Specialized roles for cysteine cathepsins in health and disease

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Cathepsins were originally identified as proteases that act in the lysosome. Recent work has uncovered nontraditional roles for cathepsins in the extracellular space as well as in the cytosol and nucleus. There is strong evidence that subspecialized and compartmentalized cathepsins participate in many physiologic and pathophysiologic cellular processes, in which they can act as both digestive and regulatory proteases. In this review, we discuss the transcriptional and translational control of cathepsin expression, the regulation of intracellular sorting of cathepsins, and the structural basis of cathepsin activation and inhibition. In particular, we highlight the emerging roles of various cathepsin forms in disease, particularly those of the cardiac and renal systems.

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Specialized roles for cysteine cathepsins in health and disease

Jochen Reiser,1 Brian Adair,2 and Thomas Reinheckel3,4

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Cathepsins were originally identified as proteases that act in the lysosome. Recent work has uncovered nontraditional roles for cathepsins in the extracellular space as well as in the cytosol and nucleus. There is strong evidence that subspecialized and compartmentalized cathepsins participate in many physiologic and pathophysiologic cellular processes, in which they can act as both digestive and regulatory proteases. In this review, we discuss the transcriptional and translational control of cathepsin expression, the regulation of intracellular sorting of cathepsins, and the structural basis of cathepsin activation and inhibition. In particular, we highlight the emerging roles of various cathepsin forms in disease, particularly those of the cardiac and renal systems.

The maintenance of a healthy organism largely relies upon controlled biosynthesis, maturation, function, and terminal breakdown of proteins. Proteolytic enzymes contribute to these processes by irreversibly cleaving peptide bonds. This can result in destruction of the substrate protein, its maturation, or modulation of the biologic activities of the cleavage products. To accomplish the multitude of selective and well-controlled proteolytic events that keep us healthy, the human genome encodes more than 550 proteases and more than 200 endogenous protease inhibitors (1, 2).

The so-called “catheptic activity” (derived from the Greek word kathépsein, meaning to digest or to boil down) was first described in the gastric juice during the 1920s (3). Today, cathepsins are classified based on their structure and catalytic type into serine in the gastric juice during the 1920s (3). Today, cathepsins are classified based on their structure and catalytic type into serine, cysteine, aspartic, and metalloproteases. This classification has helped to guide the discovery and characterization of these enzymes. Cathepsins are a family of proteases that are synthesized as precursors and are secreted or released from the lysosome upon activation. The so-called “catheptic activity” (derived from the Greek word kathépsein, meaning to digest or to boil down) was first described in the gastric juice during the 1920s (3). Today, cathepsins are classified based on their structure and catalytic type into serine, cysteine, aspartic, and metalloproteases. This classification has helped to guide the discovery and characterization of these enzymes. Cathepsins are a family of proteases that are synthesized as precursors and are secreted or released from the lysosome upon activation.

The roles of cathepsins in many physiologic and disease processes have been covered by recent comprehensive reviews (17–20). Here, we focus on the recently discovered roles of cathepsins in organ diseases, with a special emphasis on the ubiquitously expressed cysteine endopeptidase cathepsin L. In particular, we discuss how the different functions of a cysteine cathepsin depend on the cell type in which it is expressed and the cellular compartment in which the protease is localized. We address the homeostatic function of cathepsin L in the heart and its potential role in cardiac regeneration, the reciprocal processing function of cathepsins B and L in ectopic trypsinogen activation during the onset of acute pancreatitis, and the emerging roles of cystolic and nuclear cathepsin L variants in proteinuric kidney disease and stem cell physiology, respectively.

