

Review

The Intertwining of Autophagy and the Ubiquitin Proteasome System in Podocyte (Patho)Physiology

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Key Words

Podocyte • Autophagy • Lysosome • Proteasome • Ubiquitin proteasome system • Crosstalk • Diabetic nephropathy • Membranous nephropathy • Focal segmental glomerulosclerosis • Lupus nephritis • Ageing

Abstract

Protein homeostasis strongly depends on the targeted and selective removal of unneeded or flawed proteins, of protein aggregates, and of damaged or excess organelles by the two main intracellular degradative systems, namely the ubiquitin proteasomal system (UPS) and the autophagosomal lysosomal system. Despite representing completely distinct mechanisms of degradation, which underlie differing regulatory mechanisms, growing evidence suggests that the UPS and autophagy strongly interact especially in situations of overwhelming and impairment, and that both are involved in podocyte proteostasis and in the pathogenesis of podocyte injury. The differential impact of autophagy and the UPS on podocyte biology and on podocyte disease development and progression is not understood. Recent advances in understanding the role of the UPS and autophagy in podocyte biology are reviewed here.

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Introduction

Our smallest renal filtration unit, the glomerulus comprises four cell types, which form a functional syncytium: The visceral epithelial cells (podocytes), the parietal epithelial cells (PECs) of Bowman's capsule, the fenestrated and highly glycosylated glomerular endothelial cells (GEnCs), and a type of specialized pericytes termed mesangial cells (MCs) [1]. Podocytes and endothelial cells of the glomerular capillaries are separated by the glomerular basement membrane and form the 3-layered glomerular filtration barrier, over which blood filtration ultimately occurs in a size and charge selective manner. Mesangial cells modulate the size of the filtration area and parietal epithelial cells prevent leakage of the primary urinary filtrate into the tubulointerstitium. Of all glomerular cells, podocytes, as terminally differentiated

cells, exhibit the most elaborate morphology with primary processes arising from the podocyte cell body, which form an intricate network of interdigitating foot processes with neighboring podocytes. A specialized form of adherens junction, the slit membrane, interconnects foot processes. With its unique protein repertoire, the slit membrane represents a flexible non-clogging barrier to proteins, with proposed mechano-sensitive properties. Thereby, the slit membrane proteins nephrin and neph1 bridge the slit membrane and podocin generates a signaling hub in lipid rich domains of the podocyte foot process mediating mechano-sensation. The glomerulus can become a target of immune attack by both the humoral and cellular immune system resulting in different forms of glomerulonephritis. Depending on the site of glomerular injury, glomerulonephritis can present as either a nephrotic or as a nephritic syndrome. Nephrotic syndrome is defined by four components: high range proteinuria, hypoalbuminemia, edema, and hypertriglyceridemia and is prototypic for the injury of podocytes. Podocyte injury ensues in a uniform morphological injury pattern combining different degrees of podocyte hypertrophy, podocyte foot process effacement and podocyte loss to the urine, ultimately leading to dialysis necessitating renal insufficiency. As a general theme, these morphological changes appear to involve reactivation of developmental programs such as those engaged by Notch [2], Wnt [3-6], and mTOR pathways [7]. Over-activation, imbalance and impairment of these central intracellular signaling pathways disrupt normal podocyte energy metabolism [8] and protein homeostasis [9] thus initiating a mostly irreversible dedifferentiation process. The significance of proteostatic pathway alterations in podocytes will be the focus of this review, which is based on many excellent expert reviews. We apologize to those many researchers, whose work could not be cited in this context.

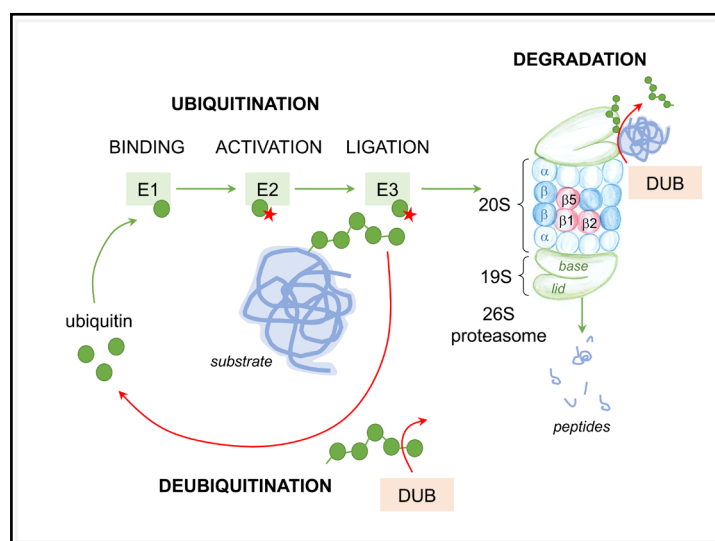
Proteostatic systems

The set of proteins, which define the proteome of a given cell, is controlled by a mechanism called proteostasis. Proteostasis comprises cellular processes such as the translation (synthesis) of proteins, the precise folding and localization of proteins following synthesis, and the degradation of flawed and of unneeded proteins by cellular degradation systems. About 240 g protein are synthesized and degraded daily in a 60 kg adult, the majority being intracellular proteins [10]. Failure to accurately regulate these cellular processes ultimately leads to disease. Several degradation systems exist, of which the ubiquitin proteasomal system (UPS) and the autophagosomal lysosomal pathway (ALP) represent the most important systems for intracellular protein degradation. Both systems degrade proteins into small polypeptides and help maintain amino acid pools and energy (ATP) balance either during acute starvation by the UPS or in the course of chronic starvation by autophagy [11] and both are responsible for protein quality control. Thereby, the UPS is responsible for degrading 80-90% of proteins including many regulated, short lived, abnormal, denatured, or in general damaged proteins. Autophagy is primarily responsible for the degradation of most long-lived proteins, but also degrades aggregated proteins as well as cellular organelles.

The Ubiquitin proteasome system

Among the multitude of biological processes regulated by the UPS are the cell cycle, gene transcription and translation, cell survival and apoptosis, cell metabolism and protein quality control, and inflammation through the degradation of specific protein components of these processes. The UPS combines the highly specific mechanisms of 1) substrate tagging and of 2) substrate protein degradation within a multi-protein complex called the proteasome (Fig. 1). Substrate tagging is achieved by the covalent attachment of the conserved protein ubiquitin to one or more lysine residues of the substrate protein via a hierarchical ATP consuming multi-enzymatic process mediated by E1, E2 and E3 enzymes. One single E1 enzyme is responsible for ubiquitin activation. Thereafter, the ubiquitin conjugating E2 enzymes (about 50 known) participate in the transfer of the activated ubiquitin to the protein substrate. In a vast majority of cases, a member of the E3 ubiquitin ligase family

Fig. 1. Ubiquitin proteasome system (UPS). Covalent attachment of (poly)ubiquitin to a lysine residue of the substrate protein is carried out via the concerted action of three different ubiquitin (Ub) enzymes (E1 = Ub binding; E2 = Ub activating; E3 = Ub ligation) at the expense of ATP. A chain of four or more Ubs is generally both necessary and sufficient to target the substrate to the proteasome for degradation, an ATP consuming process. The proteasome is a barrel shaped proteolytic organelle comprised of a 20S central core particle (CP) with the three proteolytic subunits $\beta 1$, $\beta 2$ and $\beta 5$, and one (26S proteasome) or two 19S regulatory particles (30S proteasome). The 19S regulatory particle (RP) comprises the ATPase containing base and the non-ATPase containing lid and regulates proteasomal degradation by binding to cargo-loaded shuttling proteins, deubiquitination of the substrates, unfolding and channeling them into the inner core of the 20S CP, where the substrate is degraded and recycled. Ubiquitination is counterbalanced by deubiquitination, a hydrolytic reaction carried out by 5 different classes of deubiquitinating enzymes (DUBs).



also participates in the conjugation process. E3 ligases serve as docking proteins that bring the substrate protein and the E2 carrier protein with the activated ubiquitin together. E3 enzymes are grouped into three major families, namely RING, HECT and RBR families, based on structural similarities and the functional classes of substrates they recognize. Each E3 enzyme recognizes specific proteins destined for ubiquitination, therefore this step is a major mechanism ensuring specificity toward substrate proteins and is performed by over 1000 known E3 ligases. The pattern of ubiquitin conjugation (termed the ubiquitin code) determines the fate of ubiquitinated proteins. Different kinds of ubiquitin linkages can occur, ranging from ubiquitin conjugation to target proteins either as monomers (mono-ubiquitination) or as di-, oligo- and poly-ubiquitin chains (poly-ubiquitination). Depending on the lysine (K) within the ubiquitin protein used for the generation of polyubiquitin chains, one distinguishes between K6-, K11-, K27-, K29-, K33-, K48-, and K63-polyubiquitin linkages. The level of complexity of the ubiquitin code is enhanced through posttranslational phosphorylation and acetylation of ubiquitin, and by the generation of mixed ubiquitin chains through incorporation of ubiquitin-like modifiers such as SUMO, NEDD8 or FAT10. Deubiquitinating enzymes, furthermore, are able to modify the length and the branching of polyubiquitin chains, thereby altering the fate of the substrate protein. The small number (<100) of DUBs might at first suggest a low degree of selectivity; however, DUBs are subject to multiple layers of regulation that modulate both their activity and their specificity [12]. Four main families of deubiquitinating enzymes are present in mammalian cells: 58 ubiquitin-specific proteases (USPs), 4 ubiquitin C-terminal hydrolases (UCHs), 5 Machado-Josephin domain proteases (MJDs), 14 ovarian tumor proteases (OTU), and Jab1/Mov34/Mpr1 Pad1 N-terminal+ (MPN+) (JAMM) domain-containing genes [13]. Deubiquitinating enzymes cleave ubiquitin from proteins and disassemble polyubiquitin chains that are released from substrates before proteasomal degradation. The activities of deubiquitinating enzymes are important for the recycling of ubiquitin for subsequent ubiquitination reactions, for the prevention of proteasome congestion, and for the control of specific protein turnover by modifying/removing ubiquitin or polyubiquitin chains from the targeted protein and by this changing the fate of the protein, i.e. prevention of proteasomal degradation.

