exocytosis of secretory lysosomes. All of these diseases indicate that specialized mechanisms are used to sort and secrete proteins in cells that have secretory lysosomes. Analysis of the defects that are seen in these diseases has helped to further define the differences between conventional and secretory lysosomes.

Table Box 1 | **Genetic diseases that cause defects in secretory lysosomes**

Human disease	Human gene	Mouse mutation	Protein	Function of protein	Phenotype product
Chediak–Higashi syndrome (CHS)	*CHS1*	*beige*	Lyst/Beige	Lysosome fission.	Enlarged lysosomes. In CTLs, unable to fuse with membrane.
Hermansky–Pudlak syndrome 1 (HPS1)	*HPS1*	*pale ear*	HPS1	?	
Hermansky–Pudlak syndrome 2 (HPS2)	*ADTB3A (HPS2)*	*pearl*	AP3 β3A subunit	Sorting of membrane proteins to secretory and conventional lysosomal proteins.	Mis-sorting of some lysosomal proteins.
	ADTD	*mocha*	AP3δ subunit	As above.	As above.
	PA	*pallid*	Pallidin	t-SNARE regulation.	
	RABGGTA	*gunmetal*	Rab geranylgeranyl transferase	Rab GTPase prenylation.	Reduced secretion of granules in CTLs.
Hermansky–Pudlak syndrome 3 (HPS3)	*HPS3*	*cocoa*	HPS3	?	?
Griscelli's syndrome	*RAB27A*	*ashen*	Rab27a	Movement of melanosomes, CTL granule secretion.	No secretion of CTLs, or melanocyte granules.
	ND	*leaden*	Melanophorin	Movement of melanonsomes.	No secretion of melanosomes.
	MyoVA	*dilute*	Myosin Va	Movement of melanosomes.	No secretion of melanosomes.

reach the secretory lysosome from the *trans*-Golgi network (TGN) by specific sorting pathways (discussed later).

The biogenesis of the multilamellar/dense core structure of mature secretory lysosomes has been best studied in CTLs[14]. In culture, resting human CTL clones do not contain secretory lysosomes. Only after stimulation by the T-cell receptor do they express detectable levels of the protein components of secretory lysosomes[29], including lytic proteins (such as perforin and granzyme A[30]) and lysosomal proteins (such as LAMP-2). The appearance and maturation of the secretory lysosomes correlates with the killing ability of the activated CTLs, both of which increase after activation. The initial structures are small, multi-vesicular endosomes, similar to the MVBs that are seen in other cell types. Over time, these compartments increase both in size and in the number of inner vesicles. At later stages, a dense core appears, the size of which also increases with time, so that the mature secretory lysosome in CTLs can consist of predominantly electron-dense structures that are surrounded by a few vesicles. This indicates that the biogenesis of secretory lysosomes in CTLs is very similar to that of lysosomes in other cells. Intriguingly, the secretory lysosomes of CTLs become autophagic in appearance with age, which indicates that autophagy might function in the turnover of this organelle.

Evolution of secretory lysosomes

As secretory lysosomes seem to share many characteristics with conventional lysosomes, it seems probable that the secretory lysosome has evolved from the conventional lysosome. During evolution, the process of lysosome secretion might have been enhanced in some cell types, thereby resulting in the development of secretory lysosomes. This process probably occurred early on, as secretory lysosomes are also found in evolutionarily distant organisms such as *Dictyostelium discoideum*[31] and *Caenorhabditis elegans* (Rab3, REF. 32).

Secretory lysosomes, however, might also be distantly related to secretory granules. Indeed, specialized secretory cells contain a post-Golgi intermediate of their secretory granules — the immature granules (FIG. 1) — which are acidic and contain both lysosomal and

