REVIEW ARTICLE

Endolysosomal proteolysis and its regulation

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The endolysosomal system comprises a unique environment for proteolysis, which is regulated in a manner that apparently does not involve protease inhibitors. The system comprises a series of membrane-bound intracellular compartments, within which endocytosed material and redundant cellular components are hydrolysed. Endocytosed material tends to flow vectorially through the system, proceeding through the early endosome, the endosome carrier vesicle, the late endosome and the lysosome. Phagocytosis and autophagy provide alternative entry points into the system. Late endosomes, lysosome/late endosome hybrid organelles, phagosomes and autophagosomes are the principal sites for proteolysis. In each case, hydrolytic competence is due to components of the endolysosomal system, i.e. proteases, lysosome-associated membrane proteins, H^+-ATPases and possibly cysteine transporters. The view is emerging that lysosomes are organelles for the storage of hydrolases, perhaps in an inactivated form. Once a substrate has entered a proteolytically competent environment, the rate-limiting proteolytic steps are probably effected by cysteine endoproteinases. As these are affected by pH and possibly redox potential, they may be regulated by the organelle luminal environment. Regulation is probably also affected, among other factors, by organelle fusion reactions, whereby the meeting of enzyme and substrate may be controlled. Such systems would permit simultaneous regulation of a number of unrelated hydrolases.

Key words: autophagy, endosome, lysosome, proteolysis.

INTRODUCTION

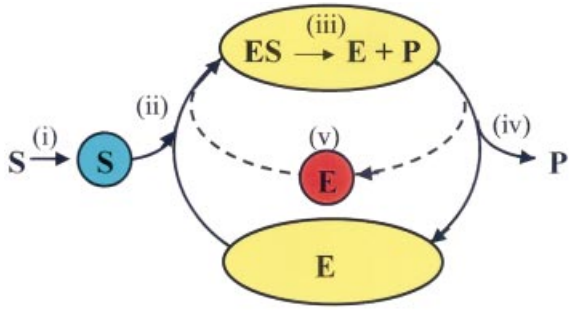
Protein degradation within the cell is principally effected by the endolysosomal, proteosome–ubiquitin and calpain systems. In contrast with the proteosome–ubiquitin and calpain systems, the endolysosomal system largely carries out non-specific bulk proteolysis. The endolysosomal membrane creates a sealed limited environment that allows for optimum functioning of its hydrolases, and yet prevents inappropriate autodegradation. The membrane also houses transporters that remove amino acids, generated by proteolysis, to the cytoplasm.

Apart from degradation, the endolysosomal system is also involved in related functions, such as regulation of signal transduction, antigen presentation and storage. These functions are divided among the various organelles making up the system, which may be thought of as distinct, but connected environments. At the most basic level, the operation of the endolysosomal system of all cells types consists of enzymes that are responsible for degrading substrate that enters the endolysosomal system. The substrate, in turn, must be channelled to the enzymes. Both the enzyme and substrate are maintained in (separate) sealed compartments prior to proteolysis. These sealed environments must be capable of meeting, with the creation of an environment that allows for optimal degradation of the substrate.

Reactions between enzymes (E) and their substrates (S) in solution are described by the reaction (where P is the product)

$$E + S \leftrightarrow ES \rightarrow E + P$$

and can be analysed by conventional kinetic methods. Such enzymes are often regulated by soluble effector molecules. Enzyme reactions in the endolysosomal system, however, can be described by the reactions shown in Scheme 1. As shown, there exists the possibility of regulation at five points: (i) at the point of acquisition of substrate by the endolysosomal system, (ii) at the meeting of E and S by fusion of their respective compartments, (iii) at the establishment of conditions supporting proteolysis (or not) within the (fused) organelle and, possibly (iv) at the point of egress of product from the system. Point (v) represents the possibility of an enzyme being retained in a lysosome for a greater or lesser period (perhaps in an inactive form, but in any event separate from substrate). In terms of flux through the



Scheme 1 Points at which the endolysosomal system may be regulated

Regulation may occur at: (i) the point of acquisition of substrate (S) by the endolysosomal system, (ii) the meeting of enzyme (E) and substrate by fusion of their respective compartments, (iii) the establishment of conditions supporting proteolysis (or not) and, (iv) the point of egress of product from the system. Enzyme may be retained in a lysosome for a greater or lesser period, possibly in an inactive state (v). The colours turquoise, yellow and orange, in that order, represent declining pH.

Abbreviations used: AVd, autophagic vacuole; AV*i*, nascent autophagic vesicle; CHO, Chinese-hamster ovary; ECV, endosome carrier vesicle; ER, endoplasmic reticulum; GILT, γ-interferon-inducible lysosomal thiol reductase; ([^{125}I]tyn-SS-PDL, [^{125}I]iodotyramine linked to poly(D-lysine) by a 3-(propionyldithio)-propionic acid spacer; LAMP, lysosome-associated membrane protein; MPR, mannose-6-phosphate receptors; PDI, protein disulphide isomerase; V-ATPase, vacuolar ATPase.

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system, regulation at (i) and (ii) could be called 'upstream regulation', whereas regulation at (iii), (iv) and (v) could be termed 'downstream regulation'.

Regulation of substrate acquisition into the endolysosomal system and of organelle fusion/budding events [Scheme 1, steps (i) and (ii)] is effected by several signalling networks (reviewed in [1–9]). The signalling networks may include small G-proteins and their signalling cascades, the actin network, and/or phosphatidylinositol 3-phosphate and associated kinases [1–9]. These signalling networks interact with the endolysosomal system through interface molecules that are part of both the endocytosis and the signalling networks [3,7,8]. In the present review, attention will be focused largely on possible regulatory mechanisms operating at points (iii) and (v), i.e. on factors that may affect the activity of the enzymes.

ORGANIZATION OF THE ENDOLYSOSOMAL PATHWAY

The basic organization of the endolysosomal pathway in a mammalian epithelial cell is depicted in Figure 1, although details may vary with cell type. Currently, there are two models to explain how the organelles within the endolysosomal pathway are related to each other [10,11]. Although the two theories differ on the temporal organization of the pathway, the spatial and functional aspects are very similar.

The 'maturation model' suggests that each organelle along the endocytic pathway is a transient, but distinct, compartment that matures into the next organelle along the pathway. In this model, the early endosome is envisioned as being formed *de novo* by the fusion of uncoated primary endosomes derived from the plasma membrane. This transient compartment then matures into a transient late endosome, which in turn matures into a lysosome, the terminal organelle [10]. Each maturation stage has its own unique set of biochemical markers associated with it. These markers and membrane components are recycled by carrier vesicles during maturation [10]. In a related model, proposed by Thilo et al. [12], maturation occurs from the primary endosome until a pre-lysosomal compartment is formed. This compartment then communicates with the lysosome through vesicular traffic.

In the 'pre-existing compartment model', the early and late endosomes are considered to be stable specialized compartments linked by vesicular traffic [11]. The early and late endosomes are regarded as 'compartments', which are stable and do not undergo maturation, but are capable of homotypic fusion. 'Vesicles', unlike compartments, are considered incapable of homotypic fusion. Compartments are considered to be more structurally complex and to have more specialized functions compared with vesicles [11].

The first organelle along the endolysosomal pathway, the early endosome (Figure 1), is a major sorting station. Based on the recycling behaviour of ligand–receptor complexes, the early endosome population may be subdivided into at least two subpopulations, the sorting and recycling endosomes [13]. The luminal pH within the sorting endosome is between pH 6.3–6.5, and is generated by an H^+-ATPase and regulated electrogenically by an Na^+,K^+-ATPase [14,15]. At this slightly acidic pH, ligands, such as low-density lipoprotein and α_2-macroglobulin, dissociate from their cognate receptors. The receptors are recycled to the

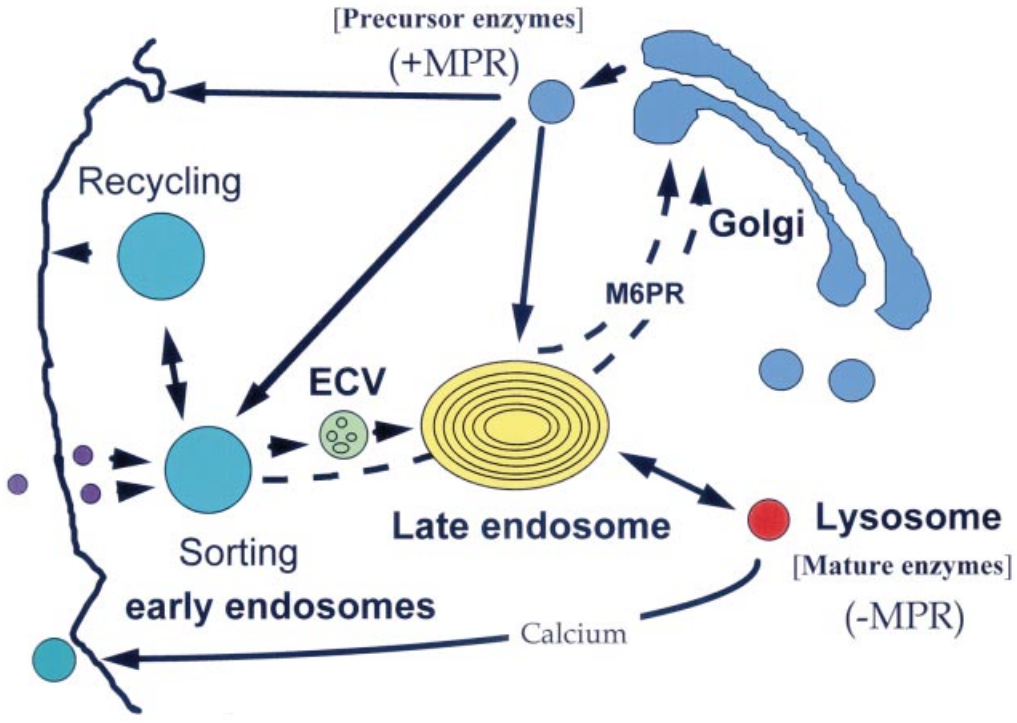


Figure 1 Representation of the endolysosomal system

The major organelles of the endosomal system, the early endosome, late endosome and lysosome, are shown. Solid lines between these organelles may represent vesicular traffic, maturation or direct fusion. Broken lines indicate the recycling of the MPR. Additional components of the endolysosomal system, i.e. phagosomes and autophagic vesicles, are not shown in this simplified diagram, but these organelles interact with the endolysosomal pathway, gaining components that make them proteolytically competent. The colours blue, turquoise, light green, yellow and orange, in that order, represent the declining pH of the intra-luminal conditions. Organelles in different colours may also differ in their redox potential (see the text for details).

plasma membrane, whereas their ligands, low-density lipoprotein and α_2-macroglobulin, are trafficked to the late endosome (reviewed in Mellman [16]). Some receptors, such as the transferrin receptor, can be recycled to the plasma membrane directly from the sorting endosome (fast cycle), or directed to the recycling endosome and then to the plasma membrane (slow cycle) [17]. The recycling endosome may also play a role in directing receptors to the leading edge of migrating cells [17].

The next stage on the endolysosomal pathway is the endosome carrier vesicle (ECV) or multivesicular body. ECVs appear to be large (0.4–0.7 μm in diameter), usually spherical vesicles, often with a membrane-enriched luminal content (reviewed in [11,18,19]), and are responsible for transferring material from early to late endosomes [11].

