Endoplasmic reticulum storage diseases

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Summary

The endoplasmic reticulum represents the cell's quality control site for accurate folding of secretory and membrane proteins. Quality control is achieved through the association of ER chaperones with unfolded or misfolded polypeptide chains. In the ER stress response, upregulation of chaperones occurs as a consequence of misfolded proteins accumulating in the ER lumen; if these proteins fail to assume their native structure, they are retained in the ER and targeted for degradation by the proteasome. ER storage diseases (ERSDs) are a group of genetically based disorders in which mutant proteins fail to pass the ER quality control. Because all eukaryotic cells contain the ER, the clinical phenotype of ERSDs is very heterogeneous. Disease may result from the mere lack of the mutant protein in question and/or may be caused indirectly by toxic effects of the misfolded protein or aggregates thereof on the cell. Additionally, the cell's reaction to the ER stress may include signaling pathways which are ultimately detrimental.

Experimentally, ERSDs serve as models to study the cellular reactions to a variety of perturbations. In particular, understanding the links between ER stress and cell degeneration may give valuable insights into the pathogenesis of other diseases where the accumulation of indigestible toxic material leads to cell injury.

Key words: endoplasmic reticulum; storage disease; quality control; protein folding; protein degradation

Storage diseases: defects in degradation or in biosynthesis

Storage diseases are caused by the intracellular accumulation of endogenous compounds which directly or indirectly impair cellular functions and may even lead to cell death. A well-known group of these disorders includes the *lysosomal storage diseases* which are caused by the accumulation of molecules from the cell exterior in lysosomes. As part of their continuous turnover, proteins, glycans, and lipids from the cell surface are internalised and transported via the endocytic pathway (clathrin-coated vesicles – early endosomes – late endosomes) to lysosomes for degradation. Incomplete degradation due to defective or missing hydrolases results in an expansion of lysosomes filled with indigestible degradation intermediates, damaging cellular function and thus causing disease. For example, in Fabry's disease (OMIM[1] entry 301 500), mutations in the X-chromosomal gene α galactosidase A cause functional defects of an enzyme involved in glycolipid breakdown. This in turn leads to lysosomal accumulation of galactosylgalactosylglucosylceramide in various tissues, eventually causing organ dysfunction and premature death of the patients. Other examples of this group of disorders include Tay-Sachs, Gaucher's, and Niemann-Pick diseases.

Endoplasmic reticulum (ER) storage diseases (ERSDs) [1], in contrast, are characterized by the accumulation of proteins in the very first compartment of their normal biography because of defects early in biogenesis. Synthesis of all but a few mitochondrial proteins is initiated at cytosolic ribosomes. Proteins normally functional in the ER or the Golgi apparatus, at the plasma membrane or in the endosomal-lysosomal system, or which are destined for secretion from the cell, are first targeted to the ER, the major site of insertion of proteins into the lipid bilayer and of translocation across the membrane. Figure 1 illustrates the major routes of membrane transport originating from the ER in eukaryotic cells.