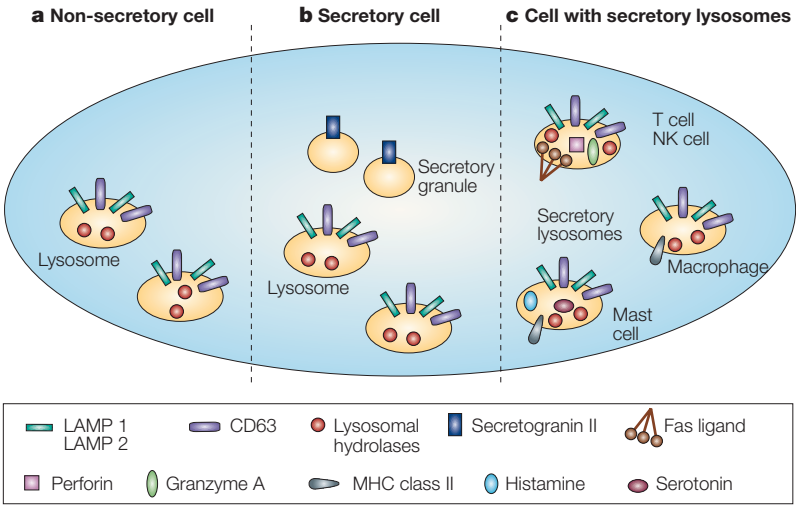

Figure 2 | **Lysosomal contents in cells with conventional lysosomes, secretory granules and secretory lysosomes. a** | All cell types contain a lysosomal compartment, which contains all the relevant lysosomal machinery, including acid hydrolases and lysosomal membrane proteins. **b** | Secretory cells contain an additional compartment — the secretory granule — which contains a cell-type-specific set of secretory proteins. **c** | Cells that have secretory lysosomes package both lysosomal and secretory proteins in one compartment. Cells store cell-type-specific secretory components in their secretory lysosomes, such as Fas ligand and the soluble protein granzyme A in T- and natural killer (NK) cells, MHCII (major histocompatibility complex, class II) in macrophages or histamine in mast cells.

ADAPTOR COMPLEX (refers to AP1, AP2, AP3 and AP4). A heterotetrameric cytosolic complex that is involved in recruitment of cargo and accessory proteins to vesicles that are involved in transport.

secretory proteins[33,34]. These features are strongly reminiscent of secretory lysosomes, which indicates that immature granules might represent a secretory-lysosomal equivalent in cells, in which the secretory and lysosomal functions are separated.

Preventing degradation of resident proteins

As lysosomes are normally degradative, how can some proteins resist degradation and remain stable within this compartment? Kundra and Kornfeld[35] have shown that asparagine-linked glycans can have a crucial role in protecting a subset of lysosomal membrane proteins (including LAMPs and lysosomal integral membrane proteins (LIMPs)) from the proteolytic environment of the lysosome. This could explain how other membrane proteins of secretory lysosomes, such as Fas ligand (FasL), can escape degradation. As for soluble, secretory proteins, such as the granzymes, storage of these proteins in the dense cores of the secretory lysosome could protect them from the action of the hydrolases.

Box 2 | **Exosomes**

Exosomes were first discovered in reticulocytes[95]. A wide range of cell types, from B lymphocytes[25,96] to platelets[97], secrete exosomes. Although the precise physiological target and function of these small (40–90-nm) vesicles is yet to be resolved for some cell types, exosomes that are secreted by dendritic cells can elicit a strong T-cell response, as they contain both major histocompatibility complex class I and II molecules, and CD86 (a co-stimulatory molecule for T cells[26]). Exosomes that are derived from tumour-peptide-loaded dendritic cells have been shown to stimulate a strong antitumour response *in vivo*[26].

Another clue comes from work on dendritic cells, in which recent data indicate that the proteolytic potential of secretory lysosomes might be regulated. Dendritic cells present extracellular antigen in complexes with MHC class II molecules to T- and B cells. To form antigen–MHC complexes, immature dendritic cells endocytose antigen and transport it to the MHC-class-II-positive secretory lysosome. Intriguingly, neither the antigen nor the MHC class II molecules that reside in the lysosomes of immature dendritic cells are degraded, and MHC-class-II–peptide complexes do not form until the dendritic cell receives a maturation signal[36,37]. This indicates that dendritic cells can regulate the proteolytic potential of their lysosomal compartment. Gregers *et al.* [38] have proposed that the invariant chain (Ii), which is associated with MHC class II before the MHC class II–peptide complex forms, might have a role in this. It is still unclear whether other factors, such as upregulation of lysosomal enzymes or an alteration in the lysosomal pH, contribute to regulated degradation[39]. If other cell types that have secretory lysosomes were to share this regulatory mechanism described in dendritic cells, it would provide another solution to the problem of degradation of resident proteins.

