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Protein quality control: the who’s who, the where’s and therapeutic escapes

Jürgen Roth · Gary Hin-Fai Yam · Jingyu Fan · Kiyoko Hirano · Katarina Gaplovska-Kysela · Valerie Le Fourn · Bruno Guhl · Roger Santimaria · Tania Torossi · Martin Ziak · Christian Zuber

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Abstract In cells the quality of newly synthesized proteins is monitored in regard to proper folding and correct assembly in the early secretory pathway, the cytosol and the nucleoplasm. Proteins recognized as non-native in the ER will be removed and degraded by a process termed ERAD. ERAD of aberrant proteins is accompanied by various changes of cellular organelles and results in protein folding diseases. This review focuses on how the immunocytochemical labeling and electron microscopic analyses have helped to disclose the in situ subcellular distribution pattern of some of the key machinery proteins of the cellular protein quality control, the organelle changes due to the presence of misfolded proteins, and the efficiency of synthetic chaperones to rescue disease-causing trafficking defects of aberrant proteins.

Keywords ERAD · Protein folding disease · Glucosidase II · Glucosyltransferase · EDEM1 · Endomannosidase · Chemical chaperones

Introduction

Folding and assembly of proteins and their function depend on each other. Like in industrial production lines, in cells the quality of newly synthesized proteins is monitored in regard to proper folding and correct assembly in the early secretory pathway, the cytosol and the nucleoplasm (Bennett et al. 2005; Bukau et al. 2006; Dobson 2003; Ellgaard and Helenius 2003; Park et al. 2007; Ravid et al. 2006; Roth 2002; Sitia and Braakman 2003; Zhang and Kaufman 2006). Protein quality control is a basic cellular phenomenon through which aberrant proteins become eliminated. Aberrant proteins can occur as waste products at a certain rate during de novo synthesis, or are caused by cellular stress, or due to disease-causing mutations (Aridor and Hannan 2002; Gregersen et al. 2006; Kim and Arvan 1998; Kopito 2000; Lukacs et al. 1994; Petäjä-Repo et al. 2000; Schubert et al. 2000; Turner and Varshavsky 2000; Ward and Kopito 1994). Once recognized as non-native or incompletely assembled, those proteins will be removed and degraded by a process generally termed ERAD, for ER-associated degradation (Hirsch et al. 2004; Meusser et al. 2005).

For secretory and membrane proteins, the molecular machinery involved in the recognition, retention and dislocation of aberrant proteins has been identified to a certain
Histochem Cell Biol (2008) 129:163–177

Wicoproteins are considered, conservation of the ERAD

Volution of electron microscopic

midipati et al. 2006

Yos9p (mammalian orthologues OS-9 and XTP3-B) (Bha-

and Ng identi-

clession, di-

et al. Mnl1p) (Hosokawa et al. 2005). In addition to various chaperones aiding pro-

teins to achieve their proper conformation, various machin-

ery proteins are involved in the recognition and retention of

aberrant proteins. For glycoproteins, the importance of spe-

ific oligosaccharidic structures generated initially by trim-

ming glucosidase II and UDP-glucose:glycoprotein

glucosyltransferase and later on by ER-mannosidase I has

been recognized (Helenius and Aebi 2004; Parodi 2000;

Roth 2002; Roth et al. 2002). Glycoproteins bearing mono-

glucosylated oligosaccharides will be bound by calnexin

calreticulin. If the aberrant glycoproteins are considered,

binding to calnexin or calreticulin will protect them tempo-

rarily from degradation. The complete deglucosylation by

glucosidase II will result in their exit from the calnexin/cal-

reticulin cycle. Subsequent trimming of mannose residue(s)

of the oligosaccharide B branch by ER-mannosidase I

opens the gate to dislocation and degradation of aberrant

proteins. The link between the calnexin/calreticulin cycle

dislocation process is apparently provided by two lectin-like proteins: EDEM1 (yeast ortholog Htm1p/

Mnl1p) (Hosokawa et al. 2001; Jakob et al. 2001; Kanehara et al. 2007; Nakatsukasa et al. 2001; Oda et al. 2003) and

Yos9p (mammalian orthologues OS-9 and XTP3-B) (Bha-

midipati et al. 2005; Buschhorn et al. 2004; Gauss et al. 2006; Kanehara et al. 2007; Kostova and Wolf 2005; Szath-

mary et al. 2005).

Depending on the type of protein and the location of the

lesion, different ERAD dislocation pathways have been

identified (Carvalho et al. 2006; Denic et al. 2006; Ismail and Ng 2006; Schuberth and Buchberger 2005). Aberrant

luminal proteins and membrane proteins with a defect in

their luminal domain undergo the ERAD-L pathway, which

is defined by the E3 ubiquitin ligase Hrd1p complex. The

Hrd1p complex consists of several proteins including

Hrd3p, an E2 complex (Ubc7p and its membrane-anchoring

factor Cue1p), the Cdc48p complex (AAA-ATPase Cdc48p

or p97, the Ufd1 and Npl4 cofactors, and the Ubx2p mem-

brane anchor), Der1p, Yos9p, Kar2p (BiP) and Usa1p. It

should be noted that the actual function of some of those

proteins in the complex remains to be established. Aberrant

membrane proteins with lesions in their cytosolic domain

enter the ERAD-C pathway organized by the E3 ubiquitin

ligase Doa10p complex. This complex is comparably sim-
pal and consists in addition to Doa10p only of the E2 com-
plex and the Cdc48p complex. The ERAD-M pathway is

followed by membrane proteins with a lesion in their trans-

membrane domain and involves only Hrd1p and Hrd3p.

These dislocation pathways were established for yeast cells

but most probably will apply to higher eukaryotes as well

because of the evolutionary conservation of the ERAD

pathways. The Doa10p complex also operates in the poly-

ubiquitination of aberrant cytosolic and nuclear proteins,

in addition to the ERAD-C pathway (Neuber et al. 2005;

Ravid et al. 2006; Swanson et al. 2001).

This review will focus on how immunocytochemical

labeling and electron microscopic analysis have helped to
disclose the in situ subcellular distribution pattern of some

of the key machinery proteins of the protein quality control,

the organelle changes due to the presence of misfolded pro-
teins, and the efficiency of synthetic chaperones to rescue
disease-causing trafficking defects of aberrant proteins.

Machinery proteins of the protein quality control

reside beyond the ER

For the quality control of glycoprotein folding, glucosidase

II (Gls II) and UDP-glucose:glycoprotein glucosyltrans-

ferase (GT) in connection with the calnexin/calreticulin cycle

are of eminent importance (Helenius and Aebi 2004; Parodi 2000; Roth 2002). Gls II is a luminal glycoprotein, which

exists in two isoforms (Pelletier et al. 2000; Ziaik et al.

2001) and does not contain known ER retention signals of

disease-causing tra-

cing defects of aberrant proteins.

2

b (Roth et al. 2001).

By confocal immunofluorescence, Gls II not

unexpectedly exhibited a pattern typically observed for the

ER as shown in Fig. 1a. Gls II acts second to glucosidase I by removing the two inner z1,3-

linked glucose residues (Brada and Dubach 1984; Burns and Touster 1982). The presence of three or two glucose

residues on oligosaccharides can be considered to represent a trimming glyco-code whereas one glucose residue repre-
sents a trimming as well as folding glyco-code (Fig. 1b)

(Jakob et al. 1998b). The involvement of Gls II and of

mono-glycosylated oligosaccharides generated by the

enzyme in the protein quality control is well documented

(Hammond et al. 1994; Hebert et al. 1995; Jakob et al.

1998a, b). By confocal immunofluorescence, Gls II not

unexpectedly exhibited a pattern typically observed for the

ER as shown in Fig. 2b (Roth et al. 2003; Zuber et al.

2001). By high-resolution immunoelectron microscopy, ER

localization of Gls II could be definitely established

(Lucocq et al. 1986; Zuber et al. 2000, 2001). In addition to

the rough ER including the nuclear envelope and the transi-
tional ER, the smooth ER was also positive for Gls II. How-
ever, with the superior resolution of electron microscopic

immunogold labeling, Gls II was additionally found in

tubulovesicular clusters between transitional ER and the cis

Golgi apparatus. They represent pre-Golgi intermediates

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involved in antero- and retrograde transport of cargo (Appenzeller-Herzog and Hauri 2006; Bannykh and Balch 1997, 1998; Hammond and Glick 2000; Palade 1975; Saraste et al. 1987; Schweizer et al. 1988).