Correctly folded proteins exit in transport vesicles which bud from the ER and congregate at the ER-Golgi-intermediate compartment (ERGIC). By vesicles or tubules they are delivered to the *cis*-side of the Golgi apparatus and move (by vesicular transport or cisternal maturation) through the Golgi cisternae to the *trans*-Golgi network (TGN). From here, different pathways diverge: (1) constitutive secretory vesicles to the cell surface; (2) secretory granules of the regulated secretory pathway in endocrine or exocrine cells; (3)
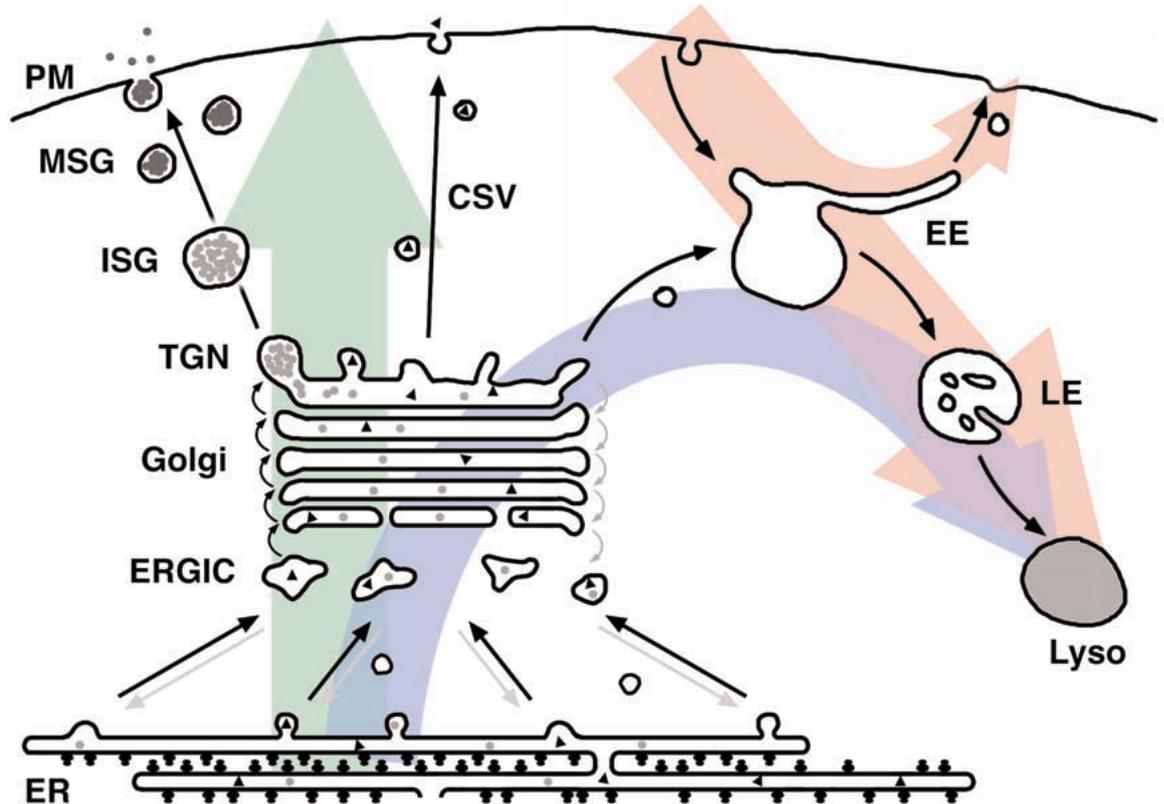
vesicles to endosomes containing mannose-
6-phosphate receptors and their cargo, lysosomal
enzymes that have been tagged by a mannose-
6-phosphate modification on N-glycans.

In contrast, proteins unable to acquire their
native conformation accumulate in the ER, at least
to some extent. In most cases, after some lag pe-
riod, they are degraded by a process called *ER-
associated degradation* (ERAD; see figure 2). The
machinery for ERAD is located in the cytosol.

Functional defects could occur at any of the
transport and sorting steps described above. For
example, mutations in the signal sequence affect-
ing membrane translocation or signal processing
may cause familial hypoparathyroidism [2, 3], co-
agulation factor X deficiency [4, 5], Crigler-Najjar
syndrome type II [6], familial central diabetes in-
sipidus [7, 8] or chronic pancreatitis [9]. Only few
instances of impaired protein exit from the Golgi
apparatus are known and the term "Golgi storage
disease" is not commonly used. One example is au-

tosomal dominant nephrogenic diabetes insipidus
(discussed in more detail below). In adult autoso-
mal dominant polycystic kidney disease (OMIM
entries 173 900, 601 313, 173 910) the polarized
traffic of proteins from the trans-Golgi network to
the basolateral membrane in proximal tubular cells
is impeded. This sorting and transport defect ap-
pears to be caused by a deranged cytoarchitecture,
resulting from complex interactions of proteins in-
volved in cell-cell adhesion with mutated proteins
named polycystins [10].

The vast majority of mutations, however, af-
fect protein folding in the ER. ER retention may
occur in any cell type, explaining the wide variety
of the clinical spectrum of the ERSDs. In this re-
view we will summarize the structure and function
of the ER and introduce the heterogeneous group
of ER-associated disorders, focusing on some clin-
ically relevant examples. We will also discuss data
from our own research dealing with a rare but rep-
resentative member of the ERSD family.

The endoplasmic reticulum: protein folding and quality control

The steps of protein synthesis, transport and
degradation discussed in this section are illustrated
in figure 2.

Protein translocation and folding

All eukaryotic cells contain an ER, an inter-
connected tubular membrane network continuous
with the outer nuclear membrane. It is the site of
synthesis of membranes and proteins which even-

tually form the organelles of the secretory and
endocytic pathways, the plasma membrane, and
the extracellular matrix. The lumen of the ER
corresponds topologically to the cell exterior and
presents intracellularly the conditions prevailing
outside of the cell.