Sorting to secretory lysosomes

Proteins can reach lysosomes by both biosynthetic and endocytic pathways (FIG. 3). Generally, membrane proteins are sorted by signals in their cytoplasmic tails that are recognized by components of the appropriate sorting machinery. This leads to their recruitment into budding vesicles. Some lysosomal-membrane proteins such as CD63, LAMP-1 and LAMP-2, have a tyrosine-based motif, which can be recognized by the ADAPTOR COMPLEXES AP1, AP2 and AP3, which are found at the TGN, plasma membrane and the TGN–endosomal system, respectively[40]. Several proteins that are found exclusively in secretory lysosomes, such as the granule-membrane protein of 17-kDa (GMP-17)[41] and cytotoxic T-lymphocyte antigen 4 (CTLA-4)[42] are transported to the secretory lysosome using a similar signal.

Soluble lysosomal proteins, such as the lysosomal hydrolases, do not have sorting signals in their peptide sequences. Instead, they are modified during biosynthesis by the addition of a mannose-6-phosphate moiety, which is recognized by mannose-6-phosphate receptors (MPR). These transmembrane receptors cycle between the TGN and late endosomes, carrying soluble proteins to lysosomes. Soluble granzymes, which are secreted from secretory lysosomes during apoptosis, are also modified by mannose 6-phosphate and, like lysosomal hydrolases, they are sorted to lysosomes by the MPR.

Several lines of evidence, however, indicate that, in addition to the well-characterized sorting pathways to lysosomes[43], some proteins might also reach secretory lysosomes by other pathways that are unique to cells that contain this organelle. The first evidence for this emerged from studies on cells that were derived from patients with I-cell disease, in which the phosphotransferase that is required for the mannose-6-phosphate

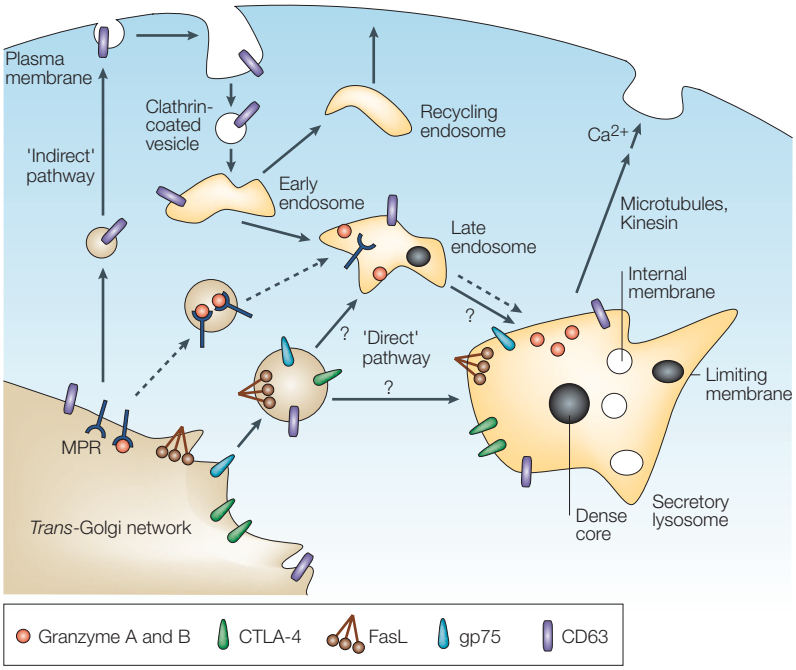

Figure 3 | **Sorting pathways to the secretory lysosome.** Soluble proteins of the secretory lysosome, such as granzyme A, are modified by a mannose-6-phosphate moiety at the *trans*-Golgi network (TGN), which is recognized by the mannose-6-phosphate receptor (MPR). The modified proteins are transported to the late endosomes (shown by dashed arrows) where they dissociate from the receptors and are then delivered to the secretory lysosome. The MPRs are recycled to the TGN from the late endosome. Membrane proteins are typically recognized by a sorting signal in their cytoplasmic tail, such as a di-leucine or tyrosine-based motif — as occurs for the cytotoxic T-lymphocyte antigen 4 (CTLA-4). Membrane proteins can reach the secretory lysosome by two main routes: direct or indirect (as shown in the figure). CD63, which is a resident lysosomal protein, reaches the lysosome by both routes. A protein that takes the indirect route travels through the default pathway to the plasma membrane, where it is internalized and reaches the secretory lysosome by the endocytic pathway. It is still unclear whether the direct route is indeed direct (from the TGN to the secretory lysosome) or if the pathway involves an intermediate step through the endosomal pathway. Fas ligand and gp75 are sorted to the secretory lysosome by virtue of their polyproline and Ser–Val–Val motifs, respectively. Recognition of these motifs by an Src-homology-3 (SH3)-domain-containing protein and a PDZ-DOMAIN-containing protein is thought to mediate their sorting either directly to the secretory lysosome or through an endosomal compartment.

