Biochimica et Biophysica Acta 1843 (2014) 182–196

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Protein quality control and elimination of protein waste: The role of the ubiquitin–proteasome system☆

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ARTICLE INFO

Article history:
Received 3 May 2013
Received in revised form 28 June 2013
Accepted 29 June 2013
Available online 10 July 2013

Keywords:
Protein quality control
Misfolded proteins
Ubiquitin–proteasome system
Chaperones
Protein degradation

ABSTRACT

Mistakes are part of our world and constantly occurring. Due to transcriptional and translational failures, genomic mutations or diverse stress conditions like oxidation or heat misfolded proteins are permanently produced in every compartment of the cell. As misfolded proteins in general lose their native function and tend to aggregate several cellular mechanisms have been evolved dealing with such potentially toxic protein species. Misfolded proteins are mostly recognized by chaperones on the basis of their exposed hydrophobic patches and, if unable to refold them to their native state, are targeted to proteolytic pathways. Most prominent are the ubiquitin–proteasome system and the autophagic vacuolar (lysosomal) system, eliminating misfolded proteins from the cellular environment. A major task of this quality control system is the specific recognition and separation of the misfolded from the correctly folded protein species and the folding intermediates, respectively, which are on the way to the correct folded state but exhibit properties of misfolded proteins. In this review we focus on the recognition process and subsequent degradation of misfolded proteins via the ubiquitin–proteasome system in the different cell compartments of eukaryotic cells. This article is part of a Special Issue entitled: Ubiquitin–Proteasome System. Guest Editors: Thomas Sommer and Dieter H. Wolf.

1. Introduction

The proteins constitute the major workhorses of every cell. Their functions are manifold: signaling, movement, transport, membrane fusion, cell protection, regulation or catalysis are only some of them [1]. The three-dimensional structure that proteins acquire after ribosomal synthesis of their amino acid chain is crucial to their function. This function has to be maintained throughout the lifetime of every protein. Already during synthesis folding of the polypeptide chain starts [2–4]. Even though the amino acid sequence of a protein determines its final conformation and folding is thermodynamically favored, the protein folding process is energetically costly: A complex network of chaperones assists in folding at the expense of ATP hydrolysis. The chaperones recognize exposed hydrophobic amino acid patches of unfolded and yet not completely folded proteins and prevent protein aggregation during the folding process [5–8], (Fig. 1). However, despite the costly folding assistance of chaperones, statistic folding mistakes happen. Furthermore, proper function of a protein requires conformational flexibility, resulting in rather poor thermodynamic stability of certain conformations. In addition, mutations, heat, oxygen radicals, heavy metal ions and other stresses can disturb proper folding of a protein and even lead to misfolding of already properly folded proteins. This results in dysfunction of the respective protein and creates the danger of protein aggregation [9,10], (Fig. 1). In humans such protein aggregation leads to severe diseases of which Alzheimer's disease, Parkinson's disease, Huntington's disease, Creutzfeldt–Jakob disease or type 2 diabetes are prominent examples. Also aging and cancer are thought to be connected to protein misfolding and aggregate formation [10–14].

To minimize the danger that misfolded proteins pose on a cell, nature has evolved a variety of protein quality control mechanisms that maintain protein homeostasis (also known as proteostasis). Central to these quality control mechanisms is the constant surveillance of proteins by chaperones and the action of two protein degradation systems, the ubiquitin–proteasome system (UPS) and autophagy driven vacuolar (lysosomal) proteolysis [9,10,15–21]. While it was previously thought that chaperones are solely responsible for the folding process of newly synthesized polypeptides and the refolding process of functional proteins that suffered damage in response to various stresses, it has become clear recently, that chaperones accompany also terminally misfolded proteins to their disposal machinery [22]. Obviously a kinetically controlled triage mechanism decides whether a protein acquires a functional life or is degraded.

Indication that the ubiquitin–proteasome system is central in clearing misfolded proteins from the cell came from studies on

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http://dx.doi.org/10.1016/j.bbamcr.2013.06.031

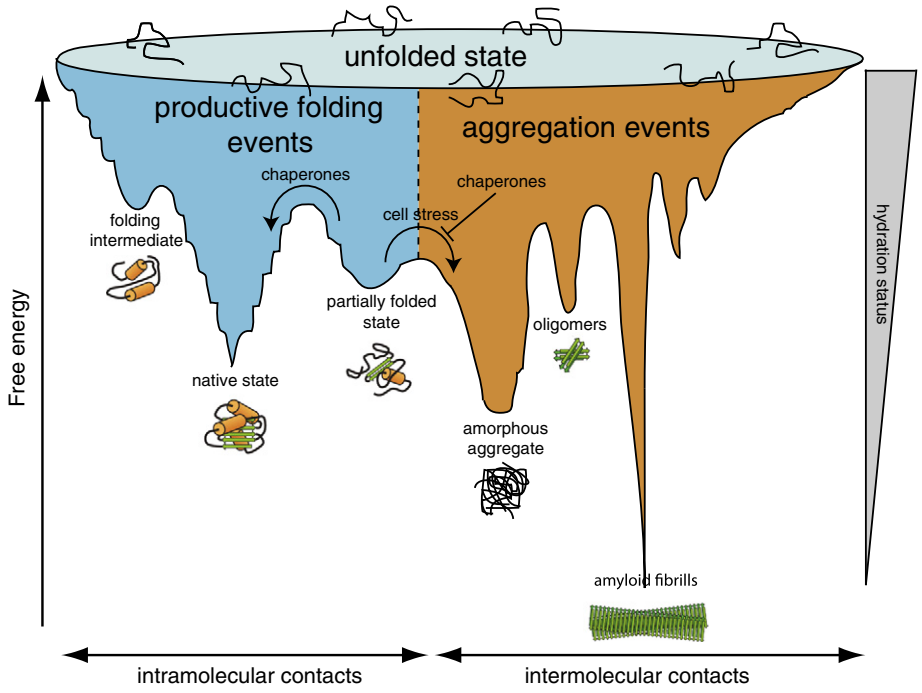

Fig. 1. Protein folding, misfolding and aggregation. Scheme of the free energy landscape that proteins traverse from their synthesis to their final folded state. Kinetically trapped conformations have to overcome free-energy barriers to enter an energetically favorable downhill folding path. These events are facilitated *in vivo* by molecular chaperones. However danger waits on the way: When several protein molecules in an compartment fold at the same time, intermolecular contacts may form which, if not disrupted by chaperones, lead to amorphous aggregates, toxic oligomers or amyloid fibrils (see refs. [6,8,10]).

proteasome mutants: Induction of protein misfolding by canavanine in mutant cells defective in the proteasome led to dramatically reduced degradation rates and the accumulation of ubiquitinated proteins [23].

Tagging of substrates with the 76 amino acid polypeptide ubiquitin is achieved by the coordinated action of a cascade of three enzyme species: at the expense of energy in form of ATP, ubiquitin activating enzymes (E1) form an energy-rich thioester bond with the C-terminal glycine residue of ubiquitin and the active site cysteine of the enzyme. Subsequently the ubiquitin residue is transferred to the active site cysteine residue of an ubiquitin-conjugating enzyme from where, with the help of ubiquitin ligases (E3), ubiquitin is linked to lysine side chains of the protein to generate an isopeptide bond. Polyubiquitin chains, mostly via internal K48 of ubiquitin, are built up. Such chains lead to recognition by the proteasome and degradation of the ubiquitin labeled protein [24,25]. More recently also other ubiquitin chain linkages and even monoubiquitination have been found to represent proteasomal degradation signals. Also ubiquitination on residues other than lysine of the protein (cysteine, serine, threonine) can serve as proteasomal degradation signal [25].

This review will concentrate on protein quality control systems acting in different compartments of the eukaryotic cell and the elimination of terminally misfolded proteins by the UPS. As eukaryotic model organism the yeast *Saccharomyces cerevisiae* will be in closer focus. With this organism many of the basic discoveries in the field have been made which served as a blueprint for similar processes in higher eukaryotes.

2. Cytoplasmic protein quality control

Ribosomes represent the primary sites of protein biosynthesis in cells. Already here the nascent polypeptides are subjected to protein quality control processes avoiding the emergence and accumulation of aberrant proteins in this early stage of a protein's life. Several E3 ligases are responsible for the ubiquitination of nascent polypeptides which have arisen from defective mRNA like nonstop mRNA causing translational arrest and stalling of the corresponding polypeptides on the ribosomes respectively (Fig. 2A). Translated nonstop mRNA causes a 3′poly (A) tail which results in C-terminal poly (Lys) tracts on corresponding polypeptides [26]. Nonstop mRNA results from DNA mutations, transcriptional mistakes or premature polyadenylation events [27,28]. In yeast, the E3 ligase Ltn1 in complex with the two highly conserved proteins Tae2 and Rqc1 [29,30], and the AAA+ ATPase Cdc48 [31] seem to be involved in recognition of the stalled ribosome, ubiquitination and extraction of the polypeptides emerging from the ribosome exit tunnel for subsequent proteasomal degradation [30,32–34]. In a recent study the ribosome bound E3 ligase Hel2 was discovered to have overlapping functions with Ltn1 in the ubiquitination process [35]. The E3 ligase Not4 as component of the CCR4/NOT complex has also an influence on the cotranslational protein quality control process. The CCR4/NOT complex is important for mRNA integrity [36]. Deletion of Not4 causes an increased amount of defective mRNA and therefore generation of a massive amount of aberrant nascent polypeptides. These have to be titrated away from the cellular environment through ubiquitination by the above-mentioned ribosome bound E3 ligases and subsequent proteasomal degradation [35]. A previous study has indicated that Not4 itself is involved in ubiquitination of polypeptides translated from nonstop mRNA [26], (Fig. 2A). Cotranslational protein folding is supported by the heterodimeric nascent polypeptide-associated complex (NAC) [37,38] which interacts with nascent polypeptides preventing them from forming incorrect interactions. NAC interacts in addition with the ribosome-associated Hsp70/Hsp40-chaperone system composed of a RAC complex (Hsp70 chaperone Ssz1, Hsp40 chaperone Zuo1) and the Hsp70 chaperone Ssb1 [38–43]. More recent studies revealed a colocalization of these folding mediators with aggregation prone proteins like PolyQ proteins, finally preventing accumulation of aggregates [44].

The next level of protein quality control is introduced when fully synthesized proteins are released from the ribosomes which do not contain signal sequences for entering the secretory pathway,

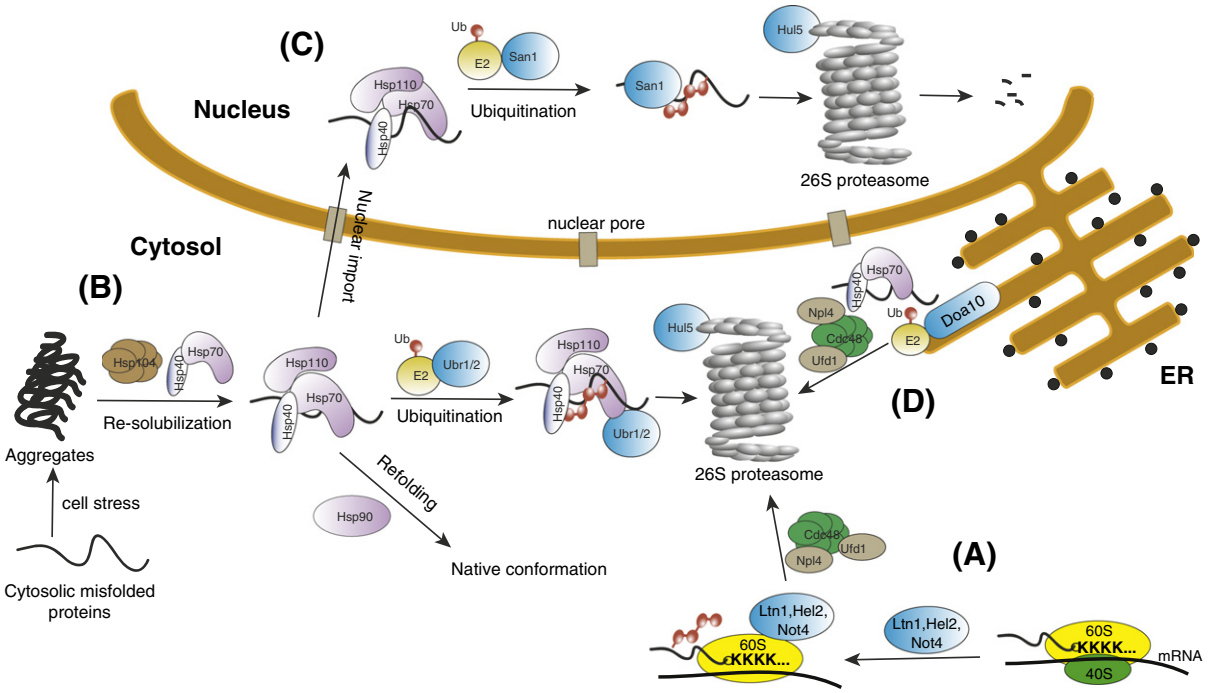

Fig. 2. Cytoplasmic protein quality control and degradation. Misfolded cytoplasmic proteins can be ubiquitinated by a set of E3 ligases which are localized to different cell compartments. Chaperones and cochaperones are involved in either preventing substrate aggregation or in disaggregation of existing aggregates for E3 recognition. (A) Nascent polypeptides translated from defective mRNA like nonstop mRNA containing C-terminal polybasic stretches are stalled on the ribosomes and attached with ubiquitin for degradation by the E3 ligases Ltn1, Hel2 and Not4. The Cdc48 machinery provides the force for the extraction of corresponding ubiquitinated substrates out of the ribosome exit tunnel. (B) The major E3 ligase responsible for ubiquitination of misfolded proteins in the cytosol is the E3 ligase Ubr1. Ubr2, the paralogue of Ubr1 has a minor role. (C) Some cytoplasmic substrates can be transported into the nucleus via chaperones where ubiquitination occurs through the action of the E3 ligase San1. (D) The ER membrane localized E3 ligase Doa10 together with ERAD components like the Cdc48 machinery which normally act in ubiquitination of ERAD substrates are also able to target some cytoplasmically localized substrates for proteasomal degradation. The 19S proteasome associated E3/E4 ligase Hul5 extends already existing ubiquitin chains. For details, see text.

peroxisomes or mitochondria. In yeast, the cytosolic Hsp70 chaperones of the Ssa type (Ssa1-4) are the main players which work in this cytosolic quality control. They act in concert with Hsp40 or J-proteins and a set of nucleotide exchange factors (NEFs) [45–48]. Besides mediating folding of client proteins in an ATP dependent reaction cycle, the cytosolic Hsp70 system prevents aggregation of not yet properly folded proteins by shielding hydrophobic patches on their surface. Smaller aggregates can be actively dissolved by the Hsp70 system which is shown both for the Hsp70 chaperones of the Ssa type and the Hsp40 chaperone Ydj1 [48]. Additionally this system functions in protein trafficking of some selected proteins [49]. Recently, the Hsp70–Hsp40 chaperone system has been found to be involved in proteasomal degradation of terminally misfolded proteins. Loss of Hsp70 function causes nearly complete stabilization of misfolded cytosolic proteins [47,48]. The absence of Hsp70 does not influence the ubiquitination of corresponding substrates but causes their sequestration into insoluble inclusions [50]. In contrast to smaller aggregates which can be dissolved by the Hsp70–Hsp40 chaperone system, larger aggregates can only be efficiently dissolved and the corresponding polypeptides reactivated, if Hsp104, an AAA-ATPase chaperone of the Hsp100 family [51] acts in concert with Hsp70 [52–54]. In contrast to Hsp70, Hsp104 does not act in preventing stress induced protein aggregation but only acts in disaggregation processes [52,55–57]. Metazoa lack Hsp100 disaggregases. Instead, disaggregation of protein aggregates executed by the Hsp70 system is stimulated by the nucleotide exchange activity of Hsp110 chaperones which represent a subclass of Hsp70 without exhibiting refolding activity [58–60]. An additional chaperone system, Hsp90, acts downstream of the Hsp70 system. Hsp90 is highly abundant in eukaryotic cells even under non-stress conditions [61–63]. It is responsible for folding and conformational regulation of many signaling proteins making this chaperone family a promising target for cancer therapy [64–66]. Hsp90 is also able to bind early folding intermediates [67], or denatured proteins to keep them in a folding-competent state. Further folding is finally exerted by the Hsp70 system [68,69]. The cytosol also possesses small heat shock proteins (sHsps) belonging to the class of ATP-independent chaperones. In yeast, the two most prominent members are Hsp42 and Hsp26. Hsp42 functions both in stressed and unstressed cells and prevents protein aggregation [70], but is also involved in targeting of misfolded proteins to peripheral aggregate deposits in cells [71]. Hsp26 is stress induced and can promote Hsp104 and Hsp70 mediated disaggregation of protein aggregates [72,73].