The information necessary to target a protein
to the ER is contained in its sequence, as originally
proposed in Blobel and Dobberstein's *signal hy-*

Figure 2

Into and out of the ER. (1) Proteins are targeted to the ER by interaction of the hydrophobic signal sequence with signal recognition particle (SRP) and (2) of the complex to the SRP receptor. (3) The nascent polypeptide is translocated through the Sec61 translocation pore. Concomitantly, (4) the signal may be cleaved by signal peptidase and (5) the protein may be glycosylated by oligosaccharyltransferase. (6) Chaperones interact with the as yet unfolded polypeptide. (7) Upon folding into the native conformation, the protein is released to exit the ER. (8) Misfolded proteins are kept back and eventually retrotranslocated via the Sec61 translocation pore into the cytoplasm. (9) The retrotranslocated protein is ubiquitinated by specific ubiquitin ligases and glycans are removed by a cytosolic N-glycanase. (10) The ubiquitin-tagged protein is a substrate for degradation by the cytosolic proteasome.
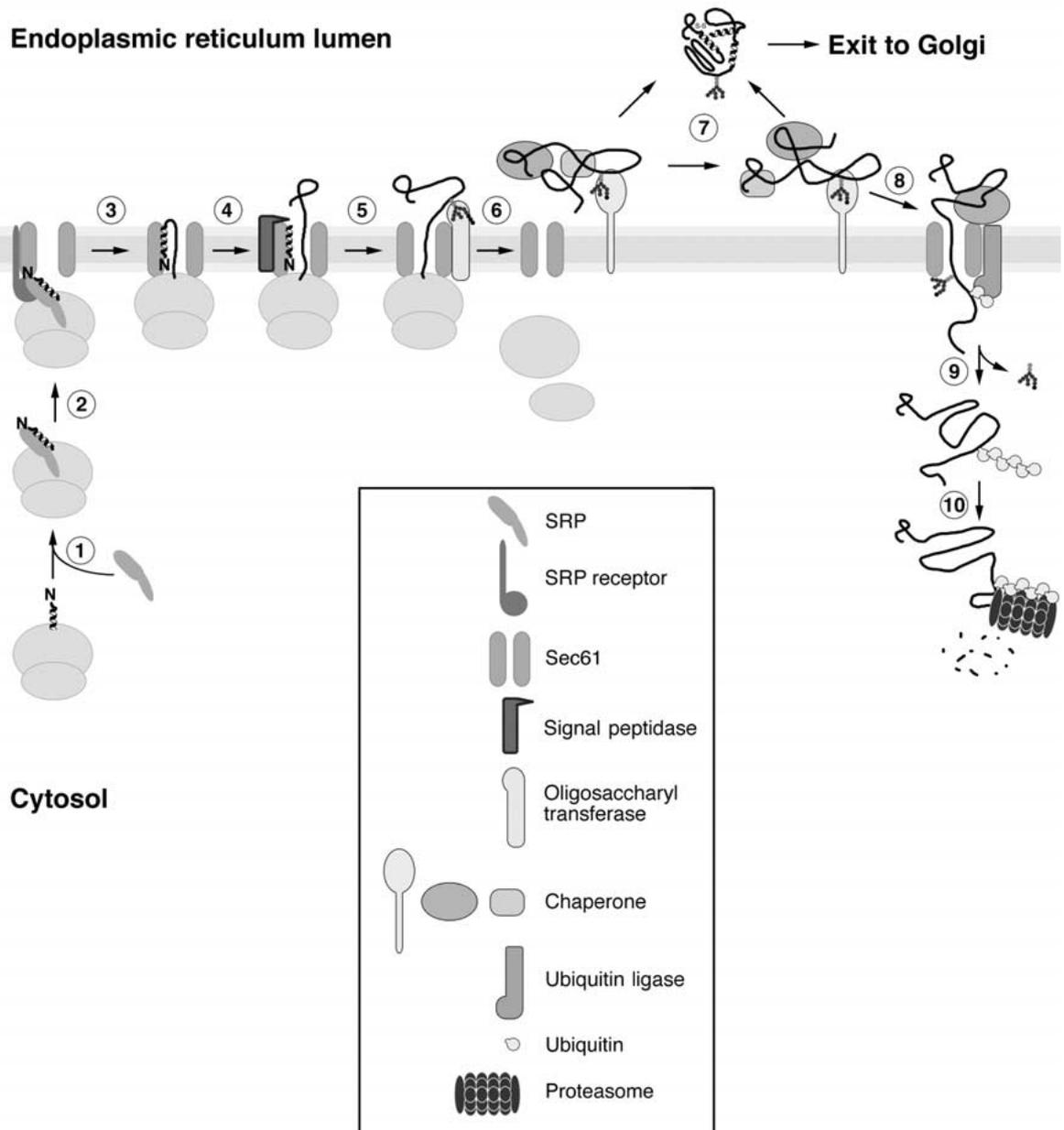
pothesis [11]. A hydrophobic signal peptide, typically at the N-terminus, serves as an "address tag" to direct the growing polypeptide with its translating ribosome to the ER by interaction with the cytosolic signal recognition particle (SRP) and the SRP receptor in the ER membrane [12]. The nascent protein is threaded through an aqueous pore in the membrane, the translocon, formed by the heterotrimeric Sec61 complex. Cotranslationally, i.e. while translation is still ongoing, the nascent polypeptide is modified by translocon-associated proteins: the signal sequence is cleaved by signal peptidases, and N-glycans are added by oligosaccharyltransferases.