modification is absent or defective (reviewed in REF. 44). In these cells, lysosomal hydrolases are not modified by mannose 6-phosphate, so they cannot be recognized by the MPR and are therefore secreted from the cell. However, early studies showed that, in such patients, the deficit of lysosomal hydrolases is less acute in tissues that are derived from the haematopoietic lineage, such as the spleen[45,46]. Kornfeld and colleagues[47] later identified an additional motif that was different from mannose-6-phosphate modification in these lysosomal enzymes, which functions in B cells that are derived from the haematopoietic lineage. This sorting signal does not occur as a result of carbohydrate modification, but requires a motif in the polypeptide sequence of the carboxy-terminal lobe of the protein. This allows the protein to be specifically recognized, and shares some similarity to the recognition motif for phosphotransferase. Other sorting motifs that operate in melanocytes and haematopoietic cells (TABLE 2) have subsequently been found, which supports the idea that cells with secretory lysosomes also have unique sorting mechanisms. These new sorting signals do not seem to have any common features with one another and, as yet, have not been identified in other proteins. Some of the best characterized of these pathways are outlined in the next sections.

PDZ DOMAIN
A domain that is found in many cytosolic proteins and is named after the founding members of this protein family (Psd-95, discs-large and ZO-1). This is an ~90-residue structural motif, which regulates protein–protein interactions, largely by binding to a specific tripeptide motif.

Fas ligand. FasL is one of the main membrane-bound mediators of apoptosis that is used by activated T- and natural killer cells to kill target cells, and therefore its presence at the cell surface is carefully controlled[48]. This control is achieved by sorting newly synthesized FasL to secretory lysosomes, which then fuse with the plasma membrane only under conditions of cell degranulation[49]. However, when expressed in cells that only have conventional lysosomes, FasL follows the default biosynthetic pathway to the plasma membrane. This indicates that FasL contains a lysosomal sorting signal that is recognized only in cells that have secretory lysosomes. This motif is a proline-rich domain (PRD) that is present in the cytoplasmic tail of FasL[50], and probably binds to a Src-homology-3 (SH3) domain-containing protein — perhaps a component of the sorting machinery. This motif is not required for endocytosis from the plasma membrane, as its deletion does not alter the kinetics of internalization[50]. So, this shows that FasL is sorted from the Golgi to secretory lysosomes without transiting through the plasma membrane (FIG. 4). So far, FasL is the only protein that has been shown to use this pathway.

gp75. gp75 — also called tyrosinase-related protein-1 (TRP-1) — and other members of the tyrosinase family are involved in melanin synthesis in melanosomes[51,52]. The functional domain of gp75 is present in the lumenal amino-terminus, whereas the sorting signals are in the cytoplasmic tail. One of these sorting signals is a classical di-leucine-based signal[53], which has been proposed to interact with AP3 adaptors to mediate sorting to melanosomes[54,55]. A second, unconventional signal sequence (Ser–Val–Val) has been found, which interacts transiently with a PDZ-domain-containing protein, RGS-GAIP-interacting protein, carboxy-terminus (GIPC)/SemF cytoplasmic domain-associated protein 1 (SEMCAP-1)[56]. Endogenous GIPC seems to bind only to newly synthesized gp75 in a transient interaction that occurs immediately after the protein exits the Golgi. GIPC could have a role in post-Golgi sorting of gp75 to pre-melanosomes, but no data are yet available to confirm this hypothesis.