The mammalian E3 ligase CHIP (C-terminus of Hsc70-interacting protein) is the best-characterized protein linking Hsp70 chaperone activity to protein degradation. CHIP itself interacts directly with both Hsp70 and Hsp90 chaperones via the tetratricopeptide repeat (TPR) domain [74]. Substrates bound to the chaperones can be ubiquitinated by CHIP mediated by a U-Box domain responsible for CHIP activity and binding of the ubiquitin-conjugating enzyme UbcH5 [75]. Furthermore CHIP itself possesses chaperoning activity through direct binding of client proteins by this preventing their aggregation [76]. The ubiquitination activity of CHIP is in addition influenced by the BAG class of Hsp70 cochaperones. They bind to Hsp70 and can either cause delivery of proteins to the proteasome (BAG-1) [77], negatively regulate CHIP ubiquitination activity (BAG-2, BAG-5) [78,79] or mediate substrate delivery to the lysosome (BAG-3) [80]. HspBP1 is an inhibitor of the CHIP ligase exerting an important role in the maturation process of the cystic fibrosis transmembrane conductance regulator (CFTR), a plasma membrane protein folded in the ER membrane. Folding of CFTR is slow and inefficient. Thus inhibition of the CHIP ligase activity by HspBP1 can provide more time for the folding of CFTR [81].

Yeast does not possess a CHIP homolog and therefore uses other E3 ligases for mediating degradation of cytosolic misfolded proteins (Fig. 2B). The main E3 playing a role in cytoplasmic protein quality control in yeast is Ubr1 [82–84], which was first discovered as the

E3 ligase of the N-end rule pathway. There it is responsible for ubiquitination of substrates containing a degradation signal (degron) composed of an N-terminal type 1 destabilizing residue (Arg, Lys, His) or type 2 destabilizing residues (Leu, Phe, Trp, Tyr or Ile) respectively, an internal lysine residue and an unstructured N-terminal extension [85,86]. In the clearance process of cytosolic misfolded proteins Ubr1 seems to work independently of the N-end rule pathway [83,84]. The ubiquitination of Ubr1 substrates is directly dependent on Hsp70 and the Hsp110 chaperone Sse1, the latter acting as NEF in the Hsp70 reaction cycle. In contrast to *in vivo* conditions, Ubr1 is able to ubiquitinate denatured luciferase *in vitro* independently of chaperones. However, simultaneous incubation of the substrate with purified Hsp70 stimulates the ubiquitination activity of Ubr1 [83,84]. A recent study using a short lived version of GFP (slGFP) as a terminally misfolded substrate revealed the Hsp40 chaperone Sis1 as being pivotally involved in proteasomal degradation of slGFP. In this degradation pathway Sis1 acts together with Ubr1 and Hsp70 which all coimmunoprecipitate together with the substrate slGFP [87]. The Hsp40 chaperone Ydj1 also interacts with slGFP but functions together with Hsp70 in preventing slGFP aggregation and not in the Ubr1 mediated degradation pathway. Consequently Ydj1 could not be detected in Ubr1 containing complexes together with the Hsp40 chaperone Sis1 [87]. Using the unstable Dihydrofolate reductase (DHFR) variant $DHFR^{MUTD}$-URA3 as substrate, the targeting process to the UPS is also promoted by the nucleotide exchange factor Fes1 which can cause the release of substrates from Hsp70 and facilitate the interaction with Ubr1 [88].

The E2 enzymes functioning together with Ubr1 in transferring ubiquitin to the substrates seem to be Ubc2 – the ubiquitin-conjugating enzyme, also active in the N-end rule pathway – and in addition the stress inducible E2 enzymes Ubc4 and Ubc5 [48,83,89]. Ubr2, the paralog of Ubr1, does not recognize N-degrons. It was shown that Ubr2 has overlapping functions with Ubr1 in the clearance of unfolded cytoplasmic substrates [83]. However the most prominent substrate of Ubr2 is native Rpn4 [90], a stress induced transcription factor stimulating the expression of proteasomal genes [90–93]. Surprisingly, a role in the cytoplasmic quality control pathway was also discovered for the E3 ligase San1 which was first shown to be involved in ubiquitination of aberrant nuclear proteins [94]. It has been observed that several misfolded cytosolic proteins become localized to the nucleus and are thereafter ubiquitinated by San1 [84,95,96], (Fig. 2C). Efficient shuttling of the substrates to the nucleus is dependent on Hsp70 chaperones of the Ssa type and the Hsp110 chaperone Sse1 [84]. In a recent study the truncated form of the nucleotide binding domain of the pheromone a-factor transporter Ste6 called NBD2* was used as model substrate. It was shown that the degradation of this protein is also San1 dependent. The Hsp70 chaperone Ssa1 promotes NBD2* binding to San1 [97]. The physiological reason of shuttling misfolded proteins to the nucleus for proteasomal degradation might rest in the fact that almost 80% of the proteasomes are localized to the nucleus. This guarantees an effective and fast clearance of cytosolic misfolded proteins [98]. Besides the involvement of nuclear and cytoplasmic components, also ERAD components have been described to be involved in degradation of cytosolic substrates. URA3-CL1 consisting of the cytosolic Ura3 enzyme fused to the 16 amino acid CL1 degron [99,100] is – dependent on ERAD-C components – a target for proteasome-mediated degradation. These ERAD-C components include the E3 ligase Doa10, the E2 enzymes Ubc6 and Ubc7 and the Cdc48 machinery. In addition the cytosolic Hsp40 and Hsp70 chaperones are required [99–101], (Fig. 2D). Doa10 was also shown to be involved in a branch of the N-end rule pathway recognizing N-terminally acetylated amino acids in cytosolic substrates [102].

Once ubiquitinated, the processivity of proteasomal substrate degradation can be enhanced by the action of Hul5 which represents a 19S proteasome associated E3 and/or E4 enzyme. It antagonizes the deubiquitinating enzyme Ubp6 by extending already existing ubiquitin chains on substrates which are stalled on the proteasome [103–106]. Hul5 is the major ligase for mediating proteasomal degradation of cytosolic proteins misfolded by heat shock and characterized by low solubility like the prion-like protein Pin3. The physiological importance of Hul5 becomes visible in *HUL5* deleted cells growing at elevated temperatures. They show a significant growth defect [107].

The degradation process of misfolded proteins in the cytosol not only involves chaperones, E3 ligases and the proteasome. Also spatial organization in separated quality control compartments within the cytosol was observed [108]. IPOD (insoluble protein deposit) is a perivacuolar region which seems to serve as storage compartment for insoluble aggregates built up of mostly non-ubiquitinated misfolded and amyloid-forming proteins which might be toxic for the cell. Obviously the removal from the cellular environment serves a cytoprotective role. IPOD does not colocalize with proteasomes. In yeast IPOD colocalizes with Atg8, an ortholog of mammalian LC3 [109–111], a ubiquitin-like adaptor protein important for vacuole delivery. Atg8 gets conjugated to phosphatidylethanolamine via a ubiquitin-like conjugation system. Through this lipid linkage Atg8 promotes membrane fusion events in autophagosome formation [109,112]. The localization of IPOD to the vacuolar periphery indicates subsequent sequestration of these potentially toxic aggregates into vacuoles for degradation. In addition, the chaperone Hsp104 colocalizes with the IPOD compartment indicating a role in disaggregation of aggregates [51] or in preventing inheritance of potentially toxic aggregates [113]. JUNQ (juxtanuclear quality control) represents a quality control compartment containing ubiquitinated soluble misfolded proteins which can be refolded with the help of chaperones or degraded via the UPS. Ubiquitination is an important modification for keeping substrates soluble and directing them to JUNQ. This compartment is found exclusively in stressed cells. JUNQ is localized to the cytosolic side of the ER. It contains chaperones like Hsp104 and represents a compartment with a high proteasome density. Because the UPS function is often impaired under stress conditions, sequestration of misfolded proteins to the JUNQ compartment serves as a mechanism shielding the cytosolic environment from these proteins to avoid stalling of the UPS function by overloading the proteasomes [108]. As mentioned above, selective autophagy is another proteolytic system important for targeting misfolded proteins and aggregates for degradation. Here, cytoplasmic constituents are engulfed into double membrane containing vesicles (autophagosomes) which fuse with the lysosome (vacuole) for degradation [109]. Autophagy becomes important when the UPS function is impaired. As in the UPS, ubiquitinated substrates destined for lysosomal degradation are recognized in higher eukaryotes via two prominent UBA domain containing adaptor proteins, p62 [114,115] and Nbr1 [116]. These adaptors bind protein aggregates preferentially decorated with K63 polyubiquitin chains. p62 and Nbr1 bind to LC3, the mammalian ortholog of yeast Atg8 [110,111], directly. This binding causes shuttling of the corresponding substrates to autophagosomes for lysosomal degradation [114,116–118]. An additional pathway in higher eukaryotes involves the formation of a specialized perinuclear compartment called aggresome which forms prior to lysosomal degradation [119]. HDAC6 (ubiquitin-binding histone deacetylase 6) binds ubiquitinated aggregates and loads the cargo onto dynein motor proteins for shuttling along microtubules to the perinuclear aggresomes [120–123]. The link between UPS and the autophagic machinery described above is not observed in yeast where corresponding UBA domain containing cargo adaptors have not been discovered yet. There is also a ubiquitin-independent route to aggresomes. This route makes use of BAG-3 which delivers non-ubiquitinated substrates bound to Hsp70 via the microtubule system to the aggresomes [124]. In contrast to BAG-3 the cochaperone BAG-1 guides substrates to proteasomal degradation.

Whether a substrate is targeted to the proteasomal or to the autophagic pathway can also be induced by a different E2 usage of the ubiquitin ligases CHIP and PARKIN. Depending on the E2 enzyme used both E3 ligases can attach either K48 or K63 ubiquitin

chains onto substrates. Proteins carrying K48 chains are targeted to proteasomal degradation, proteins carrying K63 chains enter the autophagic pathway [125–127].

3. Endoplasmic reticulum associated degradation (ERAD) of misfolded proteins

About 30% of a cell's protein equipment consists of proteins of the secretory pathway. Their function is connected to the endoplasmic reticulum (ER), the Golgi apparatus, the lysosome (vacuole), the plasma membrane and to the exterior of the cell. The major entry site of these proteins into the secretory pathway is the ER into which they are imported in an unfolded state. The transfer across the ER membrane occurs via an aqueous channel, Sec61, which is part of a large multiprotein complex accomplishing this complicated task. Protein import can occur either in a cotranslational or posttranslational fashion [128–130]. For gain of function the proteins have to be folded in the ER from where they are exported to their site of action. Consequently, the ER is packed with proteins which constitute a folding factory for secretory proteins. These consist of chaperones, co-chaperones, oxido-reductases, glycan-modifying enzymes and lectins which fold and scan proteins for proper folding in a dynamic fashion. During entry into the ER and during the folding process many proteins are modified. This includes cleavage of the signal sequence by signal peptidase, glycosylation by the oligosaccharyl transferase (OST) complex, disulfide bond formation by protein disulfide isomerases or lipid conjugation [21,131–138]. As stated in Section 1 mutations or folding errors which are dramatically enhanced by cellular stresses will finally lead to the accumulation of misfolded proteins which the ER must deal with. The primary answer to the accumulation of an overload of unfolded and misfolded proteins in the ER is the unfolded protein response (UPR) by which – depending on the organism – metazoa or yeast, a cell can handle this stress with different tools. These include decrease of the translational rate and import of secretory proteins into the ER, increase of chaperones and folding supportive proteins as well as increase of the ER volume. The UPR of yeast *S. cerevisiae* solely relies on a program of transcriptional upregulation of ER components. Prominently, many proteins of the ERAD machinery degrading the permanently misfolded proteins are upregulated [139–142]. The ERAD pathway rescues the cell from smothering in its secretory protein waste: unfolded proteins can form inactive aggregates which subsequently end up in protein precipitates; they may bind to other proteins and disturb their action. In addition, altered protein conformations may be secreted and form extracellular amyloids. Consequently, a mistaken ERAD pathway leads to a multitude of diseases in humans [143,144].

It is an exceptional feature of ERAD that misfolded proteins are not degraded by some proteolytic machinery within the ER but that they are retrograde transported out of the ER instead and eliminated by the cytoplasmic UPS. This feature and the ERAD system itself has been reviewed many times starting from its discovery until today (as a selection of reviews, see refs. [21,145–154]. We will therefore only briefly summarize known facts here and extend to novel discoveries and crucial questions. It came to a big surprise when it was found in yeast that an ER-imported, fully glycosylated soluble mutant protein of carboxypeptidase Y (CPY*) which was thought to be on its way to the vacuole, was found to be retrotranslocated out of the ER back into the cytoplasm, polyubiquitinated and degraded by the proteasome [155]. Retrotranslocation of a mutated alpha factor pheromone in yeast [156] and, upon viral intervention, membrane located MHC class I heavy chain of mammalian cells [157] with subsequent degradation by the proteasome hinted at the fact, that retrograde transport and degradation of ER proteins in the cytoplasm might be a general mechanism to eliminate unwanted proteins of this secretory organelle. As we know today, this process does not only include misfolded and virally expelled proteins as substrates, but also regulated proteins of the ER membrane, HMG-CoA reductase being a prominent example [158].

Our knowledge of the ERAD mechanism of yeast is most advanced and serves as blueprint also for the mammalian process (Fig. 3). During and after translocation through the Sec61 channel in the ER membrane most of the soluble protein species are liberated from the signal sequence and many of the imported soluble and membrane proteins are N-glycosylated. Folding in the ER is surveyed by chaperones of the Hsp70 and Hsp40 classes and chaperone-like proteins as well as lectins [132,159–162]. While the hydrophobic patches of the nascent protein are occupied by Hsp70 chaperones (Kar2 in yeast, BiP in mammals) to support folding, the N-linked carbohydrate on the protein consisting of triple branched trees of Glc3-Man9-GlcNAc2, the “glyco-code” of the ER, displays the folding status [134]. During trimming of the three glucose residues by glucosidases I and II the protein should reach its final folding status. If this is not the case, in mammalian cells calnexin and calreticulin together with the associated oxidoreductase ERp57 bind to the folding polypeptide and a folding sensor, the UDP-glucose:glycoprotein glucosyltransferase (UGT1) re-glucosylates the Man9 residue on the A-branch of the Man9GlcNAc2 tree, allowing for another round of folding. Repetitive association with calnexin and calreticulin, re-glucosylation by UGT1 and de-glucosylation by glucosidase II allows a certain time frame for folding of a protein in mammalian cells. Yeast cells are devoid of this calnexin–calreticulin–UGT1 cycle. After proper folding together with disulfide bond formation proteins are allowed to exit the ER for further delivery to their site of action [134,138,163–165]. The trimming of mannose residues by alpha-mannosidase I and the lectin Mnl1/Htm1 in yeast – (EDEM1 in mammals) – interrupts futile folding cycles and channels these non-properly folded proteins into ERAD [134,166–169]. Misfolded proteins of the ER are distinguished as three topologically different classes in yeast: proteins exposing their misfolded domain (i) in the ER lumen (ERAD-L substrates), (ii) in the ER membrane (ERAD-M substrates) or (iii) in the cytoplasm (ERAD-C substrates) [170,171]. ERAD-L and ERAD-M substrates on the one hand and ERAD-C substrates on the other are directed to two different RING ubiquitin ligase systems residing in the ER membrane, the Hrd1/Der3 ligase (ERAD-L and ERAD-M) [172,173] or the Doa10 ligase (ERAD-C) [174], respectively. In mammalian cells the equivalent of Hrd1/Der3 is represented by two ubiquitin ligases, HRD1 and gp78. Doa10 is represented by the ubiquitin ligase TEB4 (MARCH-IV) in mammalian cells [175]. No such clear distinction between different ERAD pathways is possible for mammalian cells. Also in yeast not all substrates are exclusively dependent on one or the other ERAD branch. There exists some flexibility [176,177]. For polyubiquitination of the retrograde transported substrate to occur, the two ER membrane integrated ubiquitin ligases in yeast work together with the ubiquitin-conjugating enzymes Ubc6 and Ubc7. To some extent the Hrd1/Der3 ligase also works together with cytosolic Ubc1 [178]. Ubc6 is tail-anchored in the ER membrane, while Ubc7 is a soluble conjugating enzyme of the cytoplasm which is recruited to the ER membrane and activated by binding to the membrane anchor protein Cue1 [155,179,180], (for reviews see [21,145–154]). The Ubc7-binding region (U7BR) of Cue1, a multi-helix E2-interacting domain, enhances the loading of Ubc7 by Uba1 with ubiquitin and the subsequent transfer of ubiquitin to K48 acceptor ubiquitins on the substrate [181]. The CUE1 domain possessing ubiquitin binding properties causes stabilization of growing ubiquitin chains by this enhancing the efficiency of the degradation of ERAD substrates [182]. The mammalian orthologs of Ubc6 and Ubc7 are Ube2j1/2 and Ube2g1/2 respectively [175].