The current concept is that ubiquitination is required for the initiating association of the ubiquitinated proteins with the proteasome complex. This high affinity binding to the proteasome is easily reversible by competition with other ubiquitin binding proteins and does not depend on ATP. Subsequently, the second tight binding step that commits the substrate to proteasome degradation requires ATP hydrolysis and a loosely folded region in the substrate [14]. After ubiquitinated proteins bind to the proteasome complex, proteasomes disassemble and release the ubiquitin chain, engage the deubiquitinated substrate into the proteolytic chamber and degrade the substrate.

The proteasome is a 2.5 MegaDalton protease consisting of over 60 subunits, which are assembled as a hybrid between the 20S catalytic core particle (CP) and the 19S regulatory particle (RP). The 20S CP is a barrel shaped structure of 28 subunits arranged as a cylindrical stack of two outer α -rings and two inner β -rings, composed of 7 structurally similar α or β subunits. The inner two β -rings form the proteolytic chamber, whereas the outer two α -rings serve as a gate for substrate entry into the chamber. The $\beta 1$, $\beta 2$, $\beta 5$ subunits of the constitutive CP harbor the distinct proteolytic activities: The $\beta 5$ subunit harbors the chymotrypsin-like activity; the $\beta 2$ subunit the trypsin-like activity; the $\beta 1$ subunit the caspase-like activity, which degrade the engaged substrates to peptides. The 19S regulatory particle binds to the cylinder end of the CP, and opens a channel located centrally within the cylinder end. The RP contains 19-20 subunits grouped in a base (ATPases) and a lid (non-ATPases), which in a complex interplay mediate substrate binding, substrate deubiquitination, and substrate entry into the proteolytic CP. Of note, the proteasome does not degrade proteins to amino acids, but instead produces a highly heterogeneous mixture of peptides from a given protein [15], which then either serve as raw material for adaptive cell-mediated immunity [16] or which are further processed by cytosolic peptidases to amino acids for *de novo* protein synthesis. The complexity of the proteasome system is further achieved by the fact that cells can alter their proteolytic capacity and specificity by modulating the amount and diversity of proteasomes. Thus, different types of proteasomes exist based on the types of proteolytic subunits incorporated within the β -rings of the 20S core complex or based on the regulatory caps associated with the 20S core particle. The immunoproteasome (i26S) harbors the $\beta 1i$, $\beta 2i$, $\beta 5i$ subunits within its 20S CP, which are thought to mediate a more efficient and / or alternative peptide cleavage ideal for MHC-class 1 presentation [17]. The association of so-called proteasome activators, such as PA28 α/β , PA28 γ , or PA200, which represent alternative regulatory particles to the 20S CP enhance the open probability of the proteasome and thereby peptide hydrolysis (reviewed in [18]).

The autophagosomal lysosomal pathway

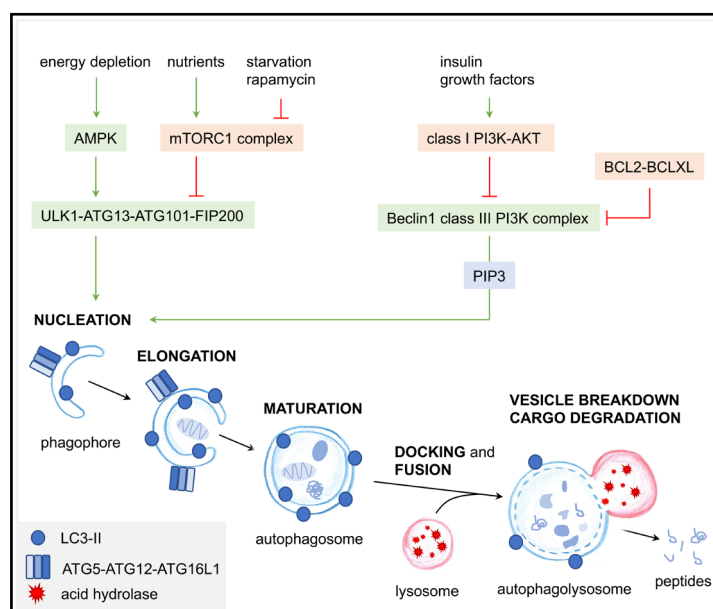
Autophagy is a predominantly nonspecific highly conserved “self-eating” process, which was discovered in the late 1950’s [19]. As one arm of the endocytic system, autophagy is essential for the maintenance of cellular homeostasis and cellular stress response with its main function to provide metabolic precursors for survival in conditions of stress and to serve like the UPS as a protein quality control system by clearing misfolded proteins and other cellular debris. Three types of autophagy are known, which differ in their mechanisms and functions: Microautophagy, chaperone mediated autophagy, and macroautophagy. In microautophagy, small cytoplasmic cargoes are engulfed within lysosomal membrane invaginations [20]. Chaperone mediated autophagy requires the recruitment of KFERQ-motive-bearing proteins to the lysosome via binding to chaperones such as heat shock cognate protein 70 (Hsc70) and co-chaperones BAG1, Hip, Hop and Hsp40/DNAJB1 [21]. The KFERQ motive is present in 30% of cellular proteins. The chaperone then binds to a specific lysosomal receptor LAMP-2A on the lysosomal membrane [21], the cargo protein is unfolded and translocated to the lysosome for degradation. Macroautophagy (hereafter termed “autophagy”) is believed to be the major mode of autophagy and is the most described form of autophagy. The principle of macroautophagy is that mainly bulky cargo such as less rapidly degraded proteins, organelles or protein aggregates are removed by engulfing them in a double membrane compartment called the autophagosome, which fuses with

lysosomes [22] for final degradation. Lysosomal degradation is highly efficient, as this membrane-enclosed organelle contains numerous proteases of low specificity [23]. Depending on the substrates degraded, macroautophagy is subdivided in mammalian cells into a bulk non-selective form and into selective forms governing the removal of mitochondria (mitophagy), of lipid droplets (lipophagy), of peroxisomes (pexophagy), of ribosomes (ribophagy), of proteasomes (proteophagy), and of lysosomes (lysophagy) [24]. Selectivity of autophagy is controlled by autophagy receptors, which associate simultaneously with cargo and the autophagosomal membrane proteins LC3 (ATG8-or microtubule-associated protein 1A/1B-light chain 3) and its homologous protein GABARAP (γ -aminobutyric acid receptor-associated protein-like). The most studied autophagy receptors are sequestome 1 (SQSTM1)/p62 and neighbor of breast cancer 1 gene (BRCA1) 1, which sequester aggregated proteins to form SQSTM1/p62 bodies for autophagy.

The process of autophagy (Fig. 2) can mechanistically be divided into distinct steps: 1) initiation by cell signaling pathways, 2) nucleation, 3) elongation, and 4) vesicle fusion to lysosomes. The understanding of the molecular mechanisms of autophagy has ensued following the initial discovery of the autophagy related genes (ATG) from genetic studies in yeast [22]. Sixteen ATG proteins comprise the conserved core ATG machinery that catalyzes formation of small vesicles made of a double membrane called phagophore (or isolation membrane) at a specialized site called the phagophore assembly site. In mammals, initiation is associated with an endoplasmic reticulum (ER) subdomain enriched for the lipid phosphatidylinositol 3-phosphate (PI(3)P), known as the omegasome [25]. The origin of the membrane used for phagophore formation may involve different sources, such as Golgi, ER, plasma membrane, recycling endosomes, mitochondria, and lipid droplets [26, 27].

