It’s all about talking: two-way communication between proteasomal and lysosomal degradation pathways via ubiquitin

Martina P. Liebl and Thorsten Hoppe*
Institute for Genetics and Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany

Liebl MP, Hoppe T. It’s all about talking: two-way communication between proteasomal and lysosomal degradation pathways via ubiquitin. Am J Physiol Cell Physiol 311: C166–C178, 2016; doi:10.1152/ajpcell.00074.2016.—Selective degradation of proteins requires a fine-tuned coordination of the two major proteolytic pathways, the ubiquitin-proteasome system (UPS) and autophagy. Substrate selection and proteolytic activity are defined by a plethora of regulatory cofactors influencing each other. Both proteolytic pathways are initiated by ubiquitylation to mark substrate proteins for degradation, although the size and/or topology of the modification are different. In this context E3 ubiquitin ligases, ensuring the covalent attachment of activated ubiquitin to the substrate, are of special importance. The regulation of E3 ligase activity, competition between different E3 ligases for binding E2 conjugation enzymes and substrates, as well as their interplay with deubiquitylating enzymes (DUBs) represent key events in the cross talk between the UPS and autophagy. The coordination between both degradation routes is further influenced by heat shock factors and ubiquitin-binding proteins (UBPs) such as p97, p62, or optineurin. Mutations in enzymes and ubiquitin-binding proteins or a general decline of both proteolytic systems during aging result in accumulation of damaged and aggregated proteins. Thus further mechanistic understanding of how UPS and autophagy communicate might allow therapeutic intervention especially against age-related diseases.

ubiquitin; autophagy; proteasome; UPS; proteostasis; aging

SELECTIVE DEGRADATION OF DAMAGED and aggregated proteins contributes to cellular protein quality control and maintenance of protein homeostasis (proteostasis). Multiple physiological situations affect the proteome or challenge proteostasis including developmental changes, adaptation to environmental stress conditions, as well as the accumulation of misfolded or damaged proteins. The latter usually increase during aging and comprise a particular burden in so called protein aggregation diseases [e.g., Amyotrophic Lateral Sclerosis (ALS), Alzheimer’s Disease (AD), Parkinson’s Disease (PD), Huntington’s Disease (HD)]. There are two major routes in higher organisms ensuring protein turnover, namely the ubiquitin-proteasome system (UPS) and a lysosomal degradation pathway termed macroautophagy (hereafter autophagy). The UPS is regarded as the major degradation route for small and short-lived proteins (101). Larger proteins or aggregates are at risk to block the narrow proteolytic chamber of the 20S proteasomal core particle and therefore might cause sustained damage (17). Thus the major proteolytic route for large proteins as well as aggregates is autophagy (95).

The 26S proteasome is a multicatalytic protease complex localized in the cytoplasm and the nucleoplasm. The abundance of proteasomal subunits is controlled by different transcription factors (e.g., RPN4, NRF1/NRF2/SKN-1, or FoxO/DAF-16). UPS activity is regulated by the regulatory proteasomal subunits, posttranslational modifications, and factors that only temporarily interact with the proteasome (100). During autophagic degradation, proteins, but also intracellular pathogens, whole organelles, or parts of the endoplasmic reticulum (ER) are sequestered by a newly forming double-layered membrane structure, the autophagosome, which fuses with late endosomal compartments to form amphisomes before fusion with the lysosome. In the lysosomal lumen the content is finally digested by acidic hydrolases. The rather unspecific degradation of proteins in response to starvation (bulk autophagy) clearly differs from selective autophagy that requires specific cargo-recognizing autophagy receptors and plays an important role in the clearance of misfolded proteins and aggregates (50). Autophagy is regulated at multiple levels and by a plethora of effectors and posttranslational modifications (96). Biogenesis and enclosure of autophagosomes is regulated by the ULK1 (unc-51 like kinase 1) complex and the BECLIN1 complex, which are under control of the mTOR complex 1 [mechanistic target of rapamycin complex 1 (mTORC1)], a major cellular regulator repressing autophagy. Moreover, the autophagy-related protein 9 (ATG9) trafficking system and two ubiquitin-like conjugation systems are important for autophagosome generation. In this context, ATG7 and ATG10 display E1- and E2-like enzymatic activity, respectively, and conjugate with ATG16L1 to form a multimeric complex required for the