The discovery and retrotranslocation of lumenal misfolded proteins (ERAD-L) seems to be the most complicated process of the ERAD branches. In yeast, CPY*, a mutant form of the glycoprotein CPY of the vacuole [155,183] served as a model substrate for this ERAD branch. After translocation into the ER and N-glycosylation, proteins which finally discover misfolded protein species and initiate their retrotranslocation

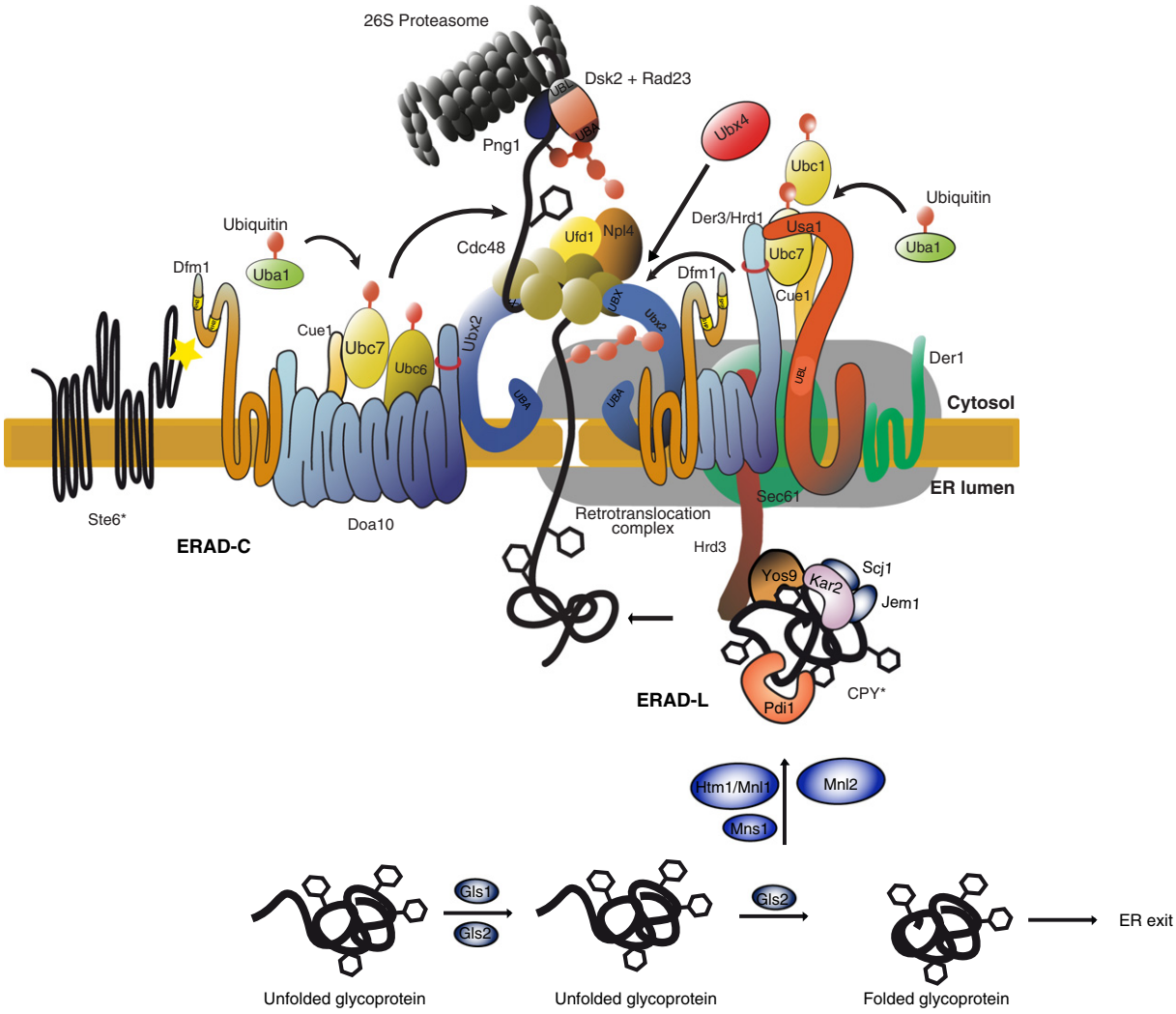

Fig. 3. Endoplasmic reticulum associated protein degradation (ERAD). Misfolded proteins of the secretory pathway are targeted for ubiquitination and degradation by the ERAD pathway which consists of several branches depending on the localization of the misfolded domain of the corresponding protein. The ERAD-L pathway targets lumenal misfolded proteins to proteasomal degradation in the cytoplasm. This includes retrotranslocation of the substrate across the ER membrane, ubiquitination by the E3 ligase Hrd1/Der3, extraction of the ubiquitinated substrates out of the ER membrane by the Cdc48 machinery and shuttling to the proteasome. The ERAD-C pathway targets ER membrane proteins with misfolded cytosolic domains for proteasomal degradation. The E3 ligase responsible for ubiquitination of these substrates is Doa10. For details see text.

include the Hsp70 chaperone Kar2 together with its Hsp40 partners Jem1 and Scj1 [159–161] as well as protein disulfide isomerase [184,185]. At the same time the carbohydrate chain is trimmed by two glucosidases and two mannosidases (alpha-mannosidase I [166,167] and the lectin/mannosidase Htm1/Mnl1 [184,186,187]) to yield the glyco-code for protein removal from the ER, Man7-GlcNAc2. This code carrying an alpha 1,6 terminal mannose is read by the lectin Yos9 [186–191] which in itself is bound to the membrane anchored chaperone Hrd3 [192]. Sequentially or together they deliver the misfolded protein to the ubiquitin ligase Hrd1/Der3 which is able to recognize misfolded protein [192,193]. Additional modification of the glyco-code for most efficient degradation of N-glycosylated proteins may be necessary: Recently a novel putative mannosidase, Mnl2, was discovered in yeast, deletion of which diminished the reduced degradation rate of CPY* in Mnl1/Htm1 deficient cells even further [194]. The glyco-code is not an essential mark for degradation of lumenal misfolded ER proteins. As an example, unglycosylated CPY* is degraded by the ERAD-L machinery as well [195–197]. Interestingly, Yos9 also binds and influences the degradation of non-glycosylated CPY* indicative of a chaperone function besides its lectin function [188,195,196]. Recently O-mannosylation by the Pmt1/Pmt2 enzyme complex was found to terminate failed folding attempts of slowly and improperly folding proteins. O-mannosylation obviously reduces the engagement of such modified substrates with the Hsp70 chaperone Kar2 and allows their subsequent degradation [198]. One may speculate that termination of folding by O-mannosylation of substrates fills in when the glyco-code based on N-glycosylation is not sufficiently functional or absent.

The central ligase of the ERAD-L branch, Hrd1/Der3 with its six membrane spanning topology [173], is in contact with several ER membrane located partner proteins: Hrd3, Usa1, Der1 and Ubx2 [171,199–202]. While, as indicated above, Hrd3 reaches into the ER lumen and together with Yos9 delivers the misfolded substrate to the Hrd1/Der3 ligase, Ubx2 makes contact to the trimeric AAA-ATPase complex Cdc48–Ufd1–Npl4 in the cytosol which, after polyubiquitination of the substrate is responsible for its delivery to the proteasome (see below). Usa1 forms a bridge between Der1 and the Hrd1/Der3 ligase. Der1 [203,204], the blueprint of the mammalian Derlins, is required for the degradation of soluble lumenal ER proteins [171,200,204] but not for membrane proteins of the ERAD-M branch which are also targets of Hrd1/Der3 ligase ubiquitination [171,200]. Usa1 furthermore induces oligomerization of Hrd1/Der3, a prerequisite for degradation of ERAD-M substrates such as Sec61-2p and HMG-CoA reductase, but not for

degradation of soluble substrates [200]. The function of Der1 has remained an enigma until now. It has been shown that N-terminal acetylation of Der1 is required for its stability. Preventing Der1 acetylation leads to its degradation via the Hrd1/Der3 ligase with a subsequent cessation of elimination of substrates dependent on Der1 [205].

Recently, the mammalian Der1 ortholog Derlin-1 has been shown to be a rhomboid pseudo-protease [206]. Rhomboids constitute a conserved superfamily of polytopic membrane proteins which recognize a broad range of substrates and clients. The family of rhomboid proteases cleave their substrates in the membrane [207]. It was recently uncovered that a rhomboid protease is required for ERAD of some membrane proteins in mammalian cells [208]. The family of rhomboid pseudoproteases including Der1 and the Derlins bind and regulate the fate of their clients without proteolytic cleavage. Der1 may help in the unfolding of aberrant solvated protein domains in the vicinity of the membrane to make respective proteins competent for retrotranslocation into the cytosol [207]. The mechanism of how the substrates are retro-translocated across the ER membrane to reach the site of ubiquitination catalysis formed by the RING ligase Hrd1/Der3 complexed with the ubiquitin-conjugating enzymes (preferentially Ubc7 but also Ubc6 or Ubc1) is still under debate [149]. Retrotranslocation through the ER import channel Sec61 has been proposed for some substrates [157,159,180,209–212] but not for others [176,213,214]. The membrane embedded Der1/Derlin rhomboid pseudoprotease as well as the polytopic ubiquitin ligases Hrd1/Der3 and Doa10 themselves have been suggested to form the channel [215–218]. However, Der1 is not required for degradation of ERAD-M substrates [213]. Thus, use of such a channel would be limited. Nevertheless, crosslinking studies with a truncated CPY* construct showed interaction with inter-bilayer residues of Hrd1/Der3 which could be interpreted with a channel function of the ligase [215]. However, interaction of substrates with the "body" of the ligase must be expected for the substrate recognition process in general. It is therefore still an open question if any of the canonical membrane embedded ER ligases act as a channel. Retrotranslocation without channel has also been suggested [219]. The process of lipid droplet formation at the outer ER leaflet and sequestration into the cytosol could constitute a delivery mechanism for membrane proteins. However, in yeast at least such a mechanism is not operating [220].

Discovery and handling of ER-lumenal (ERAD-L) substrates require a rather complicated lumenal machinery. In contrast, discovery of misfolded substrates of the ER membrane exposing their misfolded domain into the cytoplasm (ERAD-C substrates) is by far more simple. As it is the case with lumenal substrates, their recognition depends on a Hsp70 machinery. Here members of the cytosolic Ssa class are in charge [176,221]. The major ER ligase tagging membrane substrates with ubiquitin is Doa10 [174,216]. For their translocation across the ER membrane all the questions discussed for ER lumenal substrates apply. Interestingly for degradation of one of the ER membrane model substrates, Ste6*, a Der1 homolog, Dfm1 is required [222]. One may suggest that also Dfm1 is a pseudo-rhomboid, facilitating retrotranslocation of the substrate [207]. Interestingly, via its SHP box Dfm1 is also able to recruit the Cdc48 motor to the ER membrane [222,223]. Cdc48 has been shown to be an ATP-driven disaggregase [31].

Delivery of all ERAD substrates (ERAD-L, ERAD-M and ERAD-C) to the proteasome for degradation merges in the cytoplasm at the ATP driven motor Cdc48 complexed to the two adapter proteins Ufd1 and Npl4. The trimeric motor complex is recruited to the ER membrane by the membrane anchor Ubx2 [201,202]. After polyubiquitination of the substrate the Ubx2 anchored Cdc48–Ufd1–Npl4 machine pulls the ubiquitinated proteins most likely out and away from the ER membrane [224–228]. Modulation of the process can be achieved by additional adapters, for detailed review see [229]. In mammalian cells a ubiquitin ligase-associated chaperone complex consisting of Bag6, Ubl4A and Trc35 has been found which acts downstream of the Cdc48/p97 machinery in ERAD. Bag6 as the core of the complex represents a holding chaperone for keeping substrates harboring long hydrophobic stretches like membrane proteins soluble on the way to the proteasome [230]. Rather the function of Bag6 in ERAD and not its previously published function in the biosynthesis of tail-anchored proteins of the ER seem to be the primary cellular role of this holdase [230–232]. Also secretory proteins which fail to translocate into the ER and therefore become mislocalized to the cytosol can be captured by the Bag6 complex [233]. In yeast, with the help of ubiquitin receptors (Dsk2, Rad23), the substrates are finally delivered to the proteasome for degradation [234–236].

Prior to proteasomal degradation deglycosylation of N-glycosylated substrates by cytosolic peptide:N-glycanase (Png1) occurs [237], (Fig. 3). Interestingly, complete degradation of some membrane substrates requires the activity of the ubiquitin chain elongating ligase Hul5 in addition to the canonical ERAD ligase while degradation of others does not. The data available indicate that complete membrane extraction of the Hul5 dependent substrates is interrupted when Hul5 is missing [106].

The ERAD process as elucidated in yeast serves as a blueprint for mammalian ERAD. The basic steps of the process, (i) substrate recognition, (ii) retrotranslocation across the ER membrane onto the cytoplasmic side of the ER, (iii) polyubiquitination and solubilization by the Cdc48 motor and (iv) recognition and degradation by the proteasome are identical, including the basic tools of the ERAD process. However, due to a higher complexity of mammalian cells there are some variations in the detail. For reviews, see [130,238].

4. Nuclear protein quality control

Yeast nuclear protein quality control is mainly dependent on the E3 RING ligase San1 [239]. It is nucleus localized due to a bipartite nuclear localization signal (NLS). In conjunction with the E2 enzymes Cdc34 [240] and Ubc1 [241] it catalyzes the ubiquitination of mutated nuclear proteins [94]. Interestingly, the San1 dependent nuclear protein quality control system of *Schizosaccharomyces pombe* does not use the orthologous E2 enzymes of *S. cerevisiae* Ubc1 and Cdc34 but Ubc4 and Ubc5 instead. In spite of the different E2 usage, fission yeast San1 is also functional in *S. cerevisiae* [242]. In contrast to other yeast E3 ligases San1 possesses a large random coil structure outside of its RING domain. Because of these disordered regions the protein can probably adopt multiple conformations, therefore being highly flexible in binding substrates [243,244]. This property of carrying an intrinsically disordered structure is also known from some chaperones like the small heat shock proteins (sHsps) which also use these disordered regions for interaction with client proteins [245–247]. Unlike other E3 ligases like Ubr1 which requires Hsp70 and Hsp110 chaperones for substrate ubiquitination [83,84], San1 directly interacts with its misfolded nuclear substrates and ubiquitinates them without the help of chaperones [244]. This is in contrast to San1 dependent degradation of cytoplasmic substrates which seems to be Hsp70 dependent [84,97]. The similarity of chaperones and San1 in binding substrates raises the question whether there is a competition in substrate binding. Actually it has been found that the Hsp110 chaperone Sse1 negatively interferes with the ubiquitination efficiency of San1 *in vitro* [84]. As a consequence, upregulation of nuclear chaperones may shift San1 dependent degradation towards chaperone dependent repair of non-native proteins. The degron recognized by San1 in substrates consists of a minimal window with the threshold of hydrophobicity equivalent to that of 5 contiguous exposed hydrophobic residues [243]. Overall hydrophobicity of a substrate does not seem to play a role in San1 recognition [243]. The feature of San1 in recognizing the degron is paralleled by Hsp70 chaperones which also recognize a small window of hydrophobic residues, consisting of only 4 contiguous hydrophobic amino acids, however [248]. In addition, the type of hydrophobic residues within the recognized window is an important factor for San1 mediated proteasomal degradation. San1 prefers those hydrophobic residues within the window which correlate with the tendency of the residues to cause aggregation and insolubility

of corresponding substrates [249]. In an *in vitro* experiment San1 is only able to ubiquitinate denatured luciferase if San1 is added during the denaturation step and not if added after complete denaturation of luciferase [244]. It can be hypothized that San1 targets highly aggregation prone proteins for degradation which are not yet fully aggregated. When degrons are buried in aggregates they cannot be recognized anymore by San1. Keeping these substrates soluble requires upstream acting factors which maintain the accessibility of corresponding substrates for San1 recognition. Some substrates require the binding of the Hsp70 chaperones Ssa1 and Ssa2 for San1 mediated degradation [84,95,250], (Fig. 4A). Recently it has also been discovered that the AAA-ATPase Cdc48 is involved in keeping substrates soluble for San1 recognition [249]. The yeast Slx5–Slx8 SUMO targeted ubiquitin ligase (STUbL) is involved in a nuclear ubiquitination pathway which functions in genome maintenance and in control of sumoylation [251–253]. Recent studies indicate that Slx5–Slx8 plays a role in protein quality control in a fashion different from San1 [254], (Fig. 4B). STUbLs preferentially target SUMO conjugates for ubiquitination and subsequent proteasomal degradation. The SUMO E3 ligases Siz1 and Siz2 together with the SUMO-conjugating enzyme Ubc9 are involved in SUMO conjugation of corresponding substrates [255]. The SUMOylated transcription factor Mot1 is slowly degraded via the Slx5–Slx8 system, but when mutated, degradation is rapid [254]. The responsible E2 enzymes involved in the ubiquitination of this substrate are Ubc4 and Ubc5. Canavanine, an arginine analog which causes generation of abnormal proteins, also induces rapid degradation even of the non mutated Mot1 [254].