To initiate autophagy at the phagophore assembly site, a range of signaling processes converges on two main kinase protein complexes: the ULK1 (unc51-like autophagy activating kinase 1) protein kinase complex and the Beclin1-PI3KC3-C1 (class III phosphatidylinositol 3-kinase complex I) lipid kinase complex [28]. Multiple levels of (auto)phosphorylation and protein-protein interactions are necessary for activation of the two complexes, with ULK1 and Beclin1 being the respective major sites of regulation. Modification (phosphorylation) of ULK1 and ATG13 results in the recruitment of the activated ULK1 complex (with its components ULK1-ATG13-ATG10-FIP200) to the phagophore assembly site [29], a process also influenced by LC3. ULK1 then transduces pro-autophagic signals by phosphorylating many substrate proteins [29] including itself, subunits of the ULK1 complex and other elements of the core autophagy machinery such as Beclin1 and AMBRA1 (Autophagy and Beclin1 Regulator 1) and hence stimulates the activity of the Beclin1-PI3KC3-C1 complex (with its components Beclin1-VPS34-ATG14L-p150) [30, 31]. The Beclin1-PI3KC3-C1 complex is driven to the phagophore association site by its unique ATG14L subunit. As an essential early event in autophagy initiation, the activated PI3 kinase Vps34 of the Beclin1 complex produces PI3P at the phagophore membrane. The PI3P binding WIPI1-4 proteins and the two ubiquitin like conjugation systems then function downstream and drive phagophore elongation. The first conjugation system comprises LC3-ATG4-ATG7-ATG3 which conjugate phosphatidylethanolamine to free LC3 (LC3-I) in the cytosol. This lipidated LC3 (LC3-II) then redistributes to autophagic vesicles. Therefore, LC3-II is commonly used as a marker for autophagy. The second conjugation system involves the assembly of an ATG12-ATG5-ATG16 complex, which is required for the targeting of LC3 to the double membrane of the expanding phagophore, hence allowing further autophagosome growth, closure and maturation into autolysosomes. LC3-II also mediates the interaction with the cargo-loaded autophagy receptor p62 [32]. In selective autophagy, the cargo itself templates the size and shape of the phagophore. After completed elongation and closure, the outer membrane of the autophagosome fuses with the lysosome to form the autophagolysosome, a process controlled by SNARE proteins [33]. At this stage the inner membrane and all its content are degraded.

Fig. 2. Autophagy pathway. The autophagic pathway responds to signals from the environment, such as nutrient status (inhibitory through mTOR signaling pathway), to insulin and other growth factors (inhibitory through class I PI3K-AKT), and to low energy status (activating through AMPK). Autophagy is initiated by the formation and elongation of a double layered phagophore that later develops into an autophagosome by enwrapping a portion of cytoplasm. This process is carried out by the combined action of several autophagy related genes (ATG) that are responsible for forming molecular complexes that work in a sequential order. The ULK1-



ATG13-FIP200-ATG101 complex is responsible for the induction of autophagosome formation downstream of the mechanistic target of rapamycin (mTOR) signaling pathway activated in response to nutrients. The Beclin1 class III PI3K complex (which constitutes Beclin1, VPS34, ATG14L, VPS15 and AMBRA1) generates PIP3, which promotes vesicle nucleation in coordination with the ULK1-ATG13-FIP200-ATG101 complex. Two ubiquitin-like conjugation systems regulate cargo recruitment, membrane elongation, and autophagosome maturation. One of the conjugation systems forms an ATG5-ATG13 conjugate that is regulated by E1-like ATG7 and E2-like ATG10. The second system conjugates ATG8/LC3 to the lipid phosphatidylethanolamine (PE) to form LC3-II, a process mediated by ATG7 and E2-like ATG3. LC3-II remains attached to the lipid while the ATG5-ATG12 conjugate is removed after autophagosomes are formed, making LC3-II a reliable marker of autophagosomes. After being transported along microtubules, SNARE-like protein complexes facilitate the fusion of autophagosomes and lysosomes into autophagolysosomes, where the cargo is degraded by lysosomal acid hydrolases. (Figure adapted from Lenoir, et al. 2016; see [114]).

Genetic alterations of autophagy and proteasomal genes

In line with their diverging roles and regulation within a cell, genetic alterations of autophagy genes do not compromise cell viability [34], which is in strong contrast to genes encoding proteasome subunits, which are essential [35]. Genetic evidence indicates that both the UPS and autophagy are both implicated in immune modulation. Polymorphisms in autophagy genes are associated with immune diseases involving the kidney, such as polymorphisms in autophagy related 5 gene (ATG5) with systemic lupus erythematosus [36] and the PR domain 1 gene (PRDM1)-ATG5 intergenic region with rheumatoid arthritis [37]. A spectrum of disorders referred to as proteasome-associated auto-inflammatory syndrome (PRAAS) [38, 39] have their origin in mutations of constitutive core particle subunit genes such as PSMA3 ($\alpha 7$) and PSMB4 ($\beta 7$), in immunoproteasome subunit genes PSMB8 ($\beta 5i$) and PSMB9 ($\beta 1i$), and in the general core particle assembly chaperone gene POMP (UMP1) [40]. The common finding in PRAAS is impaired proteasome activity due to CP assembly defects especially in immunoproteasome expressing cells leading to a hyper-inflammatory phenotype with elevated IL-6 and IFN signaling. No renal phenotype in PRAAS has been described so far.

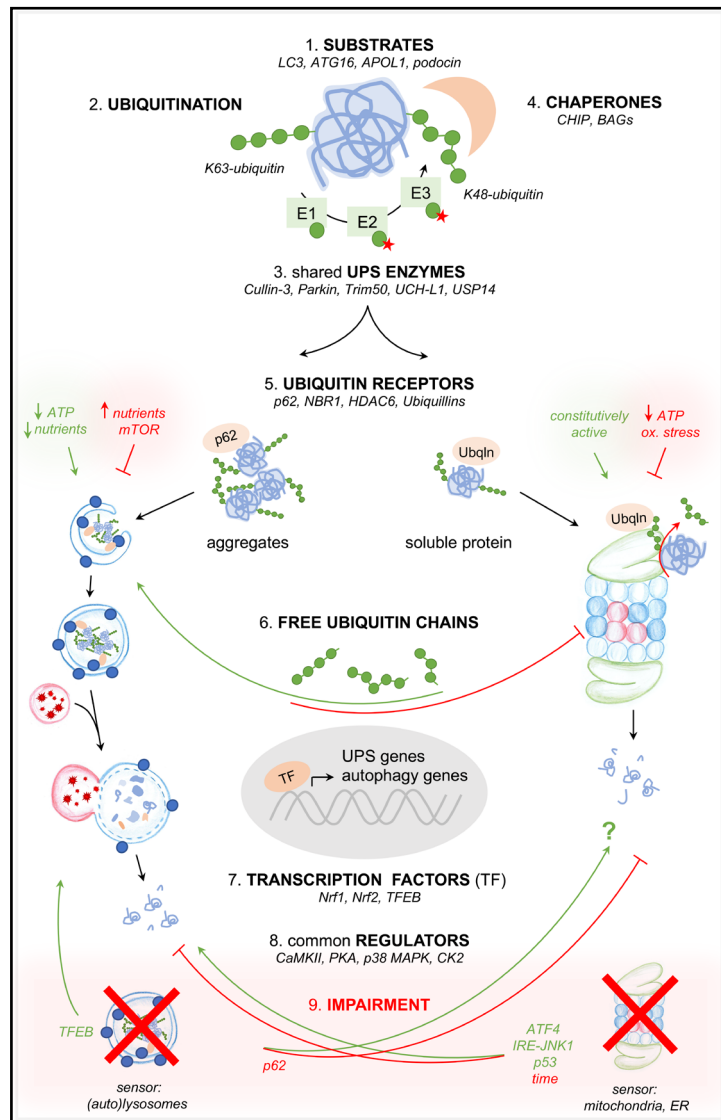
Crosstalk between the two degradation systems

The UPS and autophagy have for a long time been considered to be independent mechanisms of protein degradation. However, the existence of a strong crosstalk has been described between both pathways mainly in the brain [41, 42] but also in podocytes (Fig. 3). Thereby, when the UPS is overwhelmed, inhibited, or when cellular ATP levels are reduced (proteasomal degradation is a highly ATP-consuming process) autophagy steps in [9] to eliminate the accumulating aberrant proteins, which are then sequestered by autophagosomes and delivered to lysosomes for degradation. This cellular reaction is mediated by the unfolded protein response (UPR) through activation of the transcription factor ATF4 [43], by activation of the IRE1-JNK pathway that releases the inhibitory break of Bcl-2 on Beclin1 through phosphorylation [44], and by p53 which accumulates upon proteasomal impairment and thereafter activates AMPK [45] to induce autophagy. The cellular sensors for impaired proteasomal degradation are mitochondria and ER. Thereby, the accumulation of unfolded or damaged proteins alters the mitochondrial proteome, leading to a burst of mitochondrial reactive oxygen species, which activate AMPK to induce autophagy [46]. Additionally, reduced levels of ATP result in enhanced levels of AMP which in turn activate AMPK and autophagy [47]. It has, however, also been reported that continuous impairment of the proteasome can result in an impairment of autophagy [48]. Whether the UPS is activated to compensate for impaired autophagy is debated. The general consensus is that autophagosomal substrates are too large to be channeled through the barrel of the proteasome [49], and that accumulation of p62 and its sequestration to ubiquitinated proteins delays their delivery to proteasomes [50], thereby playing a negative factor in UPS activity [51]. Despite this general consensus, experimental data in podocytes suggest that the UPS is activated in the early course of autophagy impairment (due to genetic ATG5-deficiency) as a compensatory attempt [52]. Only with age does proteasomal impairment add up to the impaired autophagy resulting in proteinuria [52]. Further challenging this general consensus are the findings that proteasomes can indeed clear insoluble protein aggregates, a process mediated by the ubiquitin receptor Ubiquitin 2 in conjunction with the chaperones HSP70-HSP110 i.e. in the nucleus, where autophagy is not present for the removal of protein aggregates [53]. Further, the 20S proteasome is indeed capable of degrading LC3, after detaching from its 19S regulatory particles [54].