Coupling of translation and translocation prevents premature protein folding and assures that the polypeptide is released directly into the exoplasmic environment with oxidizing conditions and high calcium concentration. In addition, the ER provides a machinery to assist protein folding and to assure that only correctly folded proteins

can move on along the secretory pathway [13]. Part of this quality control mechanism are *chaperones*, ER resident folding assistants which associate with the unfolded or misfolded substrates, preventing their aggregation and thus aiding them to achieve their native conformation [14]. As long as folding is incomplete and the proteins are bound to chaperones, they are retained in the ER.

Chaperones comprise a family of conserved molecules found in bacteria, yeast and higher eukaryotes, in the cytosol as well as in mitochondria and the ER. They are expressed constitutively, but are induced by stress conditions like heat shock or glucose starvation; hence the synonyms heat shock proteins (HSPs) or glucose-regulated proteins (GRPs). GRP78 (also called BiP) [15], a prominent chaperone of the ER, was shown to bind to hydrophobic domains temporarily exposed during the folding process of substrate proteins. Binding and release of substrates is accompanied by ATP hydrolysis and thus energy driven. Calnexin and

calreticulin are lectins binding to monoglucosylated intermediates of N-linked glycans. The carbohydrate structures are continuously deglucosylated and reglucosylated, but reglucosylation stops when the protein is properly folded [16]. The formation of correct disulfide bonds, which are present in many exoplasmic proteins and stabilise their native tertiary structure, is promoted by protein disulfide isomerase (PDI) [17]. In addition, PDI acts as a nonenzymatic chaperone independently of oxidisable cysteines [18, 19]. Protein prolyl isomerase catalyses the *cis/trans* isomerisation of prolines, one of the rate-limiting steps in protein folding. The interaction of chaperones with newly synthesised proteins is neither specific nor exclusive, i.e. a particular chaperone interacts with a variety of different proteins, and different chaperones may bind to a nascent chain sequentially during the ongoing folding process.

ER exit *vs.* ER-associated degradation (ERAD)

Once the protein has reached its correct structure, it is free to leave the ER by membrane vesicles to the ER-Golgi intermediate compartment, ERGIC [20], and further along the secretory pathway. ER exit has long been assumed to occur by bulk flow. However, recently it has been discovered that cargo receptors facilitate ER exit for at least a subset of proteins. A prominent example is ERGIC-53 [21], a transmembrane lectin that cycles between ER, ERGIC, and Golgi. ERGIC-53 binds certain glycoproteins in a calcium-dependent manner [22]. Upon mutation of ERGIC-53, ER exit of a small number of proteins is significantly reduced, in particular coagulation factors V and VIII ([23], see below), resulting in a congenital bleeding disorder.