Interestingly the polytopic ER membrane ligase Doa10 which is involved in the ERAD-C pathway and also in cytosolic misfolded protein degradation, in addition plays a role in degradation of a mutant form of the nuclear kinetochore protein Ndc10 [256], (Fig. 4C). This is possible because a portion of Doa10 also localizes to the inner nuclear envelope [257] and therefore is able to get in contact with mutated nuclear Ndc10-2. The degradation signal that is recognized by Doa10 is composed of a helical hydrophobic surface of an amphipathic helix and a hydrophobic C-terminal tail called DegAB [258,259]. The Hsp70 chaperone Ssa1 together with its cochaperone Ydj1 are essential in the Doa10 mediated degradation pathway of the Ndc10-2 mutant protein [259]. Recently it was shown that the Hsp40 chaperone Sis1 targets Ndc10-2 and even other proteins like GFP containing the DegAB degron to the Doa10 mediated ubiquitination machinery by facilitating substrate binding to Doa10 [50]. In mammals no San1 homolog with its characteristic structure has been discovered yet.

In contrast to San1 the mammalian nuclear E3 RING ligase URHF-2 does not possess disordered regions but acts as E3 enzyme in nuclear protein quality control [260]. It has been shown that UHRF-2 promotes the degradation of polyglutamine-expanded huntingtin (Htt) and therefore suppresses the cytotoxicity caused by Htt. Interestingly yeast San1 expressed in mammalian cells is able to accelerate the degradation of nuclear Htt like UHRF-2, raising the suspicion that another nucleus based degradation pathway may exist in higher eukaryotes [260].

In addition to the already mentioned compartmentalization of cytoplasmic protein quality control substrates it has been reported that polyQ-expansion proteins also accumulate in inclusions in neuronal nuclei, often observed in patients with Huntington's disease [261,262]. These inclusions often colocalize with so called PML bodies representing multiprotein complexes of the promyelocytic leukemia protein PML [263–266]. Clastosomes represent a subset of PML-bodies and are suggested to be the sites of protein degradation in the nucleus because they concentrate the components of the UPS [267]. The isoform PMLIV manages the formation of clastosomes by recruitment of polyQ proteins and UPS components and by this promotes degradation of these substrates [268].

5. Mitochondrial protein quality control

Mitochondria are organelles in eukaryotic cells which have emerged through endosymbiotic processes. The mitochondrion has diverse functions; among the most important is the generation of most of the cell's energy in the form of ATP. The organelle is surrounded by a double

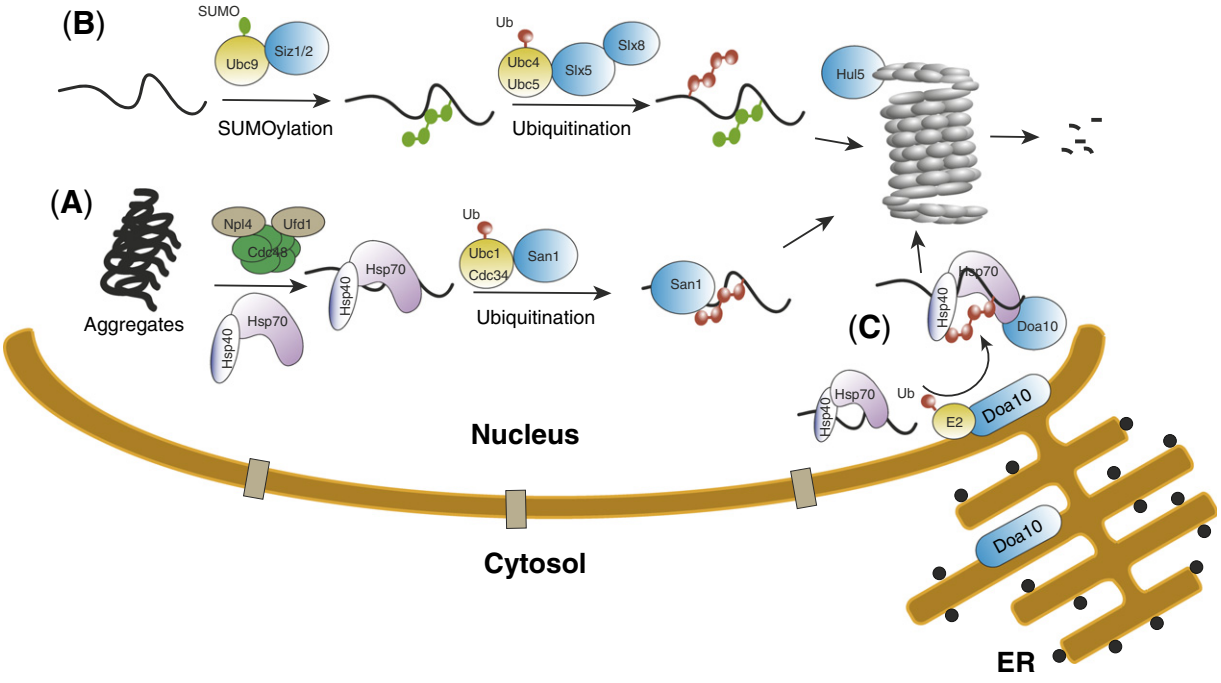

Fig. 4. Nuclear protein quality control and degradation. (A) Aberrant nuclear proteins are mainly recognized by the E3 ligase San1. (B) The Slx5–Slx8 SUMO targeted ubiquitin ligase ubiquitinates previously SUMOylated substrates for proteasomal degradation. (C) Also the E3 ligase Doa10, involved in the ERAD-C pathway, can take part in nuclear protein quality control as some Doa10 protein also localizes to the inner nuclear envelope therefore getting in contact with some nuclear substrates and causing their ubiquitination. For details see text.

membrane. Therefore the matrix proteins are not directly connected to the UPS. Due to production of reactive oxygen species (ROS) which can cause mitochondrial DNA damage and production of aberrant proteins, it is essential for the cell to possess a mitochondrial protein quality control system able to manage this oxidative stress. Mechanistically the mitochondrial system is closely related to bacterial systems due to the endosymbiontic origin of this organelle.

Translocation of newly synthesized mitochondrial proteins into the mitochondrial matrix and folding is dependent on the Hsp70 chaperone Ssc1 with its cochaperones Mdj1 and Mge1 which are related to the bacterial DnaK, DnaJ and GrpE species [269,270]. As in the case for the cytosolic counterpart Ssa1 with its cochaperones their main function is preventing protein aggregation. The chaperonin Hsp60 (homolog of GroEL in bacteria) together with the co-chaperonin Hsp10 promotes folding of substrates by providing a reaction space shielded from the environment [271]. The Hsp100 chaperone Hsp78 represents the yeast homolog of bacterial ClpB, possessing disaggregase activity similar to cytosolic Hsp104. All these species belong to the AAA-ATPase class of proteins [57,272]. The responsible protease for final degradation of misfolded and damaged mitochondrial matrix proteins in this protein quality control system is Pim1, the homolog of the bacterial LON protease [273–275], (Fig. 5A). The protease Pim1 is strongly induced under oxidative stress [276] indicating the high toxicity of oxidatively damaged proteins for the cell and therefore the necessity for their final degradation. In a recent study it was discovered that deletion of *PIM1* does not only negatively influence the turnover of mitochondrial proteins but also leads to a significant decrease of the activity of the proteasome in the cytosol, caused by accumulation of cytosolic aggregates of oxidized and ubiquitinated proteins [277]. These oxidized protein species are thought to act as proteasome inhibitors.

Inner mitochondrial membrane proteins are particularly heavily damaged by oxidative stress because of the proximity to the ROS generating respiratory chain complexes, therefore making an inner membrane located quality control system necessary. This system consists of the two ATP-consuming proteases *m*-AAA and *i*-AAA, both located in the inner membrane and responsible for degradation of inner membrane non-assembled and misfolded proteins respectively [278–281], (Fig. 5B).

Meanwhile there is evidence of the UPS being involved in mitochondrial protein quality control. Two different mechanisms have been discovered which mediate this connection. In response to oxidative stress in yeast, Vms1, a mitochondrial adaptor protein of Cdc48 and localized in the cytosol, recruits Cdc48/p97 together with its cofactor Npl4 to mitochondria where the Cdc48 complex provides the force for extracting and retrotranslocating damaged mitochondrial proteins to the cytosol for subsequent proteasomal degradation [282], (Fig. 5C). The other mode of proteasome action rests in proteasome recruitment to mitochondria in

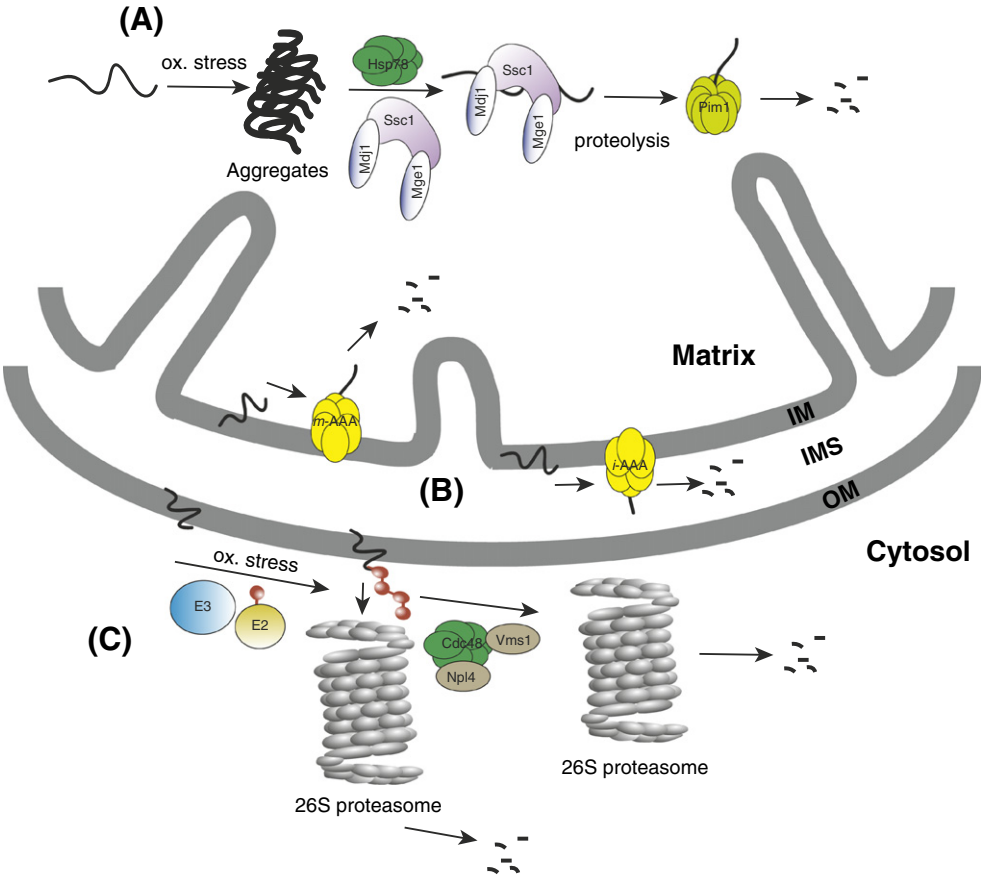

Fig. 5. Mitochondrial protein quality control and degradation. (A) Misfolded mitochondrial matrix proteins are chaperone dependently degraded by the heptameric protease Pim1. (B) An additional inner membrane located degradation machinery consisting of the 2 ATP dependent proteases, *m*-AAA and *i*-AAA, target misfolded inner membrane proteins for degradation. (C) Also the ubiquitin–proteasome system is involved in mitochondrial protein quality control. Some substrates can be retrotranslocated out of the mitochondria, similar to the ERAD pathway. The action of the Cdc48 machinery, but also both, extraction and degradation exerted by the proteasome, was observed for some substrates. For details see text.

mammalian cells where the proteasome extracts, binds and unfolds a previously ubiquitinated substrate directly [283], (Fig. 5C). This process was discovered for the short lived inner mitochondrial membrane protein UCP2 which is involved in development of many pathologies [283]. Even mitochondrial matrix proteins of mammalian cells can be connected to proteasomal degradation in the cytosol. The F1F0-ATPase subunit OSCP (oligomycin sensitivity conferring protein) is stabilized after proteasome inhibition in an ubiquitinated form in the outer mitochondrial membrane, also indicating the presence of a similar retrotranslocation system as found in ERAD [284]. The UPS is also involved in general maintenance of mitochondrial function by mediating degradation of major players of mitochondrial dynamics which – when present – can induce autophagosomal degradation of the whole organelle, called mitophagy [285–287]. Besides the function of the mammalian ubiquitin ligase PARKIN in ubiquitination of the unfolded ER membrane protein PaeI and its role in ER stress induced cell death [288,289], PARKIN together with a kinase called PINK is recruited to damaged mitochondria and ubiquitinates proteins of the outer mitochondrial membrane which function in fusion/fission events and therefore regulate mitophagy [290–292]. Another E3 ligase in mammals called MITOL, and located in the outer mitochondrial membrane is also involved in regulation of mitochondrial fission events through ubiquitination [293]. Also, MITOL directly ubiquitinates misfolded superoxide dismutase 1 (mSOD1) which is involved in familial amyotrophic lateral sclerosis (ALS) [294,295].

6. Conclusions and perspectives

When comparing our knowledge of the mechanistic aspects of the ubiquitin–proteasome system in protein quality control in the different cellular compartments it seems obvious that this knowledge is most advanced in the case of ERAD. However, also here many crucial questions remain: What drives retrotranslocation of proteins across the ER membrane? Lumenal proteins may be partially folded, many contain carbohydrate. How are they threaded into a channel of the ER membrane (if retrotranslocation occurs through a channel)? Is there energy required? Do all misfolded proteins cross the ER membrane via one and the same mechanism or are there different devices for different proteins to escape? How does the Cdc48 machine handle the polyubiquitinated substrates, what is the mechanism? How are the different unfolded proteins with their partly hydrophobic amino acid stretches safely delivered to the proteasome without aggregation and precipitation in the cytoplasm?

At first glance the recognition, delivery and degradation of terminally misfolded proteins seem to be a simple affair. However the multiple and diverse degradation pathways visible already in the different cell compartments lead to the suspicion that we have just scratched the surface. We may suspect that many more chaperones, co-chaperones, recruiting factors, ubiquitin ligases, etc. will be found when we dive deeper into the subject. Why is the machinery so diverse and sophisticated when just hydrophobic patches on the surface of a protein may be the major sign of misfolding and the recognition site for starting the elimination process? Obviously, as in cellular regulation, a diversified and on many levels of protein recognition and delivery to the proteasome fine-tuned process furnishes the cell with the best possibilities to react to the many different challenges posed by protein misfolding. We still have to learn and understand.

Acknowledgments

Work of the authors was supported by the Deutsche Forschungsgemeinschaft, Bonn and the European network of excellence RUBICON.