Regulated exocytosis of secretory lysosomes

Exocytosis of secretory lysosomes, like other regulated secretion events, involves several distinct steps. First, a signal, such as binding of a cell-surface receptor (for example TcR in CTLs), is required to stimulate exocytosis. This results in Ca^{2+} mobilization within the cell, which acts as a signal to the granules to mobilize themselves for degranulation[57]. Once mobilized, secretory lysosomes are transported to the site of stimulation. In CTLs, this movement is highly polarized to the area between the CTL and target cell. This polarized movement occurs along microtubules

Table 2 | **Sorting motifs for conventional and secretory lysosomes**

Protein	Protein function	Sorting motif
Cathepsins*	Lysosomal hydrolases	M6P and other
CD63*	Unknown	Tyrosine based
LAMP 1 and 2*	Unknown	Tyrosine based
Granzymes≠	Serine proteases involved in apoptosis	M6P
Tyrosinase≠	Melanin synthesis	Di-leucine and tyrosine based
CTLA-4≠	Negative regulator of T cell activation	Tyrosine based
Fas ligand≠	Apoptosis	Proline-rich domain
Trp-1 (gp75)≠	Melanin synthesis	Di-leucine based and S-V-V

Examples of motifs for sorting both soluble and membrane-bound proteins to conventional and secretory lysosomes*, or secretory lysosomes≠ are shown. Tyrosine-based motifs conform to either NPXY or YXXθ (where θ is a bulky hydrophobic residue, N represents asparagine, P is proline, X is any amino acid and Y represents tyrosine). CTLA-4, cytotoxic T-lymphocyte antigen 4; LAMP-1, -2, lysosomal-associated membrane protein-1, -2; M6P, mannose-6-phosphate; S–V–V, serine–valine–valine; Trp-1, tyrosinase related protein-1.

towards the microtubule organizing centre (MTOC), and is driven by a motor of the KINESIN FAMILY[58]. Once near the cell periphery, the lysosome uses actin-based movement to travel the final short distance to the docking site at the plasma membrane[59]. Melanosomes have been shown to use members of the myosin family as the motor proteins for movement along the actin cytoskeleton[60,61]. Once the secretory lysosome has docked at the plasma membrane, its soluble contents are released into the extracellular space and components of the membrane fuse with the plasma membrane (FIG. 5).

So, which components of the exocytic pathway are used by secretory lysosomes? Secretory lysosomes seem to use unique components that are found only on themselves, and common components that are shared with other secretory granules. The following sections will outline briefly the proteins (both unique and common) that have been identified as important components of secretory lysosome exocytosis.

KINESIN FAMILY
The kinesin superfamily contains a range of motor proteins that have similarities to classical 'vesicle' kinesin in the conserved microtubule-binding motor region. The motor can be at the amino-terminus, in the middle, or at the carboxyl terminus (in which case the protein usually migrates to the minus end of the microtubule).

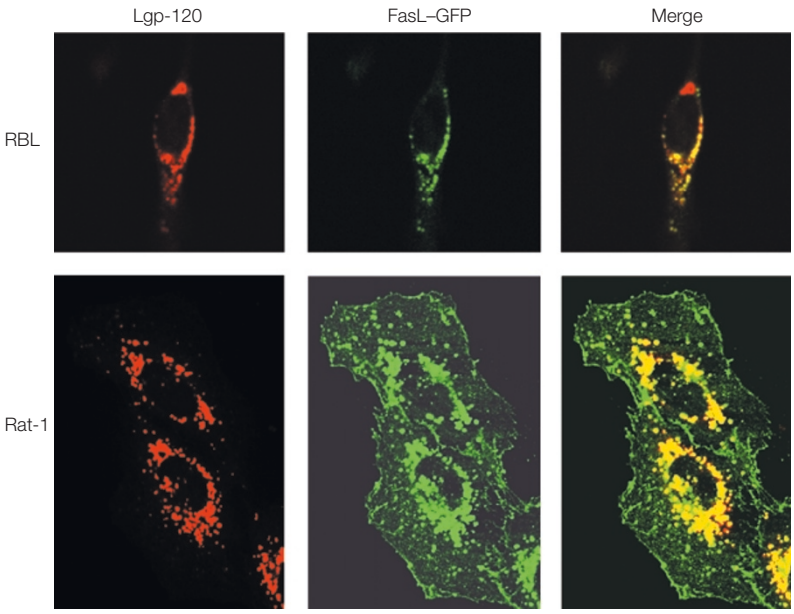

Figure 4 | **Differential sorting of Fas ligand in cells with and without secretory lysosomes.** Fas ligand (FasL) is sorted directly to the secretory lysosome in the mast-cell line, RBL-2H3 (RBL), but appears at the plasma membrane in the epithelial cell line, Rat-1, from which it is internalized to the lysosome. Cells were transfected with green-fluorescent-protein-tagged FasL (FasL–GFP; green channel) and co-stained with a rat lysosomal marker, lysosomal-associated membrane protein 1 (Lgp-120; red channel). The yellow channel shows co-localization of FasL–GFP with Lgp-120.