A primer on cathepsin biology

Cathepsin L transcription and translation. Substantial work has been done to analyze the promoter regions of the human cathepsin L gene (CTSL) promoter as well as to understand the regulation of different splice variants within the S' untranslated region of the transcript (21, 22). Of note, one of the splice variants contains a functional internal ribosomal entry site that enables ongoing translation of human cathepsin L under stress conditions, and hypoxia can shut down cap-dependent translation initiation (23). More recent work has focused on the regulation of cathepsin L alternative translation. According to the presence of different forms of cathepsin L in distinct subcellular and extracellular compartments, cathepsin L proteins can be initiated from downstream AUG sites (10), omitting the signal peptide that is normally present at the N terminus of lysosomal cathepsin L that routes the protein to the ER during its synthesis (Figure 2) (10, 24–26).

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The understanding of the nature of cathepsin propeptide inhibitory function as specific inhibitors of the parent cathepsin in the case of cathepsin L, human cathepsin V, and human cathepsin L are compared with each other and with human and mouse cathepsin B in order to demonstrate the phylogenetic distance to other prototypic members of the cysteine cathepsin family.

**Figure 1**
Phylogeny and nomenclature of cathepsins. Phylogenetic tree of mouse and human cathepsin proenzymes. Mouse cathepsin L, human cathepsin V, and human cathepsin L are compared with each other and with human and mouse cathepsin B.

**Sidebar 1**
**Structural features of cathepsins**

The name cathepsin is used to designate a diverse number of intracellular acidic proteases, including serine proteases (cathepsins A and G), aspartic proteases (cathepsin D and E), and the more numerous cysteine proteases (Figure 1). Based on their primary and tertiary structures, the cysteine cathepsins are members of the clan CA, C1a family of cysteine peptidases. Historically, cysteine cathepsins have been termed papain-like proteases, because they resemble the overall fold of papain, the major protease of the papaya fruit (reviewed in refs. 30 and 108). The mature proteins are generally small (20–30 kDa) and mostly monomeric, with the exception of tetrameric cathepsin C, which contains a C-terminal extension for assembly (109). Posttranslational processing generally results in cleavage of the enzyme into disulfide-linked heavy and light chains (110). Members of the cysteine cathepsin family papain superfamily are composed of two folding domains, comparable in size, divided by a solvent-accessible cleft, containing the enzyme active site. The cleft between the two domains serves as a binding site for the target polypeptide chain of the protein substrate in a generally extended conformation. The extended binding site allows some sequence specificity for different cathepsins. Thus, while the structurally similar cathepsins L, K, and S have similar binding sites and specificities, cathepsin L favors aromatic residues, particularly Phe, at the second position before the cleaved bond, while cathepsins K and S will take any hydrophobic residue. Cathepsin K is unusual in accepting a proline at this position, allowing it to cleave collagen (111). The active site cysteine is part of a conserved “catalytic triad” formed by the cysteine pairing with a histidine, which in turn is paired with an asparagine. The configuration is similar to what is seen in serine proteases and is believed to function by allowing the formation of a thiolate-imidazolium ion pair between the cysteine and the histidine side chains. The thiolate ion from the cysteine subsequently performs a nucleophilic attack on the peptide carbonyl carbon.
This results in an active, single chain form of the protein. Upon arrival at the lysosome, further processing cleaves the protein into two chains. Active cathepsins may also be recruited from late endosomes or lysosomes for secretion into the extracellular space via Ca\(^{2+}\)-mediated fusion of these organelles with the plasma membrane. In addition, a minor population of cathepsins (approximately 5%) does not travel to the lysosome but is instead secreted as a proenzyme. Furthermore, alternative splicing and exon skipping can lead to cathepsin forms that lack the signal peptide, and these can subsequently localize to the nucleus and mitochondrial matrix (Figure 3) (21, 45). Recent data suggest that truncated forms of cathepsin L are important in regulating angiostatin-like factors in podocytes: dynamin (7) and synaptopodin (8). In addition, CD2-associated protein (CD2AP) in podocytes is protected from puromycin-induced degradation in the absence of cathepsin L, suggesting the possibility that CD2AP in podocytes is protected from puromycin-induced degradation in the absence of cathepsin L (9).