References

[1] B. Alberts, Molecular biology of the cell, Fifth ed. Garland Science, New York, 2008.
[2] A.I. Bartlett, S.E. Radford, An expanding arsenal of experimental methods yields an explosion of insights into protein folding mechanisms, Nat. Struct. Mol. Biol. 16 (2009) 582–588.
[3] C.M. Dobson, A. Sali, M. Kaplus, Protein folding: a perspective from theory and experiment, Angew. Chem. Int. Ed. Engl. 37 (1998) 868–893.
[4] J.M. Berg, J.L. Tymoczko, L. Stryer, Biochemistry, seventh ed., Freeman, New York, 2012.
[5] F.U. Hartl, Molecular chaperones in cellular protein folding, Nature 381 (1996) 571–579.
[6] F.U. Hartl, M. Hayer-Hartl, Converging concepts of protein folding *in vitro* and *in vivo*, Nat. Struct. Mol. Biol. 16 (2009) 574–581.
[7] F.U. Hartl, M. Hayer-Hartl, Molecular chaperones in the cytosol: from nascent chain to folded protein, Science 295 (2002) 1852–1858.
[8] T.R. Jahn, S.E. Radford, The Yin and Yang of protein folding, FEBS J. 272 (2005) 5962–5970.
[9] J. Tyedmers, A. Mogk, B. Bukau, Cellular strategies for controlling protein aggregation, Nat. Rev. Mol. Cell Biol. 11 (2010) 777–788.
[10] F.U. Hartl, A. Bracher, M. Hayer-Hartl, Molecular chaperones in protein folding and proteostasis, Nature 475 (2011) 324–332.
[11] F. Chiti, C.M. Dobson, Protein misfolding, functional amyloid, and human disease, Annu. Rev. Biochem. 75 (2006) 333–366.
[12] R. Kayed, E. Head, J.L. Thompson, T.M. McIntire, S.C. Milton, C.W. Cotman, C.G. Glabe, Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis, Science 300 (2003) 486–489.
[13] A. Ben-Zvi, E.A. Miller, R.I. Morimoto, Collapse of proteostasis represents an early molecular event in *Caenorhabditis elegans* aging, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 14914–14919.
[14] C.A. Ross, M.A. Poirier, Protein aggregation and neurodegenerative disease, Nat. Med. 10 (2004) S10–S17, (Suppl.).
[15] M. Akerfelt, R.I. Morimoto, L. Sistonen, Heat shock factors: integrators of cell stress, development and lifespan, Nat. Rev. Mol. Cell Biol. 11 (2010) 545–555.
[16] C. Voisine, J.S. Pedersen, R.I. Morimoto, Chaperone networks: tipping the balance in protein folding diseases, Neurobiol. Dis. 40 (2010) 12–20.
[17] E.K. Fredrickson, R.G. Gardner, Selective destruction of abnormal proteins by ubiquitin-mediated protein quality control degradation, Semin. Cell Dev. Biol. 23 (2012) 530–537.
[18] C. He, D.J. Klionsky, Regulation mechanisms and signaling pathways of autophagy, Annu. Rev. Genet. 43 (2009) 67–93.
[19] V. Kirkin, D.G. McEwan, I. Novak, I. Dikic, A role for ubiquitin in selective autophagy, Mol. Cell. 34 (2009) 259–269.
[20] H. Nakatogawa, K. Suzuki, Y. Kamada, Y. Ohsumi, Dynamics and diversity in autophagy mechanisms: lessons from yeast, Nat. Rev. Mol. Cell Biol. 10 (2009) 458–467.
[21] A. Stolz, D.H. Wolf, Endoplasmic reticulum associated protein degradation: a chaperone assisted journey to hell, Biochim. Biophys. Acta 1803 (2010) 694–705.
[22] C. Esser, S. Alberti, J. Hohfeld, Cooperation of molecular chaperones with the ubiquitin/proteasome system, Biochim. Biophys. Acta 1695 (2004) 171–188.
[23] W. Heinemeyer, J.A. Kleinschmidt, J. Saidowsky, C. Escher, D.H. Wolf, Proteinase yscE, the yeast proteasome/multicatalytic-multifunctional proteinase: mutants unravel its function in stress induced proteolysis and uncover its necessity for cell survival, EMBO J. 10 (1991) 555–562.
[24] D.H. Wolf, W. Hilt, The proteasome: a proteolytic nanomachine of cell regulation and waste disposal, Biochim. Biophys. Acta 1695 (2004) 19–31.
[25] Y. Kravtsova-Ivantsiv, T. Sommer, A. Ciechanover, The lysine48-based polyubiquitin chain proteasomal signal: not a single child anymore, Angew. Chem. Int. Ed. Engl. 52 (2013) 192–198.
[26] L.N. Dimitrova, K. Kuroha, T. Tatematsu, T. Inada, Nascent peptide-dependent translation arrest leads to Not4p-mediated protein degradation by the proteasome, J. Biol. Chem. 284 (2009) 10343–10352.
[27] N. Akimitsu, Messenger RNA surveillance systems monitoring proper translation termination, J. Biochem. 143 (2008) 1–8.
[28] S. Ito-Harashima, K. Kuroha, T. Tatematsu, T. Inada, Translation of the poly(A) tail plays crucial roles in nonstop mRNA surveillance via translation repression and protein destabilization by proteasome in yeast, Genes Dev. 21 (2007) 519–524.
[29] M. Alamgir, V. Erukova, M. Jessulat, A. Azizi, A. Golshani, Chemical–genetic profile analysis of five inhibitory compounds in yeast, BMC Chem. Biol. 10 (2010) 6.
[30] O. Brandman, J. Stewart-Ornstein, D. Wong, A. Larson, C.C. Williams, G.W. Li, S. Zhou, D. King, P.S. Shen, J. Weibezahn, J.G. Dunn, S. Rouskin, T. Inada, A. Frost, J.S. Weissman, A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress, Cell 151 (2012) 1042–1054.
[31] A. Stolz, W. Hilt, A. Buchberger, D.H. Wolf, Cdc48: a power machine in protein degradation, Trends Biochem. Sci. 36 (2011) 515–523.
[32] M.H. Bengtson, C.A. Joazeiro, Role of a ribosome-associated E3 ubiquitin ligase in protein quality control, Nature 467 (2010) 470–473.
[33] M.A. Braun, P.J. Costa, E.M. Crisucci, K.M. Arndt, Identification of Rkr1, a nuclear RING domain protein with functional connections to chromatin modification in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 27 (2007) 2800–2811.
[34] J. Lu, C. Deutsch, Electrostatics in the ribosomal tunnel modulate chain elongation rates, J. Mol. Biol. 384 (2008) 73–86.
[35] S. Duttler, S. Pechmann, J. Frydman, Principles of cotranslational ubiquitination and quality control at the ribosome, Mol. Cell. 50 (2013) 379–393.

[36] M.A. Collart, O.O. Panasenko, The Ccr4–not complex, Gene 492 (2012) 42–53.
[37] S. Rospert, Y. Dubaquie, M. Gautschi, Nascent-polypeptide-associated complex, Cell Mol. Life Sci. 59 (2002) 1632–1639.
[38] B. Wiedmann, H. Sakai, T.A. Davis, M. Wiedmann, A protein complex required for signal-sequence-specific sorting and translocation, Nature 370 (1994) 434–440.
[39] E.A. Craig, H.C. Eisenman, H.A. Hundley, Ribosome-tethered molecular chaperones: the first line of defense against protein misfolding? Curr. Opin. Microbiol. 6 (2003) 157–162.
[40] H.A. Hundley, W. Walter, S. Bairstow, E.A. Craig, Human Mpp11 J protein: ribosome-tethered molecular chaperones are ubiquitous, Science 308 (2005) 1032–1034.
[41] C. Conz, H. Otto, K. Peisker, M. Gautschi, T. Wolfle, M.P. Mayer, S. Rospert, Functional characterization of the atypical Hsp70 subunit of yeast ribosome-associated complex, J. Biol. Chem. 282 (2007) 33977–33984.
[42] P. Huang, M. Gautschi, W. Walter, S. Rospert, E.A. Craig, The Hsp70 Ssz1 modulates the function of the ribosome-associated J-protein Zuo1, Nat. Struct. Mol. Biol. 12 (2005) 497–504.
[43] S. Preissler, E. Deuerling, Ribosome-associated chaperones as key players in proteostasis, Trends Biochem. Sci. 37 (2012) 274–283.
[44] H. Olzscha, S.M. Schermann, A.C. Woerner, S. Pinkert, M.H. Hecht, G.G. Tartaglia, M. Vendruscolo, M. Hayer-Hartl, F.U. Hartl, R.M. Vabulas, Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions, Cell 144 (2011) 67–78.
[45] M.P. Mayer, Gymnastics of molecular chaperones, Mol. Cell. 39 (2010) 321–331.
[46] H.H. Kampinga, E.A. Craig, The HSP70 chaperone machinery: J proteins as drivers of functional specificity, Nat. Rev. Mol. Cell Biol. 11 (2010) 579–592.
[47] A.J. McClellan, M.D. Scott, J. Frydman, Folding and quality control of the VHL tumor suppressor proceed through distinct chaperone pathways, Cell 121 (2005) 739–748.
[48] S.H. Park, N. Bolender, F. Eisele, Z. Kostova, J. Takeuchi, P. Coffino, D.H. Wolf, The cytoplasmic Hsp70 chaperone machinery subjects misfolded and endoplasmic reticulum import-incompetent proteins to degradation via the ubiquitin–proteasome system, Mol. Biol. Cell 18 (2007) 153–165.
[49] J. Becker, W. Walter, W. Yan, E.A. Craig, Functional interaction of cytosolic hsp70 and a DnaJ-related protein, Ydj1p, in protein translocation *in vivo*, Mol. Cell. Biol. 16 (1996) 4378–4386.
[50] A. Shiber, W. Breuer, M. Brandeis, T. Ravid, Ubiquitin conjugation triggers misfolded protein sequestration into quality-control foci when Hsp70 chaperone levels are limiting, Mol. Biol. Cell 24 (2013) 2076–2087.
[51] D.A. Parsell, A.S. Kowal, M.A. Singer, S. Lindquist, Protein disaggregation mediated by heat-shock protein Hsp104, Nature 372 (1994) 475–478.
[52] J.R. Glover, S. Lindquist, Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins, Cell 94 (1998) 73–82.
[53] P. Goloubinoff, A. Mogk, A.P. Zvi, T. Tomoyasu, B. Bukau, Sequential mechanism of solubilization and refolding of stable protein aggregates by a bichaperone network, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 13732–13737.
[54] J. Winkler, J. Tyedmers, B. Bukau, A. Mogk, Hsp70 targets Hsp100 chaperones to substrates for protein disaggregation and prion fragmentation, J. Cell Biol. 198 (2012) 387–404.
[55] S. Lindquist, G. Kim, Heat-shock protein 104 expression is sufficient for thermotolerance in yeast, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 5301–5306.
[56] S. Diamant, A.P. Ben-Zvi, B. Bukau, P. Goloubinoff, Size-dependent disaggregation of stable protein aggregates by the DnaK chaperone machinery, J. Biol. Chem. 275 (2000) 21107–21113.
[57] S.M. Doyle, S. Wickner, Hsp104 and ClpB: protein disaggregating machines, Trends Biochem. Sci. 34 (2009) 40–48.
[58] Z. Dragovic, S.A. Broadley, Y. Shomura, A. Bracher, F.U. Hartl, Molecular chaperones of the Hsp110 family act as nucleotide exchange factors of Hsp70s, EMBO J. 25 (2006) 2519–2528.
[59] H. Rampelt, J. Kirstein-Miles, N.B. Nillegoda, K. Chi, S.R. Scholz, R.I. Morimoto, B. Bukau, Metazoan Hsp70 machines use Hsp110 to power protein disaggregation, EMBO J. 31 (2012) 4221–4235.
[60] H. Raviol, H. Sadlish, F. Rodriguez, M.P. Mayer, B. Bukau, Chaperone network in the yeast cytosol: Hsp110 is revealed as an Hsp70 nucleotide exchange factor, EMBO J. 25 (2006) 2510–2518.
[61] B.T. Lai, N.W. Chin, A.E. Stanek, W. Keh, K.W. Lanks, Quantitation and intracellular localization of the 85 K heat shock protein by using monoclonal and polyclonal antibodies, Mol. Cell. Biol. 4 (1984) 2802–2810.
[62] S.K. Wandinger, K. Richter, J. Buchner, The Hsp90 chaperone machinery, J. Biol. Chem. 283 (2008) 18473–18477.
[63] J. Li, J. Soroka, J. Buchner, The Hsp90 chaperone machinery: conformational dynamics and regulation by co-chaperones, Biochim. Biophys. Acta 1823 (2012) 624–635.
[64] L. Whitesell, S.L. Lindquist, HSP90 and the chaperoning of cancer, Nat. Rev. Cancer 5 (2005) 761–772.
[65] J. Trepel, M. Mollapour, G. Giaccone, L. Neckers, Targeting the dynamic HSP90 complex in cancer, Nat. Rev. Cancer 10 (2010) 537–549.
[66] M. Taipale, D.F. Jarosz, S. Lindquist, HSP90 at the hub of protein homeostasis: emerging mechanistic insights, Nat. Rev. Mol. Cell Biol. 11 (2010) 515–528.
[67] U. Jakob, H. Lilie, I. Meyer, J. Buchner, Transient interaction of Hsp90 with early unfolding intermediates of citrate synthase. Implications for heat shock *in vivo*, J. Biol. Chem. 270 (1995) 7288–7294.
[68] R.J. Schumacher, W.J. Hansen, B.C. Freeman, E. Alnemri, G. Litwack, D.O. Toft, Cooperative action of Hsp70, Hsp90, and DnaJ proteins in protein renaturation, Biochemistry 35 (1996) 14889–14898.
[69] B.C. Freeman, R.I. Morimoto, The human cytosolic molecular chaperones hsp90, hsp70 (hsc70) and hdj-1 have distinct roles in recognition of a non-native protein and protein refolding, EMBO J. 15 (1996) 2969–2979.
[70] M. Haslbeck, N. Braun, T. Stromer, B. Richter, N. Model, S. Weinkauf, J. Buchner, Hsp42 is the general small heat shock protein in the cytosol of *Saccharomyces cerevisiae*, EMBO J. 23 (2004) 638–649.
[71] S. Specht, S.B. Miller, A. Mogk, B. Bukau, Hsp42 is required for sequestration of protein aggregates into deposition sites in *Saccharomyces cerevisiae*, J. Cell Biol. 195 (2011) 617–629.
[72] M. Haslbeck, A. Miess, T. Stromer, S. Walter, J. Buchner, Disassembling protein aggregates in the yeast cytosol. The cooperation of Hsp26 with Ssa1 and Hsp104, J. Biol. Chem. 280 (2005) 23861–23868.
[73] A.G. Cashikar, M. Duennwald, S.L. Lindquist, A chaperone pathway in protein disaggregation. Hsp26 alters the nature of protein aggregates to facilitate reactivation by Hsp104, J. Biol. Chem. 280 (2005) 23869–23875.
[74] C.A. Ballinger, P. Connell, Y. Wu, Z. Hu, L.J. Thompson, L.Y. Yin, C. Patterson, Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions, Mol. Cell. Biol. 19 (1999) 4535–4545.
[75] J. Jiang, C.A. Ballinger, Y. Wu, Q. Dai, D.M. Cyr, J. Hohfeld, C. Patterson, CHIP is a U-box-dependent E3 ubiquitin ligase: identification of Hsc70 as a target for ubiquitylation, J. Biol. Chem. 276 (2001) 42938–42944.
[76] M.F. Rosser, E. Washburn, P.J. Muchowski, C. Patterson, D.M. Cyr, Chaperone functions of the E3 ubiquitin ligase CHIP, J. Biol. Chem. 282 (2007) 22267–22277.
[77] J. Luders, J. Demand, J. Hohfeld, The ubiquitin-related BAG-1 provides a link between the molecular chaperones Hsc70/Hsp70 and the proteasome, J. Biol. Chem. 275 (2000) 4613–4617.
[78] Q. Dai, S.B. Qian, H.H. Li, H. McDonough, C. Borchers, D. Huang, S. Takayama, J.M. Younger, H.Y. Ren, D.M. Cyr, C. Patterson, Regulation of the cytoplasmic quality control protein degradation pathway by BAG2, J. Biol. Chem. 280 (2005) 38673–38681.
[79] L.V. Kalia, S.K. Kalia, H. Chau, A.M. Lozano, B.T. Hyman, P.J. McLean, Ubiquitinylation of alpha-synuclein by carboxyl terminus Hsp70-interacting protein (CHIP) is regulated by Bcl-2-associated athanogene 5 (BAG5), PLoS One 6 (2011) e14695.
[80] M. Gamerdinger, P. Hajieva, A.M. Kaya, U. Wolfrum, F.U. Hartl, C. Behl, Protein quality control during aging involves recruitment of the macroautophagy pathway by BAG3, EMBO J. 28 (2009) 889–901.
[81] S. Alberti, K. Bohse, V. Arndt, A. Schmitz, J. Hohfeld, The cochaperone HspBP1 inhibits the CHIP ubiquitin ligase and stimulates the maturation of the cystic fibrosis transmembrane conductance regulator, Mol. Biol. Cell 15 (2004) 4003–4010.
[82] F. Eisele, D.H. Wolf, Degradation of misfolded protein in the cytoplasm is mediated by the ubiquitin ligase Ubr1, FEBS Lett. 582 (2008) 4143–4146.
[83] N.B. Nillegoda, M.A. Theodoraki, A.K. Mandal, K.J. Mayo, H.Y. Ren, R. Sultana, K. Wu, J. Johnson, D.M. Cyr, A.J. Caplan, Ubr1 and Ubr2 function in a quality control pathway for degradation of unfolded cytosolic proteins, Mol. Biol. Cell 21 (2010) 2102–2116.
[84] J.W. Heck, S.K. Cheung, R.Y. Hampton, Cytoplasmic protein quality control degradation mediated by parallel actions of the E3 ubiquitin ligases Ubr1 and San1, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 1106–1111.
[85] B. Bartel, I. Wunning, A. Varshavsky, The recognition component of the N-end rule pathway, EMBO J. 9 (1990) 3179–3189.
[86] A. Varshavsky, The N-end rule pathway and regulation by proteolysis, Protein Sci. 20 (2011) 1298–1345.
[87] D.W. Summers, K.J. Wolfe, H.Y. Ren, D.M. Cyr, The Type II Hsp40 Sis1 cooperates with Hsp70 and the E3 ligase Ubr1 to promote degradation of terminally misfolded cytosolic protein, PLoS One 8 (2013) e52099.
[88] N.K. Gowda, G. Kandasamy, M.S. Froehlich, R.J. Dohmen, C. Andreasson, Hsp70 nucleotide exchange factor Fes1 is essential for ubiquitin-dependent degradation of misfolded cytosolic proteins, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 5975–5980.
[89] C. Byrd, G.C. Turner, A. Varshavsky, The N-end rule pathway controls the import of peptides through degradation of a transcriptional repressor, EMBO J. 17 (1998) 269–277.
[90] L. Wang, X. Mao, D. Ju, Y. Xie, Rpn4 is a physiological substrate of the Ubr2 ubiquitin ligase, J. Biol. Chem. 279 (2004) 55218–55223.
[91] D. Ju, Y. Xie, Proteasomal degradation of RPN4 via two distinct mechanisms, ubiquitin-dependent and -independent, J. Biol. Chem. 279 (2004) 23851–23854.
[92] G. Mannhaupt, R. Schnall, V. Karpov, I. Vetter, H. Feldmann, Rpn4p acts as a transcription factor by binding to PACE, a nonamer box found upstream of 26S proteasomal and other genes in yeast, FEBS Lett. 450 (1999) 27–34.
[93] M.K. London, B.I. Keck, P.C. Ramos, R.J. Dohmen, Regulatory mechanisms controlling biogenesis of ubiquitin and the proteasome, FEBS Lett. 567 (2004) 259–264.
[94] R.G. Gardner, Z.W. Nelson, D.E. Gottschling, Degradation-mediated protein quality control in the nucleus, Cell 120 (2005) 803–815.
[95] R. Prasad, S. Kawaguchi, D.T. Ng, A nucleus-based quality control mechanism for cytosolic proteins, Mol. Biol. Cell 21 (2010) 2117–2127.
[96] F. Khosrow-Khavar, N.N. Fang, A.H. Ng, J.M. Winget, S.A. Comyn, T. Mayor, The yeast ubr1 ubiquitin ligase participates in a prominent pathway that targets cytosolic thermosensitive mutants for degradation, G3 (Bethesda) 2 (2012) 619–628.
[97] C.J. Guerriero, K.F. Weiberth, J.L. Brodsky, Hsp70 targets a cytoplasmic quality control substrate to the San1p ubiquitin ligase, J. Biol. Chem. 288 (2013) 18506–18520.
[98] S.J. Russell, K.A. Steger, S.A. Johnston, Subcellular localization, stoichiometry, and protein levels of 26S proteasome subunits in yeast, J. Biol. Chem. 274 (1999) 21943–21952.