Mono-glucosylated oligosaccharides of glycoproteins are targeted by the calnexin/calreticulin cycle and after being deglucosylated by Gls II, will be targeted by GT if not correctly folded (Fig. 1b). GT apparently senses exposed patches of charged amino acids and reglucosylates the aberrant glycoproteins, which is followed by their re-entry in the calnexin/calreticulin cycle (Parodi et al. 1983; Sousa and Parodi 1995; Trombetta and Parodi 2003). When the subcellular distribution of GT was studied by confocal immunofluorescence, its labeling pattern (Fig. 2a) was alike that observed for Gls II (Fig. 2b; Zuber et al. 2001). By immunoelectron microscopy, GT was detectable in the rough ER including the nuclear envelope and the transitional ER as well as the smooth ER (Fig. 2d). Unlike Gls II, for which the labeling intensity over rough and smooth ER was equal, labeling intensity for GT over smooth ER was only 11% that of the rough ER. GT immunolabeling was also discovered in the pre-Golgi intermediates (Fig. 2d). Notably, the pre-Golgi intermediate immunolabeling for GT was approximately twice that of rough ER (Zuber et al. 2001). Double immunogold labeling for GT combined with the pre-Golgi intermediate marker ERGIC-53 and the COPII component sec23p (Hughes and Stephens 2008) proved the identity of the GT-labeled structures. Interestingly, specific immunogold labeling for calreticulin was also observed in the pre-Golgi intermediates (Zuber et al. 2000, 2001).

Together, these results provided new insight into the in situ subcellular organization of some key elements of the protein quality control machinery. Gls II, GT and calreticulin were not only present in the rough ER, as expected, but also in the smooth ER and unequivocally present in pre-Golgi intermediates. This pattern was found in different rat cell lines and tissues as well as Drosophila tissue and cell lines. The presence of three functionally closely associated proteins is a strong evidence for the involvement of pre-Golgi intermediates in protein quality control. Of course, immunolocalization provides no direct evidence for the functionality of the detected protein at a certain location. However, there is no reason to assume that Gls II, GT and calreticulin would be only functional in the ER. Studies in yeast have provided strong evidence that multiple, sequentially acting quality control checkpoints exist along the secretory pathway extending as far as to the Golgi apparatus (Arvan et al. 2002; Caldwell et al. 2001; Sayeed and Ng 2005; Taxis et al. 2002; Vashist et al. 2001; Vashist and Ng...
2004; Younger and Chen 2006). In mammalian and insect cells, protein quality control is apparently not restricted to the ER, and the pre-Golgi intermediates appear to be involved in this fundamental cellular process as well. As will be discussed later, pre-Golgi intermediates represent not only a quality control checkpoint, but are also sites of accumulation of aberrant proteins.

The ERAD factor EDEM1 defines a novel vesicular ER exit pathway

As mentioned in the “Introduction”, an impressive body of molecular and functional data exists concerning the macromolecular assemblies involved in the various ER pathways. The current well-founded basic conception of ERAD in yeast and higher eukaryotes is that the aberrant proteins after being removed from folding cycles are dislocated to the cytosol and eventually degraded by the 26S proteasome, and that this occurs in the ER. It is not clear whether this is a randomly occurring event or a more structured affair. Recent studies on EDEM1 in mammalian cells have provided preliminary evidence for a high level of subcellular organization.

The discovery that the Man, B isomer oligosaccharide was actively involved in ERAD-L (Fig. 1c) in yeast (Jakob et al. 1998a) and mammalian cells (Liu et al. 1999) paved the way to the identification of a lectin-like protein with sequence similarity to class 1 α,2-mannosidases in yeast -Htm1p/Mnl1p- and mammalian cells -EDEM1- (Hosokawa et al. 2001; Jakob et al. 2001; Nakatsukasa et al. 2001). EDEM1 of mammalian cells is a soluble glycoprotein (Olivari et al. 2005; Zuber et al. 2007), which is regulated by the unfolded protein response (Hosokawa et al. 2001) and seems to connect the calnexin/calreticulin cycle to the dislocation process (Molinarì et al. 2003; Oda et al. 2003). EDEM1 appears to exist in complex with the dislocation proteins Derlin-2 and -3, and the AAA ATPase p97 (Oda et al. 2006). It is not fully understood how EDEM1 interacts with aberrant proteins. However, there is evidence for interaction with ER-mannosidase I-trimmed oligosaccharides such as depicted in Fig. 1c (Hosokawa et al. 2003). Overexpression of EDEM1 has been shown to prevent formation of dimers of misfolded Null Hong Kong variant of alpha 1-antitrypsin (Hosokawa et al. 2006). Notably, EDEM1 and ER-mannosidase I do not exist in complexes, which can be immunoprecipitated (Hosokawa et al. 2003).

Recently, the subcellular distribution of endogenous EDEM1 in various mammalian cell types was established with a specific anti-peptide antibody (Zuber et al. 2007). Unexpectedly, its immunofluorescence pattern did not correlate with that of calnexin and other ER marker proteins. Rather, an unusual pattern of well distributed punctate structures along with some localized finger-like structures was revealed (Fig. 3a–c). The distribution patterns of endogenous EDEM1 and that of overexpressed tagged EDEM1 were dramatically different: instead of a punctate, non-ER pattern, a typical reticular ER pattern plus punctate staining was observed (Zuber et al. 2007). This striking difference in subcellular distribution between endogenous EDEM1 and overexpressed tagged EDEM1 was confirmed by Optiprep density gradients. Endogenous EDEM1 was restricted to the densest fractions, whereas tagged EDEM1 showed the same broad distribution as observed for calnexin, sec61β, and Derlin-1 and -2 (Zuber et al. 2007). In this context, it needs to be emphasized that previous biochemical analyses of EDEM1 interaction with quality control machinery proteins and ERAD substrates were performed with cells transiently overexpressing tagged EDEM1 (Hosokawa et al. 2003; Molinarì et al. 2003; Oda et al. 2003). The nature of the EDEM1 immunofluorescence pattern was clarified by immunogold labeling and serial section analysis (Fig. 3d–h). It revealed the presence of EDEM1-reactive buds along rough ER cisternae which apparently gave raise to ~150 nm vesicles. These buds and vesicles were devoid of a COPI coat, formed outside the canonical ER exit sites of the transitional ER and were not found in the tubulovesicular clusters of pre-Golgi intermediates (Fig. 3i). Occasionally, EDEM1 luminal immunolabeling in limited parts of distended ER cisternae was observed, which accounted for approximately 11% of the immunogold labeling for GT. Double confocal immunofluorescence for endogenous EDEM1 in rat hepatoma clone 9 cells stably expressing the Null Hong Kong variant alpha 1-antitrypsin showed co-distribution of the two proteins (Zuber et al. 2007). Together, these data revealed the existence of a vesicular transport pathway out of the rough ER through which the ERAD factor EDEM1 and an ERAD substrate became sequestered from the early secretory pathway. Through this pathway potentially harmful aberrant luminal proteins can be removed. These findings also indicate that the Gls II and GT containing pre-Golgi intermediates appear to be not involved in the dislocation of an ERAD-L substrate.

Endomannosidase assigns glucose trimming function to the Golgi apparatus

It is generally assumed the glucose trimming occurs exclusively by Gls I and II and, therefore, is limited to the ER and pre-Golgi intermediates. However, under conditions of inhibition of trimming glucosidases, formation of mature oligosaccharides has been observed. This apparent paradox could be explained by the existence of an alternate glucose-trimming pathway by neutral endo-alpha-mannosidase (Lubas and Spiro 1987, 1988; Moore and Spiro 1990, 1992; Spiro...
Endomannosidase is currently the only known endo-glycosidase. In contrast to the trimming Gls I and II, it cleaves internally between the glucose-substituted mannose and the remaining oligosaccharide (Fig. 4a). Its substrate specificity (Fig. 4a) is basically that of Gls I and II (Moore and Spiro 1990, 1992; Rabouille and Spiro 1992). However, unlike Gls I and II, ER-mannosidase I trimmed mono-glucosylated oligosaccharides are a substrate of endomannosidase. The resulting Man$_{6-5}$GlcNAc isomer A is the specific product of endomannosidase. It should be noted that this oligosaccharide is no more a substrate for reglucosylation by GT. Biochemically, activity for endomannosidase was found to be enriched in Golgi membranes (Lubas and Spiro 1987). By immunofluorescence (Dong et al. 2000; Zuber et al. 2000), endomannosidase exhibited a crescent-shaped perinuclear staining and fine punctate staining throughout the cytoplasm which partially overlapped with immunofluorescence for Glc Golgi mannosidase II (Fig. 4b–d). High-resolution immunoelectron microscopy demonstrated endomannosidase in the peripheral and Golgi-associated pre-Golgi intermediates as well as cis and medial cisternae of the Golgi apparatus (Fig. 4e) (Zuber et al. 2000). Trans cisternae of the Golgi apparatus and the trans Golgi network were unreactive. Quantification revealed ~85% of the immunogold labeling for endomannosidase in the Golgi apparatus and ~15% in pre-Golgi intermediates. Although, both endomannosidase and Gls II could be detected in pre-Golgi intermediates by double immunogold labeling, they labeled different elements of the vesiculotubular clusters (Fig. 4e). Thus, endomannosidase and Gls I and II exhibited non-overlapping subcellular distributions (Roth et al. 2003; Zuber et al. 2000). Functionally, the presence of endomannosidase in the ER would interfere with the action of glucosyltransferase by preventing the reglucosylation of misfolded glycoproteins. Together, these findings demonstrating a predominantly Golgi apparatus localization of endomannosidase strongly indicated that glucose trimming of N-linked oligosaccharides is not limited to the ER.