Besides this compensatory crosstalk in the setting of UPS or ALP impairment, both systems are intertwined at 8 further different levels: 1) by common substrates, mechanistically 2) by substrate ubiquitination, 3) by common (de)ubiquitinating enzymes, 4) by chaperones, 5) by ubiquitin receptors, 6) by common transcription factors, and 7) by common regulating kinases. Besides regulating kinases, the effectiveness of both degradation systems is modulated 8) by free ubiquitin chains, which on the one hand impair (clog) the proteasome and thus decrease proteasomal degradation [55], and on the other hand enhance the aggregation of substrates, making them more amenable to autophagy [56, 57].

The intertwining between the UPS and ALP orchestrates the function of the two most basic cellular degradation systems and is therefore relevant to podocyte biology even though not many investigations exist in this specific cell type. Known podocyte-specific proteins that represent common substrates to the UPS and ALP are α -actinin-4, podocin, and nephrin as detailed in paragraph "*The UPS and autophagy in the regulation of podocyte identity*". Interestingly, also the Parkinson's disease associated α -synuclein [58, 59], and the amyloid precursor protein APP, which is cleaved in Alzheimer disease to CTF β and A β [60-62] are common UPS and ALP substrates, which aggregate and accumulate in neurodegenerative diseases. Both substrates are also expressed in the kidney and in podocytes, however their involvement in renal disease is not described to date. From the autophagy perspective, the crosstalk between both degradation systems has been described at the level of the second ubiquitin-like ATG12-ATG5-ATG16 complex required for the lipidation of LC3 and its recruitment to the phagophore or isolation membrane. Thereby, ATG16 binds ubiquitin, is a substrate of the proteasome, and modulates proteasomal activity. Further, ATG16

Fig. 3. The intertwining of the UPS and autophagy is achieved at 9 different levels: 1) by common substrates, mechanistically 2) by substrate ubiquitination, 3) by common E1-E2-E3 ubiquitinating and deubiquitinating enzymes, 4) by chaperones, which direct substrates to degradation, 5) by ubiquitin receptors, which bind and sort ubiquitinated cargo, 6) by free ubiquitin chains, which on the one hand impair (clog) the proteasome and thus decrease proteasomal degradation, and on the other hand enhance the aggregation of substrates, making them more amenable to autophagy, 7) by common transcription factors inducing the transcription of UPS and autophagy genes, and 8) by common regulating kinases. 9) Impairment of the proteasome is sensed in mitochondria and the ER and results in the induction of autophagy via the unfolded protein response (ATF4), via IRE-JNK pathway, and via p53. Over time, continuous proteasome impairment results in impaired autophagy. Defective autophagosomes are sensed at (auto)lysosomes and result in an auto-induction of the ALP through the transcription factor TFEB. Further defective autophagy induces p62-mediated proteasome impairment.



Whether defective autophagy can be counterbalanced by proteasome activation is not clear to date, is however suggested to occur in murine models of podocytes with genetically defective autophagy or lysosomal function.

determines the neddylation of the E3 ligase Cullin-3 (Cul3), which mediates ubiquitination and proteasomal degradation of p62 [63]. A further prominent autophagy-regulating substrate for ubiquitination is the ULK1 (and PI3KC3-C1), which following activation, can be ubiquitinated by the Cul3-KLHL20 ligase complex and degraded [64], thereby switching off the autophagy-initiating signal.

Ubiquitin, a well-known signal for the degradation of polypeptides in the proteasome, also plays an important role in the recognition of cargoes destined for degradation by autophagy [65]. For a long time, the kind of ubiquitin linkage was thought to decide whether a substrate is degraded by the UPS or by autophagy. However, the initial categorization that K48-linked polyubiquitin chains direct proteins to the proteasome and K63-linked polyubiquitin chains direct proteins to autophagy [41] has been questioned, as K48 and K63-polyubiquitin chains are both recognized by autophagy receptors [66, 67] and both bind with comparable affinities to purified proteasomes [68]. Newest studies have elucidated that after substrate ubiquitination, competing ubiquitin receptors harboring either proteasome- or LC3-binding modules determine, which degradative pathway is chosen. Proteasome

pathway receptors bind ubiquitin moieties more efficiently, but autophagy receptors gain the upper hand following substrate aggregation and receptor bundling [69]. This enables a triage process, which is thought to depend on the monomeric or oligomeric physical states of these receptors rather than on specific ubiquitin moieties on the substrate [69, 70].

Chaperone proteins such as CHIP (c-terminus of Hsp-70-interacting protein) and BAG (BCL-2-associated anthanogenes) 1 and 3 contain domains, which can direct substrates either to degradation by the proteasome or the autophagosome. Thereby the tetratricopeptide repeat domain of CHIP directs substrates for degradation by the UPS and the U-box domain by the lysosome [71]. BAGs interact with CHIP to channel protein degradation by the UPS [72].

Generally, the autophagosomal degradation of protein aggregates (aggresomes), mitochondria (mitophagy), bacteria (xenophagy), peroxisomes (pexophagy), zymogen (zymophagy), proteasomes (proteaphagy), midbodies, and nucleic acids are considered to represent ubiquitin-dependent mechanisms of autophagy (reviewed in [65]), which are orchestrated by a plethora of so-called ubiquitin receptors. The best-known ubiquitin receptors, which act as bridging molecules to link ubiquitin to autophagy, are p62 [73, 74], neighbor of BRCA1 gene 1 (NBR1), and histone deacetylase 6 (HDAC6) [75, 76]. These proteins contain ubiquitin associated (UBA) domains specialized for binding to ubiquitin and an additional domain dedicated to linkage to autophagosomes, such as the LC3 interacting region (LIR) that facilitates adaptor protein binding to LC3 [77]. Examples for ubiquitin receptors that mainly target ubiquitinated proteins to the proteasome are Rpn13 [78], Rpn10 [79], Rpn11 [80], or Ubiquitin 2 for the clearance of protein aggregates by the proteasome [53]. Interestingly, Rpn11, Ubiquitin 2 and p62 have been shown to also mediate substrate degradation by both autophagy and the UPS [57, 81, 82]. Thereby p62 was the first autophagy receptor described to be essential for clearance of ubiquitinated substrates in mammals, especially in the setting of UPS inhibition [83, 84]. In situations of ubiquitin stress, that is in situations of ubiquitin overexpression, heat shock or prolonged proteasomal inhibition, p62 undergoes E2-dependent ubiquitination liberating its ability to recognize polyubiquitinated cargoes for selective autophagy [85]. P62 is upregulated by the transcription factor Nrf2 [86] and binds ubiquitin via its UBA domain [87]. Following oligomerization, p62 targets ubiquitinated substrates to autophagy [83], a process (not necessarily [88]) mediated by its association with LC3 [89] and with GABARAP subfamilies on the autophagosome [73, 83, 89]. Additionally, p62 can also target ubiquitinated proteins to the 19S RP of the proteasome for proteasomal degradation [81]. The ubiquitin receptor Rpn11 on the other hand, normally involved with the deubiquitination of the vast majority of proteins that enter the 20S CP, has been shown to target aggresomes in a coordinate action with unanchored K63-chains to the microtubule-organizing center for degradation by autophagy [12, 56]. Unanchored K63-ubiquitin chains are further important for the fusion of autophagosomes with lysosomes [56, 57].

The UPS and autophagy share enzymes involved in the (de)ubiquitination process. As such, the E3 ligase parkin targets proteasomal substrates with K48-polyubiquitin chains [90] and lysosomal substrates with K27-polyubiquitin chains [91]. Parkin senses the functional state of mitochondria and directs damaged mitochondria for disposal via mitophagy by building ubiquitin chains on the mitochondrial outer membrane proteins, where they act to recruit autophagy receptors such as NDP52 and optineurin [92, 93]. Both systems share the E3 ligase TRIM50, which is necessary for the clearance of aggresomes [94]. Besides common E3 enzymes both systems share deubiquitinating enzymes, such as UCH-L1 and USP14. UCH-L1 is thought to maintain the pool of monoubiquitin required for ubiquitination procedures [95], and is also involved in the lysosomal degradation of BACE1, the β -secretase cleaving APP to CTF β [96, 97]. USP14 associates with the proteasome 19S RP and deubiquitinates substrates prior to their proteasomal degradation, thereby regulating proteasomal activity [98]. Upon proteasome inhibition, association of USP14 with the 19S RP is disrupted resulting in an enhanced interaction of USP14 with the autophagy protein GABARAP and enhanced autophagy [99]. USP14 also regulates autophagy by suppressing the K63 ubiquitination of Beclin1 [100].

Finally, posttranslational modifications achieved by kinases have been demonstrated to represent an additional level of regulation at which the crosstalk between the UPS and autophagy is orchestrated. The most prominent kinases, which influence both proteasomal and autophagy activity in a concordant or inverse direction, are the serine/threonine kinase casein kinase 2 (CK2), the Ca²⁺/calmodulin-dependent protein kinase II (CaMKII), protein kinase A (PKA) and p38 MAPK [42]. Thereby, CK2 phosphorylates the $\alpha 7$ subunit of the proteasome and thereby regulates proteasome assembly and opening [101, 102]. CK2 also modulates autophagy by phosphorylation of ATG32, a process essential for mitophagy [103]. CaMKII enhances proteasomal activity by phosphorylation of the proteasomal rpt6 subunit of the 19S RP [104, 105]. CaMKII activates autophagy by direct phosphorylation of Beclin1 to promote K63-linked ubiquitination of Beclin1 [106]. Activation of PKA through increased cAMP levels enhances phosphorylation of Rpn6 and Rpt6 subunits of the 19S RP. These post-translational modifications are required for the correct assembly of the 26S proteasome by interaction of rpt6 with the $\alpha 2$ subunit of the 20S core particle [107] and for an enhanced proteasomal activity [108]. Autophagy is suppressed by elevated levels of PKA activity at the ATG1/ATG13 kinase complex [109], and a feedback loop of autophagy to PKA regulation is suggested [110].