[99] T. Gilon, O. Chomsky, R.G. Kulka, Degradation signals for ubiquitin system proteolysis in *Saccharomyces cerevisiae*, EMBO J. 17 (1998) 2759–2766.
[100] T. Gilon, O. Chomsky, R.G. Kulka, Degradation signals recognized by the Ubc6p–Ubc7p ubiquitin-conjugating enzyme pair, Mol. Cell. Biol. 20 (2000) 7214–7219.
[101] M.B. Metzger, M.J. Maurer, B.M. Dancy, S. Michaelis, Degradation of a cytosolic protein requires endoplasmic reticulum-associated degradation machinery, J. Biol. Chem. 283 (2008) 32302–32316.
[102] C.S. Hwang, A. Shemorry, A. Varshavsky, N-terminal acetylation of cellular proteins creates specific degradation signals, Science 327 (2010) 973–977.
[103] S. Aviram, D. Kornitzer, The ubiquitin ligase Hul5 promotes proteasomal processivity, Mol. Cell. Biol. 30 (2010) 985–994.
[104] B. Crosas, J. Hanna, D.S. Kirkpatrick, D.P. Zhang, Y. Tone, N.A. Hathaway, C. Buecker, D.S. Leggett, M. Schmidt, R.W. King, S.P. Gygi, D. Finley, Ubiquitin chains are remodeled at the proteasome by opposing ubiquitin ligase and deubiquitinating activities, Cell 127 (2006) 1401–1413.
[105] M. Koegl, T. Hoppe, S. Schlenker, H.D. Ulrich, T.U. Mayer, S. Jentsch, A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly, Cell 96 (1999) 635–644.
[106] S. Kohlmann, A. Schafer, D.H. Wolf, Ubiquitin ligase Hul5 is required for fragment-specific substrate degradation in endoplasmic reticulum-associated degradation, J. Biol. Chem. 283 (2008) 16374–16383.
[107] N.N. Fang, A.H. Ng, V. Measday, T. Mayor, Hul5 HECT ubiquitin ligase plays a major role in the ubiquitylation and turnover of cytosolic misfolded proteins, Nat. Cell Biol. 13 (2011) 1344–1352.
[108] D. Kaganovich, R. Kopito, J. Frydman, Misfolded proteins partition between two distinct quality control compartments, Nature 454 (2008) 1088–1095.
[109] T. Yorimitsu, D.J. Klionsky, Autophagy: molecular machinery for self-eating, Cell Death Differ. 12 (Suppl. 2) (2005) 1542–1552.
[110] Y. Kabeya, N. Mizushima, T. Ueno, A. Yamamoto, T. Kirisako, T. Noda, E. Kominami, Y. Ohsumi, T. Yoshimori, LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing, EMBO J. 19 (2000) 5720–5728.
[111] A. Kuma, M. Matsui, N. Mizushima, LC3, an autophagosome marker, can be incorporated into protein aggregates independent of autophagy: caution in the interpretation of LC3 localization, Autophagy 3 (2007) 323–328.
[112] H. Nakatogawa, Y. Ichimura, Y. Ohsumi, Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion, Cell 130 (2007) 165–178.
[113] M.A. Rujano, F. Bosveld, F.A. Salomons, F. Dijk, M.A. van Waarde, J.J. van der Want, R.A. de Vos, E.R. Brunt, O.C. Sibon, H.H. Kampinga, Polarised asymmetric inheritance of accumulated protein damage in higher eukaryotes, PLoS Biol. 4 (2006) e417.
[114] G. Bjorkoy, T. Lamark, A. Brech, H. Outzen, M. Perander, A. Overvatn, H. Stenmark, T. Johansen, p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death, J. Cell Biol. 171 (2005) 603–614.
[115] S. Pankiv, T.H. Clausen, T. Lamark, A. Brech, J.A. Bruun, H. Outzen, A. Overvatn, G. Bjorkoy, T. Johansen, p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy, J. Biol. Chem. 282 (2007) 24131–24145.
[116] V. Kirkin, T. Lamark, Y.S. Sou, G. Bjorkoy, J.L. Nunn, J.A. Bruun, E. Shvets, D.G. McEwan, T.H. Clausen, P. Wild, I. Bilusic, J.P. Theurillat, A. Overvatn, T. Ishii, Z. Elazar, M. Komatsu, I. Dikic, T. Johansen, A role for NBR1 in autophagosomal degradation of ubiquitinated substrates, Mol. Cell 33 (2009) 505–516.
[117] N.N. Noda, H. Kumeta, H. Nakatogawa, K. Satoo, W. Adachi, J. Ishii, Y. Fujioka, Y. Ohsumi, F. Inagaki, Structural basis of target recognition by Atg8/LC3 during selective autophagy, Genes Cells 13 (2008) 1211–1218.
[118] Y. Ichimura, T. Kumanomidou, Y.S. Sou, T. Mizushima, J. Ezaki, T. Ueno, E. Kominami, T. Yamane, K. Tanaka, M. Komatsu, Structural basis for sorting mechanism of p62 in selective autophagy, J. Biol. Chem. 283 (2008) 22847–22857.
[119] J.A. Johnston, C.L. Ward, R.R. Kopito, Aggresomes: a cellular response to misfolded proteins, J. Cell Biol. 143 (1998) 1883–1898.
[120] U.B. Pandey, Z. Nie, Y. Batlevi, B.A. McCray, G.P. Ritson, N.B. Nedelsky, S.L. Schwartz, N.A. DiProspero, M.A. Knight, O. Schuldiner, R. Padmanabhan, M. Hild, D.L. Berry, D. Garza, C.C. Hubbert, T.P. Yao, E.H. Baehrecke, J.P. Taylor, HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS, Nature 447 (2007) 859–863.
[121] R.R. Kopito, Aggresomes, inclusion bodies and protein aggregation, Trends Cell Biol. 10 (2000) 524–530.
[122] J.A. Johnston, M.E. Illing, R.R. Kopito, Cytoplasmic dynein/dynactin mediates the assembly of aggresomes, Cell Motil. Cytoskeleton 53 (2002) 26–38.
[123] Y. Kawaguchi, J.J. Kovacs, A. McLaurin, J.M. Vance, A. Ito, T.P. Yao, The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress, Cell 115 (2003) 727–738.
[124] M. Gamerdinger, A.M. Kaya, U. Wolfrum, A.M. Clement, C. Behl, BAG3 mediates chaperone-based aggresome-targeting and selective autophagy of misfolded proteins, EMBO Rep. 12 (2011) 149–156.
[125] J.A. Olzmann, L. Li, M.V. Chudaev, J. Chen, F.A. Perez, R.D. Palmiter, L.S. Chin, Parkin-mediated K63-linked polyubiquitination targets misfolded DJ-1 to aggresomes via binding to HDAC6, J. Cell Biol. 178 (2007) 1025–1038.
[126] M. Zhang, M. Windheim, S.M. Roe, M. Peggie, P. Cohen, C. Prodromou, L.H. Pearl, Chaperoned ubiquitylation–crystal structures of the CHIP U box E3 ubiquitin ligase and a CHIP-Ubc13–Uev1a complex, Mol. Cell 20 (2005) 525–538.
[127] Y. Zhang, J. Gao, K.K. Chung, H. Huang, V.L. Dawson, T.M. Dawson, Parkin functions as an E2-dependent ubiquitin–protein ligase and promotes the degradation of the synaptic vesicle-associated protein, CDCrel-1, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 13354–13359.
[128] N.G. Haigh, A.E. Johnson, Protein sorting at the membrane of the endoplasmic reticulum, in: R. Dalbey, G. Von Heijne (Eds.), Protein Targeting, Transport, and Translocation, Academic Press, London, 2002, pp. 74–106.
[129] T.A. Rapoport, Protein translocation across the eukaryotic endoplasmic reticulum and bacterial plasma membranes, Nature 450 (2007) 663–669.
[130] K. Araki, K. Nagata, Protein folding and quality control in the ER, Cold Spring Harb. Perspect. Biol. 4 (2012) a015438.
[131] E. van Anken, I. Braakman, Versatility of the endoplasmic reticulum protein folding factory, Crit. Rev. Biochem. Mol. Biol. 40 (2005) 191–228.
[132] B.R. Pearse, D.N. Hebert, Lectin chaperones help direct the maturation of glycoproteins in the endoplasmic reticulum, Biochim. Biophys. Acta 1803 (2010) 684–693.
[133] A. Helenius, M. Aebi, Roles of N-linked glycans in the endoplasmic reticulum, Annu. Rev. Biochem. 73 (2004) 1019–1049.
[134] M. Aebi, R. Bernasconi, S. Clerc, M. Molinari, N-glycan structures: recognition and processing in the ER, Trends Biochem. Sci. 35 (2010) 74–82.
[135] R.B. Freedman, T.R. Hirst, M.F. Tuite, Protein disulphide isomerase: building bridges in protein folding, Trends Biochem. Sci. 19 (1994) 331–336.
[136] B.P. Tu, J.S. Weissman, Oxidative protein folding in eukaryotes: mechanisms and consequences, J. Cell Biol. 164 (2004) 341–346.
[137] C. Appenzeller-Herzog, L. Ellgaard, The human PDI family: versatility packed into a single fold, Biochim. Biophys. Acta 1783 (2008) 535–548.
[138] I. Braakman, N.J. Bulleid, Protein folding and modification in the mammalian endoplasmic reticulum, Annu. Rev. Biochem. 80 (2011) 71–99.
[139] M. Schroder, R.J. Kaufman, The mammalian unfolded protein response, Annu. Rev. Biochem. 74 (2005) 739–789.
[140] A. Korennykh, P. Walter, Structural basis of the unfolded protein response, Annu. Rev. Cell Dev. Biol. 28 (2012) 251–277.
[141] P. Walter, D. Ron, The unfolded protein response: from stress pathway to homeostatic regulation, Science 334 (2011) 1081–1086.
[142] P. Kimmig, M. Diaz, J. Zheng, C.C. Williams, A. Lang, T. Aragon, H. Li, P. Walter, The unfolded protein response in fission yeast modulates stability of select mRNAs to maintain protein homeostasis, Elife 1 (2012) e00048.
[143] C.J. Guerriero, J.L. Brodsky, The delicate balance between secreted protein folding and endoplasmic reticulum-associated degradation in human physiology, Physiol. Rev. 92 (2012) 537–576.
[144] D.N. Hebert, M. Molinari, In and out of the ER: protein folding, quality control, degradation, and related human diseases, Physiol. Rev. 87 (2007) 1377–1408.
[145] R. Bernasconi, M. Molinari, ERAD and ERAD tuning: disposal of cargo and of ERAD regulators from the mammalian ER, Curr. Opin. Cell Biol. 23 (2011) 176–183.
[146] J.L. Brodsky, A.A. McCracken, ER protein quality control and proteasome-mediated protein degradation, Semin. Cell Dev. Biol. 10 (1999) 507–513.
[147] A. Buchberger, B. Bukau, T. Sommer, Protein quality control in the cytosol and the endoplasmic reticulum: brothers in arms, Mol. Cell. 40 (2010) 238–252.
[148] J.H. Claessen, L. Kundrat, H.L. Ploegh, Protein quality control in the ER: balancing the ubiquitin checkbook, Trends Cell Biol. 22 (2012) 22–32.
[149] R.Y. Hampton, T. Sommer, Finding the will and the way of ERAD substrate retrotranslocation, Curr. Opin. Cell Biol. 24 (2012) 460–466.
[150] Z. Kostova, D.H. Wolf, For whom the bell tolls: protein quality control of the endoplasmic reticulum and the ubiquitin–proteasome connection, EMBO J. 22 (2003) 2309–2317.
[151] B. Meusser, C. Hirsch, E. Jarosch, T. Sommer, ERAD: the long road to destruction, Nat. Cell Biol. 7 (2005) 766–772.
[152] R.K. Plemper, D.H. Wolf, Retrograde protein translocation: ERADication of secretory proteins in health and disease, Trends Biochem. Sci. 24 (1999) 266–270.
[153] T. Sommer, D.H. Wolf, Endoplasmic reticulum degradation: reverse protein flow of no return, FASEB J. 11 (1997) 1227–1233.
[154] S.S. Vembar, J.L. Brodsky, One step at a time: endoplasmic reticulum-associated degradation, Nat. Rev. Mol. Cell Biol. 9 (2008) 944–957.
[155] M.M. Hiller, A. Finger, M. Schweiger, D.H. Wolf, ER degradation of a misfolded luminal protein by the cytosolic ubiquitin–proteasome pathway, Science 273 (1996) 1725–1728.
[156] E.D. Werner, J.L. Brodsky, A.A. McCracken, Proteasome-dependent endoplasmic reticulum-associated protein degradation: an unconventional route to a familiar fate, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 13797–13801.
[157] E.J. Wiertz, D. Tortorella, M. Bogyo, J. Yu, W. Mothes, T.R. Jones, T.A. Rapoport, H.L. Ploegh, Sec61-mediated transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction, Nature 384 (1996) 432–438.
[158] R.Y. Hampton, R.G. Gardner, J. Rine, Role of 26S proteasome and HRD genes in the degradation of 3-hydroxy-3-methylglutaryl-CoA reductase, an integral endoplasmic reticulum membrane protein, Mol. Biol. Cell 7 (1996) 2029–2044.
[159] R.K. Plemper, S. Bohmler, J. Bordallo, T. Sommer, D.H. Wolf, Mutant analysis links the translocon and BiP to retrograde protein transport for ER degradation, Nature 388 (1997) 891–895.
[160] J.L. Brodsky, E.D. Werner, M.E. Dubas, J.L. Goeckeler, K.B. Kruse, A.A. McCracken, The requirement for molecular chaperones during endoplasmic reticulum-associated protein degradation demonstrates that protein export and import are mechanistically distinct, J. Biol. Chem. 274 (1999) 3453–3460.
[161] S.I. Nishikawa, S.W. Fewell, Y. Kato, J.L. Brodsky, T. Endo, Molecular chaperones in the yeast endoplasmic reticulum maintain the solubility of proteins for retrotranslocation and degradation, J. Cell Biol. 153 (2001) 1061–1070.
[162] D.N. Hebert, M. Molinari, Flagging and docking: dual roles for N-glycans in protein quality control and cellular proteostasis, Trends Biochem. Sci. 37 (2012) 404–410.