Synaptotagmins. Synaptotagmins are membrane proteins that bind phospholipid membranes in a Ca^{2+}-dependent manner. They are important in regulated exocytosis of secretory granules[62]. A specific set of synaptotagmins — SytII, SytIII and SytV — are expressed endogenously in the rat mast-cell line, RBL-2H3 (REF. 63). SytII is the most abundant of the three proteins and seems to be a negative regulator of Ca^{2+}-dependent exocytosis. Although not proven, the authors propose that SytIII might be a positive regulator of this process, as overexpression of a neuronal-derived Syt, SytI, potentiated the Ca^{2+}-dependent response[64]. So it seems, at least in mast cells, that secretory lysosomes use various calcium sensors during their secretion step, but these Syt proteins are not specific to secretory lysosomes.

Membrane fusion machinery: SNAREs. Fusion of secretory lysosomes with the plasma membrane is mediated by a set of specific proteins. Pairing of different members of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) family provides the first level of specificity to different cellular membrane fusion events[65]. Fusion between the vesicle (v) and target (t) membranes occurs when specific combinations of v-SNARE and t-SNARE interactions occur.

Evidence that SNAREs are involved in secretory-lysosome exocytosis comes from work on RBL-2H3 mast cells, which degranulate their secretory lysosomes in response to stimulation of their high-affinity IgE receptors. Paumet *et al.*[68] showed that overexpression of the SNARE syntaxin 4 inhibited IgE-receptor-stimulated degranulation. This was specific for syntaxin 4, as overexpression of syntaxin 2 or syntaxin 3 had no effect.

Several other SNARE proteins have been found that are associated with secretory lysosomes in various cell types, such as synaptosomal-associated protein 23 (SNAP-23), vesicle-associated membrane protein 2 (VAMP2) and VAMP7 in mast cells[68–70], syntaxin 3, syntaxin 4, VAMP2 and secretory carrier membrane protein (SCAMP) in neutrophils[71]. However, the functional significance of these associations is not yet fully understood and these SNAREs are also found on conventional secretory granules in exocrine and neuronal cells, so they could represent a common fusion mechanism with secretory granules. Different pairings of these SNAREs might regulate secretory-lysosome fusion events, which provides a different recognition event to that which occurs between secretory granules.

Membrane fusion machinery: Rabs. The process of fusion between the vesicle and target membranes is further regulated by small GTPases of the RAB FAMILY[66], which provide a second level of specificity to the fusion event, as each Rab associates with a different cellular