Since glucose trimming is indispensable for the synthesis of mature oligosaccharide side chains, deglucosylation by endomannosidase in the Golgi apparatus ensures that this important process is not blocked. Further biochemical and morphological analyses demonstrated that Golgi apparatus localized endomannosidase-processed oligosaccharides of alpha 1-antitrypsin irrespective of their folding state (Torossi et al. 2006). From the literature, it is well known that disease-causing misfolded glycoproteins to a certain extent might escape the protein quality control and become secreted (Cox 2001; Desnick et al. 2001). As a case in point, in humans suffering from alpha 1-antitrypsin deficiency, the Z-variant of alpha 1-antitrypsin not only becomes partially secreted, but also is active as serine protease inhibitor (Cabral et al. 2002; Teckman and Perlmutter 1996). As experimentally shown for the Z-variant of alpha 1-antitrypsin (Torossi et al. 2006),...
endomannosidase provided a back-up mechanism for its de-glucosylation en route through the Golgi apparatus. Processing of its oligosaccharides to mature ones is apparently important for their proper trafficking and correct functioning.

**Organelle changes due to intracellular accumulation of misfolded proteins**

As a general rule, misfolded proteins become targeted by the protein quality control and following polyubiquitination will be degraded by proteasomes (Eisele et al. 2006; Hochstrasser 1996; Jarosch et al. 2002; McCracken and Brodsky 2005; Wolf and Hilt 2004; Zwickl et al. 2002). Depending on various factors such as the efficiency of the ubiquitin–proteasome system, the intracellular amounts of misfolded glycoproteins and their biophysical properties as well as interactions with other proteins, a whole spectrum of organelle changes can be observed in protein folding diseases.

For some protein folding diseases, no significant structural aberrations of the early secretory pathways could be
observed. An example is Fabry’s disease, an inherited deficiency of lysosomal alpha-galactosidase A (alpha-Gal A), which causes progressive lysosomal glycosphingolipid accumulation (mainly globotriosylceramide Gb3) (Desnick et al. 2001). Disease-causing mutant alpha-Gal A could be shown by immunofluorescence to be retained in the ER where it existed in complexes with the chaperone BiP (Yam et al. 2005, 2006). From this, we concluded that recognition and ER-retention of the mutant alpha-Gal A by the protein quality control machinery constituted the mechanism leading to lysosomal deficiency in alpha-Gal A. Electron microscopic analysis of cultured fibroblast from Fabry patients harboring different mutations did not reveal significant changes of the morphology of the ER and the pre-Golgi intermediates. As expected, the fibroblasts contained numerous large lysosomes with characteristic multilamellar inclusions. Thus, the intracellularly retained mutant alpha-Gal A apparently became dislocated and was efficiently degraded by the ubiquitin–proteasome system. A similar situation was observed for a polypeptide membrane protein, aquaporin-2, whose folding mutants can cause renal diabetes insipidus (Canfield et al. 1997; Morello and Bichet 2001). The T126M mutant aquaporin-2 was found to be retained in the ER and efficiently degraded by proteasomes without causing ER dilatation (Hirano et al. 2003). ER retention and rapid proteasomal degradation are also hallmarks of the pulmonary form of alpha 1-antitrypsin deficiency (Lomas and Parfrey 2004; Sifers et al. 1988). However, other types of protein folding diseases have been shown to result in the distention of the ER cisternae. One example is the congenital hypothyroid goiter in which the mutant thyroglobulin is misfolded (Kim et al. 1996; Kim and Arvan 1998; Kim et al. 1998, 2000; Medeiros Neto et al. 1996). In disorders of procollagen biosynthesis, distended ER cisternae were also observed (Bogaert et al. 1992). Other examples are represented by LDL receptor class 2 mutants (Lehrman et al. 1987; Pathak et al. 1988).

There are protein folding diseases associated with both distended ER cisternae and enlarged pre-Golgi intermediates. A missense mutation of the insulin 2 gene (Cys96Tyr) in Akita mice disrupting one of the two interchain disulfide bonds is associated with intracellular accumulation of misfolded proinsulin (Wang et al. 1999). This resulted in a significant increase of the volume density of dilated ER profiles and of the pre-Golgi intermediates (Fig. 5a, b) (Fan et al. 2007; Zuber et al. 2004). For the latter, a significant increase of the tubular elements was observed. Although the mutant proinsulin was degraded through proteasomes (Wang et al. 1999), its accumulation in the early secretory pathway caused an activation of the unfolded protein response and induced apoptosis (Oyadomari et al. 2002a; Oyadomari et al. 2002b). Other mutant proteins such as the cystic fibrosis (Kopito 1999; Riordan 1999) causing delta F508 variant of the chloride channel (Gilbert et al. 1998) and misfolded major histocompatibility complex class I protein (Hsu et al. 1991; Raposo et al. 1995), have been shown to accumulate in the expanded pre-Golgi intermediates.

Certain other misfolded proteins are accompanied by the formation of insoluble aggregates in the lumen of the ER, which physically precludes dislocation to the cytosol and exposure to proteasomes. The stress-induced so-called intracisternal granules in the pancreas of starved guinea pigs (Palade 1956), which are composed of aggregated proenzymes (Fig. 6a) (Geuze and Slot 1980; Pavelka and Roth 2005), form a classical example. For the liver-disease-causing alpha 1-antitrypsin Z variant, about 15% of the non-secreted mutant protein is polymersogenic and thus forms insoluble aggregates in the ER lumen, which cannot be degraded (Lomas et al. 1992, 2004) The Glu342Lys substi-

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**Fig. 5** Details of an insulin-producing pancreatic beta cell from Akita mice. The presence of misfolded proinsulin results in the local distention of rough ER cisternae (RER*). Arrows point to regions of transition of non-distended ER (RER) to distended ER (RER*). In addition, the pre-Golgi intermediates (pGI) are greatly enlarged. G: Golgi apparatus, TE: transitional ER. In (B), the organelle changes in terms of differences of their volume density (Vv) and differences in proinsulin distribution pattern (LI) are schematically shown (from Zuber et al. 2004).
tution of the Z-variant results in a spontaneous loop-sheet polymerization of the protein. In contrast to the above-mentioned protein folding diseases, which all have in common a loss-of-function pathogenesis, the Z-variant-caused alpha 1-antitrypsin deficiency seems to involve a pathologic gain-of-function pathogenesis (Hidvegi et al. 2005). The ER inclusions described above belong to the category of Russell bodies. Russell bodies represent subregions of the rough ER in which insoluble proteins accumulate (Fig. 6b) (Alanen et al. 1985; Kopito and Sitia 2000; Valetti et al. 1991). They are typically found in cells synthesizing mutant immunoglobulins (Alanen et al. 1985; Kopito and Sitia 2000; Mattioli et al. 2006; Valetti et al. 1991) but also in cells synthesizing, for instance, mutant myocilin. Mutations of the myocilin gene are associated with primary open-angle glaucoma (Tamm 2002). Mutant myocilins are secretion-incompetent and have been shown biochemically to form intracellular detergent-insoluble complexes (Gobeil et al. 2004; Jacobson et al. 2001; Sohn et al. 2002). In cultured cells transfected to express both mutant and wild-type myocilin, heteromeric, detergent-insoluble protein complexes were formed which were segregated into typical Russell bodies (Yam et al. 2007c). Thus, myocilin-caused open-angle glaucoma represents a protein folding disease. Its pathogenesis involves a pathological gain-of-function mechanism because of the interaction and complex formation of mutant with wild-type myocilin (Gobeil et al. 2004; Joe et al. 2003; Sohn et al. 2002; Yam et al. 2007c). As a consequence, unfolded protein response factors and pro-apoptotic factors were up-regulated and cells underwent apoptosis (Yam et al. 2007c) as detected by the appearance of labeled nuclei and the TUNEL assay (Taatjes et al. 2008).