Mouse cathepsin L

Most of the human cysteine cathepsins possess a highly homologous mouse counterpart, which makes the generation and analysis of cathepsin-knockout mice a relatively straightforward approach to understanding the in vivo functions of these proteases. However, because of a series of tandem duplications of an ancestral cathepsin L gene, the mouse genome, but not that of the human, encodes an additional set of 8 closely related cysteine cathepsins that are all exclusively expressed in the placenta of mice (112). It is even more striking that in human phylogeny cathepsin L evolved into two closely related proteases, namely the “classic” human cathepsin L and human cathepsin V (Table 1 and Figure 1). All three enzymes — mouse cathepsin L, human cathepsin L, and human cathepsin V — are highly homologous, with about 75% amino acid identities. Nevertheless, the three proteases vary in their expression pattern and some (but not all) biochemical properties (Table 1). Consequently, commonly used biochemical assays for activity measurement do not distinguish between human cathepsins L and V, and a serious note of caution is required for conclusions on human cathepsin L drawn from analyses of Ctsl\(^{-/-}\) mice. Inhibitors selective for cathepsin L and V are just emerging (113) and will prove useful in determining their individual functions. Furthermore, the search for disease associations with expression or genetic polymorphisms of cathepsins (114–116), together with functional analysis of human cells and mouse experiments combining gene knockouts with transgenic expression of the human proteases, have already provided valuable insights into the specific in vivo functions of human cathepsins L and V (74, 117, 118). In summary, the complex phylogeny, the widespread expression of cathepsin L–like enzymes, and the multiple phenotypes of the gene knockout mice highlight the great importance of the cathepsin L–like proteases in physiology and disease processes. Defining specific in vivo functions for human cathepsins L and V and testing their individual potential as diagnostic markers or drug targets represents a considerable challenge for further investigations.

**Sidebar 2**

**Cysteine cathepsins in humans and mice**

Most of the human cysteine cathepsins possess a highly homologous mouse counterpart, which makes the generation and analysis of cathepsin-knockout mice a relatively straightforward approach to understanding the in vivo functions of these proteases. However, because of a series of tandem duplications of an ancestral cathepsin L gene, the mouse genome, but not that of the human, encodes an additional set of 8 closely related cysteine cathepsins that are all exclusively expressed in the placenta of mice (112). It is even more striking that in human phylogeny cathepsin L evolved into two closely related proteases, namely the “classic” human cathepsin L and human cathepsin V (Table 1 and Figure 1). All three enzymes — mouse cathepsin L, human cathepsin L, and human cathepsin V — are highly homologous, with about 75% amino acid identities. Nevertheless, the three proteases vary in their expression pattern and some (but not all) biochemical properties (Table 1). Consequently, commonly used biochemical assays for activity measurement do not distinguish between human cathepsins L and V, and a serious note of caution is required for conclusions on human cathepsin L drawn from analyses of Ctsl\(^{-/-}\) mice. Inhibitors selective for cathepsin L and V are just emerging (113) and will prove useful in determining their individual functions. Furthermore, the search for disease associations with expression or genetic polymorphisms of cathepsins (114–116), together with functional analysis of human cells and mouse experiments combining gene knockouts with transgenic expression of the human proteases, have already provided valuable insights into the specific in vivo functions of human cathepsins L and V (74, 117, 118). In summary, the complex phylogeny, the widespread expression of cathepsin L–like enzymes, and the multiple phenotypes of the gene knockout mice highlight the great importance of the cathepsin L–like proteases in physiology and disease processes. Defining specific in vivo functions for human cathepsins L and V and testing their individual potential as diagnostic markers or drug targets represents a considerable challenge for further investigations.