Proteins that failed to fold and/or to oligomerize correctly are retained in the ER and ultimately degraded. So far, no degradative pathway within the ER itself has been found. Rather, the protein is retrotranslocated from the ER lumen to the cytosol through the same pore-forming protein complex that serves to translocate nascent chains into the ER [24–27]. Interchain disulfide bonds are reduced and complexes dissociated before retrotranslocation [28]. As illustrated in Figure 2, ER-associated degradation actually takes place in the cytosol using to a large part the machinery for degradation of cytosolic proteins [29, 30]. First, multiple activated ubiquitin molecules (a 76-amino acid protein) are coupled as a chain to the protein substrate by specific ER-associated ubiquitin-conjugating enzymes. The polyubiquitinated protein is then hydrolysed by the 26S proteolytic complex, a large multisubunit structure composed of a 20S proteolytic core (*proteasome*) and two 19S regulator complexes. Besides mutant misfolded proteins, proteasomal substrates also include viral proteins that are processed to polypeptides for subsequent MHC-associated presentation at the cell surface, and wild-type proteins that fail to fold properly due to translational or post-translational errors. In fact, it has been estimated that in various eukaryotic cell types, 30% or more of newly synthesised proteins are defective and need to be degraded [31].

ER stress response

A number of stimuli which perturb ER functions induce a specific alteration of the cell's gene expression pattern. ER stress may be caused by conditions that interfere with glycosylation (glucose starvation, glycosylation inhibitors) or with protein folding (reducing agents, heavy metals), or by the accumulation of mutant proteins in the organelle. In addition, overload of the ER with wild-type proteins also confers ER stress. How does the cell respond when the capacity of the folding and sorting machineries of the ER are exceeded? In the past years a number of specific signaling pathways from the ER to the nucleus have been described [32] which help the cell to deal with the stress, or else drive it into cell death.

The unfolded protein response (UPR)

This pathway has been elucidated in great detail in yeast. Accumulation of incorrectly folded proteins in the ER lumen leads to upregulated expression of UPR target genes encoding ER resident chaperones such as BiP or PDI [32]. Hereby, synthesis of the transcription factor Hac1p is induced by differential splicing of HAC1 mRNA through a novel pathway involving the ER membrane protein kinase Ire1p [33–35]. Recent data suggest that the lumenal domain of Ire1p, which binds BiP, may have a role as "stress sensor" [36]. Hac1p binds to an upstream response sequence common to the UPR target genes, thereby upregulating their transcription to increase ER folding capacity. In mammalian cells, IRE1 alpha initiates splicing of the transcription factor XBP1 mRNA to generate a potent transcriptional activator of UPR genes [37].

The ER overload response (EOR)

This cellular response partly overlaps with, but is distinct from the UPR [32]. Certain, but not all of the conditions that evoke the UPR will also trigger the EOR. In contrast to the UPR, the EOR activates the nuclear transcription factor NFκB [38]. The promoter sequences of the ER chaperones BiP and grp94 do not contain NFκB binding sites; accordingly, the EOR-associated NFκB induction does not increase their expression levels [38]. NFκB is a mediator of inflammatory and immune responses. Target genes include those encoding β-interferon, interleukins-1 and -8, TNF-α, MHC class I, or $β_2$-microglobulin. Since the EOR can be triggered by high levels of wild-type (e.g. viral) or mutant proteins processed through or retained in the ER, it is believed to have a role in a broad unspecific antiviral host response [32, 39]. Whether the inflammatory reactions accompanying ER storage diseases such as cystic fibrosis or $α_1$-anti-

trypsin deficiency are also mediated by NFκB is unknown.

ER stress and its link to programmed cell death

Cellular stress, implicated either through DNA damage (e.g. by alkylating agents) or ER stress, induces a nuclear transcription factor called CHOP [40, 41]. Recent experiments in mice have shown that elimination of the gene encoding CHOP markedly diminishes apoptotic and regenerative responses of proximal tubular cells to intraperitoneal tunicamycin injection [42]. This drug causes the clinical and histological picture of acute tubular necrosis in both CHOP-knockout and wild-type animals. In this model, CHOP may thus have a role in effective disposal of injured cells and subsequent tissue regeneration. Target genes of CHOP have also been identified that support

the concept of CHOP being involved in programmed cell death [43]. So far, however, little is known about mediators of cell injury associated with ER storage diseases.

The UPR and ERAD are intimately linked. A recent study in yeast using a DNA microarray technique showed that induction of the UPR upregulates not only genes essential for vesicular transport, lipid metabolism, posttranslational modification and chaperone synthesis, but also multiple genes involved in ERAD/proteasomal protein degradation [44]. Moreover, defects in ERAD constitutively activate the UPR, and simultaneous deficiency of both pathways decreases cell viability. Thus, these pathways cooperate to protect the cell from detrimental effects implicated by the accumulation of misfolded proteins.