[163] J.J. Caramelo, A.J. Parodi, Getting in and out from calnexin/calreticulin cycles, J. Biol. Chem. 283 (2008) 10221–10225.
[164] M. Molinari, N-glycan structure dictates extension of protein folding or onset of disposal, Nat. Chem. Biol. 3 (2007) 313–320.
[165] C.K. Barlowe, E.A. Miller, Secretory protein biogenesis and traffic in the early secretory pathway, Genetics 193 (2013) 383–410.
[166] M. Knop, N. Hauser, D.H. Wolf, N-Glycosylation affects endoplasmic reticulum degradation of a mutated derivative of carboxypeptidase yscY in yeast, Yeast 12 (1996) 1229–1238.
[167] C.A. Jakob, P. Burda, J. Roth, M. Aebi, Degradation of misfolded endoplasmic reticulum glycoproteins in *Saccharomyces cerevisiae* is determined by a specific oligosaccharide structure, J. Cell Biol. 142 (1998) 1223–1233.
[168] C.A. Jakob, D. Bodmer, U. Spirig, P. Battig, A. Marcil, D. Dignard, J.J. Bergeron, D.Y. Thomas, M. Aebi, Htm1p, a mannosidase-like protein, is involved in glycoprotein degradation in yeast, EMBO Rep. 2 (2001) 423–430.
[169] N. Hosokawa, I. Wada, K. Hasegawa, T. Yorihuzi, L.O. Tremblay, A. Herscovics, K. Nagata, A novel ER alpha-mannosidase-like protein accelerates ER-associated degradation, EMBO Rep. 2 (2001) 415–422.
[170] S. Vashist, D.T. Ng, Misfolded proteins are sorted by a sequential checkpoint mechanism of ER quality control, J. Cell Biol. 165 (2004) 41–52.
[171] P. Carvalho, V. Goder, T.A. Rapoport, Distinct ubiquitin-ligase complexes define convergent pathways for the degradation of ER proteins, Cell 126 (2006) 361–373.
[172] N.W. Bays, R.G. Gardner, L.P. Seelig, C.A. Joazeiro, R.Y. Hampton, Hrd1p/Der3p is a membrane-anchored ubiquitin ligase required for ER-associated degradation, Nat. Cell Biol. 3 (2001) 24–29.
[173] P.M. Deak, D.H. Wolf, Membrane topology and function of Der3/Hrd1p as a ubiquitin–protein ligase (E3) involved in endoplasmic reticulum degradation, J. Biol. Chem. 276 (2001) 10663–10669.
[174] R. Swanson, M. Locher, M. Hochstrasser, A conserved ubiquitin ligase of the nuclear envelope/endoplasmic reticulum that functions in both ER-associated and Matalpha2 repressor degradation, Genes Dev. 15 (2001) 2660–2674.
[175] Z. Kostova, Y.C. Tsai, A.M. Weissman, Ubiquitin ligases, critical mediators of endoplasmic reticulum-associated degradation, Semin. Cell Dev. Biol. 18 (2007) 770–779.
[176] G. Huyer, W.F. Piluek, Z. Fansler, S.G. Kreft, M. Hochstrasser, J.L. Brodsky, S. Michaelis, Distinct machinery is required in *Saccharomyces cerevisiae* for the endoplasmic reticulum-associated degradation of a multispanning membrane protein and a soluble luminal protein, J. Biol. Chem. 279 (2004) 38369–38378.
[177] A. Gnann, J.R. Riordan, D.H. Wolf, Cystic fibrosis transmembrane conductance regulator degradation depends on the lectins Htm1p/EDEM and the Cdc48 protein complex in yeast, Mol. Biol. Cell 15 (2004) 4125–4135.
[178] R. Friedlander, E. Jarosch, J. Urban, C. Volkwein, T. Sommer, A regulatory link between ER-associated protein degradation and the unfolded-protein response, Nat. Cell Biol. 2 (2000) 379–384.
[179] T. Biederer, C. Volkwein, T. Sommer, Role of Cue1p in ubiquitination and degradation at the ER surface, Science 278 (1997) 1806–1809.
[180] E.M. Rubenstein, S.G. Kreft, W. Greenblatt, R. Swanson, M. Hochstrasser, Aberrant substrate engagement of the ER translocon triggers degradation by the Hrd1 ubiquitin ligase, J. Cell Biol. 197 (2012) 761–773.
[181] M.B. Metzger, Y. Liang, R. Das, J. Mariano, S. Li, J. Li, Z. Kostova, R.A. Byrd, X. Ji, A.M. Weissman, A Structurally Unique E2-Binding Domain Activates Ubiquitination by the ERAD E2, Ubc7p, Through Multiple Mechanisms, 2013.
[182] K. Bagola, M. von Delbrück, G. Dittmar, M. Scheffner, I. Ziv, M.H. Glickman, A. Ciechanover, T. Sommer, Ubiquitin Binding by a CUE Domain Regulates Ubiquitin Chain Formation by ERAD E3 Ligases, 2013.
[183] A. Finger, M. Knop, D.H. Wolf, Analysis of two mutated vacuolar proteins reveals a degradation pathway in the endoplasmic reticulum or a related compartment of yeast, Eur. J. Biochem. 218 (1993) 565–574.
[184] R. Gauss, K. Kanehara, P. Carvalho, D.T. Ng, M. Aebi, A complex of Pdi1p and the mannosidase Htm1p initiates clearance of unfolded glycoproteins from the endoplasmic reticulum, Mol. Cell 42 (2011) 782–793.
[185] P. Gillece, J.M. Luz, W.J. Lennarz, F.J. de La Cruz, K. Romisch, Export of a cysteine-free misfolded secretory protein from the endoplasmic reticulum for degradation requires interaction with protein disulfide isomerase, J. Cell Biol. 147 (1999) 1443–1456.
[186] S. Clerc, C. Hirsch, D.M. Oggier, P. Deprez, C. Jakob, T. Sommer, M. Aebi, Htm1 protein generates the N-glycan signal for glycoprotein degradation in the endoplasmic reticulum, J. Cell Biol. 184 (2009) 159–172.
[187] E.M. Quan, Y. Kamiya, D. Kamiya, V. Denic, J. Weibezahn, K. Kato, J.S. Weissman, Defining the glycan destruction signal for endoplasmic reticulum-associated degradation, Mol. Cell 32 (2008) 870–877.
[188] A. Bhamidipati, V. Denic, E.M. Quan, J.S. Weissman, Exploration of the topological requirements of ERAD identifies Yos9p as a lectin sensor of misfolded glycoproteins in the ER lumen, Mol. Cell 19 (2005) 741–751.
[189] B.A. Buschhorn, Z. Kostova, B. Medicherla, D.H. Wolf, A genome-wide screen identifies Yos9p as essential for ER-associated degradation of glycoproteins, FEBS Lett. 577 (2004) 422–426.
[190] W. Kim, E.D. Spear, D.T. Ng, Yos9p detects and targets misfolded glycoproteins for ER-associated degradation, Mol. Cell 19 (2005) 753–764.
[191] R. Szathmary, R. Bielmann, M. Nita-Lazar, P. Burda, C.A. Jakob, Yos9 protein is essential for degradation of misfolded glycoproteins and may function as lectin in ERAD, Mol. Cell 19 (2005) 765–775.
[192] R. Gauss, E. Jarosch, T. Sommer, C. Hirsch, A complex of Yos9p and the HRD ligase integrates endoplasmic reticulum quality control into the degradation machinery, Nat. Cell Biol. 8 (2006) 849–854.
[193] B.K. Sato, D. Schulz, P.H. Do, R.Y. Hampton, Misfolded membrane proteins are specifically recognized by the transmembrane domain of the Hrd1p ubiquitin ligase, Mol. Cell 34 (2009) 212–222.
[194] E. Martinez Benitez, A. Stolz, A. Becher, D.H. Wolf, Mnl2, a novel component of the ER associated protein degradation pathway, Biochem. Biophys. Res. Commun. 414 (2011) 528–532.
[195] E.M. Benitez, A. Stolz, D.H. Wolf, Yos9, a control protein for misfolded glycosylated and non-glycosylated proteins in ERAD, FEBS Lett. 585 (2011) 3015–3019.
[196] L.A. Jaenicke, H. Brendebach, M. Selbach, C. Hirsch, Yos9p assists in the degradation of certain nonglycosylated proteins from the endoplasmic reticulum, Mol. Biol. Cell 22 (2011) 2937–2945.
[197] Z. Kostova, D.H. Wolf, Importance of carbohydrate positioning in the recognition of mutated CPY for ER-associated degradation, J. Cell Sci. 118 (2005) 1485–1492.
[198] C. Xu, S. Wang, G. Thibault, D.T. Ng, Futile protein folding cycles in the ER are terminated by the unfolded protein O-mannosylation pathway, Science 340 (2013) 978–981.
[199] R. Gauss, T. Sommer, E. Jarosch, The Hrd1p ligase complex forms a linchpin between ER-lumenal substrate selection and Cdc48p recruitment, EMBO J. 25 (2006) 1827–1835.
[200] S.C. Horn, J. Hanna, C. Hirsch, C. Volkwein, A. Schutz, U. Heinemann, T. Sommer, E. Jarosch, Usa1 functions as a scaffold of the HRD-ubiquitin ligase, Mol. Cell 36 (2009) 782–793.
[201] O. Neuber, E. Jarosch, C. Volkwein, J. Walter, T. Sommer, Ubx2 links the Cdc48 complex to ER-associated protein degradation, Nat. Cell Biol. 7 (2005) 993–998.
[202] C. Schuberth, A. Buchberger, Membrane-bound Ubx2 recruits Cdc48 to ubiquitin ligases and their substrates to ensure efficient ER-associated protein degradation, Nat. Cell Biol. 7 (2005) 999–1006.
[203] R. Hitt, D.H. Wolf, Der1p, a protein required for degradation of malfolded soluble proteins of the endoplasmic reticulum: topology and Der1-like proteins, FEMS Yeast Res. 4 (2004) 721–729.
[204] M. Knop, A. Finger, T. Braun, K. Hellmuth, D.H. Wolf, Der1, a novel protein specifically required for endoplasmic reticulum degradation in yeast, EMBO J. 15 (1996) 753–763.
[205] D. Zattas, D.J. Adle, E.M. Rubenstein, M. Hochstrasser, N-terminal acetylation of the yeast Derlin Der1 is essential for Hrd1 ubiquitin-ligase activity toward luminal ER substrates, Mol. Biol. Cell 24 (2013) 890–900.
[206] E.J. Greenblatt, J.A. Olzmann, R.R. Kopito, Derlin-1 is a rhomboid pseudoprotease required for the dislocation of mutant alpha-1 antitrypsin from the endoplasmic reticulum, Nat. Struct. Mol. Biol. 18 (2011) 1147–1152.
[207] M.K. Lemberg, Sampling the membrane: function of rhomboid-family proteins, Trends Cell Biol. 23 (2013) 210–217.
[208] L. Fleig, N. Bergbold, P. Sahasrabudhe, B. Geiger, L. Kaltak, M.K. Lemberg, Ubiquitin-dependent intramembrane rhomboid protease promotes ERAD of membrane proteins, Mol. Cell 47 (2012) 558–569.
[209] M. Pilon, R. Schekman, K. Romisch, Sec61p mediates export of a misfolded secretory protein from the endoplasmic reticulum to the cytosol for degradation, EMBO J. 16 (1997) 4540–4548.
[210] A. Schafer, D.H. Wolf, Sec61p is part of the endoplasmic reticulum-associated degradation machinery, EMBO J. 28 (2009) 2874–2884.
[211] D.C. Scott, R. Schekman, Role of Sec61p in the ER-associated degradation of short-lived transmembrane proteins, J. Cell Biol. 181 (2008) 1095–1105.
[212] M. Willer, G.M. Forte, C.J. Stirling, Sec61p is required for ERAD-L: genetic dissection of the translocation and ERAD-L functions of Sec61P using novel derivatives of CPY, J. Biol. Chem. 283 (2008) 33883–33888.
[213] R.M. Garza, P.N. Tran, R.Y. Hampton, Geranylgeranyl pyrophosphate is a potent regulator of HRD-dependent 3-Hydroxy-3-methylglutaryl-CoA reductase degradation in yeast, J. Biol. Chem. 284 (2009) 35368–35380.
[214] J. Wahlman, G.N. DeMartino, W.R. Skach, N.J. Bulleid, J.L. Brodsky, A.E. Johnson, Real-time fluorescence detection of ERAD substrate retrotranslocation in a mammalian *in vitro* system, Cell 129 (2007) 943–955.
[215] P. Carvalho, A.M. Stanley, T.A. Rapoport, Retrotranslocation of a misfolded luminal ER protein by the ubiquitin-ligase Hrd1p, Cell 143 (2010) 579–591.
[216] S.G. Kreft, L. Wang, M. Hochstrasser, Membrane topology of the yeast endoplasmic reticulum-localized ubiquitin ligase Doa10 and comparison with its human ortholog TEB4 (MARCH-VI), J. Biol. Chem. 281 (2006) 4646–4653.
[217] B.N. Lilley, H.L. Ploegh, A membrane protein required for dislocation of misfolded proteins from the ER, Nature 429 (2004) 834–840.
[218] Y. Ye, Y. Shibata, C. Yun, D. Ron, T.A. Rapoport, A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol, Nature 429 (2004) 841–847.
[219] H.L. Ploegh, A lipid-based model for the creation of an escape hatch from the endoplasmic reticulum, Nature 448 (2007) 435–438.
[220] J.A. Olzmann, R.R. Kopito, Lipid droplet formation is dispensable for endoplasmic reticulum-associated degradation, J. Biol. Chem. 286 (2011) 27872–27874.
[221] K. Nakatsukasa, G. Huyer, S. Michaelis, J.L. Brodsky, Dissecting the ER-associated degradation of a misfolded polytopic membrane protein, Cell 132 (2008) 101–112.
[222] A. Stolz, R.S. Schweizer, A. Schafer, D.H. Wolf, Dfm1 forms distinct complexes with Cdc48 and the ER ubiquitin ligases and is required for ERAD, Traffic 11 (2010) 1363–1369.
[223] B.K. Sato, R.Y. Hampton, Yeast Derlin Dfm1 interacts with Cdc48 and functions in ER homeostasis, Yeast 23 (2006) 1053–1064.
[224] N.W. Bays, S.K. Wilhovsky, A. Goradia, K. Hodgkiss-Harlow, R.Y. Hampton, HRD4/NPL4 is required for the proteasomal processing of ubiquitinated ER proteins, Mol. Biol. Cell 12 (2001) 4114–4128.