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**Table 1**

Summary of nomenclature of human and mouse cathepsins L and V, together with genomic localization, expression pattern, and some examples of substrate specificity

<table>
<thead>
<tr>
<th>Cathepsin</th>
<th>Symbols(^a) and alleles</th>
<th>Genomic locus</th>
<th>Expression</th>
<th>Substrate specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human cathepsin L</td>
<td>CTSL, CTS1L, CATL, MEP</td>
<td>9q21/22 (no synteny in mouse genome)</td>
<td>ubiquitous, expression levels vary among cell types</td>
<td>cleaves z-PheArg-AMC(^b); does not cleave elastin; does not generate angiostatin-like peptides from plasminogen</td>
</tr>
<tr>
<td>Human cathepsin V</td>
<td>CTSV, CTS2L, CATL2, CTSU</td>
<td>9q22.2 (syntenic to mouse chromosome 13: 30.0 cM, i.e., the Cts/locus)</td>
<td>restricted to macrophages, thymus, testis, cornea, keratinocytes, some cell cancer types</td>
<td>cleaves z-PheArg-AMC(^b); cleaves elastin; generates angiostatin-like peptides from plasminogen</td>
</tr>
<tr>
<td>Mouse cathepsin L</td>
<td>Ctsl, Cts1L, MEP, nkt, fs</td>
<td>Chromosome 13: 30.0 cM (syntenic to human chromosome 9q22.2, i.e., the CTSV/locus)</td>
<td>ubiquitous, expression levels vary among cell types</td>
<td>cleaves z-PheArg-AMC(^b); cleaves elastin</td>
</tr>
</tbody>
</table>

\(^{a}\)Symbols in common use are underlined. \(^b\)The fluorogenic peptide z-PheArg-AMC is frequently used for determination of cathepsin L activities. These assays require inhibition of cathepsin B by a specific inhibitor such as CA074.
Despite enteropeptidase, a highly selective trypsinogen-cleaving protease, trypsinogen or impair the inhibition/degradation of active trypsin located at the luminal site of duodenal cells (26). The proteolytic ensemble of cathepsins, due to inefficient trafficking from the trans-Golgi network to the endosomal/lysosomal compartment via the m6p/m6p receptor pathway (56). It has been estimated that approximately 5%–10% of lysosomal proteases are missorted into extracellular matrices. The activation of trypsinogen begins in vesicular organelles that are acidic (55), raising the possibility that the colocalization of cathepsin B and trypsinogen has been shown by subcellular fractionation and immunogold labeling of EM sections in pancreata from patients and rodents with pancreatitis (reviewed in ref. 54).

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### Table 2

<table>
<thead>
<tr>
<th>Inherited syndrome</th>
<th>Cysteine cathepsin</th>
<th>Description</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillon-Lefèvre and Haim-Munk syndromes</td>
<td>Cathepsin C</td>
<td>Loss-of-function mutations</td>
<td>Hyperkeratosis palmoplantaris with periodontosis in humans. The severe periodontitis causes early loss of primary and permanent teeth, due to destruction of alveolar bone. Ctsc-null mice show impaired activation of granzymes and other immune cell serine proteases. This may be the cause for dysregulation of localized polymorphonuclear leucocyte response in the inflamed periodontal tissues of patients with Papillon-Lefèvre/Haim-Munk disease.</td>
</tr>
<tr>
<td>Pycnodysostosis</td>
<td>Cathepsin K</td>
<td>Loss-of-function mutations</td>
<td>Pycnodysostosis is characterized by osteopetrosis, caused by impaired degradation of collagen I in the bone matrix in humans, with an identical phenotype in Ctsk-null mice. Ctsk inhibitors are in trials for slowing turnover of bone in osteoporosis.</td>
</tr>
</tbody>
</table>

### Opposing roles of cathepsins B and L in acute pancreatitis

**Physiological activation of digestive proteases.** The acinar cells of the exocrine pancreas produce and secrete a wide variety of potent proteolytic enzymes essential for intestinal digestion of nutrient proteins. However, these digestive enzymes are potentially harmful. Therefore, these proteases are produced as precursors (i.e., zymogens) within pancreatic acinar cells and are only activated in the duodenum. The key step in this activation process is the conversion of inactive trypsinogen to active trypsin by limited proteolysis by enteropeptidase, a highly selective trypsinogen-cleaving protease located at the luminal side of duodenal cells (26). The proteolytically active trypsin initiates an activation cascade of proteolytic enzymes within the duodenum, thereby ensuring the high proteolytic capacity needed for food digestion (26).