All the mutant proteins discussed above are luminal or membrane proteins. What happens to aberrant cytosolic and nuclear proteins? Same like ER proteins, one extreme situation is that they become efficiently degraded by cytosolic and nuclear proteasomes subsequent to polyubiquitination (Schubert et al. 2000; Turner and Varshavsky 2000). Thus, cytosolic and nuclear quality control in normal cells suppresses the formation of aggregates of aberrant proteins by degrading them. The other extreme is represented by the formation of cytosolic and nuclear inclusion bodies due to inefficient degradation of aberrant proteins by the ubiquitin–proteasome system. Cytosolic, non-membrane bounded inclusion bodies are generally called aggresomes (Corboy et al. 2005; Kopito and Sitia 2000). They consist of pericentriolar protein aggregates surrounded by a cage of intermediate (vimentin) filaments that are the most consistent component of aggresomes in addition to ubiquitin, proteasomes and molecular chaperones. Aggresomes can be induced experimentally by forced overexpression of aggregation-prone mutant proteins or by experimentally inhibiting proteasomes (Fig. 7) (Anton et al. 1999; Fan et al. 2007; Johnston et al. 1998; Wigley et al. 1999). On the other hand, it has been shown that protein aggregates can directly impair the function of the ubiquitin–proteasome system (Bence et al. 2001). The formation of aggresomes is a multi-step process, which depends on the intact microtubules. Aggresomes are formed by the coalescence of small protein aggregates transported from the cell’s periphery along microtubules to centrioles (Garcia-Mata et al. 1999; Johnston et al. 1998; Kawaguchi et al. 2003; Vidair et al. 1966; Wigley et al. 1999; Wojcik et al. 1996). In the nucleus, the inclusion bodies can be found in association with the promyelocytic leukemia oncogenic domains (Anton et al. 1999).

Inclusion bodies have been observed in association with a number of chronic neurodegenerative diseases such as Parkinson’s disease, Huntington’s disease, Alzheimer’s disease and amyotrophic lateral sclerosis (Johnston et al. 2000; Kabashi and Durham 2006; Rubinsztein 2006; Selkoe 2003; Shults 2006; Soto 2003). Inclusion bodies named Lewy bodies are a morphological hallmark of Parkinson’s disease and other neurodegenerative disorders (McNaught et al. 2002b; Olanow et al. 2004; Shults 2006). Lewy bodies in the dopaminergic neurons resemble aggresomes and represent spherical bodies commonly composed of a core of granular material and peripheral radiating filaments. They contain a variety of proteins such as alpha-synuclein, the alpha-synuclein-binding protein synphilin-1 torsin A, neurofilaments, ubiquitin, proteasomal subunits and various heat shock proteins as well as ubiquitin-activating enzyme, ubiquitin-conjugating enzyme, ubiquitin ligase enzymes and proteasome activators. Furthermore, they contain centrosome-related gamma-tubulin and pericentrin. Thus, it has been proposed that the formation of Lewy bodies represents an aggresome-like response in dopaminergic neurons (McNaught et al. 2002c). Considering the observed impair-
Many attempts have been made to at least partially correct the protein misfolding in order to overcome their trafficking defect and to alleviate ER stress. Among other approaches, small molecule synthetic chaperones have been used in order to shift the folding equilibrium of mutant proteins towards a more native state (Arakawa et al. 2006; Chaudhuri and Paul 2006; Cohen and Kelly 2003; Papp and Csermely 2006; Perlmutter 2002). Chemical chaperones include osmotically active substances such as DMSO, glycerol, polyols or deuterated water, and other compounds such as 4-phenylbutyric acid (Burrows et al. 2000; Lim et al. 2004; Liu et al. 2004; Pedemonte et al. 2005; Rubenstein and Zeitlin 2000; Tamarappoo and Verkman 1998; Tveten et al. 2007; Welch and Brown 1996). Other substances such as enzyme inhibitors (Fan et al. 1999; Matsuda et al. 2003; Sawkar et al. 2002) and receptor ligands or antagonists (Petäjä-Repo, 2002 #16211; Egan, 2002 #12283) have been shown to function as pharmacological chaperones.

Here, we have chosen two examples from our work to demonstrate how immunocytochemistry and microscopy in combination with biochemical analyses can be applied to demonstrate the functionality of a chemical and a pharmacological chaperone in rescuing the consequence of disease-causing protein misfolding. It has been mentioned above that open-angle glaucoma-causing mutant myocilin forms insoluble protein aggregates in the ER lumen (Russel bodies), which result in ER stress and apoptotic cell death (Yam et al. 2007b). Among the other tested chemical chaperones, treatment with sodium 4-phenylbutyrate significantly reduced the amount of intracellular detergent-insoluble myocilin aggregates and thereby the number of Russel bodies in the cells (Fig. 8a–c), diminished mutant myocilin interaction with calreticulin and restored the secretion of mutant myocilin. As a consequence, the ER stress was released and most interesting, the apoptosis rate was reduced close to levels observed in control cells expressing wild-type myocilin (Fig. 8d). Thus, sodium

**Fig. 7** Formation of pericentriolar aggresomes following proteasome inhibition by lactacystin in CHO cells stably expressing misfolded proinsulin. Irregularly shaped, electron dense flocculent material is present in the cytoplasm and surrounded by intermediate filaments (a). At higher magnification, the spatial relationship between the protein aggregates and a centriole can be seen (b). Micrographs from Fan et al. (2007)

The various protein folding diseases mentioned above can be classified based on the pathogenetic mechanism. Efficient proteasomal degradation of the misfolded protein is characteristic of the loss-of-function pathogenesis. This is the case in protein folding diseases such as cystic fibrosis, the lung form of alpha 1-antitrypsin deficiency, aquaporin 2-caused renal diabetes insipidus, Gaucher’s disease and Fabry’s disease. Here, the missing function of the degraded protein alone can be the cause of the clinical symptoms, or secondary effects due to substrate accumulation like in lysosomal storage diseases. Intracellular accumulation due to inefficient proteasomal degradation of misfolded proteins is representative of a pathological gain-of-function mechanism, which is combined with a loss of function. Intracellular accumulation of misfolded proteins associated or not with protein aggregation can result in the activation of the unfolded protein response leading to ER stress and apoptosis. A pathological gain-of-function mechanism can be also the cause of a dominant clinical course when the wild-type protein in complexes with the mutant protein is retained inside the cells. Examples for pathological gain-of-function pathogenesis-associated protein folding diseases are myocilin-caused open-angle glaucoma, familial hypophyseal diabetes insipidus, Parkinson’s disease and Huntington’s disease.

**Synthetic chaperones for treatment of protein folding disease**

Many attempts have been made to at least partially correct the protein misfolding in order to overcome their trafficking defect and to alleviate ER stress. Among other approaches, small molecule synthetic chaperones have been used in order to shift the folding equilibrium of mutant proteins towards a more native state (Arakawa et al. 2006; Chaudhuri and Paul 2006; Cohen and Kelly 2003; Papp and Csermely 2006; Perlmutter 2002). Chemical chaperones include osmotically active substances such as DMSO, glycerol, polyols or deuterated water, and other compounds such as 4-phenylbutyric acid (Burrows et al. 2000; Lim et al. 2004; Liu et al. 2004; Pedemonte et al. 2005; Rubenstein and Zeitlin 2000; Tamarappoo and Verkman 1998; Tveten et al. 2007; Welch and Brown 1996). Other substances such as enzyme inhibitors (Fan et al. 1999; Matsuda et al. 2003; Sawkar et al. 2002) and receptor ligands or antagonists (Petäjä-Repo, 2002 #16211; Egan, 2002 #12283) have been shown to function as pharmacological chaperones.

Here, we have chosen two examples from our work to demonstrate how immunocytochemistry and microscopy in combination with biochemical analyses can be applied to demonstrate the functionality of a chemical and a pharmacological chaperone in rescuing the consequence of disease-causing protein misfolding. It has been mentioned above that open-angle glaucoma-causing mutant myocilin forms insoluble protein aggregates in the ER lumen (Russel bodies), which result in ER stress and apoptotic cell death (Yam et al. 2007b). Among the other tested chemical chaperones, treatment with sodium 4-phenylbutyrate significantly reduced the amount of intracellular detergent-insoluble myocilin aggregates and thereby the number of Russel bodies in the cells (Fig. 8a–c), diminished mutant myocilin interaction with calreticulin and restored the secretion of mutant myocilin. As a consequence, the ER stress was released and most interesting, the apoptosis rate was reduced close to levels observed in control cells expressing wild-type myocilin (Fig. 8d). Thus, sodium
4-phenylbutyrate exerts a beneficial effect by protecting the cells from the deleterious effects of mutant myocilin. Since sodium 4-phenylbutyrate is a tissue and cell-permeable molecule, it holds the potential for topical administration in the treatment of myocilin-caused primary open-angle glaucoma.