Protein degradation systems in podocyte physiology

The relative contribution of autophagy and of the proteasome system in podocyte proteostasis is unclear as the mere histological detection of UPS and ALP players does not assess the overall activity state of the respective degradative systems. The proteasome abundance, proteasome degradative capacity and selectivity are not fixed within a cell. They are precisely regulated by transcription factors, by proteasomal assembly chaperones and by posttranslational modifications to adjust to physiological needs. It is presumed that cells contain an excess of unengaged proteasomes, which can be assembled and mobilized when needed [111]. Some regulators affect proteasomal activity by associating with it, others modify the proteasome post-translationally, and still others promote the transcription of proteasomal genes [112]. Triggers that lead to changes in proteasomal abundance and subtypes are multiple and include: 1) cytokines, with IFN γ being the most potent one, 2) oxidative stress 3) ER stress activating ER associated degradation system, and 4) enhanced protein synthesis. In contrast to the proteasome, the cellular levels of autophagy vary considerably depending on the cell type and on the nutrient supply, with energy deficiency and starvation being major inducers of autophagy aiming at restoring intracellular ATP and nutrient supply such as amino acids for protein synthesis [113]. It is generally accepted that (if nutrients are abundant), a cell can survive without autophagy, as the amino acid supply is constitutively provided by the actions of the UPS whose protein turnover is constantly high. Autophagy is regulated by 4 main growth-signaling pathways converging either at the ULK1 complex or at the Beclin1-PI3KC3-C1 complex: 1) AMP-activated protein kinase (AMPK) initiates autophagy in the setting of energy depletion, 2) mammalian target of rapamycin (mTOR) complex 1 (mTORC1) inhibits autophagy in the setting of high nutrient, amino acid glucose supply, 3) the anti-apoptotic factor B-Cell lymphoma 2 (Bcl-2) inhibits autophagy, and 4) class I PI3K-AKT inhibits autophagy following stimulation with insulin and/or growth factors. Besides energy and nutrient supply, laboratory and clinical evidence highlight ER stress and hypoxia as strong modulators of autophagy, excellently reviewed in [114-116].

Cellular levels of UPS and autophagy activity in podocytes

Podocytes are terminally differentiated cells and therefore their capacity to regenerate is limited [117]. Similarly to neurons, they require efficient cellular mechanisms to remove flawed proteins that accumulate over their lifetime, cellular debris and damaged organelles. Autophagy seems to be one of these cleaning mechanisms that ensure podocyte maintenance, especially with age. In line, podocytes are sensible to autophagosomal

inhibition [118]. Experiments with GFP-LC3 reporter mice suggest that podocytes have high levels of basal autophagy [51, 119] in comparison to other intraglomerular cell types. Nonetheless, levels of lysosomes and activity of lysosomal acid hydrolases required for the degradation of autophagosomal content after autophagolysosome formation have repeatedly been shown to be higher in glomerular endothelial and mesangial cells than in podocytes in unstressed conditions in multiple pioneer studies [120-122]. Despite this conundrum, prevention of autophagosome formation through podocyte-specific knockout of ATG5 results in proteinuria, loss of podocytes and age-related glomerulosclerosis in mice aged older than 7 months. Interestingly, prior to the development of these symptoms, younger podocyte-specific ATG5-deficient mice upregulate the UPS in podocytes, compensating for the loss of autophagy. With 7 months however, the UPS becomes impaired, as proteasomal activity decreases and polyubiquitinated proteins accumulate, a finding coinciding with the manifestation of podocyte injury in podocyte-specific ATG5-deficient mice [52] demonstrating that both degradative systems are imperative for podocyte maintenance. To which extent is however unclear. In some instances of ALP impairment, podocytes exhibit severe injury and loss, while in others, podocyte injury is mild to absent possibly due to a compensatory upregulation of proteostatic stress reducing pathways. For instance, mice with a podocyte-specific deficiency of Vps34, which in yeast is essential for the sorting of hydrolases to yeast vacuoles [123] and in mammals (as mVps34 (class III PI3K)) has been implicated in the regulation of autophagy [124], develop severe glomerulosclerosis with enlarged vacuoles and increased autophagosomes in podocytes and die at 9 weeks of age [125]. This phenotype appears to be the result of a direct lysosomal impairment rather than of a primary defect in autophagy. On the other hand, mice with the lysosomal storage disease mucopolidosis type II or III do not exert overt podocyte injury. This lysosomal storage disease is due to mutations of the Golgi resident GlcNAc-1-phosphotransferase. Mutations in *GNPTAB* result in inactive GlcNAc-1-phosphotransferase cause the severe MLII disease, whereas a few mutations in *GNPTAB* and mutations in *GNPTG*, which are accompanied by a residual GlcNAc-1-phosphotransferase activity, cause the less progressive diseases MLIII alpha/beta or MLIII gamma, respectively [126]. Consequently, in cells from patients with MLII and MLIII, the generation of M6P targeting signals on lysosomal enzymes is impaired and leads to their missorting and hypersecretion into the extracellular compartment [127]. The subsequent intracellular deficiencies of multiple lysosomal enzymes result in the accumulation of various non-degradable macromolecules in dysfunctional lysosomes in all glomerular cell types [128]. Interestingly, as in ATG5 deficiency, the glomerular UPS compensates for cellular homeostasis in MLIII mice, whereas in MLII with the severe lysosomal enzyme missorting protein synthesis through the integrated stress response was decreased to alleviate for the disturbance in proteostasis [128].

In cultured murine and human podocytes, proteomic studies demonstrate a strong proteolytic shift from a predominant proteasomal activity to a lysosomal activity in the course of podocyte differentiation [129, 130], which coincides with a globally increased stability of mitochondrial, cytoskeletal, and membrane proteins in differentiated podocytes [130]. How far this finding can be transferred to the physiologic *in vivo* situation still needs to be determined, as possible skewers such as culture conditions could explain the proteolytic system shift from undifferentiated to differentiated podocytes. Recent data from our lab suggest that podocytes have an exceptional high dependence on a functioning proteasomal system *in vivo*, already at young age. Thereby, a new technique enabling a reporter-free bulk separation of glomerular cell-types from wildtype mice for protein biochemical investigations shows that podocytes express higher levels of proteasomal proteins in comparison to mesangial and glomerular endothelial cells [131] in 8 to 10-week-old mice. The strong podocyte dependence on the proteasome for regular proteostasis is further stressed by the finding that podocyte-specific deficiency of rpt3, a subunit of the 19S RP of the proteasome leads to early onset podocyte injury (albuminuria at 4 weeks of age) and loss, with atrophic kidneys at 14 weeks of age [132]. More investigations are required to dissect the individual contribution of the UPS and ALP for podocyte proteostasis.

However, sub summation of the current literature lets us suggest that podocytes exhibit a basal dependence on the proteasome system, which steps in to alleviate proteostatic stress in the setting of ALP impairment, whereas autophagy appears to be especially relevant for podocyte homeostasis in aging and in the setting of podocyte stress.

The UPS and autophagy in the regulation of podocyte identity

Accumulating evidence suggests that homeostatic levels of podocyte-specific proteins are dependent on the UPS [18], in some cases in tight interplay with the endocytic / autophagosomal system. The development and maintenance of podocyte identity is carried out by the major podocyte transcription factor WT1, which is responsible for regulation of an extensive transcriptional network of podocyte-specific proteins [133]. The UPS is involved in WT1 ubiquitination and degradation in a β -catenin dependent manner [134], thereby strongly affecting podocyte identity. Proteomic analyses have further identified endogenous ubiquitination sites on podocyte-specific proteins such as Neph1 (also known as KIRREL1), α -actinin 4, and synaptopodin suggesting an involvement of the UPS in their levels and distribution [135]. In line, levels of synaptopodin, α -actinin 4, and nestin can be stabilized by proteasomal inhibition in parietal epithelial cells (which share common lineage to podocytes) [136] or in podocytes in the setting of injury [9]. Induction of autophagy was shown to regulate podocalyxin levels in parietal epithelial cells [136]. Further underscoring an involvement of the proteasome in the maintenance of homeostatic levels of podocyte essential proteins, the UPS was shown to be necessary for the ubiquitination and removal of mutant α -actinin-4 [137] and podocin [138]. However, again an intact interplay with the ALP was shown to be necessary. In case of mutant α -actinin-4, autophagy is increased in glomerular epithelial cell cultures, presumably to relieve ER stress and remove α -actinin-4 aggregates [139] secondary to proteasome impairment [137]. Podocin and nephrin are both ubiquitinated after growth factor stimulation in CD2AP negative podocytes, a process dependent on CIN85/RUK_L, a mediator of ubiquitin-mediated endocytosis. This process is thought to destabilize the slit membrane in the absence of CD2AP resulting in endocytosis of nephrin [140]. Nephrin endocytosis is widely accepted as a means for nephrin regulation at the slit membrane [141]. To enhance nephrin stability at the slit membrane, modification of nephrin by the ubiquitin-like protein SUMO at the intracellular tail was shown to be essential. SUMO binds at lysine residues to block ubiquitination of the same site leading to stabilization of the target protein [142]. Podocin on the other hand is polyubiquitinated by the E3 ligase Ubr4 [135]. Interestingly, both K48- and K63-polyubiquitination of podocin have been observed resulting in unfolding and proteasomal as well as lysosomal degradation of podocin [135]. Synaptopodin regulates the podocyte actin cytoskeletal dynamics by associating with two E3 ligases. First, synaptopodin regulates stress fiber formation by interfering with the binding of the E3 ligase Smurf1 to the actin-cytoskeleton regulator RhoA, thereby preventing its ubiquitination and degradation [143]. Second, synaptopodin also competes with the E3 ligase Cbl for binding to the adaptor protein Nck1 located at the slit membrane, which is involved in the phosphorylation and endocytosis of nephrin. The interaction of synaptopodin with Nck1 decreases the ubiquitination and proteasomal degradation of Nck1 [144]. These findings underscore a primary function of the UPS in maintaining a podocyte identity by regulating homeostatic levels of podocyte-specific proteins, in some cases in tight interplay with the endocytic / autophagosomal system.