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Address for reprint requests and other correspondence: T. Hoppe, Institute for Genetics and Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), Univ. of Cologne, Joseph-Stelzmann Str. 26, 50931 Cologne, Germany (e-mail: thorsten.hoppe@uni-koeln.de).
It has been recently shown that autophagy can even degrade anistically balanced and coordinated at multiple levels. In fact, contrast to the former concept of separate proteolytic pathways tylation plays a major role in both degradation pathways. In unknown. This is further complicated by the fact that ubiqui- substrates to one particular degradation route remained largely LC3-II (50).

ubiquitin-binding autophagy receptors bridge ubiquitylated mole autophagic clearance (25, 51, 81). In selective autophagy, K63-linked polyubiquitin chains or monoubiquitylation pro- tate proteasomal degradation in vivo (125). Moreover, in some all non-K63-mediated ubiquitin linkages were found to facili- tence of a high number of unconventional ubiquitin chains and spectrometric analysis of the yeast proteome proved the exis- ubiquitin residues linked via K11 or K48. However, mass-

degradation is a polyubiquitin chain comprised of four to six fate. The canonical signal targeting a substrate for proteasomal ubiquitin chain attached to a substrate is crucial to its cellular proces-

Attachment of multiple ubiquitin residues to a substrate, a process termed polyubiquitylation, is highly important in the context of both proteolytic routes. Ubiquitin is a 76-residue, globular and highly conserved protein that can be covalently attached to proteins by the orchestrated action of a complex enzymatic machinery mediating ATP-dependent activation, transfer and conjugation of ubiquitin to a substrate protein. More than 600 E3 ubiquitin ligases encoded by the human genome link the ubiquitylation machinery to distinct target proteins and therefore play a major role for substrate specific-

Ubiquitin chains can be conjugated by peptide bonds between each of the seven lysine residues of ubiquitin (K6, K11, K27, K31, K33, K48, and K63) or via the ubiquitin amino-terminal methionine residue. Ubiquitylation can be re- versed by deubiquitylating enzymes (DUBs) that remove ubi-

quin from either an ubiquitin chain or a substrate. The type of ubiquitin chain attached to a substrate is crucial to its cellular fate. The canonical signal targeting a substrate for proteasomal degradation is a polyubiquitin chain comprised of four to six ubiquitin residues linked via K11 or K48. However, mass-
spectrometric analysis of the yeast proteome proved the exis-
tence of a high number of unconventional ubiquitin chains and all non-K63-mediated ubiquitin linkages were found to facili-
tate proteasomal degradation in vivo (125). Moreover, in some cases multiple monoubiquitylation of a substrate is sufficient to target it to the 26S proteasome (105). In contrast to other linkage types, increasing evidence suggests that attachment of K63-linked polyubiquitin chains or monoubiquitylation pro-

mote autophagic clearance (25, 51, 81). In selective autophagy, ubiquitin-binding autophagy receptors bridge ubiquitylated cargo proteins to autophagosomes via their binding to activated LC3-II (50).

So far the key regulatory factors for targeting selected substrates to one particular degradation route remained largely unknown. This is further complicated by the fact that ubiqui- tylation plays a major role in both degradation pathways. In contrast to the former concept of separate proteolytic pathways there is growing evidence that UPS and autophagy are mechanistically balanced and coordinated at multiple levels. In fact, it has been recently shown that autophagy can even degrade proteasomes in response to starvation or proteasomal damage, a process that was termed proteophagy (18, 79) (see Fig. 3). In this context the proteasomal subunit RPN10 was suggested as an autophagy receptor, bridging the binding of the proteasome to the autophagosome (79). This is a very apparent example of how the activity of one degradation pathway directly affects the activity of the other one. Here, we discuss mechanistic aspects controlling the communication between the UPS and autophagy and its impact on organismal aging (see also Table 1 for an overview).