Formation of ECVs is dependent on the acidification of their luminal interior and can be blocked by the vacuolar ATPase (V-ATPase) inhibitor, bafilomycin A_1 [20]. Indeed, the luminal pH of the ECV drops from that of the early endosome to pH 5.0–5.5 [21]. Endocytosed material may not always be transferred to late endosomes via the ECV. In the mouse macrophage cell line J774, early and late endosomes are capable of fusing directly with each other [22]. This suggests that certain cell types may have specialized endocytic fusion machinery depending on their function.

Distal to the endosome carrier vesicle is the late endosome (sometimes called the pre-lysosomal compartment), followed by the lysosome (Figure 1). A modern definition of a lysosome is that it is 'the terminal organelle on the endocytic pathway and is devoid of recycling receptors' [11]. Complicating the field of endolysosomal proteolysis is the fact that not all authors make a distinction between the late endosome and the lysosome. In the studies of de Duve [23] 'lysosomes' were defined on the basis of two criteria: the existence of a limiting membrane and the presence within the organelle of acid hydrolases. Thus to many authors, lysosomes are any organelles containing acid hydrolases, i.e. 'lysosomal' enzymes (in referring to 'lysosomal' hydrolases here, we put the term 'lysosomal' in quotation marks because some of these enzymes are, in fact, not limited to lysosomes, but are found throughout the endolysosomal system). However, from a modern perspective, this definition encompasses early and late endosomes, lysosomes, phagosomes and autophagosomes. It has also been found that not all 'lysosomal' enzymes have an acidic pH optimum [24,25], and that not all late endosomes/lysosomes are acidic [24].

Both late endosomes and lysosomes contain 'lysosomal' hydrolases and lysosome-associated membrane proteins (LAMPs) [11], and both are enriched in lysobisphosphatidic acid [26]. Lysobisphosphatidic acid is a phospholipid found at high concentrations on the internal membranes of late endosomes, where it may play an important role in the degradation of glycolipids and the transport of membrane proteins and lipids [27]. Under certain conditions, the two organelles may also have similar distributions in density gradients. However, there are differences, for example, mannose-6-phosphate receptors (MPRs) and the regulatory (RII) domain of the cAMP-dependent protein kinase are found on late endosomes, but not lysosomes [11]. Their ultrastructural morphologies are also different. Late endosomes have a complex morphology [11], which is organized by microtubules [28]. In sections, they often have a multivesicular appearance, with intra-luminal membrane whorls (see, for example, [29,30]). In contrast, lysosomes appear as roughly spherical, electron-dense organelles with a simpler organization [11,30]. Based on their relatively simple ultrastructure and their markers, lysosomes may be closer to 'vesicles' than to 'compartments' [11]. A significant difference between lysosomes and other 'vesicles', however, is their capacity to undergo homotypic fusion [31]. To date, no specific marker has been described for lysosomes.

Late endosomes and lysosomes are apparently in dynamic equilibrium. Fluid-phase markers, like BSA-gold, are distributed between the late endosome and lysosome after extended chase periods [11]. Their concentrations of LAMPs are approximately equal, suggesting that their membranes are also in equilibrium. The mechanisms involved in establishing equilibrium may include vesicular transport [32], 'kiss and run' events [33] or direct fusion (reviewed in Luzio et al. [34]) and may vary with cell type. Despite their close association, late endosomes and lysosomes have distinct functional differences. Although containing only 20 % of the total hydrolase pool, late endosomes are the main site for proteolysis [29,30]. By contrast, lysosomes contain the bulk of the 'lysosomal' hydrolase pool but only about 20 % of total proteolysis takes place in lysosomes. It has consequently been suggested that lysosomes may act as storage organelles for these hydrolases [11,29].

Fusion of late endosomes and lysosomes produces a hybrid organelle with properties of both [34]. Bafilomycin A_1 did not decrease late endosome–lysosome fusion, suggesting that acidification was not a requirement for hybrid formation [35]. However, lysosome recondensation from the hybrid organelle was dependent on a functional V-ATPase [36]. A further requirement for hybrid organelle formation was the presence of intra-organellar Ca^{2+} and calmodulin. Intra-organellar Ca^{2+} is also required for fusion and recondensation of lysosomes to and from the hybrid organelle [36].

Although the lysosome is the terminal organelle of the endolysosomal system, lysosomes should not be viewed as dead-end organelles, as 'secretory lysosomes' may be a feature of many normal cells [37]. An increase in intracellular Ca^{2+} levels typically results in secretion of about 5–15 % (rising to 60 % in haematopoietic cells) of the total content of lysosomes. A rise in Ca^{2+} levels causes lysosomal membranes to fuse with the cell membrane, resulting in exocytosis of the lysosomal contents [37]. The process is thought to be regulated by synaptotagmin VII [38], and may constitute a mechanism for the repair of damaged plasma membrane [39]. Mechanisms for the secretion of 'lysosomal' proteases are clearly of interest in the context of cancer, where these enzymes have been purported to play an extracellular role. Many cells of haematopoietic origin are capable of exocytosing multivesicular bodies that contain internal vesicles called exosomes. These exosomes may be involved in multiple functions, including T-lymphocyte stimulation (reviewed in Denzer et al. [40]).

DELIVERY OF SUBSTRATES

Substrates destined for degradation can enter the endolysosomal lumen by three broad mechanisms: endocytosis, autophagy and phagocytosis. These mechanisms all result in environments that support proteolysis, using a common set of lysosomal hydrolases. This present review of these processes, although not exhaustive, is aimed at finding common luminal features that may provide insights into the minimal requirements for endolysosomal proteolysis and may also suggest possible modes of regulation of proteolysis.

Endocytosis

Endocytosis can be divided into three distinct mechanisms: receptor-mediated endocytosis, constitutive endocytosis and caveoli formation. Ligand–receptor complexes that enter the endo-

Table 1 Common luminal features of proteolytic organelles of the endolysosomal system

Organelle	Hydrolases	LAMPs	MPR	V-ATPase	Lumenal Ca^{2+}	Cysteine transporter	References
Early endosome	+ (cathepsin H)	−	+	+	+	?	[11,71,72]
Late endosome	+	+	+	+	+	?	[11,71,72,75]
Lysosome	+	+	−	+	+	+	[11,36,71,75,126,127]
Hybrid organelle	+	+	+	?	+	?	[30,34,36]
AVd	+	+	−	+	+	?	[52,60–63]
Phagosome	+	+	−	+	?	?	[11,13,71,72]

lysosomal system can be sorted into one of three pathways: (i) the entire ligand–receptor complex may be recycled back to the plasma membrane; (ii) the ligand–receptor complex may dissociate, with the receptor being recycled and the ligand directed further along the pathway; or (iii) the entire ligand–receptor complex may be targeted to the later stages of the pathway (see Warnock [41] and references therein). This sorting process thus occurs within the early or late endosomes.

In contrast with receptor-mediated endocytosis, substrates entering the pathway by fluid-phase endocytosis are constitutively directed to further stages along the endolysosomal pathway. Pulse-chasing fluid-phase markers, such as horseradish peroxidase and BSA labelled with gold, into a cell first labels early endosomes (5 min), and with longer chase times (<30 min) the later endosome populations become labelled (see, for example, Aniento et al. [19] and Rabinowitz et al. [42]).

Autophagy

Autophagy is responsible for the destruction of most endogenous proteins, the removal of obsolete and/or damaged organelles, cellular re-modelling during differentiation, metamorphosis and ageing [43,44]. It may be activated when amino acids are limiting *in vitro* [45] and *in vivo* [46]. There are four distinct autophagic mechanisms: macro-autophagy, micro-autophagy, crinophagy and chaperone-mediated autophagy. Micro-autophagy happens when parts of the cytoplasm are taken up directly by lysosomes, occurring when lysosomes invaginate their membranes. Crinophagy is a process whereby secretory granules fuse directly with lysosomes. Chaperone-mediated autophagy effects direct import of cytosolic polypeptides into the endolysosomal system. Micro-autophagy, crinophagy, and chaperone-mediated autophagy have been reviewed previously [44,47,48]. The regulation of autophagy has been reviewed previously [44,49].

Macro-autophagy occurs when entire regions of the cytoplasm are sequestered by a membrane and degraded. This process occurs in at least three distinct stages that are characterized by morphological and biochemical changes to the sequestered cytosol. These stages from sequestration to degradation occur rapidly, with a half-life of approx. 9 min. [50]. The first stage is referred to as sequestration, and is characterized by the formation *de novo* of an organelle referred to as a nascent autophagic vesicle (AV*i*) or the phagophore. A consistent terminology to describe the various stages of autophagy has not yet emerged, and the terminology of Dunn [51,52] will be used here. The signals responsible for the sequestration step are being resolved and may involve G-proteins [49,39–55], a novel protein-conjugation system (see Mizushima et al. [56] and Kirisako et al. [57]), protein and lipid kinases, Ca^{2+} and adenosine nucletotides, and the cytoskeleton (reviewed in Kim and Klionsky [49]).

The source of the sequestering membrane is a matter of dispute, but it may originate from the rough endoplasmic reticulum (ER) [51] or the post-Golgi region [58,59]. AV*i*s have a distinctive structure, with membrane structures and entire organelles engulfed by the forming AV*i* (see, for example Figure 1 in [60]). The AV*i* does not have lysosomal hydrolases or LAMPs associated with it [52,62,63]. It is therefore presumed that the AV*i* is not involved in proteolytic degradation of the engulfed cytoplasm.

The AV*i* apparently undergoes biochemical and morphological changes (maturation), acquiring lysosomal membrane proteins (such as LAMPs) and hydrolases that subsequently allow it to degrade the sequestered substrate [52,62,63]. The AV*i* also develops an acidic luminal interior [52,64] and becomes capable of proteolysis. The fully matured vesicle is called an autophagic vacuole (AVd) [51,52]. In contrast with the AV*i*, the AVd usually has a single membrane, the other membrane structures presumably having been degraded or recycled.

The mechanism of AVd formation from the AV*i* has not been resolved. The AV*i* may fuse directly with lysosomes, which rapidly converts it into an AVd [60]. Alternatively, it has been demonstrated that the endocytic and autophagic pathways converge after the AV*i* and early endosome stages respectively, resulting in formation of amphisomes [61,62]. In either case, the AVd acquires its lysosomal hydrolases from the endolysosomal system.

Phagocytosis

Although a number of cell types are capable of phagocytosis, the most important professional phagocytes are neutrophils and mononuclear phagocytes [65]. The discussion in this review will be limited to macrophages. Macrophages have a complement of cysteine endoproteases similar to that of most epithelial cells. Presumably, the components necessary for a fully functional proteolytic system are the same, or very similar, in both cell types. A notable exception is that macrophages contain the powerful cysteine endoprotease, cathepsin S [66].

Phagocytosed particles are usually in the size range 0.3–0.5 μm in diameter or larger. Their adhesion to the macrophage surface may depend on a number of forces: van der Waal's-, hydrophobic-, electrostatic- or receptor-mediated interactions [65]. The adhesion of a particle to the membrane surface triggers a cascade of signalling events (reviewed in May and Machesky [9], and Kwiatkowska and Sobota [67]) that lead to the formation of a phagosome. Initially, the phagosome lumen resembles the extracellular environment, but the phagosome soon undergoes a series of biochemical changes (maturation), accompanied by changes to the lumen environment. This process is Rab-dependent and involves fusion of the developing phagosome with endocytic organelles [22,68–70]. The changes to the phagosome include: V-ATPase-dependent acidification, and acquisition of Rabs, LAMPs and lysosomal hydrolases [71,72]. Some phagocytosed pathogens escape destruction by disturbing the maturation process. For example, phagosomes that ingest *Mycobacterium avium* cells fail to acidify [73], and those ingesting *M. bovis*

cells fail to incorporate Rab7 [74] and consequently do not participate in late endocytic fusion events [22,74]. Thus the pathogen escapes destruction by the hydrolases that are found in late endosomes/lysosomes.