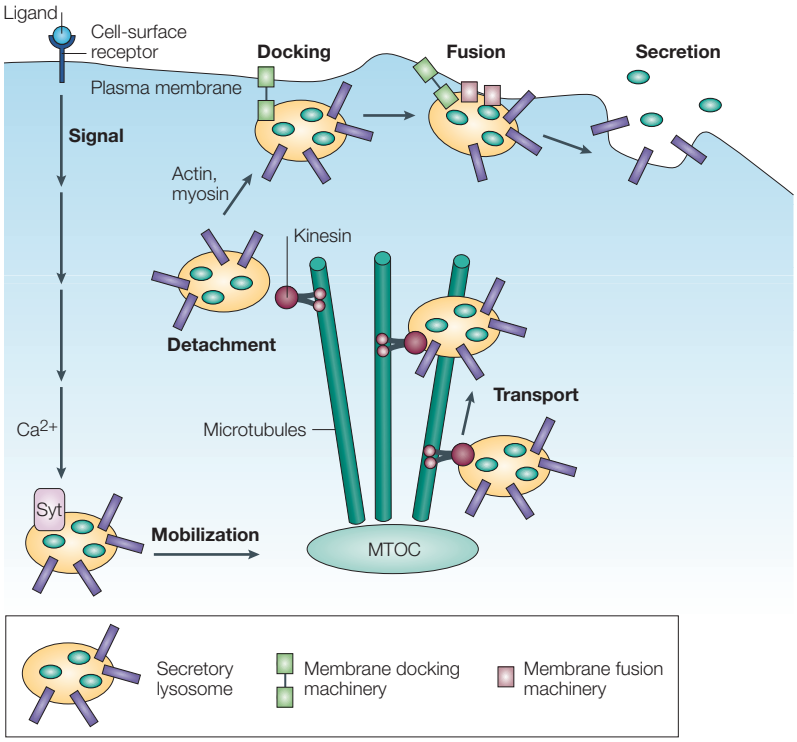

Figure 5 | **Secretion of the secretory lysosome.** Stimulation of a cell-surface receptor, such as Immunoglobulin E, on mast cells results in an increase in intracellular Ca^{2+} levels. This signal is detected by synaptotagmin (Syt), a calcium sensor on the secretory lysosome, which translates the signal to mobilize the granule towards the microtubule organizing centre (MTOC). Here, the granule associates with a kinesin motor for transport to the site of secretion. Once near the cell membrane, the granule detaches from the motor, and movement towards the plasma membrane is then actin based. The granule then associates and fuses with the plasma membrane through specific docking and fusion machinery, such as Rabs and SNARE complexes. Fusion with the membrane results in secretion of the soluble components of the granule and presentation of the granule-membrane proteins at the cell surface.

membrane[67]. Mast cells express two isoforms of Rab3 (a and d)[72] — Rab3a is predominantly found in the cytoplasmic fraction and Rab3d in the membrane fraction. Wild-type proteins or mutant proteins that were unable to hydrolyse GTP were used to investigate whether these Rab isoforms were involved in the degranulation response. Neither wild-type nor mutant Rab3a affected the degranulation response, whereas both forms of Rab3d did affect it. Rab3a has also been found to be associated with secretory lysosomes of melanoma cells[73,74]. Rab37 has also been found on secretory lysosomes in RBL-2H3 mast cells[75]. However, none of these Rabs are unique to secretory lysosomes, as they were initially found to be associated with synaptic vesicles[76] and later discovered in neuroendocrine cells[77] and specialized secretory cells[78].

Two Rabs that seem to be associated exclusively with secretory lysosomes have been identified. Rab27a has a crucial role in secretion and is absent in some patients that suffer from Griscelli's syndrome and in *ashen* mouse mutants (BOX 1). Interestingly, this disease is characterized by both immunodeficiency and a lack of pigmentation[79], which is consistent with a crucial role for Rab27a in cells that have secretory lysosomes. Studies using CTLs from *ashen* mice show that the secretory lysosomes can move along microtubules and accumulate at the MTOC near the contact site with the target cell, but they are unable to reach the plasma membrane and secrete their content[80,81], which results in immunodeficiency. The secretory lysosomes in the melanocytes from these mice are also unable to dock at the plasma membrane, and they cluster in the perinuclear region of the cell[82]. From this, it seems that Rab27a is required for the final stage of moving the secretory lysosomes from the microtubules across the actin-rich region to the plasma membrane.

A new Rab, Rab38, has been identified in melanoma cells. As expression is thought to be restricted to this cell type[83], this indicates that Rab38 might have a role in some step of secretory-lysosome movement that is specific to melanocytes.

Rab regulators. The *gunmetal* mouse, which is a model for Hermansky–Pudlak syndrome (HPS) (BOX 1), is characterized by immunodeficiency, coat-colour deficiency and decreased platelet synthesis. The mice have mutations that inactivate Rab geranylgeranyltransferase (RGGT) — an enzyme that PRENYLATES certain Rabs and thereby regulates their membrane association[84]. Although CTLs from these mice can secrete their lysosomes, they do so at a much reduced level. Few lysosomes can polarize at the contact site with the target cell[85] and most are scattered around the periphery of the cell. So, one or several so-far unidentified Rab proteins that are prenylated by RGGT are required for the microtubule-dependent transport of secretory lysosomes to the contact site. It therefore seems likely that RGGT-mutants will also identify other Rabs that might be specific to secretory lysosomes.

Another important step in the movement of lysosomes along microtubules is the recruitment of motor proteins. Although it is not known at present which kinesin is used by secretory lysosomes for the microtubule-based movement, it seems that, at least in conventional lysosomes, Rab-regulating proteins control this recruitment step. Rab7-interacting lysosomal protein (RILP) mediates the recruitment of dynactin–dynein complexes (which work in the opposite direction to kinesin) to Rab7-positive lysosomes[86], which prevents lysosomes from moving forward. Whether secretory lysosomes use this mechanism is unknown, however, it is possible that Rabs that are specific to secretory lysosomes can control the recruitment of various motor proteins.