Proteolytic systems in podocyte stress response

As differentiated post-mitotic cells, podocytes strongly depend on their ability to react to different situations of cellular stress, e.g., proteotoxic or oxidative stress. Findings implicating an involvement of proteostatic systems in podocyte injury have been reported for both the UPS and the ALP as reviewed elsewhere [9, 18, 114]. Depending on the species, type and stage of kidney disease, reported findings may be contradictory and experimental

evidence for some of the observations are still scarce/missing, partially due to the complex nature of the degradative systems. As a solidly validated general theme (Fig. 4), autophagy is induced in most situations of podocyte injury, highlighting its importance for stress-related balance of proteostasis. Impairment of autophagy has also been described in podocyte injury, there mostly as a long-term sequel rather than as a primary event. Research on proteasomal function in podocyte injury is scarce to absent. First publications, however, suggest an initial reaction of the UPS to limit podocyte injury [9]. Early UPS reactions comprise an enhancement of proteasomal activity partly by upregulating and changing proteasome subtypes from the standard 26S proteasome to the i26S immunoproteasome and an enhanced expression of ubiquitin and UPS enzymes [9, 145]. Proteasomal function appears to be rapidly impaired in stressed podocytes by unidentified factors. This is reflected by the accumulation of oxidative-modified (podocyte-specific) proteins and by K48-polyubiquitin, and by decreased proteasomal activity. Whether induction of autophagy in stressed podocytes is an independent mechanism to the UPS to limit podocyte injury, or whether it mostly occurs in crosstalk with the UPS to compensate for proteasomal impairment is not known and certainly strongly dependent of the type of inflicted podocyte stress. One aspect that is neglected in many studies is the heterogeneity of glomerular cell types. Often, protein or mRNA levels are assessed on glomerular or even renal level, making conclusions towards one specific cell type and the actual activity of the system hard to draw. In the following sections, the evidence already available for some common glomerulopathies are summarized and new developments highlighted that might facilitate new discoveries in the near future.

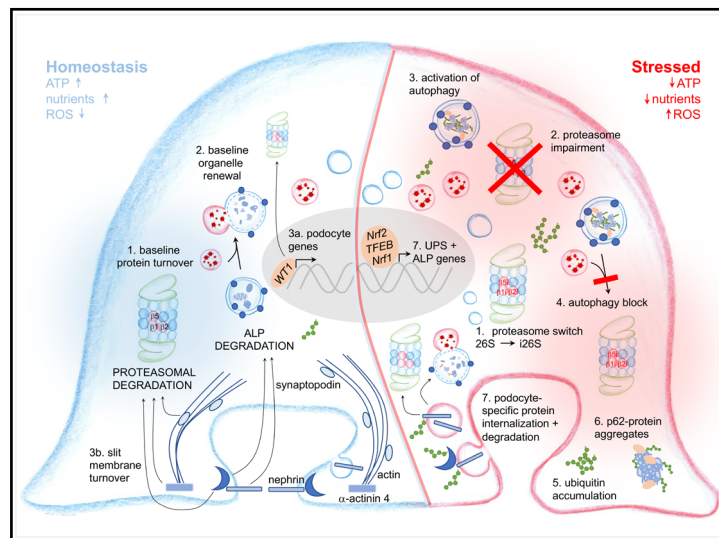
Ageing

Accumulation of damaged organelles such as mitochondria as well as protein aggregates is the common theme of ageing cells, a process from which podocytes are not exempt. Recent comparative measurements of the transcriptomic signature of young and aged murine podocytes exhibit differential regulation of cellular pathways, which affect or are involved in the regulation of autophagy and the proteasome, such as the PI3K-mTOR pathway and the p53 pathway [146]. As a main theme, however, this study revealed an increase of apoptosis and senescence related transcripts in aged podocytes [146]. Nonetheless, endocytosis transcripts were enhanced in aged podocytes [146], possibly resulting in enhanced autophagy. The downregulation of the electron transport chain transcripts and of the MAP kinase activity transcripts [146] could relate to impaired proteasome function. In line, experimental data support the importance of autophagy as an essential cleaning mechanism allowing podocyte maintenance during ageing. Atg5 knockout podocytes show typical age-related alterations, namely lipofuscin accumulation, an increase in oxidized proteins, and ubiquitin and p62 positive protein aggregates [52]. The contribution of the UPS in ageing podocytes is completely unclear. Does proteasomal impairment occur, similarly to ageing neurons? In old mice podocytes show higher levels of UPS players such as ubiquitin and UCH-L1 in comparison to younger mice, which might be indicative of both, proteasomal impairment or UPS activation. Since loss of UCH-L1 in podocytes results in an enhanced accumulation of oxidative-modified proteins and K48-polyubiquitinated proteins in 22-24 months old mice (the equivalent of 70-year-old humans), at least the upregulation of this deubiquitinating enzyme could be considered as a protective mechanism to age-related proteostatic disturbances in podocytes [145].

Diabetic nephropathy

Diabetic nephropathy (DN) is a renal complication occurring with both type 1 and type 2 diabetes that may result in end-stage kidney disease. Classical clinical symptoms like proteinuria and elevated serum creatinine levels can be observed. While the observed effects on renal function match those of other glomerulopathies, the molecular mechanisms linking high blood glucose levels to renal failure are incompletely understood. However, observations implicating both the ALP and the UPS in DN have been described and reviewed [147, 148].

Fig. 4. The UPS and autophagy in podocyte proteostasis. Left side: Healthy podocytes strongly depend on 1. the proteasome system for baseline protein turnover and amino acid supply and 2. autophagy for baseline organelle renewal and amino acid supply. Both processes are essential for a balanced podocyte proteome. 3. Proteolytic systems are involved in the maintenance of podocyte cellular identity through 3a. regulation of podocyte-specific proteins on the transcriptional level (WT1) and 3b. on the protein level by an interplay of the UPS and autophagy. Right side: Early reactions to podocyte stress



Early reactions to podocyte stress

include an initial upregulation and activation of the proteasome and of UPS enzymes and the occurrence of 1. a proteasome switch from the standard 26S proteasome to the i26S immunoproteasome, which harbors different proteolytic subunits ($\beta 1i$, $\beta 2i$, and $\beta 5i$). 2. Proteasome impairment is frequently the sequel of prolonged podocyte stress, which might be among others one reason for 3. autophagy activation. Prolonged autophagy can result in a 4. block of autophagy, which together with proteasome impairment accounts for the accumulation of 5. ubiquitin and 6. p62-containing protein aggregates. 7. Podocyte-specific proteins, especially slit membrane proteins are internalized and their degradation is achieved by crosstalk of the UPS with the ALP.

High glucose levels markedly increase the autophagic flux in glomeruli of diabetic mice and in cultured podocytes (both primary and immortalized). An impairment of this autophagic response via podocyte-specific knockout of Atg5 leads to higher apoptosis rates under high glucose conditions in cultured podocytes and exacerbates clinical parameters in a mouse model of DN. Interestingly, this initial upregulation of autophagy was reversed upon long-term high glucose conditions, resulting in lower levels of autophagy at the time of glomerular lesions forming. Podocyte-specific loss of Atg5 in mice resulted in higher apoptosis rate, increased thickening of the GBM and substantiated glomerular morphological changes under high glucose conditions [149]. Further studies confirmed these findings in kidney biopsies of DN patients and gathered evidence that serum factors are involved in the downregulation of podocyte autophagy under DN conditions. The accumulation of damaged lysosomes possibly indicate impairment of lysophagy as one crucial process. Importantly, downregulation of autophagic flux was only observed in the presence of proteinuria and not in other disease stages [150]. These findings were further substantiated by observations that induction of autophagy by resveratrol and other pharmacologic agents protects podocytes from high glucose-induced apoptosis [151, 152]. In a complex interplay between glomerular endothelial cell and podocyte damage, podocyte autophagy was shown to be protective against damage to glomerular endothelial cells. As such, inducible deletion of Atg5 in podocytes rendered podocytes more susceptible to structural damage when they were located adjacent to injured glomerular endothelial cells in a model of high fat diet [153].