Cross Talk Between the UPS and Autophagy

Evidence that the UPS and autophagy function interdependently came from studies showing that impairment of the UPS activates autophagy in mammalian cells and different model organisms (41, 91, 97, 110). In a fly model for spinobulbar muscle atrophy, a neurodegenerative disease caused by poly-

glutamine repeat expansions in the androgen receptor, au-

tophagy also inhibits proteasomal degradation. The question


glutenin to cytosolic LC3-I (microtubule-associated pro-

tein 1A/B light chain 3A-I) by ATG7 and the E2-like enzyme ATG3, resulting in LC3-II, which is then recruited to the autophagosomal membrane. Subsequent binding between LC3-II and ubiquitin-binding autophagy receptors like p62 loaded with ubiquitylated cargo proteins ensures selective degradation of the cargo. A subset of well conserved proteins originally identified in yeast comprises the core autophagic machinery and was termed autophagy related proteins (ATGs). However, for a number of additional autophagic regulators in mammals so far no counterparts in nonmammalian model organisms have been identified. Intriguingly, it has been shown that autophagy is also regulated on a transcriptional level. Two recent studies illustrated a competitive binding of the transcription factors farnesoid X receptor (FXR; inhibitory to au-

tophagy) and peroxisome proliferator-activated receptor-α (PPARα) (68) or cAMP-response element binding protein (CREB; both activating) (104) to shared binding sides within autophagic gene promoters.


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tophagy also inhibits proteasomal degradation. The question
<table>
<thead>
<tr>
<th>Common Molecules</th>
<th>Class</th>
<th>Mechanisms</th>
<th>Impacts for Aging and Degenerative Neuronal and Muscle Diseases</th>
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<tr>
<td><strong>Ubiquitin</strong></td>
<td>Ubiquitin/ubiquitin-like proteins</td>
<td>Common recognition signal for both proteasomal and autophagic degradation. Depending on the type of ubiquitylation, the protein is subjected to either the UPS or autophagy. Nonproteolytic ubiquitylation regulates activity of autophagic regulators.</td>
<td>Enrichment of aggregated polyubiquitylated proteins in multiple neurodegenerative diseases</td>
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<tr>
<td><strong>p62</strong></td>
<td>Ubiquitin binding protein/autophagy receptor</td>
<td>Protein levels are controlled by basal autophagy. p62 Bridges K63-polyubiquitylated proteins to autophagosomes. Due to a limited affinity for K48-polyubiquitylated proteins, conditions of increased p62 levels can prevent proteasomal substrates from degradation via the UPS. In this context, a direct competition between p62 and p97 for K48-polyubiquitylated proteins is important. p62 can interact with the proteasome and potentially also shuttles proteins for proteasomal degradation.</td>
<td>p62 mutations in ALS</td>
</tr>
<tr>
<td><strong>p97</strong></td>
<td>Ubiquitin binding protein/AAA + -ATPase</td>
<td>Shuttles K48-polyubiquitylated proteins to the proteasome. Directly competes with p62 and potentially other ubiquitin binding proteins for K48-polyubiquitylated substrates. Whether a polyubiquitylated substrate is recognized by p97 or by p62 can decide about the chosen proteolytic route. p97 might play a critical role for autophagosome formation.</td>
<td>p97 mutations in IBM/PFD, ALS, and a variety of myopathies</td>
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<tr>
<td><strong>RPN10</strong></td>
<td>Proteasomal subunit/autophagy receptor</td>
<td>Might serve as an autophagy receptor, bridging whole proteasomes to autophagosomes for their autophagic removal (proteophagy). By this, autophagic activity controls UPS activity directly.</td>
<td>Unknown</td>
</tr>
<tr>
<td><strong>AMBRA1</strong></td>
<td>Beclin1-interacting protein</td>
<td>Control of AMBRA1 levels via constitutive proteasomal degradation mediated by CULLIN4. Therefore, AMBRA1 accumulates upon proteasomal inhibition. AMBRA1 then activates autophagy (potentially as a compensatory mechanism ensuring proteostasis) via stabilization of DEPTOR.</td>
<td>Unknown</td>
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<tr>
<td><strong>CULLIN4</strong></td>
<td>E3 ligase</td>
<td>Controls levels of AMBRA1 by mediating its proteasomal degradation and therefore represses autophagy. Decreasing the CULLIN4-AMBRA1 interaction allows activation of autophagy via AMBRA1.</td>
<td>Unknown</td>
</tr>
<tr>
<td><strong>CULLIN5</strong></td>
<td>E3 ligase</td>
<td>Controls levels of DEPTOR and therefore represses autophagy. Competition between AMBRA1 and DEPTOR for ELONGIN-B inhibits or activates DEPTOR degradation by CULLIN5, respectively.</td>
<td>Unknown</td>
</tr>
<tr>
<td><strong>TRAF6</strong></td>
<td>E3 ligase</td>
<td>Increases proteasomal degradation by positively regulating expression of MAFBx and MuRF1. In concert with UBC13: K63-polyubiquitylation of substrates enhances their autophagic removal. Mediates nonproteolytic ubiquitylation of BECLIN1 and MTORC1, which regulates autophagy. In this context, it acts antagonistically to the DUB A20.</td>
<td>Upregulation in atrophying muscle, knockdown of TRAF6 prevents atrophy in denervated muscles.</td>
</tr>
<tr>
<td><strong>PARKIN</strong></td>
<td>E3 ligase</td>
<td>Depending on posttranslational modifications/interaction with E2 enzymes PARKIN mediates mono- and different types of polyubiquitylation. Interaction with UBC13: K63-polyubiquitylation of substrates targets them for autophagic removal. Special role for PARKIN in mitophagy is regulated by PINK1. Interaction with HSP70 supports proteasomal degradation of HSP70-bound clients.</td>
<td>Mutations in familial forms of PD</td>
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continued
CHIP  E3 ligase/cochaperone  Is recruited to HSP70 when refolding of a client cannot be achieved to mark a client for both, proteasomal or autophagic degradation.  Important for clearance of alpha-synuclein aggregates in PD and polyQ aggregates in HD