Based on their importance for endocytosis, autophagy and phagocytosis, the apparent minimal requirements for generating functional proteolytic environments within the endolysosomal system are summarized in Table 1, and are discussed in greater detail below.

COMMON FEATURES OF LUMEN PROTEOLYTIC ENVIRONMENTS

Hydrolases

Most of the soluble endolysosomal hydrolases are synthesized as pre-proenzymes [75]. The signal peptide is cleaved co-translationally and the hydrolases fold into their precursor form within the ER lumen. These precursors undergo asparagine-linked glycosylation and carbohydrate processing, which continues in the Golgi. Within the Golgi, two enzymes, *N*-acetylglucosaminylphosphotransferase and α-*N*-acetylglucosaminidase, add a mannose-6-phosphate label [75,76]. Thus the labelled precursors become ligands for the membrane bound MPRs, which direct them to the endolysosomal system (reviewed in Hasilik [75] and Kornfeld [76]). Glycosylation may also serve to protect the hydrolases from destruction within the endolysosomal system [77].

There are over 50 'lysosomal' hydrolases, and some of these enzymes show no sequence homology with each other. Their recognition by the phosphotransferase therefore involves common tertiary structural features [78–80]. There are two MPRs; the 275-kDa cation-independent MPR and the 46-kDa cation-dependent MPR [76]. These receptors have different affinities for the soluble precursors, depending on their carbohydrate structure [81]. Together, these two receptors are able to sort efficiently the diverse array of soluble precursors for targeting to the endolysosomal system. Evidence also exists for MPR-independent targeting mechanisms [75]. Within the endolysosomal system, proteolytic removal of a pro-piece serves to generate the mature active enzyme.

The endolysosomal protease pool may be divided into the endo- and exo-peptidase pools. The endoprotease pool (Table 2) is mainly made up of cysteine and aspartic proteases, and the exopeptidase pool of cysteine and serine proteases. Serine proteases, which are the most numerous and diverse hydrolase class in nature, are notably absent from the endoproteolytic pool. The serine protease cathepsin G is found exclusively in haematopoietic cells and is not a true endolysosomal enzyme (Table 2).

Table 2 Endoproteases found within the endolysosomal system

Name	Catalytic group	M_r	Operating pH*	pI	Distribution	References
Cathepsin B	Cys	27	5–6.5	5.4	Ubiquitous	[66,77,139]
Cathepsin D	Asp	42	2.8–5.0	5.5–6.5	Ubiquitous	[77,144]
Cathepsin E	Asp	100	3–3.5	4.1–4.4	Restricted	[77,145]
Cathepsin G	Ser	30	7.5	10	Neutrophils	[77,138]
Cathepsin H	Cys	28	5.0–6.5	7.1	Ubiquitous	[66,77,143]
Cathepsin L	Cys	29	4.5–6.0	5.8–6.1	Ubiquitous	[66,77,140]
Cathepsin N	Cys	34	3.5	6.2	Ubiquitous	[66,79]
Cathepsin S	Cys	24	5.0–7.5	6.3–6.9	Restricted	[66,79,141]
Cathepsin T	Cys	34	6.9	?	Restricted	[66,77,146]
Cathepsin K	Cys	27–29	6.0–6.5	?	Osteoclasts	[142]
Legumain	Cys	31	4–6	?	Ubiquitous	[66]

* 'Operating pH' is the pH at which the enzyme is stable, which may be different from the pH optimum.

Once a substrate is delivered into a proteolytically competent compartment, it is presumed that the rate-limiting steps in substrate hydrolysis are affected by endoproteases. Their action would generate peptide fragments that would serve as substrates for the lysosomal exopeptidase pool. Thus the present discussion will focus on the endopeptidases, most of which belong to the C1 family of cysteine proteases (Table 2). The C1 family all show a common fold with the archetypal protease of this family, papain. These enzymes are bi-lobular with left and right domains. These domains are highly conserved and all members show common secondary structure elements in their respective domains. Differences between these proteases are usually due to deletions or insertions in the loop regions between the conserved structural elements that comprise the papain fold [66].

The active site is in a deep cleft between the left and right domains. The active site cysteine residue, Cys^{25} (papain numbering), and histidine residue, His^{159} (papain numbering), form a thiolate–imidazolium ion-pair that is responsible for catalysis. Other highly conserved residues are Asn^{175}, which is believed to orientate the imidazolium ring, and Gln^{19}, which is part of the oxyanion hole. Substrates bind into the active site in an extended conformation, and the carbonyl carbon of the scissile bond undergoes nucleophilic attack from the active-site thiol. This results in the release of amine product. The resultant acyl-enzyme reacts with water to release the carboxyl product (deacylation), resulting in the regeneration of the free enzyme. A detailed description of the catalytic mechanism is provided by Storer and Ménard [82].

Why has this family of proteases been selected in preference to other types of proteases? Perhaps uniquely, these enzymes have the following features that make them well suited to the endolysosomal system.

- The nascent enzymes must be capable of being recognized by the phosphotransferase system within the ER. This recognizes structural features, and not a linear sequence [78–80]. This may thus place limits on the hydrolases that could be accommodated within the system. Gene duplication may have created variants of these unique enzymes within the endolysosomal system.
- The lysosomal cysteine proteases are required to cleave a wide variety of substrates and thus their active sites cannot be optimized for a specific substrate. However, the catalytic mechanism of this class of cysteine peptidases is very efficient when compared with the catalytic mechanism employed by serine proteases (see, for example, [83]). This may off-set any loss of efficiency due to a relatively non-specific active site.
- The luminal conditions of pH and redox potential within the late endolysosomal system are designed to denature substrates, allowing for increased hydrolytic efficiency. These conditions may be in a constant state of flux, allowing many different hydrolase classes to operate within the system. The cysteine proteases must be stable to these denaturing conditions and, in addition, must be capable of operating over a broad pH range. The enzymes themselves also have to be fairly resistant to proteolysis. The catalytic ion-pair of the papain superfamily appears to be active over a wide pH range [82], a requirement that cannot be met by any of the other proteolytic classes. This would allow these enzymes to operate in the dynamic endolysosomal proteolytic environment [24]. However, in contrast to other members of the papain superfamily, the endolysosomal enzymes tend to be unstable at neutral to alkaline pH values [66]. This may protect the cell against these enzymes.

The enzymes themselves appear to be stable to the denaturing conditions within the endolysosomal system and have half-lives that run from days to weeks [77].

- Some of the cysteine proteases are restricted to specific organelles. For example, cathepsin H appears to be restricted to the early endosome in J774 macrophages [72]. This suggests that this enzyme must have features that restrict it to this specific organelle, at least in this cell type.
- Finally, endolysosomal proteinases may be capable of regulation without the need for inhibitors. There is no evidence of intra-endolysosomal inhibitors, and yet proteolysis may be shut-down in (storage) lysosomes. This suggests that these enzymes have properties that allow them to be regulated within the endolysosomal system.

Thus quite apart from being efficient hydrolases, these enzymes appear to fulfil a number of requirements that allow them to operate within the endolysosomal environment. Of these additional requirements, the ability to be regulated, without the need for intra-endolysosomal inhibitors, may be amongst the most significant.

Cathepsin D is an aspartic endoprotease found within the endolysosomal system. Unlike its cysteine protease counterparts, this enzyme is capable of operating at very acidic pHs. Thus cathepsin D could effect proteolysis at pH values where the cysteine proteases may be inactivated by protonation of the active-site thiol.

LAMPs

LAMPs are the major protein constituents of late endolysosomal membranes, which is consistent with a putative role in protecting these membranes from hydrolysis and thus preventing leakage of the hydrolases into the cytoplasm. The phagosome, AVd, late endosome and lysosome are all enriched in LAMP proteins. LAMPs are ubiquitously distributed throughout mammalian cells, and make up as much as 50 % of the total protein found on 'lysosomal' membranes. LAMP-1 and LAMP-2 are evolutionarily related: both are type-I membrane proteins, with a short cytoplasmic tail, a transmembrane region and a large luminal domain. The short (10–11 residues) cytoplasmic tail contains targeting information directing the LAMPs to the late endosome/lysosome (reviewed in Peters and von Figura [84]). Proteolytic processing of this tail probably ensures that the protein is retained by these organelles [85]. Although LAMPs and MPRs are directed to the same organelles, they use different targeting machinery [86].

The large luminal domain of the LAMPs has a high carbohydrate content with 16–20 N-linked glycosylation sites, as well as O-linked glycosylation sites. The luminal domain of these proteins also has a proline-rich hinge region and four contiguous disulphide bridges [84]. The high sialic acid content of their carbohydrate moieties contributes to their low pI, which may be of functional significance. LAMPs may participate in aggregation of the soluble lysosomal contents [87]. This could be due to the low pI of the LAMPs, allowing these proteins to behave as cation exchangers. This putative regulatory mechanism will be discussed in greater detail below. When expressed on the cell surface, LAMPs may also play a role in cell adhesion processes [88].

LAMP-1- [89] and LAMP-2-deficient [90] mice have been generated recently. The LAMP-1-deficient mice were viable and fertile, and had lysosomes with properties similar to control lysosomes. The loss of LAMP-1 appeared to be compensated for by increased expression of LAMP-2 [89]. LAMP-2 mutants, on the other hand, showed increased mortality compared with control mice, and showed extensive accumulation of AV*i*s. These results suggest that LAMP-2 is necessary for maturation of the AV*i* to the AVd [90]. The LAMP-1 and LAMP-2 double mutation is lethal [89].

Cysteine transporter

The primary function of endolysosomal proteolysis is to degrade macromolecules for recycling into anabolic reactions. Egress of the products of proteolysis from this sealed environment is largely undertaken by transporters, which have been described for carbohydrate monomers, nucleosides, amino acids and ions (reviewed in Pisoni and Thoene [91]). Two of the transporters that could be involved in regulating the redox potential of the endolysosomal environment are the cystine- and cysteine-specific transporters, and the possible role of these in supporting lysosomal proteolysis will be the focus here.

Disulphide bridges are important in the structural stability of proteins, and reduction of these in the endolysosomal environment may increase the rate of proteolysis. Cysteine is believed to be the physiological reducing agent involved [91]. However, reduction of disulphide bridges by cysteine results in the generation of cystine [92], which is poorly soluble and crystallizes unless it is removed from the 'lysosome'. This occurs in the disease, cystinosis, due to a defect in the cystine transporter [91]. The properties of the cystine transporter have been evaluated *in vitro* using counter-transport and *trans*-stimulation studies, although such studies are complicated by the heterogeneity of 'lysosomal' preparations.

In contrast with other 'lysosomal' transporters, the cysteine-transporter produces a net influx of cysteine *into* 'lysosomes'. When human fibroblasts were incubated with [^{35}S]cystine, at least 50–60 % of the total radioactivity taken up by the cells was found to be associated with 'lysosomes' in the form of cysteine [93]. This uptake occurred rapidly (2–5 min) and the transporter was found to be highly specific. Transport into the 'lysosome' was stimulated when the pH outside was greater than the luminal pH [94]. Cysteine-transport activity has also been described in lysosomal fractions from macrophage and B-cell lymphoma cell lines [95]. In the B-cell lymphoma cell line, cysteine transport activity was also detected in antigen processing compartments [95], suggesting that reduction may be a feature of antigen processing.