Selected examples of ER storage diseases

ERSDs can be divided into several pathogenetic groups (see table 1). In the vast majority, the defect lies within the cargo, i.e. the secretory protein encoded by a mutated gene. ER retention

of such proteins can cause disease by different mechanisms. In the simplest case, the protein (e.g., enzyme or hormone) activity is lacking at its normal site of action, particularly in a homozygous situation. However, folding mutants may also form toxic ER lumenal or cytoplasmic aggregates called Russell bodies and aggresomes, respectively [45], or toxic degradation products and thus compromise functionality and viability of affected cells. As a result, these mutations are often dominant.

On the other hand, the transport machinery can harbour a defect, as exemplified by the ERGIC53 mutant mentioned above. Since a number of the known ER storage diseases have not been characterised in detail by expression studies in transfected cells, however, such a pathogenetic classification remains theoretical. We will therefore discuss some members of the ERSD family from which detailed data are available.

Table 1

Endoplasmic reticulum storage diseases.

1. Lack of protein due to ER retention
Cystic fibrosis and associated diseases
α₁-antitrypsin deficiency without liver disease
Congenital hypothyroidism
Thyroglobulin deficiency
Thyroid peroxidase deficiency
Thyroxin binding globulin deficiency
Protein C deficiency
Disorders of lipid metabolism
LDL receptor defect
Lipoprotein lipase deficiency
Lipoprotein(a) deficiency
Hereditary hypoparathyroidism
Nephrogenic diabetes insipidus due to mutations in AVP receptor 2 or aquaporin-2
Growth hormone receptor deficiency
Osteogenesis imperfecta
Procollagen type I, II, IV deficiency
Albinism/tyrosinase deficiency
Obesity/elevated prohormone levels: prohormone convertase 1 deficiency
2. Toxic protein or protein aggregates
Autosomal dominant neurohypophyseal diabetes insipidus
Liver disease in α₁-antitrypsin deficiency
? Creutzfeldt-Jakob disease
? Retinitis pigmentosa
3. Defective transport machinery
Combined coagulation factor V and VIII deficiency (ERGIC-53 mutation)
Abetalipoproteinemia/deficiency of microsomal triglyceride transfer protein

Cystic fibrosis (CF) (OMIM entries 219700, 602421)

CF is an autosomal recessive disorder affecting the pancreas (exocrine and endocrine insufficiencies), bronchial glands (chronic lung infections and emphysema), biliary tree (biliary cirrhosis), intestinal glands (meconium ileus), reproductive organs, and sweat glands. In our era, lung disease clearly determines the course and life expectancy in CF patients. The CF gene was identified in 1989 by positional cloning [46]. It encodes a large (~168 kDa) epithelial membrane protein called cystic fibrosis transmembrane conductance regulator (CFTR) [47], which functions as a chloride channel and regulator of other ion channels. Depending on the nature of the polarised epithelium expressing CFTR, the transporter may have a role in chloride secretion or reabsorption across the epithelial membrane, but the mechanisms by which defective ion transport and/or regulation of other

membrane transporters produce CF-associated tissue injury are still under debate. For example, whether the CFTR defect results in high salt concentration or low volume in the airway surface liquid remains unsettled [48], but mechanical (mucociliary clearance) and chemical antibacterial defense may be impeded by the altered fluid composition. Colonisation and chronic infection with *Pseudomonas aeruginosa* develop in most CF patients, accounting for their early mortality. Superinfection with *Burkholderia cepacea* or *aspergillus* spp. often occurs. Interestingly, recent data have shown that CFTR functions specifically as a receptor for lipopolysaccharide components of *P. aeruginosa*, which after binding is internalised by bronchial epithelial cells (reviewed in ref. [49]). Thus, this mechanism of host defense to reduce the bacterial burden appears to be defective in CF.