ATAXIN3  DUB  Supports proteasomal degradation by trimming K48-polyubiquitin chains more suitable for proteasomal recognition. Displays either activating or inhibitory function in p97-mediated ERAD. Enhances autophagy, potentially by trimming K63-polyUb chains to enhance their transport to the autophagic machinery. ATAXIN3 can promote autophagic degradation of PARKIN and regulate activity of CHIP. PolyQ-extensions in ATAXIN3 cause Spinocerebellar Ataxia 3/Machado-Joseph Disease (SCA3/MJD).

BAG1  BAG-protein/cochaperone  Competes with BAG3 for binding of HSP70. BAG1 facilitates proteasomal degradation of HSP70 bound clients. Unknown

BAG3  BAG-protein/cochaperone  Competes with BAG1 for binding of HSP70. BAG3 promotes clearance of HSP70 bound clients via autophagy and enhances autophagic flux. Unknown

Free amino acids  Amino acids  Levels of intracellular free amino acids depend on proteasomal activity. Shortage of amino acids inhibits mTORC1 and therefore activates autophagy as a compensatory mechanism. Unknown
In macrophages, TRAF6 promoted autophagy by polyubiquitylation of BECLIN1, which interrupted its interaction with the inhibitory protein BCL2 in response to Toll-like receptor 4 (TLR4) signaling (106). In this regard it acts antagonistically to the deubiquitylating enzyme A20 that removes TRAF6-mediated K63-polyubiquitin chains from BECLIN1 and thus suppresses autophagy. In contrast, other studies suggest an inhibitory role for TRAF6 in autophagy (13, 76). Therefore, under nutrient-rich conditions, when autophagy is repressed, TRAF6 activates mTORC1 via K63-linked polyubiquitylation to shut down autophagy (76) (see Fig. 3). In summary, TRAF6, probably depending on the cell type and the physiological requirements, promotes proteasomal degradation and either supports or suppresses autophagy, indicating that fine tuning of this E3 ligase activity directly impacts both proteolytic routes.