The second example concerns Fabry’s disease, a lysosomal storage disorder caused by a deficiency of alpha-Gal A activity in lysosomes that result in the accumulation of glycosphingolipid globotriosylceramide (Gb3). The lysosomal trafficking of mutant alpha Gal A is impaired because the enzyme is retained in the ER by the protein quality control (Yam et al. 2005). Others had demonstrated that the activity of mutant alpha-Gal A in vitro at neutral pH could be stabilized with the competitive enzyme inhibitor 1-deoxygalactonorijimycin (DGJ) (Fan et al. 1999). Treatment of cells expressing mutant alpha-Gal A with a non-inhibitory dose of DGJ enhanced the intracellular enzyme activity (Yam et al. 2005, 2006). In addition, we could demonstrate by immunofluorescence and quantitative immunogold labeling that the mutant enzyme was redistributed from the ER to lysosomes and that this trafficking was mannos-6-phosphate-dependent. The DGJ treatment resulted in release of mutant alpha-Gal A from the chaperone BiP and its conversion in the mature lysosomal form. Double confocal immunofluorescence and immunogold labeling demonstrated that the lysosomal Gb3 storage was cleared and that the size of the lysosomes became normalized (Yam et al. 2005, 2006). Together, this demonstrated that DGJ exhibited a chaperone-like effect and induced the trafficking of ER-retained mutant alpha Gal A to lysosomes where the enzyme was catalytically active. Therefore, the pharmacological chaperone DGJ potentially offers a convenient and cost-efficient therapeutic alternative to enzyme replacement therapy.

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References

Alanen A, Pira U, Lassila O, Roth J, Franklin R (1985) Mott cells are plasma cells defective in immunoglobulin secretion. Eur J Immunol 15:235–242
Anton L, Schubert U, Bacik I, Princiotto M, Warsch P, Gibbs J, Day P, Realini C, Rechsteiner M, Bennink J, Yewdell J (1999) Intracellular location of proteasomal degradation of a viral antigen. J Cell Biol 146:113–124
Appenzeller-Herzog C, Hauri HP (2006) The ER-Golgi intermediate compartment (ERGIC): in search of its identity and function. J Cell Sci 119:2173–2183
Arakawa T, Ejima D, Kita Y, Tsumoto K (2006) Small molecule pharmacological chaperones: from thermodynamic stabilization to pharmaceutical drugs. Biochimica Biophysica Acta Proteins Proteomics 1764:1677–1687
Aridor M, Haman LA (2002) Traffic jams II: an update of diseases of intracellular transport. Traffic 3:781–790
Avran P, Zhao X, Ramos-Castaneda J, Chang A (2002) Secretory pathway control operating in Golgi, plasmalemmal, and endosomal systems. Traffic 3:771–780
Bannych SL, Balch WE (1997) Membrane dynamics at the endoplasmic reticulum–Golgi interface. J Cell Biol 138:1–4
Bannych SL, Balch WE (1998) Selective transport of cargo between the endoplasmic reticulum and Golgi compartments. Histochem Cell Biol 109:463–475
Bence NF, Sampat RM, Kopito RR (2001) Impairment of the ubiquitin–proteasomal system by protein aggregation. Science 292:1552–1555
Bennett EJ, Bence NF, Jayakumar R, Kopito RR (2005) Global impairment of the ubiquitin–proteasomal system by nuclear or cytoplasmic protein aggregates precedes inclusion body formation. Mol Cell 17:351–365
Bhamidipati A, Denic V, Quan EM, Weissman JS (2005) Exploration of the topological requirements of ERAD identifies Yos9p as a lectin sensor of misfolded glycoproteins in the ER lumen. Mol Cell 19:741–751
Bogaert R, Tiller GE, Weis MA, Gruber HE, Rimoin DL, Cohn DH, Eyre DR (1992) An amino acid substitution (Gly853→Glu) in the...
Histochem Cell Biol (2008) 129:163–177

collagen alpha 1(II) chain produces hypochondrogenesis. J Biol Chem 267:22522–22526

Brada D, Dubach UC (1984) Isolation of a homogeneous glucosidase II from pig kidney microsomes. Eur J Biochem 141:149–156

Bukau B, Weissman J, Horwich A (2006) Molecular chaperones and protein quality control. Cell 125:443–451

Burns DM, Touster O (1982) Purification and characterization of glucosidase II, an endoplasmic reticulum hydrolase involved in glycoprotein biosynthesis. J Biol Chem 257:9990–10000

Burrows JA, Willis LK, Perlmutter DH (2000) Chemical chaperones mediate increased secretion of mutant alpha 1-antitrypsin (alpha 1-AT) Z: a potential pharmacological strategy for prevention of liver injury and emphysema in alpha 1-AT deficiency. Proc Natl Acad Sci USA 97:1796–1801

Buschhorn BA, Kostova Z, Medicherla B, Wolf DH (2004) A genome-wide screen identifies Yos9p as essential for ER-associated degradation of glycoproteins. FEBS Lett 577:422–426

Cabral CM, Liu Y, Moremen KW, Sifers RN (2002) Organizational diversity among distinct glycoprotein endoplasmic reticulum-associated degradation programs. Mol Biol Cell 13:2629–2650

Caldwell SR, Hill KJ, Cooper AA (2001) Degradation of endoplasmic reticulum (ER) quality control substrates requires transport between the ER and Golgi. J Biol Chem 276:23296–23303

Canfield M, Tamarappoo B, Moses A, Verkman A, Holtzman E (1997) Identification and characterization of aquaporin-2 water channel mutations causing nephrogenic diabetes insipidus with partial vasopressin response. Hum Mol Genet 4:103–107

Carvalho P, Goder V, Rapoport TA (2006) Distinct ubiquitin–ligase complexes define convergent pathways for the degradation of ER proteins. Cell 126:361–373

Chaudhuri TK, Paul S (2006) Protein-misfolding diseases and chaperone-based therapeutic approaches. FEBS J 273:1331–1349

Cohen FE, Kelly JW (2003) Therapeutic approaches to protein-misfolding disease. In: Scriver C, Beaudet A, Sly W, Valle D (eds) The metabolic and molecular bases of inherited disease. McGraw-Hill, New York, pp 3733–3774

Cox D (2001) Protein folding and misfolding. Nature 426:884–890

Dobson CM (2003) Protein folding and misfolding. Nature 426:884–890

Dong ZZ, Zuber C, Wolf DH (2004) Disproportional immunostaining patterns of two secretory proteins in guinea pig and rat exocrine pancreatic cells. An immunoferritin and fluorescence study. Eur J Cell Biol 83:219–229

Fan JY, Roth J, Zuber C (2007) Expression of mutant Ins2(C96Y) results in enhanced tubule formation causing enlargement of pre-Golgi intermediates of CHO cells. Histochem Cell Biol 128:161–173

Flura T, Brada D, Ziaik M, Roth J (1997) Expression of a cDNA encoding the glucos trimming enzyme glucosidase II in CHO cells and molecular characterization of the enzyme deficiency in a mutant mouse lymphoma cell line. Glycobiology 7:617–624

Garcia-Mata R, Bebök Z, Sorscher EJ, Szul E (1999) Characterization and dynamics of aggresome formation by a cytosolic GTP-chimeras. J Cell Biol 146:1239–1254

Gauss R, Jarosch T, Hirsch C (2006) A complex of Yos9p and the HRD ligase integrates endoplasmic reticulum quality control into the degradation machinery. Nat Cell Biol 8:849–854

Geuze HJ, Slot JW (1980) Disproportional immunostaining patterns of two secretory proteins in guinea pig and rat exocrine pancreatic cells. An immunoferritin and fluorescence study. Eur J Cell Biol 83:219–229

Gobell S, Rodrigue MA, Moisan S, Nguyen TD, Polansky JR, Morisette J, Raymond V (2004) Intracellular sequestration of hetero-oligomers formed by wild-type and glaucoma-causing myocilin mutants. Invest Ophthalmol Vis Sci 45:3560–3567

Gregersen N, Bross P, Vang S, Christensen JH (2006) Protein misfolding and human disease. Annu Rev Gen Hum Genet 7:103–124

Hammond AT, Glick BS (2000) Dynamics of transitional endoplasmic reticulum sites in vertebrate cells. Mol Biol Cell 11:3013–3030

Hammond C, Braakman I, Helenius A (1994) Role of N-linked oligosaccharide recognition, glucose trimming, and calnexin in glycoprotein folding and quality control. Proc Natl Acad Sci USA 91:913–917

Hebert DN, Foellmer B, Helenius A (1995) Glucose trimming and re-sulcosylation determine glycoprotein association with calnexin in the endoplasmic reticulum. Cell 81:425–433

Helenius A, Aebi M (2004) Roles of N-linked glycans in the endoplasmic reticulum. Annu Rev Biochem 73:1019–1049