Over 800 CFTR mutations have been identified, the most frequent (found in ~$2/3$ of all patients) being a deletion of phenylalanine at position 508 (ΔF508; ref. [50]), which causes severe disease in the homozygous patient. Pathogenesis and clinical severity differ in various mutations, depending on their functional nature, zygosity, and the genetic background in which the mutation occurs. For example, mutations leading to a premature stop in protein synthesis or to ER retention due to protein misfolding cause a severe phenotype, while others decreasing chloride conductance without completely abolishing it may present as an isolated pancreatic insufficiency. Heterozygosity (including ΔF508) or compound heterozygosity (i.e. the presence of different mutations on each of the two alleles) for a "severe" (e.g., ΔF508) and a "mild" mutation or polymorphism may cause chronic "idiopathic" pancreatitis [51, 52], CF with preserved pancreatic function, or disseminated bronchiectasis. Disease onset in such patients may be retarded to the 5th or 6th decade. Males with CF are infertile because of the absence of functional vasa deferentia. Conversely, (compound) heterozygous CFTR mutations can cause an isolated infertility syndrome called congenital bilateral aplasia of the vas deferens (OMIM entry 277180). These patients present without the other phenotypic hallmarks of CF.

A number of studies in heterologous expression systems have documented that CFTR ΔF508 and other mutations associated with CF fail to mature and are trapped in the ER [53] or ERGIC [54]. In cultured cells of various types, both wild-type and ΔF508 CFTR is associated with calnexin and other chaperones, but only the wild-type molecule can escape from this association and traffic through the Golgi to the plasma membrane. In contrast, ΔF508 CFTR fails to exit the ER [55] and after retrotranslocation to the cytosol is degraded by the ubiquitin-proteasome system [56–58]. The defect in protein maturation and ER exit appears to be temperature sensitive: if ΔF508 CFTR is expressed in cells grown at low temperatures (30 °C or below), a proportion of molecules escape and

are transported to the plasma membrane [59, 60]. The same effect can be obtained by strongly overexpressing the mutant protein [61] or by treatment of cells with so-called chemical chaperones (e.g. glycerol). Interestingly, patch-clamp studies have demonstrated that ΔF508 CFTR localised in the ER [62] or plasma membrane [61, 63] retains its function as chloride channel. This confirmed the notion that the CF phenotype associated with ΔF508 CFTR (as well as other allelic variants, see ref. [53]) appears to originate from mislocalisation and premature degradation, rather than altered functional properties or decreased stability of mutant CFTR.

Electron microscopic studies in a pancreatic cell line derived from a ΔF508 homozygous CF patient [56] and immunofluorescence experiments in primary upper airway epithelial cells from patients with various genotypes [64] localised the mutant CFTR proteins to the ER, confirming the findings of the heterologous expression studies discussed above. Thus, the sum of the available experimental data has rendered CF a classic example of an ER storage disease, and the search for agents promoting the delivery of the mutant protein to the plasma membrane has been included in therapeutic strategies. For example, treatment of cultured human respiratory epithelium CF-cells with thapsigargin leads to correct routing and plasma membrane insertion of the mutant CFTR molecule, presumably by depleting ER lumenal calcium stores and thus interfering with the chaperone machinery [64a]. Importantly, thapsigargin restores chloride channel activity in the apical plasma membrane of these cells. When given in aerosolized form to transgenic CF mice carrying the ΔF508 mutation, the drug was well tolerated and changed the membrane potential of nasal epithelium cells towards that seen in wild-type animals [64a].

The view of CF as a prototypic example of a ERSD, however, has been challenged by a recent report examining various *ex vivo* tissue samples from CF patients [65]. As expected, ΔF508 CFTR was not found at the apical membrane of eccrine sweat glands, but in the airway and intestinal epithelium, ΔF508 CFTR was localised to the plasma membrane in patterns indistinguishable from wild-type. Therefore, with the reports on ion channel function in mind [62, 63], one has difficulty to explain the severe phenotype of the ΔF508 mutation in vivo. This issue remains currently unsettled.

α₁-antitrypsin deficiency (OMIM entry 107400)

α₁-antitrypsin (or α₁-protease inhibitor; A1Pi), an acute phase protein synthesised by the liver, is the most abundant serine protease inhibitor in plasma. The clinical picture of A1Pi deficiency varies with zygosity and the type of mutation present. Heterozygous individuals may be asymptomatic carriers but can have a predisposi-