[225] S. Braun, K. Matuschewski, M. Rape, S. Thoms, S. Jentsch, Role of the ubiquitin-selective CDC48(UFD1/NPL4)chaperone (segregase) in ERAD of OLE1 and other substrates, EMBO J. 21 (2002) 615–621.
[226] E. Jarosch, C. Taxis, C. Volkwein, J. Bordallo, D. Finley, D.H. Wolf, T. Sommer, Protein dislocation from the ER requires polyubiquitination and the AAA-ATPase Cdc48, Nat. Cell Biol. 4 (2002) 134–139.
[227] E. Rabinovich, A. Kerem, K.U. Frohlich, N. Diamant, S. Bar-Nun, AAA-ATPase p97/Cdc48p, a cytosolic chaperone required for endoplasmic reticulum-associated protein degradation, Mol. Cell. Biol. 22 (2002) 626–634.
[228] Y. Ye, H.H. Meyer, T.A. Rapoport, The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol, Nature 414 (2001) 652–656.
[229] D.H. Wolf, A. Stolz, The Cdc48 machine in endoplasmic reticulum associated protein degradation, Biochim. Biophys. Acta 1823 (2012) 117–124.
[230] Q. Wang, Y. Liu, N. Soetandyo, K. Baek, R. Hegde, Y. Ye, A ubiquitin ligase-associated chaperone holdase maintains polypeptides in soluble states for proteasome degradation, Mol. Cell 42 (2011) 758–770.
[231] P. Leznicki, A. Clancy, B. Schwappach, S. High, Bat3 promotes the membrane integration of tail-anchored proteins, J. Cell Sci. 123 (2010) 2170–2178.
[232] M. Mariappan, X. Li, S. Stefanovic, A. Sharma, A. Mateja, R.J. Keenan, R.S. Hegde, A ribosome-associating factor chaperones tail-anchored membrane proteins, Nature 466 (2010) 1120–1124.
[233] T. Hessa, A. Sharma, M. Mariappan, H.D. Eshleman, E. Gutierrez, R.S. Hegde, Protein targeting and degradation are coupled for elimination of mislocalized proteins, Nature 475 (2011) 394–397.
[234] B. Medicherla, Z. Kostova, A. Schaefer, D.H. Wolf, A genomic screen identifies Dsk2p and Rad23p as essential components of ER-associated degradation, EMBO Rep. 5 (2004) 692–697.
[235] S. Raasi, D.H. Wolf, Ubiquitin receptors and ERAD: a network of pathways to the proteasome, Semin. Cell Dev. Biol. 18 (2007) 780–791.
[236] H. Richly, M. Rape, S. Braun, S. Rumpf, C. Hoege, S. Jentsch, A series of ubiquitin binding factors connects CDC48/p97 to substrate multiubiquitylation and proteasomal targeting, Cell 120 (2005) 73–84.
[237] T. Suzuki, H. Park, N.M. Hollingsworth, R. Sternglanz, W.J. Lennarz, PNG1, a yeast gene encoding a highly conserved peptide:N-glycanase, J. Cell Biol. 149 (2000) 1039–1052.
[238] J.A. Olzmann, R.R. Kopito, J.C. Christianson, The mammalian endoplasmic reticulum-associated degradation system, Cold Spring Harb. Perspect. Biol. (2012) (a013185).
[239] A. Dasgupta, K.L. Ramsey, J.S. Smith, D.T. Auble, Sir Antagonist 1 (San1) is a ubiquitin ligase, J. Biol. Chem. 279 (2004) 26830–26838.
[240] M.G. Goebl, J. Yochem, S. Jentsch, J.P. McGrath, A. Varshavsky, B. Byers, The yeast cell cycle gene CDC34 encodes a ubiquitin-conjugating enzyme, Science 241 (1988) 1331–1335.
[241] W. Seufert, J.P. McGrath, S. Jentsch, UBC1 encodes a novel member of an essential subfamily of yeast ubiquitin-conjugating enzymes involved in protein degradation, EMBO J. 9 (1990) 4535–4541.
[242] Y. Matsuo, H. Kishimoto, K. Tanae, K. Kitamura, S. Katayama, M. Kawamukai, Nuclear protein quality is regulated by the ubiquitin–proteasome system through the activity of Ubc4 and San1 in fission yeast, J. Biol. Chem. 286 (2011) 13775–13790.
[243] E.K. Fredrickson, J.C. Rosenbaum, M.N. Locke, T.I. Milac, R.G. Gardner, Exposed hydrophobicity is a key determinant of nuclear quality control degradation, Mol. Biol. Cell 22 (2011) 2384–2395.
[244] J.C. Rosenbaum, E.K. Fredrickson, M.L. Oeser, C.M. Garrett-Engele, M.N. Locke, L.A. Richardson, Z.W. Nelson, E.D. Hetrick, T.I. Milac, D.E. Gottschling, R.G. Gardner, Disorder targets misorder in nuclear quality control degradation: a disordered ubiquitin ligase directly recognizes its misfolded substrates, Mol. Cell 41 (2011) 93–106.
[245] M. Haslbeck, A. Ignatiou, H. Saibil, S. Helmich, E. Frenzl, T. Stromer, J. Buchner, A domain in the N-terminal part of Hsp26 is essential for chaperone function and oligomerization, J. Mol. Biol. 343 (2004) 445–455.
[246] N. Jaya, V. Garcia, E. Vierling, Substrate binding site flexibility of the small heat shock protein molecular chaperones, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 15604–15609.
[247] T. Stromer, E. Fischer, K. Richter, M. Haslbeck, J. Buchner, Analysis of the regulation of the molecular chaperone Hsp26 by temperature-induced dissociation: the N-terminal domail is important for oligomer assembly and the binding of unfolding proteins, J. Biol. Chem. 279 (2004) 11222–11228.
[248] S. Rudiger, L. Germeroth, J. Schneider-Mergener, B. Bukau, Substrate specificity of the DnaK chaperone determined by screening cellulose-bound peptide libraries, EMBO J. 16 (1997) 1501–1507.
[249] E.K. Fredrickson, P.S. Gallagher, S.V. Clowes Candadai, R.G. Gardner, Substrate recognition in nuclear protein quality control degradation is governed by exposed hydrophobicity that correlates with aggregation and insolubility, J. Biol. Chem. 288 (2013) 6130–6139.
[250] R. Prasad, S. Kawaguchi, D.T. Ng, Biosynthetic mode can determine the mechanism of protein quality control, Biochem. Biophys. Res. Commun. 425 (2012) 689–695.
[251] J.R. Mullen, S.J. Brill, Activation of the Slx5–Slx8 ubiquitin ligase by poly-small ubiquitin-like modifier conjugates, J. Biol. Chem. 283 (2008) 19912–19921.
[252] Y. Xie, O. Kerscher, M.B. Kroetz, H.F. McConchie, P. Sung, M. Hochstrasser, The yeast Hex3.Slx8 heterodimer is a ubiquitin ligase stimulated by substrate sumoylation, J. Biol. Chem. 282 (2007) 34176–34184.
[253] J.R. Mullen, V. Kaliraman, S.S. Ibrahim, S.J. Brill, Requirement for three novel protein complexes in the absence of the Sgs1 DNA helicase in *Saccharomyces cerevisiae*, Genetics 157 (2001) 103–118.
[254] Z. Wang, G. Prelich, Quality control of a transcriptional regulator by SUMO-targeted degradation, Mol. Cell. Biol. 29 (2009) 1694–1706.
[255] K. Maderbock, A. Pichler, Sumo control, Subcell. Biochem. 54 (2010) 158–169.
[256] T. Ravid, S.G. Kreft, M. Hochstrasser, Membrane and soluble substrates of the Doa10 ubiquitin ligase are degraded by distinct pathways, EMBO J. 25 (2006) 533–543.
[257] M. Deng, M. Hochstrasser, Spatially regulated ubiquitin ligation by an ER/nuclear membrane ligase, Nature 443 (2006) 827–831.
[258] O.S. Alfassy, I. Cohen, Y. Reiss, B. Tirosh, T. Ravid, Placing a disrupted degradation motif at the C terminus of proteasome substrates attenuates degradation without impairing ubiquitylation, J. Biol. Chem. 288 (2013) 12645–12653.
[259] N. Furth, O. Gertman, A. Shiber, O.S. Alfassy, I. Cohen, M.M. Rosenberg, N.K. Doron, A. Friedler, T. Ravid, Exposure of bipartite hydrophobic signal triggers nuclear quality control of Ndc10 at the endoplasmic reticulum/nuclear envelope, Mol. Biol. Cell 22 (2011) 4726–4739.
[260] A. Iwata, Y. Nagashima, L. Matsumoto, T. Suzuki, T. Yamanaka, H. Date, K. Deoka, N. Nukina, S. Tsuji, Intranuclear degradation of polyglutamine aggregates by the ubiquitin–proteasome system, J. Biol. Chem. 284 (2009) 9796–9803.
[261] M. DiFiglia, E. Sapp, K.O. Chase, S.W. Davies, G.P. Bates, J.P. Vonsattel, N. Aronin, Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain, Science 277 (1997) 1990–1993.
[262] S. Waelter, A. Boeddrich, R. Lurz, E. Scherzinger, G. Lueder, H. Lehrach, E.E. Wanker, Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation, Mol. Biol. Cell 12 (2001) 1393–1407.
[263] K.L. Borden, Pondering the promyelocytic leukemia protein (PML) puzzle: possible functions for PML nuclear bodies, Mol. Cell. Biol. 22 (2002) 5259–5269.
[264] K. Jensen, C. Shiels, P.S. Freemont, PML protein isoforms and the RBCC/TRIM motif, Oncogene 20 (2001) 7223–7233.
[265] D. Negorev, G.G. Maul, Cellular proteins localized at and interacting within ND10/PML nuclear bodies/PODs suggest functions of a nuclear depot, Oncogene 20 (2001) 7234–7242.
[266] L. Fu, Y.S. Gao, A. Tousson, A. Shah, T.L. Chen, B.M. Vertel, E. Sztul, Nuclear aggresomes form by fusion of PML-associated aggregates, Mol. Biol. Cell 16 (2005) 4905–4917.
[267] M. Lafarga, M.T. Berciano, E. Pena, I. Mayo, J.G. Castano, D. Bohmann, J.P. Rodrigues, J.P. Tavanez, M. Carmo-Fonseca, Clastosome: a subtype of nuclear body enriched in 19S and 20S proteasomes, ubiquitin, and protein substrates of proteasome, Mol. Biol. Cell 13 (2002) 2771–2782.
[268] A. Janer, E. Martin, M.P. Muriel, M. Latouche, H. Fujigasaki, M. Ruberg, A. Brice, Y. Trottier, A. Sittler, PML clastosomes prevent nuclear accumulation of mutant ataxin-7 and other polyglutamine proteins, J. Cell Biol. 174 (2006) 65–76.
[269] W. Voos, K. Rottgers, Molecular chaperones as essential mediators of mitochondrial biogenesis, Biochim. Biophys. Acta 1592 (2002) 51–62.
[270] A. Strub, J.H. Lim, N. Pfanner, W. Voos, The mitochondrial protein import motor, Biol. Chem. 381 (2000) 943–949.
[271] S. Walter, Structure and function of the GroE chaperone, Cell Mol. Life Sci. 59 (2002) 1589–1597.
[272] C. Leidhold, B. von Janowsky, D. Becker, T. Bender, W. Voos, Structure and function of Hsp78, the mitochondrial ClpB homolog, J. Struct. Biol. 156 (2006) 149–164.
[273] L. Van Dyck, D.A. Pearce, F. Sherman, PIM1 encodes a mitochondrial ATP-dependent protease that is required for mitochondrial function in the yeast Saccharomyces cerevisiae, J. Biol. Chem. 269 (1994) 238–242.
[274] C.K. Suzuki, K. Suda, N. Wang, G. Schatz, Requirement for the yeast gene LON in intramitochondrial proteolysis and maintenance of respiration, Science 264 (1994) 891.
[275] T. Bender, I. Lewrenz, S. Franken, C. Baitzel, W. Voos, Mitochondrial enzymes are protected from stress-induced aggregation by mitochondrial chaperones and the Pim1/LON protease, Mol. Biol. Cell 22 (2011) 541–554.
[276] T. Bender, C. Leidhold, T. Ruppert, S. Franken, W. Voos, The role of protein quality control in mitochondrial protein homeostasis under oxidative stress, Proteomics 10 (2010) 1426–1443.
[277] N. Erjavec, A. Bayot, M. Gareil, N. Camougrand, T. Nystrom, B. Friguet, A.L. Bulteau, Deletion of the mitochondrial Pim1/Lon protease in yeast results in accelerated aging and impairment of the proteasome, Free Radic. Biol. Med. 56 (2013) 9–16.
[278] M.J. Baker, T. Tatsuta, T. Langer, Quality control of mitochondrial proteostasis, Cold Spring Harb. Perspect. Biol. 3 (2011).
[279] M. Koppen, T. Langer, Protein degradation within mitochondria: versatile activities of AAA proteases and other peptidases, Crit. Rev. Biochem. Mol. Biol. 42 (2007) 221–242.
[280] C.D. Dunn, Y. Tamura, H. Sesaki, R.E. Jensen, Mgr3p and Mgr1p are adaptors for the mitochondrial i-AAA protease complex, Mol. Biol. Cell 19 (2008) 5387–5397.
[281] D. Korbel, S. Wurth, M. Kaser, T. Langer, Membrane protein turnover by the m-AAA protease in mitochondria depends on the transmembrane domains of its subunits, EMBO Rep. 5 (2004) 698–703.
[282] J.M. Heo, N. Livnat-Levanon, E.B. Taylor, K.T. Jones, N. Dephoure, J. Ring, J. Xie, J.L. Brodsky, F. Madeo, S.P. Gygi, K. Ashrafi, M.H. Glickman, J. Rutter, A stress-responsive system for mitochondrial protein degradation, Mol. Cell 40 (2010) 465–480.
[283] V. Azzu, M.D. Brand, Degradation of an intramitochondrial protein by the cytosolic proteasome, J. Cell Sci. 123 (2010) 578–585.
[284] D.H. Margineantu, C.B. Emerson, D. Diaz, D.M. Hockenbery, Hsp90 inhibition decreases mitochondrial protein turnover, PLoS One 2 (2007) e1066.

[285] K. Okamoto, N. Kondo-Okamoto, Y. Ohsumi, Mitochondria-anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy, Dev. Cell 17 (2009) 87–97.
[286] A. Tanaka, M.M. Cleland, S. Xu, D.P. Narendra, D.F. Suen, M. Karbowski, R.J. Youle, Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin, J. Cell Biol. 191 (2010) 1367–1380.
[287] N.C. Chan, A.M. Salazar, A.H. Pham, M.J. Sweredoski, N.J. Kolawa, R.L. Graham, S. Hess, D.C. Chan, Broad activation of the ubiquitin–proteasome system by Parkin is critical for mitophagy, Hum. Mol. Genet. 20 (2011) 1726–1737.
[288] Y. Imai, M. Soda, R. Takahashi, Parkin suppresses unfolded protein stress-induced cell death through its E3 ubiquitin–protein ligase activity, J. Biol. Chem. 275 (2000) 35661–35664.
[289] Y. Imai, M. Soda, H. Inoue, N. Hattori, Y. Mizuno, R. Takahashi, An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin, Cell 105 (2001) 891–902.
[290] D. Narendra, A. Tanaka, D.F. Suen, R.J. Youle, Parkin is recruited selectively to impaired mitochondria and promotes their autophagy, J. Cell Biol. 183 (2008) 795–803.
[291] Y. Kim, J. Park, S. Kim, S. Song, S.K. Kwon, S.H. Lee, T. Kitada, J.M. Kim, J. Chung, PINK1 controls mitochondrial localization of Parkin through direct phosphorylation, Biochem. Biophys. Res. Commun. 377 (2008) 975–980.
[292] N. Matsuda, S. Sato, K. Shiba, K. Okatsu, K. Saisho, C.A. Gautier, Y.S. Sou, S. Saiki, S. Kawajiri, F. Sato, M. Kimura, M. Komatsu, N. Hattori, K. Tanaka, PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy, J. Cell Biol. 189 (2010) 211–221.
[293] M. Karbowski, A. Neutzner, R.J. Youle, The mitochondrial E3 ubiquitin ligase MARCH5 is required for Drp1 dependent mitochondrial division, J. Cell Biol. 178 (2007) 71–84.
[294] D.R. Rosen, Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis, Nature 364 (1993) 362.
[295] R. Yonashiro, A. Sugiura, M. Miyachi, T. Fukuda, N. Matsushita, R. Inatome, Y. Ogata, T. Suzuki, N. Dohmae, S. Yanagi, Mitochondrial ubiquitin ligase MITOL ubiquitinates mutant SOD1 and attenuates mutant SOD1-induced reactive oxygen species generation, Mol. Biol. Cell 20 (2009) 4524–4530.