Rab effector protein. The *leaden* mouse model of HPS (BOX 1) provides a clear example of a Rab-effector protein that is involved in secretory-lysosome function. The defect in *leaden* mice has recently been identified as a mutation in the gene that encodes melanophilin (Mlph), which shows homology to Rab-effector proteins[87]. Although the identity of the Rab that Mlph is an effector for is not known, it has been proposed that it might be part of a complex with Rab27a and MyoVa (see below).

SNARE FAMILY
(soluble N-ethylmaleimide-sensitive factor attachment protein receptor). A family of membrane-tethered coiled-coil proteins that regulate fusion reactions and target specificity in the vacuolar system. They can be divided into v-SNAREs and t-SNAREs on the basis of their localization (vesicle and target, respectively), or into Q-SNAREs and R-SNAREs on the basis of a highly conserved amino acid (Gln and Arg, respectively).

RAB FAMILY
Rab proteins form the largest subfamily of small GTPases of the Ras superfamily. They regulate budding, tethering, fusion and motility at various sites within cells.

PRENYLATION
The post-translational modification of proteins by the enzymatic addition of prenyl moieties, which allows membrane attachment.

Myosin motor proteins. MyoVa is an unconventional myosin motor protein that can move across the actin cytoskeleton[88], and is expressed by most cell types. *Dilute* mice and some patients with Griscelli's syndrome have mutations in the gene that encodes MyoVa (REF. 79). Melanocytes from these mice are unable to dock their secretory lysosomes (melanosomes) at the plasma membrane, which shows that MyoVa is required to move melanosomes to the plasma membrane[60,89]. However, secretory lysosomes of CTLs from *dilute* mice are fully functional[80] and are able to reach the plasma membrane and secrete their contents. These results show that, whereas melanocytes use both Rab27a and MyoVa, only Rab27a is required for secretory-lysosome function in CTLs[90]. MyoVa seems to be dispensable for the secretory function of CTLs, perhaps because it is replaced by another, as yet unidentified, unconventional myosin in these cells.

Unique components of secretory-lysosome machinery. In addition to Rab27a, another protein, Lyst, which has a unique function in secretory-lysosome function, has been identified. Lyst is the protein product of the gene that is mutated in CHS[91] (or *beige* in the mouse[92]). This cytoplasmic protein is expressed at low levels in all cell types and its absence leads to the formation of abnormally large lysosomes in all cells[93]. However, these enlarged organelles function perfectly well as conventional lysosomes in most cell types, and only secretory lysosomes have defects in exocytosis. As cells with conventional secretory granules are unaffected by this mutation, Lyst is probably not involved in all fusion events with the plasma membrane, but only in those that involve secretory lysosomes.

Perspective

The picture that emerges from recent studies is that lysosomes are much more secretable than was previously thought. Cells with secretory lysosomes use their lysosomes as a regulated secretory organelle. This process uses some components of the machinery that are used by conventional secretory granules, but also some that are clearly unique. This is best illustrated by several genetic diseases that are characterized by albinism and immunodeficiency, in which only cells with secretory lysosomes are affected. These studies have already identified a crucial role for Rab27a in the release of secretory lysosomes. As there are more than three genes responsible for these diseases, it seems likely that these will provide an invaluable resource for identifying other essential components of the secretory-lysosome machinery.

Recent findings, however, which show that conventional lysosomes can, in some cases, also fuse with the plasma membrane, further complicate the issue. This mechanism is important for membrane repair, and SytVII is central to this secretory event. How much do these secretory mechanisms differ? By identifying the molecular machinery that is involved, it should be possible to compare the expression of proteins such as Rab27a and SytVIIa, and therefore be able to distinguish secretory lysosomes from conventional lysosomes at the molecular level.

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Online links

DATABASES

The following terms in this article are linked online to:

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
ashen | *gunmetal* | MyoVa | Rab 3a | Rab27a | Rab geranylgeranyltransferase

OMIM: http://www.ncbi.nlm.nih.gov/OMIM/
Chediak–Higashi syndrome | Griscelli's syndrome | Hermansky–Pudlak syndrome

Swiss-Prot: http://www.expasy.ch/
CD63 | CD86 | CTLA-4 | FasL | GIPC | gp75 | granzyme A | LAMP-1 | LAMP-2 | Lyst | melanophilin | P-Selectin | perforin | Rab3d | Rab37 | Rab38 | RILP | SCAMP | SNAP-23 | synaptotagmin VII | syntaxin 3 | SytII | SytIII | VAMP2 |VAMP7

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