The 'lysosomal' cysteine transporter, similar to most endolysosomal transporters, has not been isolated and therefore its intracellular location has not been directly established. However, there is evidence that disulphide reduction occurs in the late endosome compartments, rather than in early endosomes [96,97], suggesting that the cysteine transporter may not be present in early endosomes.

The presence of the cysteine transporter can be interpreted as evidence of reducing conditions within the endolysosomal environment. Indeed, the proteolytic environment may be reducing, but the situation is not as simple as often presented. That reduction does occur is evident from studies on toxins, such as diphtheria toxin, which requires reducing conditions to be active [98,99]. Reduction also appears to be an essential component of antigen processing (see, for example, Collins et al. [97] and Merkel et al. [100]). Presentation of the insulin A chain requires that the cysteine residues are in the thiolate form [101]. Furthermore, Gainey et al. [95] demonstrated that presentation of antigens with disulphide bonds requires compartments capable of cysteine transport. The effect may also be a general one, i.e. that reducing conditions enhance substrate proteolysis, which is not limited to antigen processing.

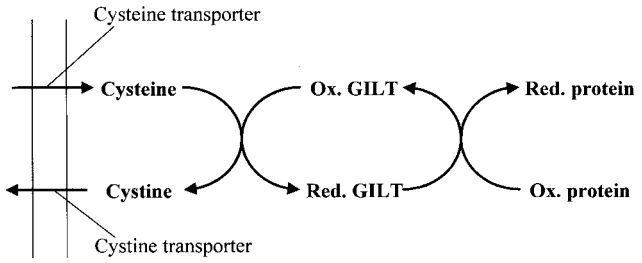
Reducing conditions may affect both proteases and their substrates. It is often assumed that a reducing environment is necessary for the activity of the lysosomal cysteine proteases. However, although isolated cysteine proteases do require reducing agents in order to be active, there is no evidence that these enzymes are oxidized *in vivo* [102–104]. Oxidation may, therefore, be an isolation artefact. In the reduced activated form, the isolated enzymes are more stable to neutral pH conditions [25]. As will be described below, the late endolysosomal environment may not always be acidic and may cycle in neutral or even alkaline conditions. A reducing environment may serve to stabilize these proteases during the purported pH changes.

Reducing conditions would also affect the substrate proteins. It was found that substrates with disulphide links are cleaved with greater efficiency by lysosomal proteases in the presence of a reducing agent [105,106]. Reducing agents are believed to work synergistically with the proteases by reducing the disulphide links, relaxing the substrate structure and exposing more sites for proteolysis. Thus a reducing endolysosomal environment does seem to confer several advantages for proteolysis. However, Lloyd [92] has pointed out that the stoichiometry of the reduction reaction does not necessitate the continuous influx of cysteine and has suggested that the cysteine transporter may simply serve an anaplerotic function, replenishing cysteine lost to auto-oxidation. It must also be questioned how endolysosomal proteins containing essential disulphide bridges (e.g. cathepsins and LAMPs) are protected against reduction.

A further question is the effect of (low) pH on the reduction potential within the endolysosomal system. Feener et al. [96] constructed a probe comprising [^{125}I]iodotyramine linked to poly(D-lysine) by a 3-(propionyldithio)-propionic acid spacer ([^{125}I]tyn-SS-PDL), which was acid stable and resistant to proteolysis, but sensitive to reduction. This probe was found to be inefficiently reduced within the endolysosomal system, which suggested that the Golgi/*trans*-Golgi may be the main site of reduction [96]. In experiments *in vitro*, the probe was inefficiently reduced by cysteine (5 mM) at low pH [96]. A basis for this result may be found by considering the effect of pH on reduction potential. At fixed cysteine/cystine concentrations, redox potential is inversely proportional to pH, so the reduction potential will be more oxidizing at lower pH. Low pH conditions within the endolysosomal system may thus generate a relatively oxidizing environment.

How may this evidence for and against a reducing endolysosomal proteolytic environment be reconciled? The extremely rapid sequestration of cysteine by 'lysosomes' [93], we believe, is inconsistent with a purely anaplerotic function for the cysteine transporter, as suggested by Lloyd [92]. Moreover, the experiments describing the synergistic effects of cysteine on proteolysis were all undertaken at acidic pH [105,106], suggesting that reduction is not precluded by low pH. To keep the reduction potential more or less constant, however, at low pH, a higher concentration of cysteine and/or a lower concentration of cystine is required. Perhaps the function of the cysteine and cystine transporters is thus to maintain the redox potential within limits by ensuring an influx of the thiol and efflux of the disulphide.

The cytosolic redox buffer is glutathione and cytosolic GSH/GSSG ratios range from 30:1 to 100:1, which corresponds to a redox potential of about −221 mV to −236 mV [107]. Protein disulphide bridges are largely formed in the ER secretory pathway by the enzymes Ero1p (in yeast) and enzymes of the protein disulphide isomerase (PDI) family [108]. The redox buffer in the ER/Golgi secretory pathway may also be glutathione, but here it is more oxidizing than the cytosol, with a GSH/GSSG ratio of 1:1 to 5:1 and a redox potential of about −160 mV to −170 mV [107]. This is in agreement with the optimum GSH/GSSG ratio for the activity of PDI *in vitro*. However, Pdi1p (the yeast analogue of PDI) occurs largely in the disulphide form *in vivo*, and Frand et al. [108] have argued that this balance point is due to efficient oxidation by Ero1p and relatively inefficient reduction by GSH. In the disulphide form, PDI-type enzymes can act as oxidases, transferring disulphides directly to oxidizable proteins.



Scheme 2 Reduction of substrate proteins in the endolysosomal system

It is hypothesized that greater specificity and efficiency of reduction of substrate proteins may be effected by the interposition of GILT-type enzymes between cysteine and the substrate protein. Ox., oxidized; Red., reduced.

An essentially opposite situation may occur in the endolysosomal system where efficient hydrolysis requires the reduction of protein disulphide bridges. Here the reducing agent is thought to be cysteine, but the cysteine/cystine concentration and ratio is unknown. Nevertheless, modelling of the Nernst equation reveals that to achieve a redox potential of −160 mV to −170 mV (i.e. the same as in the ER) at pH 5.0 with a cysteine/cystine redox buffer (at 1 mM total half-cystine), a cysteine/cystine ratio of about 180:1 to 200:1 is required, and to achieve −221 mV to −236 mV (i.e. the same as in the cytosol) requires ratios of 500:1 to 600:1. This could provide a reason for the existence of the cysteine transporter.

A lysosomal reductase, γ-interferon-inducible lysosomal thiol reductase (GILT), has been described [109], which is optimally active at acidic pH, is activated by cysteine (and not by glutathione) and is capable of reducing protein disulphides. Although details are unknown, it is possible that reduction of protein disulphides may occur in a manner such as shown in Scheme 2. The proposed interposition of GILT between cysteine and oxidized proteins (Scheme 2) is analogous to the interposition of PDI between oxidants and protein substrates in the ER, and could play a similar role in introducing greater efficiency and specificity into the reduction reactions. For example, the fact that the [^{125}I]tyn-SS-PDL probe of Feener et al. [96] was not reduced may be because GILT could not recognize this substrate. Similarly, lysosomal proteases with disulphide links may have structural features that exclude them as substrates for GILT.

Acidification

An acidic luminal environment is important for the following functions.

- Receptor-mediated endocytosis and recycling. Receptors, such as the MPR, require acidic conditions in order to discharge their ligands and recycle.
- Movement/maturation of organelles. ECV formation requires a functional V-ATPase [29], which suggests a link between the movement of substrate along the endolysosomal pathway and acidification.

- Activity of membrane transporters. 'Lysosomal' membrane transporters, such as the cystine transporter [110], have acidic pH optima that effectively ensures that amino acid transport is from the lysosome to the cytoplasm [91].
- Activation of lysosomal hydrolases and proteolysis. Many of the lysosomal hydrolases are proteolytically processed to their mature forms under acidic conditions [75], some by autoprocessing. Furthermore, many of these hydrolases have acidic pH optima and are therefore maximally active under acidic conditions. An acidic environment may also contribute to denaturation of substrate proteins, which increases the rates of proteolysis.

An acidic luminal environment is established by the V-ATPase and a redox chain [111], both of which pump protons into the lumen. The lysosomal redox chain uses cytoplasmic NADH as an electron donor in a chain that has oxygen as the final electron acceptor [111]. The V-ATPase is made up of two multi-subunit sectors: V_0, an integral membrane sector, and V_1, which is essentially a cytoplasmic sector. The V_0 sector is made up of at least nine subunits (100, 38, 19 and 17 × 6 kDa), and is responsible for proton translocation. The V_1 sector is also made up of at least nine subunits (73 kDa × 3, 58 kDa × 3, 40, 34 and 33 kDa) and is responsible for the ATPase activity. The ATP-binding regions are found on the 73-kDa A-subunits, which show a high degree of co-operativity in ATP catalysis (reviewed in Futai et al. [112]). Also found within the A subunit is a conserved region known as the P-loop. The P-loop has a cysteine residue (Cys^{254}), which is capable of undergoing thiol/disulphide exchange with Cys^{532}, allowing for redox regulation of the V-ATPase [113]. Disulphide-bond formation is believed to induce a conformational change, depressing ATPase activity and inhibiting proton translocation [113]. This mechanism provides a further link, though of a different type, between redox potential and pH.

Although the V-ATPase is responsible for translocating protons into the endolysosomal lumen environment, regulation of the pH of this environment also depends on other factors. Continuous influx of protons into an organelle would result in the generation of a net positive membrane potential that would inhibit further proton translocation. It is believed that inwardly-directed Cl^- ion channels, and perhaps cation channels also, may help regulate the pH of an organelle (reviewed in Futai et al. [112]). The Cl^- channels, in turn, may be regulated by protein kinase A-dependent phosphorylation [114]. As described above, the early endosome pH is regulated by an Na^+/H^+-exchanger that helps to create an internal positive membrane potential, inhibiting further proton uptake [14,15]. The 'lysosomal' pH may be maintained by a Donnan-type equilibrium [116,117]. The number of V-ATPase molecules found on an organelle may also influence the pH of that organelle [115]. Other regulatory features include reversible dissociation–reassembly of the V-ATPase complex, changes in the coupling efficiency between ATP hydrolysis and proton transport and low-molecular-mass activator and inhibitor proteins (reviewed in [115]).

Given the importance of an acidic environment to the proper functioning of the endolysosomal system, are all lysosomes acidic? Some of the cysteine endoproteases found within the endolysosomal system are capable of working over a broad pH range. In fact, cathepsin B has a neutral pH optimum against synthetic substrates *in vitro* [25,118–121]. Cathepsin S is also capable of operating at neutral pH [66]. Butor et al. [24] confirmed the late endolysosomal location of two enzymes, sialic acid-specific 9-*O*-acetyl-esterase and glycosyl-*N*-asparaginase, both of which have neutral to alkaline pH optima, and demonstrated further that not all 'lysosomes' were acidic. They [24] suggested that the 'lysosomal' pH may cycle between acidic and neutral pHs, allowing for the optimal activity of the different hydrolases found within the system. A similar pH cycle may also exist as a consequence of a late endosome/lysosome traffic cycle.