Upregulation of UPS activity under high glucose conditions was also reported in numerous models of DN. However, in contrast to the autophagic degradation pathway, inhibition of the UPS has been shown to ameliorate the disease course. In a streptozotocin-induced model of DN in rats, proteasomal inhibition with MG132 improved clinical parameters and relieved oxidative stress in the kidney cortex [154]. Following up on this finding, in the OVE26 mouse model of type I diabetes, non-toxic doses of MG132 resulted in lowered proteasomal degradation of the transcription factor Nrf2, which in turn increased

the expression of several enzymes with anti-oxidative activity like SOD2 and HO-1 [155]. Mediated by NF- κ B, MG132 repressed Akt-mediated inflammation and thereby protected mice against renal dysfunction in DN [156]. However, these effects were only assessed in whole kidneys and evidence for specific glomerular cell types is still lacking. Since complex interactions between glomerular cells have been observed in other contexts [1, 157], these questions need to be addressed in a glomerular cell-specific manner in future studies.

Ubiquitin independent UPS-mediated degradation of the SNARE proteins syntaxin 17 (STX17) and SNAP29 negatively regulates autophagic flux by preventing the necessary vesicle fusion between autophagosomes and lysosomes [158]. This effect might add to the compensatory upregulation of autophagy under proteasomal impairment and could add to the reasons why inhibition of the proteasome is beneficial in DN models. The decreased degradation of the transcription factor Nrf2 upon proteasome inhibition results in its nuclear translocation, increasing the transcription of, among other genes, p62 and HO-1, which could further result in increased autophagic flux [155, 159]. While several such hints exist towards an essential role for UPS-ALP crosstalk in podocytes in the development of DN, concrete evidence is still lacking and will have to be supplied by future studies. Observations that degradation of accumulating proteins via autophagy is triggered via aggregation and receptor bundling could indicate that autophagy simply takes over when the UPS has failed to discard (damaged) proteins [69]. The open conformation of K63-conjugated ubiquitin chains could facilitate aggregation and receptor bundling more easily than the compact chains formed by K48-conjugated polyubiquitin [160, 161]. Mitochondria have been described to be involved in the sensing of UPS impairment and in AMPK-mediated upregulation of autophagy [162]. Together with findings that in podocytes, autophagy is mainly regulated through the AMP-ULK1 axis and not via mTOR signalling, this indicates a possible crosstalk mechanism in podocytes [163].

Focal segmental glomerulosclerosis

Focal segmental glomerulosclerosis (FSGS) is a generic term for a histological injury pattern defined by segmental glomerular scarring that affects some but not all glomeruli with a wide range of etiological interpretations, as FSGS describes both a disease characterized by primary podocyte injury (primary FSGS), and a lesion that occurs secondarily in any type of chronic kidney disease (secondary FSGS) [164]. Up to 50 causative mutations to podocyte proteins have been identified as causes to primary FSGS [165], one of which is the lysosomal integral membrane protein (LIMP) 2 [166]. In micro-dissected human glomeruli, UPS and ALP transcript upregulation was only present in FSGS and not in minimal change disease (MCD) [9], a histological condition presenting with to FSGS comparable morphological podocyte alterations, however importantly without glomerular sclerosis. Further underlining a differential pathogenic involvement of the protein degradation systems in FSGS and MCD was the observation that the presence of autophagosomes in podocytes differentiated patients from these two groups in renal biopsies. Autophagosomes were detected (via electron microscopy) in significantly more podocytes from MCD patients than in those from FSGS patients. Analysis of LC3 and Beclin1 protein levels substantiated these findings. Repeat renal biopsies of MCD patients further hint towards a protective role of autophagy. While in patients that progressed from MCD to FSGS, autophagosome numbers decreased significantly, this was not the case in patients with persistent MCD [167].

FSGS is mimicked in rodents by inducing selective podocyte injury either by administration of chemical substances such as puromycin or by genetically inducing podocyte loss [168]. These animal models are the basis of investigations showing an upregulation of ALP and UPS players [9] and of suggesting a beneficial effect for autophagy modulation in FSGS. Thereby, inhibition of autophagic flux with 3-Methyladenine (3-MA) in a rat model of puromycin amino nucleoside (PAN)-induced podocyte damage accentuated morphological changes and proteinuria. Conversely, induction (and maintenance) of high levels of autophagy using rapamycin prevented podocyte foot process effacement and improved proteinuria [167]. Studies in cultured human podocytes revealed an initial upregulation of

autophagy upon PAN-induced podocyte damage. Interestingly, as already observed in settings of DN, autophagy declined again over time also in this model of podocytopathy. Inhibition of autophagy via knockdown of Beclin1 lead to decreased levels of podocyte marker proteins and increased podocyte apoptosis, which was also the case when autophagy was inhibited with 3-MA or chloroquine [167]. The role of the UPS in FSGS is mostly related to those FSGS forms induced by mutations of podocyte immanent proteins [165] such as α -actinin-4 and podocin described above, as their removal primarily depends on the proteasome system. Of note, proteasomal inhibition can partially redistribute the FSGS-associated mutant podocin proteins to the plasma membrane in cultured podocytes [138].

The intricate interplay of the UPS and ALP in podocyte pathophysiology is nicely observed in humans of recent African ancestry, which exhibit an increased susceptibility to developing FSGS due to implicated variants of apolipoprotein L1 (APOL1) [169]. Experimental expression of the *APOL1* risk alleles APOL1-G1 and -G2 in a podocyte-specific manner is causal for podocyte foot process effacement, proteinuria, and glomerulosclerosis [170]. As a secreted protein, APOL1 can induce lysosomal membrane depolarization resulting in a block of autophagic flux [171] due to impaired autophagosomal – lysosomal fusion ultimately leading to inflammatory-mediated podocyte death and glomerular scarring [170]. On the other hand, sequence variation at the *UBD* gene locus (also known as *FAT10*, which encodes an IFN γ -inducible ubiquitin-like protein [172]) was identified as a genetic modifier of the risk of FSGS development in individuals with a high risk *APOL1* genotype [173]. Thereby, UBD modification of APOL1-G1 and APOL1-G2 was shown to decrease their levels by proteasomal degradation and thereby mitigate their cytotoxic effects. As African ancestry at the UBD locus is associated with lower levels of UBD transcripts, the impaired proteasomal removal of APOL1-G1 and -G2 in podocytes most likely contributes to the higher risk of FSGS development [173].

Membranous nephropathy

Membranous nephropathy (MN) is an autoimmune disease caused by autoantibodies directed against podocyte foot process proteins, such as phospholipase A2 receptor 1 (PLA₂R1) [174] and thrombospondin type-1 domain-containing protein 7A (THSD7A) [175]. Morphologically, MN is characterized by subepithelial immune deposits at the glomerular basement membrane (GBM), resulting in an overall increased GBM thickness. Further, subepithelial deposition of the autoantigen, IgG (mostly IgG4), and complement factors are morphologic characteristics of MN. The mechanisms that initiate podocyte injury after autoantibody binding to foot process antigens remain obscure. Sublytic complement induced injury [176], and mechanical or antigen-related effects are discussed [177]. Downstream of these initiating events, protein degradation pathways are induced in human and rodent MN [9, 178]. Typically, podocytes exhibit extensive hypertrophy and vacuolization, which might partly be attributed to a strong induction of the ALP in MN, as mirrored by transcriptional studies in micro-dissected human glomeruli [9] and by the prominent induction of Limp2-expressing lysosomes [179], and by an enhanced expression of the lysosomal acid hydrolase cathepsin D [180]. Interestingly, lysosomal acid hydrolases are suggested to be involved in the slow degradation of the deposited IgG in MN [181]. Whether the upregulation of the ALP in MN represents a podocyte injury promoting or an attenuating event is not clear. Deficiency of lysosomal Limp2 in mice exacerbates podocyte injury and disease in the anti-podocyte nephritis model [9] (a murine model of MN [182]), suggesting that lysosomal induction might represent a disease attenuating response. In line, autophagy has been implicated in the relief of ER stress in podocytes in a rat model of MN, the passive Heymann nephritis, and in cultured murine podocytes [183]. As an indication of induced but defective autophagy in MN related podocyte injury, renal biopsies of MN patients exhibit accumulation of LC3 and p62 [184]. Studies in cultured mouse podocytes link complement, especially the C5b-9 membrane attack complex (MAC), to impaired autophagy through permeabilization of the lysosomal membrane, which leads to deacidification of the lysosomal milieu and to low activity of lysosomal degradative proteases. As a direct cause, autophagosomal flux is

impaired and autophagosomal vacuoles accumulate [184]. Interpretation of this result is complicated by the fact that the complement system was also shown to induce the UPS activity via activation of c-Jun N terminal kinases (JNKs), in the setting of impaired endoplasmic reticulum associated degradation (ERAD) [185]. The authors speculate that this indicates a higher ubiquitination activity rather than an actual increase in proteasome catalytic activity [185].