Mutations in the cytosolic E3 ligase PARKIN are a common cause of familial forms of PD. PARKIN plays multiple roles in the regulation of protein degradation pathways and can mediate monoubiquitylation as well as polyubiquitylation by K6, K27, K48, and K63 linkages (23, 31, 44, 90, 107). A variety of substrates have been identified to be marked by PARKIN for proteasomal degradation (52, 107). Upon proteotoxic stress conditions, PARKIN binds heat shock protein 70 (HSP70) to target misfolded proteins for proteasomal degradation (116).
PARKIN directly interacts with the proteasomal subunit RPN13, which was shown to increase PARKIN activity and is of specific importance in the clearance of mitochondrial proteins (1). On the other hand, PARKIN promotes autophagic degradation of aggregation-prone proteins by attaching K63-linked polyubiquitin chains (90, 127). Interestingly, a recent study shows that upon proteasomal inhibition, when autophagy is activated, K63-linked ubiquitin chains mediated by PARKIN and UBC13 were enhanced (73). The switch from proteasomal to autophagic degradation might therefore be triggered by changing the preference of substrate modification from K48 to K63 linkage. This observation likely correlates with modulation of E3 ligase activities or association with distinct E2 enzymes (Fig. 1). Additionally, phosphorylation of PARKIN by PTEN-induced putative kinase 1 (PINK1) activates and recruits PARKIN to mitochondria to assist in the clearance of mitochondrial proteins via the UPS as well as via autophagy (28, 48, 49, 57). Therefore, PARKIN posttranslational modifications, its recruitment to distinct subcellular localizations, and its association with specific binding partners (e.g., HSP70, E2 ligases, mitochondrial receptors) promote either proteasomal or autophagic substrate clearance, revealing an important role for PARKIN in the cross talk between the UPS and autophagy.

Deubiquitylating Enzymes–Agonists or Antagonists of E3 Ligases

Deubiquitylating enzymes (DUBs) can edit or remove ubiquitin residues or even complete chains from substrate proteins. Therefore, in general they either counteract E3 ligase activity by completely removing ubiquitin from the substrate and thus prevent degradation or act in concert with E3 ligases by modulating ubiquitin chain topology. For certain DUBs like A20, which antagonizes TRAF6 activity, an involvement in autophagy has been shown (106). Another DUB likely involved in the cross talk between proteolytic routes is the Machado-Joseph disease protein ATAXIN3. ATAXIN3 executes DUB activity by editing polyubiquitin chains of five or more ubiquitin residues, generating tetra-ubiquitin chains (8, 20, 60). A length of four ubiquitin residues is the minimal signal required for the degradation of a substrate via the 26S proteasome. ATAXIN3 promotes proteasomal degradation by trimming polyubiquitin chains more suitable for proteasomal degradation; however, it remained unclear whether K48, K63, or mixed linkages are the preferred substrates (87, 123). Moreover, ATAXIN3 acts in concert with the AAA-ATPase (ATPase associated with diverse cellular activities) p97 in ER-associated degradation (ERAD). However, there are controversial findings supporting either an inhibitory or an activating role of ATAXIN3 in p97-mediated proteasomal degradation (38, 60, 63, 98, 129). Ubiquitylation of the Josephin domain of ATAXIN3 increases its deubiquitylating activity and this modification increases upon proteotoxic stress, indicating that its function is indeed required in protein quality control. Besides its role in targeting proteins for proteasomal degradation, ATAXIN3 carries out a regulatory role in the clearance of protein aggregates by autophagy. It was speculated that by trimming K63-linked polyubiquitin chains on misfolded proteins, ATAXIN3 ensures recognition of these proteins by the dynein motor complex leading to their subsequent transport to aggresomes for autophagic clearance (71). Interestingly, ATAXIN3 also regulates the activity of the E3 ligase PARKIN, which is itself implicated in both degradation pathways (see above; Ref. 22).