Luminal Ca^{2+}

The role of cytoplasmic Ca^{2+} in lysosomal homotypic fusion [122], lysosome–plasma membrane fusion [37] and phagosome–lysosome fusion [123,124] has been well documented. However, the role of *luminal* Ca^{2+} in regulating proteolysis within these organelles has not been as extensively studied. To the best of our knowledge, the role of intra-organellar Ca^{2+} in regulating phagosome function is unknown. Autophagy, however, appears to depend on intracellularly sequestered Ca^{2+} [125], although the identity of the organelle(s) involved is not known. Lysosomes have been shown to be an intracellular pool for Ca^{2+} [126], and the existence of a lysosomal Ca^{2+} transporter has been demonstrated [127]. The K_m for this lysosomal Ca^{2+} transporter is approx. 5 mM [127], which is significantly higher than the luminal Ca^{2+} concentration [128]. This lysosomal Ca^{2+} transporter may therefore play a role in Ca^{2+} efflux from lysosomes, rather than influx. Calcium sequestration by lysosomes may be via endocytosis, as the extracellular Ca^{2+} concentration can be in the micromolar range [128]. As discussed above, hybrid organelle formation is dependent on Ca^{2+} release from the lumen of late endosomes and lysosomes to facilitate membrane fusion [36]. Luminal Ca^{2+} may also play a role in lysosome reformation from the hybrid organelle [36]. The effect of Ca^{2+} on lysosomal proteases has not, to the best of our knowledge, been reported.

REGULATION OF ENDOLYSOSOMAL PROTEOLYSIS

Regulation by intra-luminal redox conditions?

A conceivable control mechanism for endolysosomal cysteine proteinases is via regulation of the redox conditions in the intraluminal environment, as discussed above. Both cathepsin B and cathepsin L are stable in the range pH 4.5–6.5 and require a reducing environment for activity *in vitro* [66], which is thought to be required to maintain the active-site cysteine residue in a thiol form. However, there is no evidence that these enzymes are oxidized *in vivo*. Labelling with the inhibitor benzyloxycarbonyl-[^{125}I]iodo-Tyr-Ala-diazomethane (*Z*-[^{125}I]-Tyr-Ala-CHN_2) of cathepsin B in isolated mouse liver lysosomes did not increase with the addition of dithiothreitol or cysteine [102]. These two reducing agents also did not enhance the activity of cathepsin B in extracts from lung carcinomas and lungs [103,104]. This suggests that redox regulation of the active-site thiol may not occur in the endolysosomal proteolytic environment. Furthermore, it also suggests that these enzymes may not necessarily become oxidized upon their secretion into the extracellular environment.

Although the active site thiols of these enzymes may not be oxidized, it is possible that their ionization state may be altered. At very low pH, the activity of the cathepsins is depressed. This may be related to a pH-dependent change in the ionization state from a thiolate (RS^-) to a thiol (RSH) form. However, at low pH cathepsin D would be active (Table 2).

A study by Krepela et al. [104] suggested that cathepsin B may be inhibited by low concentrations of the thiol (RSH) form of cysteine, which acts as a competitive inhibitor. The inhibitory effect of the thiol form of cysteine could be reversed by increasing concentrations of the thiolate (RS^-) form, and was less prevalent at higher pH. These authors [104] suggested that cysteine may bind into the S1′ subsite of the enzyme, inhibiting its function.

This subsite varies between the different cathepsins [66] and may not be a general regulatory mechanism for all the enzymes. However, since cathepsin B is the most prevalent endoprotease, regulating its function may significantly affect proteolysis within the endolysosomal system.

A study of the effects of changes in redox potential on the activity of (100 % active) cathepsin B, showed that the redox potential itself had very little effect on the activity (C. S. Pillay and C. Dennison, unpublished work). It is concluded that, although the endolysosomal lumen may be reducing, changes in cysteine/cystine-induced redox potentials are probably not involved in regulating endolysosomal proteases. Moreover, an expectation of any regulatory mechanism is that it should include all hydrolases within the system, and other endolysosomal hydrolases, e.g. α-glucosidase, have no cysteine residues that could be affected by redox changes.

Unfortunately, the cysteine transporter has not been isolated and its intracellular location, e.g. lysosomes versus late endosomes, and whether it is present in autophagic vacuoles, have not been determined. Thus, at this stage, it is not possible to unequivocally state that reducing conditions are, or are not, a necessary attribute of a proteolytic compartment.

Regulation by pH

Regulation by pH changes, in contrast with redox changes, could simultaneously influence many enzyme classes. Factors that could regulate the luminal pH include: the redox state of the cysteine on the P-loop of the V-ATPase, the presence of active Cl^- transporters on the organelle, a Donnan-type equilibrium, the number of V-ATPases found on that organelle, factors that influence the reversible dissociation/reassembly of the V-ATPase complex, changes in the coupling efficiency between ATP-hydrolysis proton transport and low-molecular-mass activator and inhibitor proteins [112,115], and possibly also a membrane redox chain [111]. What is not clear is how these factors work together to establish the lumen pH. As described above the lumen pH may be dynamic. Within the early endosome, pH appears to regulated by G-proteins (reviewed in Warnock [41]), which respond to transporter entry into the early endosome. Also, Na^+/K^+ exchangers help modulate this environment. The (relatively high) pH within this environment allows cathepsin H to function optimally.

Acidification could also contribute to the storage of endolysosomal enzymes by complexation. Kostoulas et al. [129] found that elastase and other enzymes within the azurophil granules of human neutrophils bound to sulphonated glycosaminoglycans by electrostatic interactions at low pH. It was proposed that this may be a storage mechanism for these enzymes. Pryor et al. [36] suggested that lysosome condensation may be similar to secretory granule formation. A feature of secretory granules is that they contain condensed cores of aggregated proteins, a morphology similar to that of lysosomes [11]. Using bovine pituitary gland cells, it was demonstrated that the granule content proteins and the luminal domains of granule membrane proteins could aggregate at low pH (pH <5.5) [130]. A similar result [130] was obtained for bovine adrenal glands, although aggregation depended on the presence of Ca^{2+}. Proteins destined for constitutive secretion did not aggregate with the granule content and luminal membrane proteins, and this property may serve as a segregation mechanism for those proteins to be stored and those that are constitutively secreted [130].

The aggregation of lysosomal enzymes at pH 4.8 in Chinese-hamster ovary (CHO) cells has been described [131]. This aggregation was disrupted by NaCl, suggesting an electrostatic mechanism. Horseradish peroxidase that had been chased into the CHO cells failed to aggregate with the lysosomal enzymes, suggesting that aggregation was specific. Jadot et al. [87] showed that rat liver lysosomal enzymes aggregated at low pH. This aggregation occurred between pH 4.5 and 5.0, and was mediated by the integral membrane protein LAMP-2. Under these low pH conditions, lysosomal enzymes bind by electrostatic interactions to the LAMP-2 proteins, immobilizing them in a matrix. This process was specific for the 11 lysosomal hydrolases assayed during the experiment; a cytosolic extract and BSA failed to aggregate with LAMP-2 under the conditions tested. Unlike the aggregation observed in CHO cells [131], detergents affected aggregation of the rat liver lysosomal enzymes, suggesting that membrane association with LAMP-2 was vital for aggregation. Lysosomal condensation from the hybrid lysosome–late endosome organelle was shown to be dependent on intra-organellar Ca^{2+} and a functional V-ATPase [36]. If this process involved aggregation of the lysosomal hydrolases, it would be analogous to the pH- and Ca^{2+}-dependent aggregation found in bovine adrenal granules [130]. Furthermore, if this process involved LAMP-2 it may specifically aggregate the lysosomal luminal proteins [87], allowing lysosome condensation without contaminating proteins.

A pH-dependent aggregation mechanism could operate to withdraw lysosomal hydrolases out of the late endosome, and/or it could be a feature of the lysosome itself. An advantage of this complexation mechanism, as opposed to a purported redox-dependent storage mechanism, is that it could encompass several different types of lysosomal hydrolases [87]. Furthermore, as described by Griffiths [11], lysosomes viewed by electron microscopy appear to be small highly dense organelles, a morphology that could be explained by aggregation of their luminal hydrolases.

The concept of lysosomal enzymes aggregating by electrostatic interactions is a fairly old one (see, for example, Henning et al. [132]). However, the acceptance of this as a mechanism for regulating and storing lysosomal hydrolases has been limited. This may be because erroneously low pH optima were assigned to some hydrolases (see Dehrmann et al. [133]), which by chance co-incided with the low pH of lysosomes where the bulk of substrate hydrolysis was thought to occur. Subsequently, it has been found that most proteolysis occurs in late endosomes, which have a pH closer to the revised pH optima of lysosomal proteases. The hypothesis that lysosomal hydrolases may be stored by low pH-induced aggregation does not exclude the possibility that late endosomes may fluctuate between alkaline and acidic pH values, allowing for the activation of different hydrolases with individual pH optima [24]. The lysosome with its lower pH may complex the lysosomal hydrolases, effectively storing them in a precipitated matrix.

This proposed regulatory mechanism could itself be regulated by the factors discussed above that influence V-ATPase activity, i.e. the activity of the lysosomal proteases may be influenced by factors that influence the V-ATPase activity.

CONCLUSIONS AND FUTURE PERSPECTIVES

Complicating the whole field of endolysosomal proteolysis is the lack of a consistent nomenclature, especially concerning the definition of the 'lysosome'. To many authors a 'lysosome' is any organelle containing 'lysosomal' hydrolases. However, we believe that this simple definition has outlived its usefulness and that it may be more usefully replaced by a functional definition, e.g. that lysosomes are organelles mainly for the storage of 'lysosomal' hydrolases (probably in an inactivated state, due to

acid-induced complexation or simply lack of substrate). To become functional, 'lysosomal' hydrolases must be injected into an endosome, phagosome or autophagosome, which are the organelles within which hydrolysis largely occurs. This concept of a lysosome is not too different from the original concept of a 'primary lysosome' [23].

Another modern functional definition of a lysosome is that it is 'the terminal organelle in the endolysosomal pathway and is devoid of recycling receptors' [11]. In the early definition of a 'primary lysosome', this was envisioned as being a vesicle containing newly synthesised enzymes, which budded off the Golgi apparatus [23]. Griffiths [11] has noted that lysosomes are indeed vesicle-sized, so in microscopy studies they may easily be confused with 'lysosomal'-enzyme-carrier vesicles, which have a similar cargo. Unlike carrier vesicles, however, lysosomes are MPR negative.

Many hydrolytic organelles (secondary lysosomes) may be assembled *de novo* when required and this gives an opportunity to determine a minimal requirement for hydrolysis. A common theme applicable to the endolysosomal system, phagosomes and autophagosomes is that enzymes are imported by fusion with lysosomes, acidification is effected by importation of a V-ATPase and egress of products is effected by importation of membrane transporters. Some pathogens exploit the endolysosomal system and escape hydrolysis by blocking the assembly of a complete functional hydrolytic compartment. Of the proposed common factors required to generate a proteolytic luminal environment (Table 1), the least is known about the lysosomal cysteine transporter. Future studies could be directed at determining the location of this transporter within the endolysosomal system, and the role(s) it plays in proteolysis, especially in processes such as phagocytosis and autophagy.