**Cathepsin L: keeping trypsin in check.** Because of their partially overlapping substrate repertoires, it had been widely assumed that cysteine cathepsins other than cathepsin B might also contribute to trypsinogen activation in pancreatitis. Biochemical data obtained from bovine and human proteins revealed a specific cathepsin L cleavage site in trypsinogen at a position 3 amino acids C-terminal from the normal enteropeptidase/cathepsin B cleavage site (5). This cleavage prevents the generation of the N-terminal isoleucine that is essential for the active conformation of trypsin. Hence, in contrast to cathepsin B, cleavage of trypsinogen by cathepsin L results in an inactive trypsin variant. Active trypsin is not cleaved by cathepsin B, at this position but at a second cathepsin L cleavage site between amino acids E82 and G83 that inactivates trypsin (5). These data imply that cathepsin L can prevent ectopic trypsinogen activation. In keeping with this notion, Ctsl−/− mice show increased intrapancreatic trypsin activity upon pancreatitis induction (5). Of note, Ctsl−/− mice also show less severe pancreatitis, because, as a result of the less severe local and systemic inflammation, acinar cells undergo apoptosis instead of necrosis. Thus, cysteine cathepsins serve complex roles in the pancreas beyond activating and inactivating trypsinogen.

**Cathepsins and protease zymogens: how do they meet?** Despite compelling evidence for selective cleavage of trypsinogen by cathepsins B and L, an important cell biological question remains to be resolved: how can endolysosomal cathepsins meet trypsinogen, which is located in the secretory vesicles of the pancreatic acinar cell that are known as zymogen granules? Of note, colocalization of cathepsin B and trypsinogen has been shown by subcellular fractionation and immunogold labeling of EM sections in pancreata from patients and rodents with pancreatitis (reviewed in ref. 54).

The activation of trypsinogen begins in vesicular organelles that are acidic (55), raising the possibility that the colocalization of trypsinogen and cathepsins could result from the missorting of lysosomal cathepsins, due to inefficient trafficking from the trans-Golgi network to the endosomal/lysosomal compartment via the m6p/m6p receptor pathway (56). It has been estimated that approximately 5%–10% of lysosomal proteins are missorted and enter the constitutive and regulated secretory pathways, and it is clear that cathepsin L and other cysteine cathepsins can selectively effect their protease function in secretory vesicles (18, 57). For example, cathepsin L has been identified as a major enzyme involved in the generation of secreted pituitary neuropeptides (e.g., enkephalin, ACTH, α-MSH, β-endorphin) from their large
imbalance consisting of low levels of cathepsin L, which degrades trypsinogen and trypsin, and high levels of cathepsin B, which are digested by lysosomal hydrolases, including cathepsins (61). In addition, there is evidence that the degradation of glycogen. Deficiency of lysosome-associated membrane protein 2 (LAMP-2) induces the accumulation of autophagic vacuoles and immune response against microbes (62). In these processes, cysteine cathepsins cooperate within their family and with other proteases such as matrix metalloproteinases and the uPAR system.

In the search for alternative mechanisms, it was recently proposed that incompletely executed autophagy leads to the observed enzyme colocalization in early pancreatitis (60). Both starvation and pancreatitis induce an autophagic response in pancreatic acinar cells that engulfs cytoplasmic proteins and organelles, including zymogen granula (60). After fusion of autophagosomes with lysosomes, the contents of the resulting autophagolysosomes are digested by lysosomal hydrolases, including cathepsins (61). Eventually, the autophagolysosomes break down and disappear in starvation-induced autophagy. In contrast, during pancreatitis the autophagic process is impaired at the level of autophagolysosome degradation, thereby providing an acidic compartment for colocalization of trypsinogen and cathepsins (60). Furthermore, an imbalance consisting of low levels of cathepsin L, which degrades trypsinogen and trypsin, and high levels of cathepsin B, which activates trypsinogen, has been found in autophagic vesicles during pancreatitis (60). This, in turn, results in intracellular accumulation of active trypsin that activates further digestive enzymes, thereby causing damage to pancreatic cells, a hallmark event in the pathogenesis of pancreatitis.