Hidvegi T, Schmidt BZ, Hale P, Perlmutter DH (2005) Accumulation of mutant alpha1-antitrypsin Z in the endoplasmic reticulum activates caspases-4 and -12, NfKappaB, and BAP31 but not the unfolded protein response. J Biol Chem 280:39002–39015

Hirano K, Zuber C, Roth J, Ziaik M (2003) The proteasome is involved in the degradation of different aquaporin-2 mutants causing nephrogenic diabetes insipidus. Am J Pathol 163:111–120

Hirsch C, Blom D, Ploegh HL (2003) A role for N-glycanase in the cytosolic turnover of glycoproteins. EMBO J 22:1036–1046

Hirsch C, Jarosch T, Sommer T, Wolf DH (2004) Endoplasmic reticulum-associated protein degradation—one model fits all? Biochim Biophys Acta Mol Cell Res 1695:215–223

Hochstrasser M (1996) Ubiquitin-dependent protein degradation. Annu Rev Genet 30:405–439

Hosokawa N, Wada I, Hasegawa K, Yorihuzi T, Tremblay LO, Herschman T, Weissman J, Horwich A (2006) Quality control into the degradation machinery. Nat Cell Biol 8:849–854

Hosokawa N, Wada I, Natsuka Y, Nagata K (2006) EDEM accelerates ERAD by preventing aberrant dimer formation of misfolded alpha 1-antitrypsin. Genes Cells 11:465–476

Hsu VW, Yuan LC, Nuchtern JG, Lippincott-Schwartz J, Hammerling GJ, Klausner RD (1991) A recycling pathway between the endo-
plasmic reticulum and the Golgi apparatus for retention of unassembled MHC class I molecules. Nature 352:441–444
Hughes H, Stephens D (2008) Assembly, organization, and function of the COPII coat. Histochem Cell Biol 127. doi:10.1007/s00418-007-0363-x
Ismail N, Ng DT (2006) Have you HRD? Understanding ERAD is DO-Able! Cell 126:237–239
Jacobson N, Andrews M, Shepard A (2001) Nonsecretion of mutant proteins of the glaucoma gene myocilin in cultured trabecular meshwork cells and in aqueous humor. Hum Mol Genet 10:117–125
Jakob CA, Burda P, Roth J, Aebi M (1998a) Degradation of misfolded endoplasmic reticulum glycoproteins in Saccharomyces cerevisiae is determined by a specific oligosaccharide structure. J Cell Biol 142:1223–1233
Jakob CA, Burda P, teHeesen S, Aebi M, Roth J (1998b) Genetic tailoring of N-linked oligosaccharides: the role of glucose residues in glycoprotein processing of Saccharomyces cerevisiae in vivo. Glycobiology 8:155–164
Jakob CA, Bodmer D, Spiriig U, Battig P, Marcil D, Bergeron JJ, Thomas DY, Aebi M (2001) Htm1p, a mannosidase-like protein, is involved in glycoprotein degradation in yeast. EMBO Rep 2:423–430
Jarosch E, Taxis C, Volkwein C, Bordallo J, Finley D, Wolf DH, Sommer T (2002) Protein dislocation from the ER requires polyubiquitination and the AAA-ATPase Cdc48. Nat Cell Biol 4:134–139
Joe MK, Sohn S, Hur W, Choi YR, Kee C (2003) Accumulation of mutant myocilins in ER leads to ER stress and potential cytotoxicity in human trabecular meshwork cells. Biochem Biophys Res Commun 312:592–600
Johnston JA, Ward CL, Kopito RR (1998) Aggresomes: a cellular response to misfolded proteins. J Cell Biol 143:1833–1898
Johnston JA, Dalton MJ, Gurney ME, Kopito RR (2000) Formation of high molecular weight complexes of mutant Cu-Zn-superoxide dismutase in a mouse model for familial amyotrophic lateral sclerosis. Biochim Biophys Acta 1762:1038–1050
Kabashi E, Durham HD (2006) Failure of protein quality control in amyotrophic lateral sclerosis. Biochim Biophys Acta 1762:1038–1050
Kanehara K, Kawaguchi S, Ng DT (2005) The retrotranslocation protein endo-alpha-mannosidase in processing N-linked oligosaccharides. J Biol Chem 280:3990–3998
Kawaguchi Y, Kovacs JJ, McLaurin A, Vance JM, Ito A, Yao TP (2003) The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress. Cell 115:727–738
Kim PS, Arvan P (1998) Endocinopathies in the family of endoplasmic reticulum (ER) storage diseases: disorders of protein trafficking and the role of ER molecular chaperones. Endocr Rev 19:173–202
Kim PS, Kwon OY, Arvan P (1996) An endoplasmic reticulum storage disease causing congenital goiter with hypothyroidism. J Cell Biol 133:517–527
Kim PS, Hossain SA, Park YN, Lee I, Yoo SE, Arvan P (1998) A single amino acid change in the acetylatedinnerase-like domain of thyroglobulin causes congenital goiter with hypothyroidism in the ceg/cog mouse: a model of human endoplasmic reticulum storage diseases. Proc Natl Acad Sci USA 95:9909–9913
Kim PS, Ding M, Menon S, Jung CG, Cheng JM, Miyamoto T, Li BL, Agui T (2000) A missense mutation G2320R in the thyroglobulin gene causes nongoitrous congenital primary hypothyroidism in the WIC-rdw rat. Mol Endocrinol 14:1944–1953
Kopito RR (1999) Biosynthesis and degradation of CFTR. Physiol Rev 79:S167–173
Kopito R (2000) Conformation disease. Nat Cell Biol 2:E207–E209
Kopito RR, Sitia R (2000) Aggresomes and Russell bodies. Symptoms of cellular indigestion? EMBO Rep 1:225–231
Kostova Z, Wolf DH (2005) Importance of carbohydrate positioning in the recognition of mutated CPY for ER-associated degradation. J Cell Sci 118:1485–1492
Lehrman MA, Schneider WJ, Brown MS, Davis CG, Elhammer A, Russell DW, Goldstein JL (1987) The Lebanese allele at the low density lipoprotein receptor locus. Nonsense mutation produces truncated receptor that is retained in endoplasmic reticulum. J Biol Chem 262:401–410
Li GT, Zhao G, Zhou XK, Schindelin H, Lennarz WJ (2006) The AAA ATPase p97 links peptide N-glycanase to the endoplasmic reticulum-associated E3 ligase autocrine motility factor receptor. Proc Natl Acad Sci USA 103:8348–8353
Lilley BN, Ploegh HL (2004) A membrane protein required for dislocation of misfolded proteins from the ER. Nature 429:834–840
Lim M, McKenzie K, Floyd AD, Kwon E, Zeitlin PL (2004) Modulation of deltaF508 cystic fibrosis transmembrane regulator trafficking and function with 4-phenylbutyrate and flavonoids. Am J Respir Cell Mol Biol 31:351–357
Lindholm D, Wootz H, Korholen L (2006) ER stress and neurodegenerative diseases. Cell Death Differ 13:385–392
Liu Y, Choudhury P, Cabral CM, Sifers RN (1999) Oligosaccharide modification in the early secretory pathway directs the selection of a misfolded glycoprotein for degradation by the proteasome. J Biol Chem 274:5861–5867
Liu XL, Done SC, Yan K, Kilpelainen P, Pikkarainen T, Tryggvason K (2004) Defective trafficking of nephrin missense mutants rescued by a chemical chaperone. J Am Soc Nephrol 15:1731–1738
Lomas DA, Parfrey H (2004) Alpha1-antitrypsin deficiency. 4: molecular pathophysiology. Thorax 59:529–535
Lomas DA, Evans DL, Finch JT, Carrell RW (1992) The mechanism of Z alpha 1-antitrypsin accumulation in the liver. Nature 357:605–607
Lomas DA, Belorgey D, Mallya M, Onda M, Kinghorn KJ, Sharp LK, Phillips RL, Page R, Crowther DC, Miranda E (2004) Polymerisation underlies alpha1-antitrypsin deficiency, dementia and other serpinopathies. Front Biosci 9:2873–2891
Lubas WA, Spiro RG (1987) Golgi endo-alpha-n-mannosidase from rat liver, a novel N-linked carbohydrate unit processing enzyme. J Biol Chem 262:3775–3781
Lubas WA, Spiro RG (1988) Evaluation of the role of rat liver Golgi endo-alpha-n-mannosidase in processing N-linked oligosaccharides. J Biol Chem 263:3990–3998
Luocq JM, Brada D, Roth J (1986) Immunolocalization of the oligosaccharide trimming enzyme glucosidase II. J Cell Biol 102:2137–2146
Lukacs GL, Mohamed A, Karrner N, Chang XB, Riordan JR, Grinstein S (1994) Conformational maturation of CFTR but not its mutant counterpart (delta F508) occurs in the endoplasmic reticulum and requires ATP. EMBO J 13:6076–6086
Matsuda J, Suzuki O, Oshima A, Yamamoto Y, Noguchi A, Takimoto M1-gangliosidosis. Proc Natl Acad Sci USA 100:15912–15917
Mattioli L, Anelli T, Fagioli C, Taccetti C, Sitta R, Valetti C (2006) ER storage diseases: a role for ERGIC-53 in controlling the formation and shape of Russell bodies. J Cell Sci 119:2532–2541
McCracken AA, Brodsky JL (2005) Recognition and degradation of ERAD substrates to the proteasome and alternative paths for cell survival. Curr Top Microbiol Immunol 300:17–40
McNaught KSP, Olanow CW, Halliwell B, Isacson O, Jenner P (2001) Failure of the ubiquitin–proteasome system in Parkinson’s disease. Nat Rev Neurosci 2:589–594
McNaught KS, Mytilineou C, JnoBaptiste R, Yabut J, Shashidharan P, Jenner P, Olanow CW (2002a) Impairment of the ubiquitin–
proteasome system causes dopaminergic cell death and inclusion body formation in ventral mesencephalic cultures. J Neurochem 81:301–306