Emerging evidence suggests that the UPS plays a major role in the development and progression of MN. Transcripts of UPS components are upregulated in micro-dissected glomeruli of MN patients, and a podocyte accumulation of ubiquitinated proteins is seen in human MN biopsies [9, 178] and rodent MN [186]. In line with the ubiquitin accumulation in podocytes, proteasomal function was observed to become impaired following induction of anti-podocyte nephritis in UB^{G76V}-GFP transgenic mice (reporter mice that visualize proteasome activity by means of GFP accumulation). Of note, time-course analyses depicted an impairment of proteasome function prior to the development of proteinuria and prior to morphologic podocyte injury [9]. Upregulation of the ALP was a late event in anti-podocyte nephritis, suggesting a compensatory effort of the ALP to restore proteostasis in the setting of proteasomal dysfunction [9]. The UPS is involved in the degradation of oxidatively damaged proteins such as α -actinin 4 in the course of passive Heymann nephritis [9], a degradative pathway that may also apply to other disease associated podocyte foot process proteins, such as THSD7A or PLA₂R1, which accumulate in glomeruli of MN patients. Interestingly, specific UPS components could be involved in the pathogenesis of MN. Chemical inhibition of the neuronal specific deubiquitinating enzyme UCH-L1, was shown to prevent podocyte ubiquitin accumulation and to ameliorate the clinical course of MN in passive Heymann nephritis, whereas treatment with the proteasomal inhibitor MG132 further enhanced the ubiquitin accumulation and exacerbated the clinical course [186]. UCH-L1 is thought to maintain the pool of free ubiquitin in neurons [95] and is normally not expressed in healthy podocytes, however *de novo* expressed in podocyte injury, especially in MN [178, 187, 188]. Whether UCH-L1 perpetuates or attenuates podocyte injury in MN is unclear, as its effects on podocyte proteostasis vary depending on the MN model used (mouse or rat) and on the mode of UCH-L1 modulation (chemical inhibition of hydrolase function versus conditional or podocyte-specific knockout). UCH-L1 was shown to regulate TNF-induced necroptosis in cultured murine podocytes [189], to be important for the balance between proteasome subpopulations in the murine kidney [145], and to influence the glomerular filtration rate [190] of constitutive UCH-L1 deficient mice. Highlighting the importance of UCH-L1 for the regulation of glomerular cell proteostasis with ageing is the observation that a constitutive knockout of UCH-L1, as well as a podocyte-specific knockout of UCH-L1 both resulted in the accumulation of oxidatively-modified proteins under basal conditions in glomerular cells of old mice, especially in podocytes [145]. Correlative observations in rodent models of MN associate UCH-L1 with an overall decreased proteasomal activity [191] and with the development of podocyte hypertrophy [191], a feature that might be related to a described toxic-gain-of-function of this enzyme upon post-translational modification especially induced by oxidative stress [192], or with its influence on the degradation of disease relevant proteins such as the cell cycle inhibitor p27Kip1 [192].

Lupus nephritis

Culminating evidences demonstrates the effectiveness of proteasomal inhibitors in targeting B and plasma cells in antibody mediated kidney injury such as ANCA-associated vasculitis, transplant nephropathy and lupus nephritis (LN) [193], and suggest an advantage of subunit specific targeting of the immunoproteasome [194, 195]. However, analyses of the effects proteasomal inhibitors could exert on resident renal cells such as podocytes in the setting of antibody-mediated injury have not been the focus of present studies. On the other hand, direct evidence suggests a protective effect of autophagy in podocyte injury in the MRL (lpr/lpr) mouse model of LN [196]. In this genetic model of LN, autophagosomes were present in podocytes, but not in glomerular endothelial or

Table 1. Overview of reviewed findings in selected publications. Abbreviations: Ref = reference number; UPS = ubiquitin proteasomal system, ALP = autophagosomal lysosomal pathway, Δ pod = podocyte specific deletion, ER = endoplasmic reticulum; DM = diabetes mellitus

Ref	Experimental model	System	Main findings in podocytes
52, 118, 119, 163	GFP-Lc3-tg mouse; cultured mouse podocytes, <i>Rptor</i> Δ pod mouse; <i>Tsc1</i> Δ pod mouse	autophagy	High basal autophagy levels; sensible to inhibition of autophagy; autophagy mainly regulated through the AMP-ULK1 axis and not via mTOR in podocytes.
52	<i>Atg5</i> Δ pod mouse; GFP-Lc3- <i>tgXAtg5</i> Δ pod; cultured human & mouse podocytes	autophagy	Age-related glomerulosclerosis after 7 months of age; upregulation of proteasomal system for compensation; development of late and mild proteinuria, after additional impairment of the proteasomal system.
125	<i>mVsp34</i> Δ pod mouse	lysosome	Severe glomerulosclerosis with enlarged vacuoles and increased autophagosomes; death at 9 weeks of age.
128	Mucopolipidosis type II (<i>GNPTAB</i> -KO) and type III (<i>GNPTG</i> -KO) mouse model; human urine	lysosome	No overt podocyte injury; compensatory upregulation of the proteasomal system in mucopolipidosis type III; downregulation of protein synthesis in mucopolipidosis type II podocytes to balance proteostasis in this lysosomal storage disorder.
129, 130	Cultured mouse & human podocytes	UPS/ALP	Podocyte differentiation in culture involves proteolytic shift from a predominant proteasomal activity to a lysosomal activity.
131	Naïve mice, TimMEP isolated podocytes	UPS/ALP	Podocytes express high levels of UPS proteins.
132	<i>Rpt3</i> Δ pod mouse	proteasome	Early onset of podocyte injury at 4 weeks of age; atrophic kidneys at 14 weeks of age, lower number of autophagosomes.
9, 135, 136-138	Primary rat parietal epithelial cells; cultured mouse & human podocytes; rat and mouse models of podocyte injury; human biopsies; rat glomerular epithelial cells	proteasome/ autophagy	Regulation of podocyte identity through degradation of podocyte-specific proteins.
9, 145, 178	Antibody-mediated injury in naïve mice and rats; human biopsies	UPS	Early induction of proteasomal system in podocyte injury with subsequent impairment in established injury.
52, 144, 146	<i>Nphs2-rTA/tetO-cre/tdTomato</i> ; <i>Nphs1-FLPo/FRT-EGFP</i> mouse	UPS/ALP	Ageing induces upregulation of autophagy and of the proteasome in podocytes.
149, 151, 152	<i>Cdh5.CreXAtg5</i> Δ <i>fl/fl</i> mouse; <i>Atg5</i> Δ pod mouse; C57BL6 with streptozotocin DM model; primary podocytes; inducible <i>Atg5</i> Δ pod mouse with high fat diet, <i>db/db</i> mouse	autophagy	High apoptosis rate, increased GBM thickening; induction of autophagy protects podocyte from high-glucose related injury.
154-156	Streptozotocin DM rats; OVE26 mouse model of DM1	proteasome	Proteasomal inhibition protects from renal injury in diabetes.
166	SCARB2 mutations in humans	lysosome	LIMP2 mutations as a genetic cause of primary focal segmental glomerulosclerosis.
167	Puromycin nephritis in rats; cultured human podocytes; human biopsies	autophagy	Upregulation of autophagy in puromycin-related injury; inhibition of autophagic flux using 3-MA accentuates podocyte injury.
170, 173	Humans; mouse model of (inducible) podocyte expression of <i>APOL1</i> -G0 or G1 or G2; cultured human podocytes; T-Rex-293 cells	UPS/ lysosome	<i>APOL1</i> induces lysosomal membrane permeabilization and inflammatory podocyte loss and glomerular scarring; <i>FAT10</i> as a genetic modifier of the risk of FSGS development in individuals with a high risk <i>APOL1</i> genotype.
9, 145, 178-181, 183-185, 190	Human biopsies; rat and murine models of membranous nephropathy; <i>Limp2</i> -KO and <i>Uchl1</i> -KO mouse; cultured mouse & human podocytes	UPS/ALP	Induction of UPS and ALP in membranous nephropathy; accumulation of non-degraded proteins; degradation of subepithelial deposits, autophagy induction to relieve ER stress, C5b-9 as a cause of lysosomal membrane permeabilization in podocytes.
196	<i>Lpr/lpr</i> mice; cultured mouse podocytes	autophagy	Protective effect of autophagy in podocytes in lupus nephritis.

mesangial cells. Interestingly, autophagy could be induced in podocytes by treatment with IFN α and IgG isolated from LN patients in this study. Patient IgG appeared to have a direct cytotoxic effect on cultured human podocytes, as exposure to patient IgG led to an increased production of reactive oxygen species and podocyte apoptosis. Apoptosis could be prevented via stimulation of autophagy, whereas autophagy inhibition via 3-MA or bafilomycin aggravated podocyte apoptosis. Exposure to IFN α alone did not lead to increased podocyte apoptosis rates but decreased podocin levels in cultured podocytes and resulted in higher albumin leakage in an *in vitro* albumin flux assay, representing structural podocyte damage [196]. As IFN α has been shown to be secreted from tubular cells in an autocrine manner in LN and to induce the expression of genes involved in protein ubiquitination and an induction the i26S immunoproteasome [197], IFN α could be responsible for activation of proteasomal (especially of immunoproteasomal) degradation in podocytes in the setting of LN, which might be involved in the decrease of podocyte podocin levels.

Conclusion

Podocytes, as terminally differentiated cells, highly depend on intact proteolytic machineries. Sub summation (Table 1) of common knowledge about the UPS and autophagy in other cellular systems with the few investigations performed in podocytes support the notion that basal high levels of UPS activity predominantly maintain podocyte health, whereas autophagy compensates for UPS impairment during ageing and in podocyte injury, especially in situations in which ATP-depletion and oxidative stress are high in podocytes. The fact that both degradation systems are intertwined at multiple steps renders analyses and interpretations of their contribution to podocyte health and disease challenging. Future investigations in this fascinating field will open new avenues to therapeutically modulate podocyte injury.

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Disclosure Statement

The authors declare that no conflict of interest exists.

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