**Cysteine cathepsins in the heart**

Cathepsin L: a homeostatic protease within the myocardium. Cardiomyopathies represent a heterogeneous group of heart diseases that are characterized by progressive myocardial remodeling, leading to impaired pump function of the heart (62, 63). Among many other etiologies, defects in lysosomes and lysosomal hydrolases have been shown to cause myocardial heart disease (64, 65). Cardiomyopathies have also been described as a component of inherited disorders caused by deficiency of lysosomal glycosidases; for example, Pompe disease is caused by abnormal accumulation of glycogen. Deficiency of lysosome-associated membrane protein 2 (LAMP-2) induces the accumulation of autophagic vacuoles and causes Danon disease, which is characterized by severe myopathy.
of cardiac and skeletal muscles (64, 65). Of note, LAMP-2–deficient mice display a vacuolar cardioskeletal myopathy that is similar to that observed in individuals with Danon disease (66). Increased activity of lysosomal enzymes also has been found in patients with hypertensive heart failure (67, 68).

In contrast to those of these well-established lysosomal storage diseases and their causative molecules, the role of lysosomal proteases in the heart remained elusive for a long time. However, recent findings with 1-year-old Ctsl−/− mice shed light on this issue (69, 70). These aging animals develop a cardiac phenotype that displays key features of human dilated cardiomyopathy (69). As such, complete deficiency of cathepsin L causes interstitial myocardial fibrosis and the appearance of pleomorphic nuclei in cardiomyocytes, both characteristics of human cardiomyopathies. It also causes cardiac chamber dilation and impaired cardiac contraction. Moreover, at 1 year of age, Ctsl−/− mice develop supraventricular tachycardia, ventricular extrasystoles, and first-degree atrioventricular blockage (69). Deficiency of cathepsin L in mice affects the endolysosomal system of cardiomyocytes in newborn mice (69). In particular, it increases the number of acidic organelles, although these vesicles lack the accumulation of typical lysosomal storage materials and have altered morphology (70). Subsequently, the defects in the acidic cellular compartment are accompanied by complex biochemical and cellular alterations, with loss of cytoskeletal proteins and mitochondrial impairment (70). These findings raise the question of how cathepsin L deficiency and the observed alteration of the acidic cellular compartment change intracellular signaling toward induction of a hypertrophic response with subsequent dilation of the heart.

**Cathepsin L involvement in cardiac signal transduction.** In a gain-of-function approach, human cathepsin L was transgenically overexpressed in the cardiomyocytes of mice (71). The transgenic
Extracellular cathepsins in cardiac remodeling and repair. It is also worth noting that dilative cardiomyopathy–associated interstitial fibrosis in Ctsl–/– mice is the only defect in the heart that cannot be rescued by transgenic reexpression of mouse cathepsin L in cardiomyocytes of otherwise cathepsin L–deficient mice (75). These results imply that the observed cardiac fibrosis in Ctsl–/– mice is caused by the absence of cathepsin L from cardiac fibroblasts and not from cardiomyocytes. Since collagen I (Col1a1) mRNA expression is not enhanced in cathepsin L–deficient myocardium, the accumulation of collagen in the ECM is most likely due to impaired collagen turnover.