McNaught KS, Shashidharan P, Perl DP, Jenner P, Olanow CW (2002b) Aggresome-related biogenesis of Lewy bodies. Eur J Neurosci 16:2136–2148

McNaught KSP, Shashidharan P, Perl DP, Jenner P, Olanow CW (2002c) Aggresome-related biogenesis of Lewy bodies. Eur J Neurosci 16:2136–2148

McNaught KSP, Jackson T, JnoBaptiste R, Kapustin A, Olanow CW (2006) Proteasomal dysfunction in sporadic Parkinson’s disease. Neurology 66:S37–S49

Medeiros Neto G, Kim PS, Yoo SE, Vono J, Targovnik HM, Camargo R, Hossain SA, Arvan P (1996) Congenital hypothyroid goiter with deficient thyroglobulin—identification of an endoplasmic reticulum storage disease with induction of molecular chaperones. J Clin Invest 98:2838–2844

Meusser B, Hirsch C, Jarosch C, Sommer T (2005) ERAD: the long road to destruction. Nat Cell Biol 7:766–772

Molinar M, Calanca V, Galí C, Luca P, Paganetti P (2003) Role of EDEM in the release of misfolded glycoproteins from the calnexin cycle. Science 299:1397–1400

Moore SE, Sprio RG (1990) Demonstration that Golgi endo-z-mannosidase provides a glucosidase-independent pathway for the formation of complex N-linked oligosaccharides of glycoproteins. J Biol Chem 265:13104–13112

Moore SE, Sprio RG (1992) Characterization of the endomannosidase pathway for the processing of N-linked oligosaccharides in glucosidase II-deficient and parent mouse lymphoma cells. J Biol Chem 267:8443–8451

Morello JP, Bichet DG (2001) Nephrogenic diabetes insipidus. Annu Rev Physiol 63:607–630

Nakatsuaka K, Nishikawa S, Hosokawa N, Nagata K, Endo T (2001) Mnl1p, an alpha-mannosidase-like protein in yeast Saccharomyces cerevisiae, is required for endoplasmic reticulum-associated degradation of glycoproteins. J Biol Chem 276:8635–8638

Neuber O, Jarosch E, Volkwein C, Walter J, Sommer T (2005) Ubx2 links the Cdc48 complex to ER-associated protein degradation. Nat Cell Biol 7:993–998

Oda Y, Hosokawa N, Wada I, Nagata K (2003) EDEM as an acceptor of terminally misfolded glycoproteins released from calnexin. Science 299:1394–1397

Oda Y, Okada T, Yoshida H, Kaufman RJ, Nagata K, Mori K (2006) Derlin-2 and Derlin-3 are regulated by the mammalian unfolded protein response and are required for ER-associated degradation. J Cell Biol 172:383–393

Olanow CW, Perl DP, DeMartino GN, McNaught KS (2004) Lewy-body formation is an aggresome-related process: a hypothesis. Lancet Neurol 3:496–503

Olivi S, Galli C, Alanen H, Ruddock L, Molinari M (2005) A novel stress-induced EDEM variant regulating endoplasmic reticulum-associated glycoprotein degradation. J Biol Chem 280:2424–2428

Oyadomari S, Araki E, Mori M (2002a) Endoplasmic reticulum stress-mediated apoptosis in pancreatic beta-cells. Apoptosis 7:335–345

Oyadomari S, Koizumi A, Takeda K, Gotoh T, Akira S, Araki E, Mori M (2002b) Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes. J Clin Invest 109:525–532

Palade G (1956) Intracisternal granules in the exocrine cells of the pancreas. J Biophys Biochem Cytol 2:417–422

Palade G (1975) Intracellular aspects of the process of protein biosynthesis. Science 189:347–358

Papp E, Csermely P (2006) Chemical chaperones: mechanisms of action and potential use. Handb Exp Pharmacol 172:405–416

Park SH, Bolender N, Eisele F, Kostova Z, Takeuchi J, Coffino P, Wolf DH (2007) The cytoplasmic Hsp70 chaperone machinery subjects misfolded and endoplasmic reticulum import-incompetent proteins to degradation via the ubiquitin–proteasome system. Mol Biol Cell 18:153–165

Parodi AJ (2000) Protein glycosylation and its role in protein folding. Annu Rev Biochem 69:69–93

Parodi AJ, Mendelzon DH, Lederkremer GZ (1983) Transient glucosylation of protein-bound ManGlCNac2, ManGlCNac2, and ManGlCNac2 in calf thyroid cells. A possible recognition signal in the processing of glycoproteins. J Biol Chem 258:8260–8265

Pathak RK, Merkle RK, Cummings RD, Goldstein JL, Brown MS, Anderson R (1988) Immunocytochemical localization of mutant low density lipoprotein receptors that fail to reach the Golgi complex. J Cell Biol 106:1831–1841

Pavlova M, Roth J (2005) Functional ultrastructure. An Atlas of tissue biology and pathology. Springer, Vienna

Pedermon E, Lukacs GL, Du K, Caci E, Zegarra-Moran O, Galietta L, Verkman AS (2005) Small-molecule correctors of defective DeltaF508-CFTR cellular processing identified by high-throughput screening. J Clin Invest 115:2564–2571

Perelmutter DH (2002) Chemical chaperones: a pharmacological strategy for disorders of protein folding and trafficking. Pediat Res 52:832–836

Pethy-Repo U, Hogue M, Laperrière A, Walkers P, Bouvier M (2000) Export from the endoplasmic reticulum represents the limiting step in the maturation and cell surface expression of the human δ opioid receptor. J Biol Chem 275:13727–13736

Petitajo-Repo UE, Hogue M, Bhalla S, Laperrière A, Morello JP, Bouvier M (2002) Ligands act as pharmacological chaperones and increase the efficiency of delta opioid receptor maturation. EMBO J 21:1628–1637

Rabouille C, Sprio RG (1992) Nonselective utilization of the endomannosidase pathway for processing glycoproteins by human hepatoma (HepG2) cells. J Biol Chem 267:11573–11578

Raposo G, van S-HM, Leijendekker R, Geuze HJ, Ploegh HL (1995) Misfolded mutant histocompatibility complex class I molecules accumulate in an expanded ER–Golgi intermediate compartment. J Cell Biol 131:1403–1419

Ravid T, Kreft SG, Hochstrasser M (2006) Membrane and soluble substrates of the Doa10 ubiquitin ligase are degraded by distinct pathways. EMBO J 25:533–543

Riordan JR (1999) Cystic fibrosis as a disease of misprocessing of the cystic fibrosis transmembrane conductance regulator glycoprotein. Annu Rev Biochem 69:69–93

Rubinson DC (2006) The roles of intracellular protein-degradation pathways in neurodegeneration. Nature 443:780–786

Rubenstein RC, Zeitlin PL (2000) Sodium 4-phenylbutyrate downregulates Hsc70: implications for intracellular trafficking of Del-taF508-CFTR. Am J Physiol Cell Physiol 278:C259–267

Saraste J, Palade G, Farquhar M (1989) Antibodies to rat pancreas Golgi subfractions: identification of a 58-kD cis-Golgi protein. J Cell Biol 105:2021–2029