Is endolysosomal proteolysis subject to flux control? With feedback regulation, the downstream rate of proteolysis [Scheme 1, steps (iii) and (iv)] would affect upstream rates of substrate acquisition. Observations by Kominami et al. [134] of the large accumulation of hepatic autophagic vacuoles containing undigested material, after treatment of rats with leupeptin *in vivo*, suggests that there may be no feedback regulation. A second possibility is that proteolysis within the endolysosomal system is sufficiently efficient, and that regardless of the amount of substrate channelled to the proteases, the system could cope. Retention of reserves of temporarily unneeded hydrolases, by storage in lysosomes, would give the system greater elasticity and the ability to respond quickly to changes in demand. In this case, regulation of flux would best be effected in the initial stages of the process, such as substrate acquisition [Scheme 1, steps (i) and (ii)].

It has become apparent that regulation of endocytosis, as an example, is effected by complex signalling networks [1–9]. These signalling networks exert their influence through molecules that interface between the signalling and endocytic pathways. Future research efforts could be directed at attempting to unite the signalling networks, the regulation of endolysosomal fusion reactions and the components that affect the endolysosomal luminal proteolytic environment into an integrated model for proteolysis. Some progress has been made in this regard. Recently, it has been shown that p38 mitogen-activated protein kinase may accelerate the rate of endocytosis in response to oxidative stress [135], providing a direct link between the endocytic rate and the signalling pathways. It has also been shown that the E subunit of the V-ATPase may interact with the Dbl-homology domain of murine Sos1, a guanine nucleotide exchange factor involved in Rac1 activation [136]. Rac1 activation regulates various trafficking events, such as transferrin receptor-mediated clathrin-coated-vesicle formation [137]. Although in this case, the E subunit affected the mSos1–Rac1 signalling pathway [136], it does indicate that there are domains on the V-ATPase capable of interacting with signalling pathways. The activity of Cl^- channels that influence luminal pH, is regulated by protein kinase A-dependent phosphorylation [114]. Thus, it is possible that acidification of endolysosomal organelles may be connected to these signalling pathways. Regulation of conditions within endolysosomal organelles, and in turn the activity of the proteases, may therefore be integrated via signalling networks to endocytosis and changes occurring at the cellular level. In turn, the cell is regulated by the tissue and, ultimately, by the whole-body system. Endolysosomal hydrolases are thus envisioned to be the downstream effectors of a hydrolytic system, which is ultimately regulated at the whole-body level.

We thank Dr Gareth Griffiths (EMBL, Heidelberg, Germany) for a critical reading of the draft manuscript and for many helpful suggestions.

REFERENCES

1 Guallier, J.-M., Gillooly, D., Simonsen, A. and Stenmark, H. (1999) Regulation of endocytic membrane traffic by phosphatidylinositol 3-phosphate. Biochem. Soc. Trans. **27**, 666–670
2 Sechi, A. S. and Wehland, J. (2000) The actin cytoskeleton and plasma membrane connection: PtdIns(4, 5)P_2 influences cytoskeletal protein activity at the plasma membrane. J. Cell Sci. **113**, 3685–3695
3 Matozaki, T., Nakanishi, H. and Takai, Y. (2000) Small G-protein networks. Their crosstalk and signal cascades. Cell Signal. **12**, 515–524
4 Qualmann, B., Kessles, M. M. and Kelly, R. B. (2000) Molecular links between endocytosis and the actin cytoskeleton. J. Cell Biol. **150**, F111–F116
5 Stenmark, H. and Olkkonen, V. M. (2001) The Rab GTPase family. Genome Biol. **2**, 3007.1–3007.7
6 Miaczynska, M. and Zerial, M. (2002) Mosaic organisation of the endocytic pathway. Exp. Cell Res. **272**, 8–14
7 Di Fiore, P. P. and De Camilli, P. (2001) Endocytosis and signalling: an inseparable partnership. Cell (Cambridge, Mass.) **106**, 1–4
8 Clague, M. J. and Urbé, S. (2001) The interface between receptor trafficking and signalling. J. Cell Sci. **114**, 3075–3081
9 May, R. C. and Machesky, L. M. (2001) Phagocytosis and the actin cytoskeleton. J. Cell Sci. **114**, 1061–1077
10 Murphy, R. F. (1991) Maturation models for endosome and lysosome biogenesis. Trends Cell Biol. **1**, 77–82
11 Griffiths, G. (1996) On vesicles and membrane compartments. Protoplasma **193**, 37–58
12 Thilo, L., Stroud, E. and Haylett, T. (1995) Maturation of early endosomes and vasicular traffic to lysosomes in relation to membrane recycling. J. Cell Sci. **108**, 1791–1803
13 Ghosh, R. N., Gelman, D. L. and Maxfield, F. R. (1994) Quantification of low density lipoprotein and transferrin in endocytic sorting in Hep2 cells using confocal microscopy. J. Cell Sci. **107**, 2177–2189
14 Fuchs, R., Schmid, S. and Mellman, I. (1989) A possible role for the Na^+,K^+-ATPase in regulating ATP-dependent endosome acidification. Proc. Natl. Acad. Sci. U.S.A. **86**, 539–543
15 Cain, C. C., Sipe, D. M. and Murphy, R. F. (1989) Regulation of endocytic pH by the Na^+,K^+-ATPase in living cells. Proc. Natl. Acad. Sci. U.S.A. **86**, 544–548
16 Mellman, I. (1992) The importance of being acid: the role of acidification in intracellular membrane traffic. J. Exp. Biol. **172**, 39–45
17 Hopkins, C. R., Gibson, A., Shipman, M., Strickland, D. K. and Trowbridge, I. S. (1994) In migrating fibroblasts, recycling receptors are concentrated in narrow tubules in the pericentriolar area, and then routed to the plasma membrane of the leading lamella. J. Cell Biol. **125**, 1265–1274
18 Gruenberg, J., Griffiths, G. and Howell, K. E. (1989) Characterisation of the early endosome and putative endocytic carrier vesicles *in vivo* and with an assay of vesicle fusion *in vitro*. J. Cell Biol. **108**, 1301–1316
19 Aniento, F., Emans, N., Griffiths, G. and Gruenberg, J. (1993) Cytoplasmic dynein-dependent vesicular transport from early to late endosomes. J. Cell Biol. **123**, 1373–1387
20 Clague, M. J., Urbé, S., Aniento, F. and Gruenberg, J. (1994) Vacuolar ATPase activity is required for endolysosomal carrier vesicle formation. J. Biol. Chem. **269**, 21–24

21 Killisch, I., Steinlein, P., Römisch, K., Hollinshead, R., Hartmut, B. and Griffiths, G. (1992) Characterisation of early and late endocytic compartments of the transferrin cycle. Transferrin receptor antibody blocks erythroid differentiation by trapping the receptor in the early endosome. J. Cell Sci. **103**, 211–232

22 Jahraus, A., Tjelle, T. E., Berg, T., Habermann, A., Storrie, B., Ullrich, O. and Griffiths, G. (1998) *In vitro* fusion of phagosomes with different endocytic organelles from J774 macrophages. J. Biol. Chem. **273**, 30379–30390

23 de Duve, C. (1983) Lysosomes revisited. Eur. J. Biochem. **137**, 391–397

24 Butor, C., Griffiths, G., Aronson, Jr., N. N. and Varki, A. (1995) Co-localisation of hydrolytic enzymes with widely disparate pH optima: implications for the regulation of lysosomal pH. J. Cell Sci. **108**, 2213–2219

25 Dehrmann, F. M., Elliott, E. and Dennison, C. (1996) Reductive activation markedly increases the stability of cathepsins B and L to extracellular ionic conditions. Biol. Chem. Hoppe Seyler **377**, 391–394

26 Clague, M. J. (1998) Molecular aspects of the endocytic pathway. Biochem. J. **336**, 271–282

27 Gruenberg, J. (2001) The endocytic pathway: a mosaic of domains. Nat. Rev. Mol. Cell Biol. **2**, 721–730

28 Méresse, S., Gorvel, J. P. and Chavrier, P. (1995) The Rab7 GTPase resides on a vesicular compartment connected to lysosomes. J. Cell Sci. **108**, 3349–3358

29 Tjelle, T. E., Brech, A., Juvet, L. K., Griffiths, G. and Berg, T. (1996) Isolation and characterisation of early endosomes, late endosomes and terminal lysosomes: their role in protein degradation. J. Cell Sci. **109**, 2905–2914

30 Bright, N. A., Reaves, B. J., Mullock, B. M. and Luzio, J. P. (1997) Dense core lysosomes fuse with late endosomes and are re-formed from the resultant hybrid organelles. J. Cell Sci. **110**, 2027–2040

31 Ward, D. M., Leslie, J. D. and Kaplan, J. (1997) Homotypic lysosome fusion in macrophages: analysis using an *in vitro* assay. J. Cell Biol. **139**, 665–673

32 Berg, T., Gjoen, T. and Bakke, O. (1995) Physiological functions of endolysosomal proteolysis. Biochem. J. **307**, 313–326

33 Storrie, B. and Desjardins, M. (1996) The biogenesis of lysosomes: is it a kiss and run, continuous fusion and fission process? Bioessays **18**, 895–903

34 Luzio, J. P., Rous, B. A., Bright, N. A., Pryor, P. R., Mullock, B. M. and Piper, R. C. (2000) Lysosome–endosome fusion and lysosome biogenesis. J. Cell Sci. **113**, 1515–1524

35 Mullock, B. M., Bright, N. A., Fearon, C. W., Gray, S. R. and Luzio, J. P. (1998) Fusion of lysosomes with late endosomes produces a hybrid organelle of intermediate density and is NSF dependent. J. Cell Biol. **140**, 591–601

36 Pryor, P. R., Mullock, B. M., Bright, N. A., Gray, S. R. and Luzio, J. P. (2000) The role of intraorganellar Ca^{2+} in late endosome–lysosome heterotypic fusion and in the reformation of lysosomes from hybrid organelles. J. Cell Biol. **149**, 1053–1062

37 Andrews, N. W. (2000) Regulated secretion of conventional lysosomes. Trends Cell Biol. **10**, 317–321

38 Martinez, I., Chakrabarti, S., Hellevik, T., Morehead, J., Fowler, K. and Andrews, N. W. (2000) Synaptotagmin VII regulates Ca^{2+}-dependent exocytosis of lysosomes in fibroblasts. J. Cell Biol. **148**, 1141–1149

39 Reddy, A., Caler, E. V. and Andrews, N. W. (2001) Plasma membrane repair is mediated by Ca^{2+}-regulated exocytosis of lysosomes. Cell (Cambridge, Mass.) **106**, 157–169

40 Denzer, K., Kleijmeer, M. J., Heijnen, H. F. G., Stoorvogel, W. and Geuze, H. J. (2000) Exosome: from internal vesicle of the multivesicular body to intercellular signaling device. J. Cell Sci. **113**, 3365–3374

41 Warnock, D. G. (1999) Regulation of endolysosomal acidification via the G_i-type protein. Kidney Int. **55**, 2524–2525

42 Rabinowitz, S., Horstmann, H., Gordon, S. and Griffiths, G. (1992) Immunocytochemical characterisation of the endocytic and phagolysosomal compartments in peritoneal macrophages. J. Cell Biol. **116**, 95–112

43 Seglen, P. O. and Bohley, P. (1992) Autophagy and vacuolar protein degradation mechanisms. Experientia **48**, 158–172

44 Blommaart, E. F. C., Luiken, J. J. F. P. and Meijer, A. J. (1997) Autophagic proteolysis: control and specificity. Histochem. J. **29**, 365–385

45 Mortimore, G. E., Lardeux, B. R. and Adams, C. E. (1988) Regulation of microautophagy and basal protein turnover in rat liver. Effects of short-term starvation. J. Biol. Chem. **263**, 2506–2512