Cathepsin L is mainly located in the endosomal/lysosomal compartment, but a fraction of the proenzyme can be secreted and activated by other proteases such as matrix metalloproteinases. Activated extracellular cathepsin L is capable of processing ECM proteins, such as fibronectin, laminin, and types I, IV, and XVIII collagen, even at neutral pH (76–78). Cysteine cathepsins, such as cathepsin S and cathepsin B, are highly abundant in the left ventricular myocardium of patients with hypertensive heart failure and therefore have been implicated in turnover of the ECM and cardiac remodeling in this disease (68, 79).

This turns attention to another emerging aspect of extracellular cathepsin L—its involvement in cardiac repair. Endothelial progenitor cells home to ischemic areas, differentiate, and build the basis for new blood vessels, a process known as neovascularization (80). Improvement of vascularization and function of ischemic areas in the heart may represent a physiologic function of endothelial progenitor cells. The accumulation of collagen in the ECM is most likely due to impaired collagen turnover.

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activity (but not that of other major proteases) in endothelial progenitor cells and also reduces invasion of these cells in a glucose concentration–dependent manner (83). Hence, the specific impairment of cathepsin L function by hyperglycemia may explain the poor neovascularization and regeneration capacities of ischemic tissues in diabetic patients.

The role of truncated forms of cathepsin L in murine and human renal disease

The role of podocyte cathepsin L in proteinuric kidney diseases. The kidney glomerulus is a highly specialized structure that ensures the selective ultrafiltration of plasma, so that essential proteins are retained in the blood (84). Podocytes are unique cells with a complex cellular organization, consisting of a cell body, major processes, and foot processes (FPs). The FPs cover the outer aspect of the glomerular basement membrane. They form a characteristic interdigitating pattern with FPs of neighboring podocytes, leaving in between the glomerular filtration barrier. Dephosphorylation of synaptopodin by calcineurin abrogates the interaction with 14-3-3. This renders the cathepsin L cleavage sites of synaptopodin accessible and promotes the degradation of synaptopodin and dynamin, thereby promoting a motile phenotype and the development of proteinuria. The calcineurin inhibitor CsA and the cathepsin inhibitor E64 safeguard against proteinuria by stabilizing synaptopodin and dynamin protein levels in podocytes.

Cytosolic cathepsin L and its function in proteinuric kidney disease. Phosphorylation of synaptopodin by PKA or CaMKII promotes 14-3-3 binding, which protects synaptopodin and dynamin against cathepsin L–mediated cleavage, thereby contributing to a stationary podocyte phenotype and an intact glomerular filtration barrier. Dephosphorylation of synaptopodin by calcineurin protects against cathepsin L–mediated cleavage, thereby contributing to a stationary podocyte phenotype and an intact glomerular filtration barrier. Moreover, induction of proteinuria represents a migratory event in podocyte FP that involves the cleavage of the large GTPase dynamin (7) and synaptopodin (8). The clinical relevance of these findings was underscored by the observation that podocyte cathepsin L expression is increased in a variety of human proteinuric kidney diseases, ranging from minimal change disease (MCD) to diabetic nephropathy (7).

Together, these results support the notion that cathepsin L–mediated proteolysis plays a critical role in the development of various forms of proteinuria (94).

Cathepsin L–mediated degradation of dynamin leads to proteinuria in mice. Dynamin is essential for the formation of clathrin-coated vesicles at the plasma membrane during endocytosis (95). Dynamin has also been implicated in the regulation of actin dynamics in certain cell types (96). Using the Prediction of Endopeptidase Proteolytic Sites computer algorithm (PEPS) for predicting putative cathepsin L substrates (46), dynamin was identified as a target of cathepsin L (7). In mouse podocytes, dynamin is cleaved by cytoplasmic cathepsin L during LPS- or PAN-induced experimental proteinuria, and gene delivery of cathepsin L–resistant dynamin protected mice against LPS-induced proteinuria (7). The notion that dynamin is required for proper podocyte structure and function is further supported by the observation that overexpression of dominant-negative dynamin leads to a loss of podocyte stress fibers in vitro and development of proteinuria in mice (7).