Sawarkar AR, Cheng WC, Beutler E, Wong CH, Balch WE, Kelly JW (2002) Chemical chaperones increase the cellular activity of
N370S beta-glucosidase: a therapeutic strategy for Gaucher disease. Proc Natl Acad Sci USA 99:15428–15433
Sayed A, Ng DT (2005) Search and destroy: ER quality control and ER-associated protein degradation. Crit Rev Biochem Mol Biol 40:75–91
Schubert C, Buchberger A (2005) Membrane-bound Ub2 recruits Cdc48 to ubiquitin ligases and their substrates to ensure efficient ER-associated protein degradation. Nat Cell Biol 7:999–1006
Schubert U, Anton LC, Gibbs J, Norbury CC, Yewdell JW, Bennink JR (2000) Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. Nature 404:770–774
Schweizer A, Fransen JA, Bachi T, Gimsel L, Hauri HP (1988) Identification, by a monoclonal antibody, of a 53-kD protein associated with a tubulo-vesicular compartment at the cis-side of the Golgi apparatus. J Cell Biol 107:1643–1653
Selkoe DJ (2003) Folding proteins in fatal ways. Nature 426:900–904
Shults CW (2006) Body liquids. Proc Natl Acad Sci USA 103:1661–1668
Sifer RN, Brashears-Macatee S, Kidd VJ, Muensch H, Woo SL (1988) A frameshift mutation results in a truncated alpha 1-antitrypsin that is retained within the rough endoplasmic reticulum. J Biol Chem 263:7330–7335
Sitia R, Braakman I (2003) Quality control in the endoplasmic reticulum protein factory. Nature 426:891–894
Sohn S, Hur W, Joe MK, Kim JH, Lee ZW, Ha KS, Kee C (2002) Expression of wild-type and truncated myocilins in trabecular meshwork cells: their subcellular localizations and cytotoxicities. Invest Ophthalmol Vis Sci 43:3680–3685
Soto C (2003) Unfolding the role of protein misfolding in neurodegenerative diseases. Nat Rev Neurosci 4:49–60
Sousa M, Parodi AJ (1995) The molecular basis for the recognition of misfolded glycoproteins by the UDP-Glc:glycoprotein glucosyltransferase. EMBO J 14:4196–4203
Spiro RG (2000) Glucose residues as key determinants in the biosynthesis and quality control of glycoproteins with N-linked oligosaccharides. J Biol Chem 275:35657–35660
Swanson R, Locher M, Hochstrasser M (2001) A conserved ubiquitin ligase of the nuclear envelope/endoplasmic reticulum. J Cell Biol 155:355–368
Tamarappoo BK, Verkman AS (1998) Defective aquaporin-2 trafficking in nephrogenic diabetes insipidus and correction by chemical chaperones. J Clin Invest 102:2257–2267
Tamm E (2002) Myocilin and glaucoma: facts and ideas. Prog Retin Eye Res 21:395–428
Tsai B, Ye YH, Rapoport TA (2002) Retro-translocation of proteins from the endoplasmic reticulum into the cytosol. Nat Rev Mol Cell Biol 3:246–255
Turner GC, Varshavsky A (2000) Detecting and measuring cotranslational protein degradation in vivo. Science 289:2117–2120
Tveten K, Holla OL, Ranheim T, Berge KE, Leren TP, Kulseth MA (2007) 4-Phenylbutyrate restores the functionality of a misfolded mutant low-density lipoprotein receptor. FEBS J 274:1881–1893
Valetti C, Grossi CE, Milstein C, Sitia R (1991) Russell bodies: a general response of secretory cells to synthesis of a mutant immunoglobulin which can neither exit from, nor be degraded in, the endoplasmic reticulum. J Cell Biol 115:983–994
Vashist S, Ng DT (2004) Misfolded proteins are sorted by a sequential checkpoint mechanism of ER quality control. J Cell Biol 165:41–52
Vashist S, Kim W, Belden WJ, Spear ED, Barlowe C, Ng DT (2001) Distinct retrieval and retention mechanisms are required for the quality control of endoplasmic reticulum protein folding. J Cell Biol 155:355–368
Vidair C, Huang R, Doxsey S (1966) Heat shock causes protein aggregation and reduced protein solubility at the centrosome and other cytosolic locations. Int J Hyperther 12:681–695
Wang J, Takeuchi T, Tanaka S, Kubo SK, Kayo T, Lu D, Takata K, Koizumi A, Izumi T (1999) A mutation in the insulin 2 gene induces diabetes with severe pancreatic beta-cell dysfunction in the Mody mouse. J Clin Invest 103:27–37
Ward CL, Kopito RR (1994) Intracellular turnover of cystic fibrosis transmembrane conductance regulator. Inefficient processing and rapid degradation of wild-type and mutant proteins. J Biol Chem 269:25710–25718
Welch WJ, Brown CR (1996) Influence of molecular and chemical chaperones on protein folding. Cell Stress Chaperones 1:109–115
Wigley WC, Fabunmi RP, Lee MG, Marino CR, Muﬄem S, DeMartino GN, Thomas PJ (1999) Dynamic association of proteasomal machinery with the centrosome. J Cell Biol 145:481–490
Wojcik C, Schroeter D, Wilk S, Lamprecht J, Paweletz N (1996) Ubiquitin-mediated proteolysis centers in HELa cells: indication from studies of an inhibitor of the chymotrypsin-like activity of the proteasome. Eur J Cell Biol 71:311–318
Wolf DH, Hilt W (2004) The proteasome: a proteolytic nanomachine of cell regulation and waste disposal. Biochim Biophys Acta Mol Cell Res 1695:19–31
Yam GH, Zuber C, Roth J (2005) A synthetic chaperone corrects the trafficking defect and disease phenotype in a protein misfolding disorder. FASEB J 19:12–18
Yam GH, Bosshard N, Zuber C, Steinmann B, Roth J (2006) Pharmacological chaperone corrects lysosomal storage in Fabry disease caused by trafficking-incompetent variants. Am J Physiol Cell Physiol 290:C1076–1082
Yam GH, Gapalovska-Kysela K, Zuber C, Roth J (2007a) Sodium 4-phenylbutyrate acts as a chemical chaperone on misfolded myocilin to rescue cells from endoplasmic reticulum stress and apoptosis. Invest Ophthalmol Vis Sci 48:1683–1690
Yam GH, Gapalovska-Kysela K, Zuber C, Roth J (2007b) Aggregated myocilin induces Russell bodies and causes apoptosis: implications for the pathogenesis of myocilin-caused primary open-angle glaucoma. Am J Pathol 170:100–109
Yam GH, Gapalovska-Kysela K, Zuber C, Roth J (2007c) Aggregated myocilin induces Russell bodies and causes apoptosis—implications for the pathogenesis of myocilin-caused primary open-angle glaucoma. Am J Pathol 170:100–109
Ye Y, Meyer HH, Rapoport TA (2003) Function of the p97–Ufd1–Npl4 complex in retrotranslocation from the ER to the cytosol: dual recognition of nonubiquitinated polypeptide segments and polyubiquitin chains. J Cell Biol 162:71–84
Ye Y, Shibata Y, Yun C, Ron D, Rapoport TA (2004) A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol. Nature 429:841–847

Younger JM, Chen L (2006) Sequential quality-control checkpoints triage misfolded cystic fibrosis transmembrane conductance regulator. Cell 126(3):571–582

Zhang K, Kaufman RJ (2006) Protein folding in the endoplasmic reticulum and the unfolded protein response. Handb Exp Pharmacol:69–91

Ziak M, Meier M, Etter KS, Roth J (2001) Two isoforms of trimming glucosidase II exist in mammalian tissues and cell lines but not in yeast and insect cells. Biochem Biophys Res Commun 280:363–367

Zuber C, Spiro MJ, Guhl B, Spiro RG, Roth J (2000) Golgi apparatus immunolocalization of endomannosidase suggests post-endoplasmic reticulum glucose trimming: implications for quality control. Mol Biol Cell 11:4227–4240

Zuber C, Fan JY, Guhl B, Parodi A, Fessler JH, Parker C, Roth J (2001) Immunolocalization of UDP-glucose: glycoprotein glucosyltransferase indicates involvement of pre-Golgi intermediates in protein quality control. Proc Natl Acad Sci USA 98:10710–10715

Zuber C, Fan JY, Guhl B, Roth J (2004) Misfolded proinsulin accumulates in expanded pre-Golgi intermediates and endoplasmic reticulum subdomains in pancreatic beta cells of Akita mice. FASEB J 18:917–919

Zuber C, Cormier JH, Guhl B, Santimaria R, Hebert DN, Roth J (2007) EDEM1 reveals a quality control vesicular transport pathway out of the endoplasmic reticulum not involving the COPII exit sites. Proc Natl Acad Sci USA 104:4407–4412

Zwickl P, Seemuller E, Kapelari B, Baumeister W (2002) The proteasome: a supramolecular assembly designed for controlled proteolysis. In: Richards FM, Eisenberg DS, Kuriyan J (eds) Protein folding in the cell, vol 59. Academic Press Inc., San Diego, pp 187–222