Ubiquitin-Binding Proteins–Competitors in Substrate Binding

p62 is a scaffolding protein involved in a plethora of cellular processes. It can act as an autophagy receptor shuttling ubiquitylated cargo bound to its ubiquitin-associated domain (UBA) to the autophagic machinery by directly interacting with LC3-II decorating the autophagosomes as well as in an LC3-independent manner (40, 92). p62 itself is also a substrate of autophagy and its abundance is regulated via basal autophagy that keeps its level relatively low (Fig. 2A), although inhibition of autophagy causes a dramatic increase in p62 levels (Fig. 2B; Refs. 55, 56, 84). Korolchuk et al. (56) showed that the impairment of UPS activity upon blockage of autophagy depends largely on p62. In this context competition between p62 and p97 for polyubiquitylated substrates was suggested (Fig. 2, A and B). The AAA-ATPase p97 is a key regulator of the UPS that shuttles substrates marked with a canonical proteasomal degradation signal to the proteasome (53, 124). In contrast to p62, p97 does not contain an UBA-domain but binds ubiquitin via UBA-domain containing adaptors like Ubx2, p47, or Ufd1/Npl4 (83, 102). p62 can bind both K48- and K63-linked polyubiquitin chains, although K63-linked chains are preferred (Fig. 2). When upon autophagy impairment p62 is stabilized the ratio between p62 and p97 increases. Subsequently, even substrates marked with K48-linked polyubiquitin chains are more likely bound by p62 and therefore accumulate as autophagy is impaired (Fig. 2B) (56). In this context it might be of importance that p62 is able to form oligomers that can further shield the ubiquitin surface from recognition by the proteasomal subunits RPN10 or RPN13 (101). This model explains the delay of degradation of proteasomal substrates upon autophagy inhibition and is in line with the finding that proteasomal activity per se was unaffected in autophagy-deficient mice (54). This is further supported by a study in fruit flies where the additional deletion of the p62 homolog Ref(2)P in autophagy deficient flies decreased protein aggregation (86). Therefore, the competition between ubiquitin-binding proteins like p62 and p97 for polyubiquitylated substrates can be of physiological relevance when the proteasome is compromised or overloaded under conditions of severe stress. In this case a relative increase in p62 over p97 levels would relieve the proteasome by promoting degradation of proteasomal substrates via autophagy. It is of note that besides its role in proteasomal degradation, p97 likely is critical for autophagosome maturation (Fig. 3). Therefore, the strong degenerative phenotype observed in patients with IBM/PFD (inclusion body myopathy, Paget’s disease of the bone and fronto-temporal dementia) caused by mutations in p97 might be attributed at least partially to disturbed autophagy (47, 115). This function of p97 is further supported by findings that p97 interacts with poly-glutamine expanded proteins characteristic of Machado-Joseph Disease and HD that show a strong aggregation propensity that requires their degradation via autophagy rather than via the UPS (37). Moreover, p62 interacts with the 19S regulatory particle of the proteasome and might directly
shuttle proteins for proteasomal degradation (103) (Fig. 3). Such additional functions of p97 and p62 in the complex protein degradation network likely make their competitive interplay deciding on the degradation fate of a substrate even more complex. Although p62 is degraded by autophagy, it is transcriptionally induced upon sustained autophagy and by various forms of oxidative stress (39, 85, 99). p62 levels are also elevated in a variety of neurodegenerative diseases (29, 85). p62 can be phosphorylated at multiple sides and it was shown that phosphorylation of its UBA domain by the ULK1 complex increases its affinity for polyubiquitylated substrates (74). Therefore, the competition between UBA-domain containing proteins and all factors that impact their abundance, activity, or localization contribute to a fine tuned cross talk between proteasomal and lysosomal degradation. Intriguingly, mutations in both p62 and p97, as well as in the autophagy receptor optineurin, are linked to ALS, a fatal human neurodegenerative disease characterized by the loss of motor neurons in the spinal cord, the brain stem, and the motor cortex (26, 45, 46, 80). Therefore, it is of special interest whether an impaired cross talk between the UPS and autophagy plays a role in ALS and other neurodegenerative diseases.