Cyclosporine A prevents proteinuria by blocking cathepsin L–mediated degradation of synaptopodin in podocytes. Calcineurin is a ubiquitously expressed serine/threonine phosphatase (97). Its best-characterized function is the regulation of nuclear factor of activated T cells (NFAT) signaling. The immunosuppressive action of the calcineurin inhibitor cyclosporine A (CsA) stems from its inhibition of NFAT signaling in T cells (98). CsA can also induce remission of the proteinuria associated with diseases such as MCD and focal segmental glomerulosclerosis (FSGS) (99). Although T cell dysfunction is associated with some forms of proteinuria (100), including a subset of children with MCD (101), the salutary action of CsA in MCD and FSGS led to the suspicion that CsA might exert its effect, at least in part, independently of its effects on T cells, a hypothesis also supported by reports of CsA effectiveness in nonimmunological human (102) and experimental (103) Alport syndrome.

Recently, a mechanism was identified wherein CsA blocks calcineurin-mediated dephosphorylation of synaptopodin in mouse podocytes, thereby preserving the phosphorylation-dependent synaptopodin/14-3-3β interaction (8). This interaction, in turn, protects synaptopodin from cathepsin L–mediated degradation and preserves a stable filtration barrier. Moreover, inducible expression of dominant-active calcineurin in podocytes is suf-
efficient to cause the degradation of synaptopodin and dynamin, thereby inducing proteinuria (8). These data describe a calcineu-
rin/cathepsin L signaling pathway in podocytes that contributes to the regulation of kidney filter function (Figure 4). In contrast to most other calcineurin-NFAT controlled signaling events (97, 98, 104, 105), the antiproteinuric effect of CsA stems, at least in part, from its inhibition of cathepsin L–mediated degradation of synaptopodin in podocytes (Figure 4 and ref. 8).

The emerging role of nuclear cathepsin L in polycystic kidney disease. Polycystic kidney disease (PKD) represents the most common genetic renal disease in the world. PKD is inherited as an auto-
somal dominant (ADPKD) or autosomal recessive (ARPKD) trait and characterized by progressive enlargement of renal cysts (106). Cux1 is a homeobox gene that represses the cyclin kinase inhibitors p21 and p27, and transgenic mice ectopically expressing Cux1 develop renal hyperplasia (107). A 246–amino acid deletion in Cux1 accelerates PKD progression in the cpk model of ARPKD (11), and the ensuing phenotype was explained by a missing cathepsin L cleavage site in the truncated Cux1 mutant, which thereby maintains increased tubular cell proliferation and apoptosis. Cux1 is proteolytically processed by a nuclear isoform of cathepsin L (10). In both human ADPKD cells and in kidneys of mice with a targeted deletion in Pkd1, a murine model of PKD, decreased nuclear cathepsin L levels are associated with increased levels of Cux1 protein in the cystic cells in vitro and the cysts in vivo (11). These results suggest a mechanism by which reduced Cux1 processing by nuclear cathepsin L results in the accumulation of Cux1, downregulation of p21/p27, and increased cell proliferation in PKD (11). Furthermore, they provide proof of principle of the hypothesis that nuclear cathepsin L is capable of processing transcription factors that control important cellular programs, such as growth.

Outlook
Recent studies have uncovered multiple divergent roles for different cathepsins in a variety of physiologic and pathophyslogic processes. From the findings in the different organs discussed above, it has become clear that cathepsins serve as regulatory enzymes beyond acting as simple housekeeping proteases and harbor important functions outside the lysosome. Future studies are required to delineate the translational mechanisms leading to the generation of the truncated forms of cathepsin L. Structural insights should aid drug development of cathepsin inhibitors that act in an allosteric manner and, therefore, may be more specific for individual cathepsin forms than currently available inhibitors. The understanding of cathepsins and their recently identified substrates continues to be an expanding area in biology. Most importantly, they provide starting points for the development of novel selective therapeudic modalities for various human diseases.

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