46 Mortimore, G. E., Hutson, N. J. and Surmacz, C. A. (1983) Quantitative correlation between proteolysis and macro- and microautophagy in mouse hepatocytes during starvation and refeeding. Proc. Natl. Acad. Sci. U.S.A. **80**, 2179–2183

47 Cuervo, A. M. and Dice, J. F. (1998) Lysosomes, a meeting point of proteins, chaperones, and proteases. J. Mol. Med. **76**, 6–12

48 Holtzman, E. (1988) Lysosomes. pp. 263–267, Plenum Press, New York

49 Kim, J. and Klionsky, D. J. (2000) Autophagy, cytoplasm-to-vacuole targeting pathway, and pexophagy in yeast and mammalian cells. Annu. Rev. Biochem. **69**, 303–342

50 Pfeifer, U., Werder, E. and Bergeest, H. (1978) Inhibition by insulin of the formation of autophagic vacuoles in rat liver. J. Cell Biol. **78**, 152–167

51 Dunn, Jr, W. A. (1990) Studies on the mechanism of autophagy: formation of the autophagic vacuole. J. Cell Biol. **110**, 1923–1933

52 Dunn, Jr, W. A. (1990) Studies on the mechanism of autophagy: maturation of the autophagic vacuole. J. Cell Biol. **110**, 1935–1945

53 Ogier-Denis, E., Couvineau, A., Maoret, J. J., Houri, J. J., Bauvy, C., De Stefanis, D., Isidoro, C., Laburthe, M. and Codogno, P. (1995) A heterotrimeric G_{i3}-protein controls autophagic sequestration in the human colon cancer cell line HT-29. J. Biol. Chem. **270**, 13–16

54 Ogier-Denis, E., Houri, J. J., Bauvy, C. and Codogno, P. (1996) Guanine nucleotide exchange on heterotrimeric G_{i3}-protein controls autophagic sequestration in HT-29 cells. J. Biol. Chem. **271**, 28593–28600

55 Petiot, A., Ogier-Denis, E., Bauvy, C., Cluzeaud, F., Vanderwalle, A. and Codogno, P. (1999) Subcellular localisation of the $G_{\alpha i3}$ protein and Gα interacting protein, two proteins involved in the control of macroautophagy in human colon cancer HT-29 cells. Biochem. J. **337**, 289–295

56 Mizushima, N., Sugita, H., Yoshimori, T. and Ohsumi, Y. (1998) A new protein conjugation system in human. The counterpart of the yeast Apg12p conjugation system essential for autophagy. J. Biol. Chem. **273**, 33889–33892

57 Kirisako, T., Baba, M., Ishihara, N., Miyazawa, K., Ohsumi, M., Yoshimori, T., Noda, T. and Ohsumi, Y. (1999) Formation of autophagosome is traced with Apg8/Aut7p in yeast. J. Cell Biol. **147**, 435–446

58 Yamamoto, A., Masaki, R., Fukui, Y. and Tashiro, Y. (1990) Absence of cytochrome P-450 and the presence of autolysosomal membrane antigens on the isolation membranes and autophagosomal membranes in rat hepatocytes. J. Histochem. Cytochem. **38**, 1571–1581

59 Yamamoto, A., Masaki, R. and Tashiro, Y. (1990) Characterisation of the isolation membranes and the limiting membranes in autophagosomes in rat hepatocytes by lectin cytochemistry. J Histochem. Cytochem. **38**, 573–580

60 Lawrence, B. P. and Brown, W. J. (1992) Autophagic vesicles rapidly fuse with pre-existing lysosomes in cultured hepatocytes. J. Cell Sci. **102**, 515–526

61 Gordon, P. B. and Seglen, P. O. (1988) Prelysosomal convergence of autophagic and endocytic pathways. Biochem. Biophys. Res. Commun. **151**, 40–47

62 Tooze, J., Hollinshead, M., Ludwig, T., Howell, K., Hoflack, B. and Kern, H. (1990) In exocrine pancreas, the basolateral endocytic pathway converges with the autophagic pathway immediately after the early endosome. J. Cell Biol. **111**, 329–345

63 Rabouille, C., Strous, G. J., Crapo, J. D., Geuze, H. J. and Slot, J. W. (1993) The differential degradation of two cytosolic proteins as a tool to monitor autophagy in hepatocytes by immunocytochemistry. J. Cell Biol. **120**, 897–908

64 Strømhaug, P. E. and Seglen, P. O. (1993) Evidence for acidity of the prelysosomal autophagic/endocytic vacuoles (amphisomes). Biochem. J. **291**, 115–121

65 van Oss, C. J. (1986) Phagocytosis: an overview. Methods Enzymol. **132**, 3–15

66 Kirschke, H., Barrett, A. J. and Rawlings, N. D. (1998) Lysosomal Cysteine Proteases. 2nd edn, pp. 5, 15, 21 and 34–45, Oxford University Press, New York

67 Kwiatkowska, K. and Sobota, A. (1999) Signaling pathways in phagocytosis. Bioessays **21**, 422–431

68 Mayorga, L. S., Bertini, F. and Stahl, P. D. (1991) Fusion of newly formed phagosomes with the endosomes in intact cells and in a cell–free system. J. Biol. Chem. **266**, 6511–6517

69 Pitt, A., Mayorga, L. S., Schwartz, A. L. and Stahl, P. D. (1992) Transport of phagosomal components to an endolysosomal compartment. J. Biol. Chem. **267**, 126–132

70 Desjardins, M., Huber, L. A., Parton, R. G. and Griffiths, G. (1994) Biogenesis of the phagolysosome proceeds through a sequential series of interactions with the endocytic apparatus. J. Cell Biol. **124**, 677–688

71 Mellman, I., Fuchs, R. and Helenius, A. (1986) Acidification of the endocytic and exocytic pathways. Annu. Rev. Biochem. **55**, 663–700

72 Claus, V., Jahraus, A., Tjelle, T., Berg, T., Kirschke, H., Faulstich, H. and Griffiths, G. (1998) Lysosomal enzyme trafficking between phagosomes, endosomes and lysosomes in J774 macrophages. Enrichment of cathepsin H in early endosomes. J. Biol. Chem. **273**, 9842–9851

73 Sturgill-Kozycki, S., Schlesinger, P. H., Chakrabarty, P., Haddix, P. L., Collins, H. L., Fok, A. G., Allen, R. D., Gluck, S. L., Heuser, J. and Russel, D. G. (1994) Lack of acidification in mycobacterium phagosomes is produced by the exclusion of the vesicular proton-ATPase. Science (Washington, D.C.) **263**, 678–681

74 Via, L. E., Deretic, D., Ulmer, R. J., Hibler, N. S., Huber, L. A. and Deretic, V. (1997) Arrest of mycobacterial phagosome maturation is caused by a block in vesicle fusion between stages controlled by Rab5 and Rab7. J. Biol. Chem. **272**, 13326–13331

75 Hasilik, A. (1992) The early and late processing of lysosomal enzymes: proteolysis and compartmentation. Experientia **48**, 130–151

76 Kornfeld, S. (1986) Trafficking of lysosomal enzymes in normal and disease states. J. Clin. Invest. **77**, 1–6

77 Bohley, P. and Seglen, P. O. (1992) Proteases and proteolysis in the lysosome. Experientia **48**, 151–157

78 Tikkanen, R., Peltola, M., Oinonen, C., Rouvinen, J. and Peltonen, L. (1997) Several cooperating binding sites of a lysosomal enzyme with phosphotransferase. EMBO J. **16**, 6684–6693

79 Cuozzo, J. W., Tao, K., Cygler, M., Mort, J. S. and Sahagian, G. G. (1998) Lysine-based structure responsible for selective mannose phosphorylation of cathepsin D and cathepsin L defines a common structural motif for lysosomal enzyme targetting. J. Biol. Chem. **273**, 21067–21076

80 Lukong, K. E., Elsliger, M.-A., Mort, J. S., Potier, M. and Pshezhetsky, A. V. (1999) Identification of UDP-*N*–acteylglucosamine-phosphotransferase-binding sites on the lysosomal proteases, cathepsins A, B and D. Biochemistry **38**, 73–80

81 Ludwig, T., Le Borgne, R. and Hoflack, B. (1995) Roles for mannose-6-phosphate receptors in lysosomal enzyme sorting, IGF-II binding and clathrin-coat assembly. Trends Cell Biol. **5**, 202–205

82 Storer, A. C. and Ménard, R. (1994) Catalytic mechanism in papain family of cysteine peptidases. Methods Enzymol. **244**, 486–500

83 Polgar, L., Asboth, B. and Korodi, I. (1986) Mechanism of action of cysteine proteases: 1. Differences from serine proteases; 2. The thiol group of chymopapain. In Cysteine Proteinases and Their Inhibitors (Turk, V., ed.), pp. 328–338, Walter de Gruyter, New York

84 Peters, C. and von Figura, K. (1994) Biogenesis of lysosomal membranes. FEBS Lett. **346**, 108–114

85 Guarnier, F. G., Arterburn, L. M., Penno, M. B., Cha, Y. and August, J. T. (1993) The motif Tyr-X-X-hydrophobic residue mediates lysosomal membrane targeting of lysosome-associated membrane protein 1. J. Biol. Chem. **268**, 1941–1946

86 Karlsson, K. and Carlsson, S. R. (1998) Sorting of lysosomal membrane glycoproteins lamp-1 and lamp-2 into vesicles distinct from the mannose-6-phosphate receptor/γ-adaptin vesicles at the *trans*-Golgi network. J. Biol. Chem. **273**, 18966–18973

87 Jadot, M., Dubois, F., Wattiaux-De Coninck, S. and Wattiaux, R. (1997) Supramolecular assemblies from lysosomal matrix proteins and complex lipids. Eur. J. Biochem. **249**, 862–869

88 Silverstein, R. L. and Febbraio, M. (1992) Identification of lysosome-associated membrane protein-2 as an activation-dependent surface glycoprotein. Blood **80**, 1470–1475

89 Andrejewski, N., Punnonen, E.-L., Guhde, G., Tanaka, Y., Lüllman-Rauch, R., Hartmann, D., von Figura, K. and Saftig, P. (1999) Normal lysosome morphology and function in LAMP-1-deficient mice. J. Biol. Chem. **274**, 12692–12701

90 Tanaka, Y., Guhde, G., Sute, A., Eskelinen, E.-V., Hartmann, D., Lüllman-Rauch, R., Janssen, P. M. L., Blanz, J., von Figura, K. and Saftig, P. (2000) Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2 deficient mice. Nature (London) **406**, 902–906

91 Pisoni, R. L. and Thoene, J. G. (1991) The transport systems of mammalian lysosomes. Biochim. Biophys. Acta **1071**, 351–373

92 Lloyd, J. B. (1992) Lysosomal handling of cystine residues: stoichiometry of cysteine involvement. Biochem. J. **286**, 979–980

93 Pisoni, R. L., Acker, T. L., Lisowski, K. M., Lemons, R. M. and Thoene, J. G. (1990) A cysteine-specific lysosomal transport system provides a major route for the delivery of thiol to human fibroblast lysosomes: possible role in supporting lysosomal proteolysis. J. Cell Biol. **110**, 327–335

94 Pisoni, R. L. and Velilla, V. (1995) Evidence for an essential histidine residue located in the binding site of the cysteine-specific lysosomal transport protein. Biochim. Biophys. Acta **1236**, 23–30

95 Gainey, D., Short, S. and McCoy, K. L. (1996) Intracellular location of cysteine transport activity correlates with productive processing of antigen disulfide. J. Cell. Physiol. **168**, 248–254