HSPs and Associated Cofactors–BAG Proteins Decide on the Proteolytic route

HSPs are molecular chaperones supporting polypeptides to acquire their proper tertiary structure after translation or damage. If the native folding state of a substrate cannot be reached, HSPs conciliate the degradation of these substrates (4, 121). Prolonged disposition of clients that cannot reach a native folding state recruits E3 ligases like PARKIN or carboxy-terminus of HSP70-interacting protein (CHIP) to mark them for proteasomal degradation (89). Other E3 ligases involved in quality control like UBR1 or SAN1 might fulfill similar functions (33, 88). HSP70, an HSP of major cellular relevance, can bind to a variety of different cochaperones and nucleotide-exchange factors that decide whether folding or degradation of a bound substrate is promoted. In this context an interesting role for the Bcl-2-associated athanogene (BAG) proteins BAG1 and BAG3 has been described. Both interact with HSP70 via their COOH-terminal BAG domain, interrupting the cycle of folding/refolding activity of HSP70 and promoting substrate turnover (Fig. 3). However, their binding differently impacts the chosen degradation route. BAG1 is required for proteasomal degradation, which might be attributed to its direct interaction with the proteasome. The binding of BAG1 to HSC70/HSP70 facilitates degradation of HSP70-bound clients via the UPS (30, 77) (Fig. 3). In contrast, binding of BAG3 to HSP70 promotes clearance of substrates via autophagy and elevated BAG3 levels increase the autophagic flux (9, 30) (Fig. 3). Moreover, besides interaction with HSP70, BAG3 can also interact with the small chaperone heat shock protein-β1 (HSPB1) to promote autophagic turnover (9). The autophagy-promoting role of BAG3 was dependent on its interaction with p62. It was suggested that a complex of HSP70, HSPB8, BAG3, and E3 ligases like PARKIN or CHIP supports ubiquitylation to mark a client for autophagic degradation (30). Therefore, competition between BAG1 and BAG3 for their binding site on HSP70 directly impacts the degradation fate of misfolded proteins unable to reach a native conformation. It is...
noteworthy that BAG1 and BAG3 levels appeared to be regulated age dependently with a decrease of BAG1 and an increase of BAG3 during aging. Moreover, BAG1 and BAG3 levels inversely affected each other on a transcriptional level, as knockdown of BAG1 increased BAG3 levels and vice versa. This might be attributed to a direct nuclear role of BAG-proteins, which are able to bind DNA (111). Whether the so called BAG1/BAG3 switch contributes to decreased proteasomal degradation (72) and an elevated autophagic flux (30) during aging has to be further elucidated. However, it already became evident that competitive binding of different cofactors to HSP70 plays an important role in the cross talk between autophagy and the UPS. In this context it will be crucial to better understand how binding of BAG proteins to HSP70 is regulated. Posttranslational modifications such as phosphorylation of BAG-proteins would provide an obvious mechanism to quickly modulate their binding affinity for HSP70 (69).