96 Feener, E. P., Shen, W.-C. and Ryser, H. J.-P. (1990) Cleavage of disulfide bonds in endocytosed macromolecules. A processing requirement not associated with lysosomes or endosomes. J. Biol. Chem. **265**, 18780–18785

97 Collins, D. S., Unanue, E. R. and Harding, C. V. (1991) Reduction of disulfide bonds within lysosomes is a key step in antigen processing. J. Immunol. **147**, 4054–4059

98 Collier, R. J. and Kandel, J. (1971) Structure and activity of the diphtheria toxin. Thiol-dependent dissociation of a fraction of toxin into enzymatically active and inactive fragments. J. Biol. Chem. **246**, 1496–1503

99 Moskaug, J. O., Sandvig, K. and Olsnes, S. (1987) Cell-mediated reduction of the interfragment disulfide in nicked diphtheria toxin. J. Biol. Chem. **262**, 10339–10345

100 Merkel, B. J., Mandel, R., Ryser, H. J.-P. and McCoy, K. L. (1995) Characterisation of fibroblasts with a unique defect in processing antigens with disulfide bonds. J. Immunol. **154**, 128–136

101 Hampl, J., Gradehandt, G., Kalbacher, H. and Rüde, E. (1992) *In vitro* processing of insulin for recognition by murine T cells results in the generation of A chains with free CysSH. J. Immunol. **148**, 2664–2671

102 Wilcox, D. and Mason, R. W. (1992) Inhibition of cysteine proteinases in lysosomes and whole cells. Biochem. J. **285**, 495–502

103 Krepela, E., Procházka, J., Kárová, B., Cermák, J. and Roubková, H. (1997) Cathepsin B, thiols and cysteine proteinase inhibitors in squamous-cell lung cancer. Neoplasma. **44**, 219–239

104 Krepela, E., Procházka, J. and Kárová, B. (1999) Regulation of cathepsin B activity by cysteine and related thiols. Biol. Chem. **380**, 541–551

105 Kooistra, T., Millard, P. C. and Lloyd, J. B. (1982) Roles of thiols in degradation of proteins by cathepsins. Biochem. J. **204**, 471–477

106 Mego, J. L. (1984) Role of thiols, pH and cathepsin D in the lysosomal catabolism of serum albumin. Biochem. J. **218**, 775–783

107 Hwang, C., Sinskey, A. J. and Lodish, H. F. (1992) Oxidised redox state of glutathione in the endoplasmic reticulum. Science (Washington, D.C.) **257**, 1496–1502

108 Frand, A. R., Cuozzo, J. W. and Kaiser, C. A. (2000) Pathways for protein disulphide bond formation. Trends Cell Biol. **10**, 203–210

109 Arunachalam, B., Phan, U. T., Gueze, H. J. and Cresswell, P. (2000) Enzymatic reduction of disulfide bonds in lysosomes: characterisation of a γ-interferon-inducible lysosomal thiol reductase (GILT). Proc. Natl. Acad. Sci. U.S.A. **97**, 745–750

110 Gahl, W. A. and Tietze, F. (1985) pH effects on cystine transport in lysosome-rich leucocyte granular fractions. Biochem. J. **228**, 263–267

111 Gille, L. and Nohl, H. (2000) The existence of a lysosomal redox chain and the role of ubiquinone. Arch. Biochem. Biophys. **375**, 347–354

112 Futai, M., Oka, T., Moriyama, Y. and Wada, Y. (1998) Diverse roles of single membrane organelles: factors establishing the acid lumenal pH. J. Biochem. **124**, 259–267

113 Feng, Y. and Forgac, M. (1994) Inhibition of vacuolar H^+-ATPase by disulfide formation between cysteine 254 and cysteine 532 in subunit A. J. Biol. Chem. **269**, 13224–13230

114 Forgac, M. (1998) Structure, function and regulation of the vacuolar (H^+)-ATPases. FEBS Lett. **440**, 258–263

115 Forgac, M. (1999) Structure and properties of the vacuolar (H^+)-ATPases. J. Biol. Chem. **274**, 12951–12954

116 Reijngoud, D.-J. and Tager, J. M. (1977) The permeability properties of the lysosomal membrane. Biochim. Biophys. Acta **472**, 419–449

117 Moriyama, Y., Maeda, M. and Futai, M. (1992) Involvement of a non-proton pump factor (possibly Donnan-type equilibrium) in maintenance of an acidic pH in lysosomes. FEBS Lett. **302**, 18–20

118 Willenbrock, F. and Brocklehurst, K. (1985) A general framework of cysteine-proteinase mechanism deduced from studies on enzymes with structurally different analogous catalytic site residues Asp-158 and -161 (papain and actinidin), Gly-196 (cathepsin B) and Asn-175 (cathepsin H). Biochem. J. **227**, 521–528

119 Khouri, H. E., Plouffe, C., Hasnain, S., Hirama, T., Storer, A. C. and Ménard, R. (1991) A model to explain the pH-dependent specificity of cathepsin B-catalysed hydrolysis. Biochem. J. **275**, 751–757

120 Hasnain, S., Hirama, T., Tam, A. and Mort, J. S. (1992) Characterisation of recombinant rat cathepsin B and non-glycosylated mutants in yeast. J. Biol. Chem. **267**, 4713–4721

121 Moin, K., Day, N. A., Sameni, M., Hasnain, S., Hirama, T. and Sloane, B. F. (1992) Human tumor cathepsin B. Comparison with normal liver cathepsin B. Biochem. J. **285**, 427–434

122 Bakker, A. C., Webster, P., Jacob, W. A. and Andrews, N. W. (1997) Homotypic fusion between aggregated lysosomes triggered by elevated $[Ca^{2+}]_i$ in fibroblasts. J. Cell Sci. **110**, 2227–2238

123 Jaconi, M. E. E, Lew, D. P., Carpentier, J.-L., Magnusson, K. E., Sjögren, M. and Stendahl, O. (1990) Cytosolic free calcium elevation mediates the phagosome–lysosome fusion during phagocytosis in human neutrophils. J. Cell Biol. **110**, 1555–1564

124 Malik, Z. A., Iyer, S.S and Kusner, D. J. (2001) *Mycobacterium tuberculosis* phagosomes exhibit altered calmodulin-dependent signal transduction: contribution to inhibition of phagosome-lysosome fusion and intracellular survival in human macrophages. J. Immunol. **166**, 3392–3401

125 Gordon, P. B., Holen, I., Fosse, M., Rotnes, J. S. and Seglen, P. O. (1993) Dependence of hepatic autophagy on intracellularly sequestered calcium. J. Biol. Chem. **268**, 26107–26112

126 Haller, T., Völkl, H., Deetjen, P. and Dietl, P. (1996) The lysosomal Ca^{2+} pool in MDCK cells can be released by Ins(1,4,5)P_3-dependent hormones or thapsigargin but does not activate store-operated Ca^{2+} entry. Biochem. J. **319**, 909–912

127 Lemons, R. M. and Thoene, J. G. (1991) Mediated calcium transport by isolated human fibroblast lysosomes. J. Biol. Chem. **266**, 14378–14382

128 Carafoli, E. (1987) Intracellular calcium homeostasis. Annu. Rev. Biochem. **56**, 395–433

129 Kostoulas, G., Horler, D., Naggi, A., Casu, B. and Biaci, A. (1997) Electrostatic interactions between human leukocyte elastase and sulfated glycosaminoglycans: physiological implications. Biol. Chem. **378**, 1481–1489

130 Colomer, V., Kicska, G. A. and Rindler, M. J. (1996) Secretory granule content proteins and the lumenal domains of granule membrane proteins aggregate *in vitro* at mildly acidic pH. J. Biol. Chem. **271**, 48–55
131 Buckmaster, M. J., Ferris, A. L. and Storrie, B. (1988) Effects of pH, detergent and salt on the aggregation of Chinese-hamster-ovary cell lysosomal enzymes. Biochem. J. **249**, 921–923
132 Henning, R., Plattner, H. and Stoffel, W. (1973) Nature and localisation of acidic groups on lysosomal membranes. Biochem. Biophys. Acta **330**, 61–75
133 Dehrmann, F. M., Coetzer, T. H. T., Pike, R. N. and Dennison, C. (1995) Mature cathepsin L is substantially active in the ionic milieu of the extracellular medium. Arch. Biochem. Biophys. **324**, 93–98
134 Kominami, E., Hashida., S., Khairallah, E. A. and Katunuma, N. (1983) Sequesteration of cytoplasmic enzymes in an autophagic vacuole–lysosomal system induced by injection of leupeptin. J. Biol. Chem. **258**, 6093–6100
135 Cavalli, V., Vilbois, F., Corti, M., Marcote, M. J., Tamura, K., Karin, M., Arkinstall, S. and Gruenberg, J. (2001) The stress-induced MAP kinase p38 regulates endocytic traffic via the GDI·rab5 complex. Mol. Cell **7**, 421–432
136 Miura, K., Miyazawa, S., Furuta, S., Mitsushita, J., Kamijo, K., Ishida, H., Miki, T., Suzukawa, K., Resau, J., Copeland, T. D. and Kamata, T. (2001) The Sos1–Rac 1 signaling. Possible involvement of a vacuolar H^+-ATPase E subunit. J. Biol. Chem. **276**, 46276–46283
137 Lamaze, C., Chuang, T.-H., Terlecky, L. J., Bokoch, G. M. and Schmid, S. L. (1996) Regulation of receptor-mediated endocytosis by Rho and Rac. Nature (London) **382**, 177–179
138 Salvasen, G. S. (1998) Cathepsin G. In Handbook of Proteolytic Enzymes (Barrett, A. J., Rawlings, N. D. and Woessner, J. F., eds.), pp. 60–72, Academic Press, London
139 Mort, J. S. (1998) Cathepsin B. In Handbook of Proteolytic Enzymes (Barrett, A. J., Rawlings, N. D. and Woessner, J. F., eds.), pp. 609–617, Academic Press, London
140 Kirschke, H. (1998) Cathepsin L. In Handbook of Proteolytic Enzymes (Barrett, A. J., Rawlings, N. D. and Woessner, J. F., eds.), pp. 617–621, Academic Press, London
141 Kirschke, H. (1998) Cathepsin S. In Handbook of Proteolytic Enzymes (Barrett, A. J., Rawlings, N. D. and Woessner, J. F., eds.), pp. 621–624, Academic Press, London
142 Brömme, D. (1998) Cathepsin K. In Handbook of Proteolytic Enzymes (Barrett, A. J., Rawlings, N. D. and Woessner, J. F., eds.), pp. 624–628, Academic Press, London
143 Kirschke, H. (1998) Cathepsin H. In Handbook of Proteolytic Enzymes (Barrett, A. J., Rawlings, N. D. and Woessner, J. F., eds.), pp. 628–631, Academic Press, London
144 Conner, G. E. (1998) Cathepsin D. In Handbook of Proteolytic Enzymes (Barrett, A. J, Rawlings, N. D. and Woessner, J. F., eds.), pp. 828–836, Academic Press, London
145 Kay, J. and Jatnell, P. J. (1998) Cathepsin E. In Handbook of Proteolytic Enzymes (Barrett, A. J., Rawlings, N. D. and Woessner, J. F., eds.), pp. 819–823, Academic Press, London
146 Gohda, E. and Pitout, H. C. (1998) Cathepsin T. In Handbook of Proteolytic Enzymes (Barrett, A. J., Rawlings, N. D. and Woessner, J. F., eds.), pp. 774–775, Academic Press, London