Aging–Changes in Proteolytic Activity and Communication

Aging is a multifactorial physiological process affecting nearly all cellular pathways. Various factors like increased production of reactive oxygen species (ROS) by mitochondria or misregulation of transcription and translation contribute to the aging process. Therefore, aged cells have to face an increased burden of misfolded or surplus proteins. Elevated levels of proteotoxic stress are accompanied by an age-dependent collapse in proteostasis (113). In Caenorhabditis elegans,
UPS activity is impaired relatively early in adulthood (34) and it was shown that the expression of proteasomal subunits is decreased in mice during aging (66, 67). Several studies in yeast, C. elegans and fruit flies have shown that enhancing proteasomal activity increases organism life span and resistance towards oxidative stress, indicating proteasomal activity as a potential therapeutic target in the context of age-related diseases (14, 32, 58, 114, 117). Conversely, however, a recent proteomic approach in C. elegans revealed several proteasomal subunits to be upregulated during aging (119), whereas the majority of proteins remained unchanged during the course of aging (120). Apart from changes in the amount of proteasomal subunits, the age-related decline of proteasomal activity might be attributed to altered activity of regulatory factors or proteasomal subunits or by damage induced by ROS or misfolded proteins (72). Taken together, there is accumulating evidence that UPS activity is affected during aging. On the other hand it is still controversial whether autophagic activity also undergoes changes during aging (11, 30, 75, 109, 112). The discrepancy of these findings might be attributed to tissue- or context-dependent differences ensuring either the upregulation of the lysosomal pathway to relieve the proteasome or autophagic dependent differences ensuring either the upregulation of the proteasome or autophagic dependent proteolytic routes. Selective degradation of proteins was attributed to the UPS, whereas autophagy was believed to ensure protein degradation in particular during starvation, when the majority of the cellular proteome is broken down relatively unspecific to provide a source for gluconeogenesis. Another broadly accepted role of autophagy specifically in neurons was the clearance of protein aggregates that occur during aging or disease-linked mutations. This view was challenged by findings that basal autophagy occurs in all cell types including neurons. Neuron-specific knockdown of autophagy leads to neurodegeneration even in young mice (35). Moreover, a still increasing number of autophagy receptors was identified that conciliate highly specific forms of autophagy (50). Studies blocking autophagy or the UPS showed that in each case also the other degradation pathway is affected. Ubiquitin is a central mediator of such a proteolytic cross talk; the specific type of polyubiquitin chain (or even monoubiquitin) attached to a substrate decides whether it is degraded via the UPS or autophagy. Therefore, all factors that mediate and shape substrate ubiquitylation, namely E3 ligases and DUBs, actively participate in the proteolytic cross talk (Fig. 3). Another important role is provided by the ubiquitin-binding protein p62, competing with other UBDs like p97 for polyubiquitylated cargos. Moreover, the regulation of HSPs by their cofactors directly impacts the chosen degradation route of HSP-bound clients (Fig. 3). It is of major interest how such mediators are regulated during aging, either by changes in their abundance or by posttranslational modifications. Novel proteomic approaches in C. elegans allow for mass spectrometric analysis of single worms as well as for tissue-specific labeling of proteins with a noncanonical amino acid (5, 126). It is to be expected that such techniques will help in the identification of mediators of a proteolytic cross talk with respect to changing physiological demands and aging. A better understanding of how the communication between the UPS and autophagy is affected by aging can support the development of therapeutic approaches for age-dependent protein aggregation diseases as well as other diseases like cancer. Proteasome inhibitors (e.g., bortezomib, carfilzomib) are applied in therapy against multiple myeloma and lymphoma. Proteasomal inhibition activates the ERAD pathway as well as interferes with nuclear factor-kB (NF-κB) signaling which are both believed to counteract the malignant properties of the cancer cells (21, 59). However, as proteasomal inhibition also induces autophagy, related side effects have to be considered. This is of special importance regarding the observed neurodegenerative phenotypes in multiple myeloma patients treated with proteasome inhibitors (3).
Potentially, ubiquitin-binding proteins like p97 present more specific drug targets in cancer therapy and are also of remarkable interest in age-associated neurodegenerative diseases. Several p97 inhibitors have been already described and evaluated for cancer treatment (12, 15, 19). However, whether by inhibiting p97 in neurodegenerative diseases, the recognition and subsequent autophagic clearance of substrates by the p97-competitor p62 can be facilitated remains to be elucidated.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

M.P.L. and T.H. conception and design of research; M.P.L. and T.H. prepared figures; M.P.L. and T.H. drafted manuscript; M.P.L. and T.H. edited and revised manuscript; T.H. approved final version of manuscript.

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