tion to obstructive lung disease at later ages. In patients homozygous for a null mutation no A1Pi will be produced or secreted into the bloodstream. These individuals are at risk for bronchiectasis and pulmonary emphysema due to the unopposed proteolytic activity of neutrophil elastase in the lung, which leads to chronic tissue destruction. Certain other mutations lead to a change of function of the A1Pi molecule. For example, due to the replacement of arginine for methionine at position 358, A1Pi "Pittsburgh" loses its antitrypsin activity but obtains heparin-independent antithrombin activity, causing the phenotype of a severe bleeding disorder [66]. Finally, so-called deficiency mutations direct the synthesis of an aberrantly processed molecule. The most important member of this class is the allelic variant Pi Z (replacement of glutamic acid by lysine at position 342), which is the most frequent A1Pi mutation overall with an allele frequency of 0.01–0.02 in Caucasians. Plasma levels of A1Pi Z homozygous patients are greatly reduced but measurable. Homozygotes are prone to develop early pulmonary emphysema and, in a subset of patients (~15%), cirrhosis and hepatoma [67]. It was shown nearly 30 years ago that hepatocytes in patients with A1Pi deficiency contain large insoluble intracellular inclusions [68]. It is now known that the majority of Pi Z proteins (approximately 85%) are retained in the endoplasmic reticulum in patients and in transgenic mice expressing the human Pi Z gene [69]. On the molecular level, the reactive center loop, the base of which harbours the amino acid substitution, folds into a β-sheet of a neighbouring protein, thus allowing a so-called loop-sheet polymerisation [70] and leading to the formation of large aggregates. Several papers have reported that the degradation of this ER-retained material occurs through the proteasome in a ubiquitination-dependent manner [71, 72], although other data suggested that in hepatocytes degradation pathways other than the proteasome may be involved [73]. Mutants other than A1Pi Z have also been found to be ER-retained by coprecipitation with the ER chaperone calnexin [74] or immunofluorescence and immunoprecipitation experiments [75].

From these clinical and molecular data two major questions regarding the pathophysiology of α_1-antitrypsin deficiency emerge. First, why does only a relatively small percentage of patients homozygous for the Pi Z mutation develop liver disease, while all are at risk for pulmonary emphysema? Lung damage results from absence or low levels of A1Pi. There is experimental evidence that in the subset of patients which are susceptible to liver damage, ER-associated degradation of A1Pi Z is specifically delayed [76]. Additionally, hepatic net synthesis of ER stress proteins and of ubiquitin is increased selectively in A1Pi Z individuals prone to liver disease, but not in those solely developing emphysema or in patients with other mutations, e.g. the allele A1Pi S [77]. Secondly, why and how does cirrhosis develop in the susceptible

individuals? If the degradation of the insoluble material accumulating in the ER is inefficient, one would indeed propose a toxic effect of these aggregates on the hepatocyte. The mechanism by which this toxicity occurs and the factors controlling successful degradation of ER-retained A1Pi Z, however, are unknown.

Congenital goiter and hypothyroidism due to thyroglobulin deficiency (OMIM entry 188450)

Thyroglobulin (Tg) is a large (~330 kDa) homodimeric glycoprotein and forms the major protein constituent in follicular lumenal colloid, where it serves as storage pool for thyroid hormones. Upon TSH stimulation, colloid is taken up into the follicular cell by endocytosis; subsequently, Tg is hydrolysed in phagolysosomes and cleaved thyroid hormone molecules are released into the bloodstream. Tg is synthesised in the thyrocytes and secreted into the follicular lumen via the regulated secretory pathway in a TSH-dependent manner. The ER chaperones BiP, GRP94 and calnexin have all been shown to interact with Tg during the normal folding process [78–80].

In humans, autosomal-recessive Tg deficiency is one of the causes for congenital hypothyroidism and goiter. Its prevalence is approx. 1/40,000. The molecular pathogenesis of this disorder has been studied in an animal model, the so-called *cog/cog* mouse [81]. In thyrocytes cultured *ex vivo* from *cog/cog* glands, mutant Tg is not secreted into the culture medium but is retained in the ER, where levels of five different chaperones are elevated five to ten fold compared to thyrocytes isolated from wild-type control mice [82]. The cause for this ER stress response is the failure of mutant Tg to fold properly and form homodimers. Likewise, in humans with congenital Tg deficiency (some of which have identified defects in the Tg gene), Tg is retained in the ER, thyroid colloid is largely devoid of Tg, and the ER is distended when studied by electron microscopy [83]. Thus, unlike in α_1-antitrypsin deficiency with liver disease or familial neurohypophyseal diabetes insipidus (see below), the retained Tg in these instances of congenital hypothyroidism does not seem to cause cellular damage. Rather, the phenotype is explained by the mere absence of Tg and hence circulating thyroid hormone.

Diabetes insipidus

Autosomal dominant neurohypophyseal diabetes insipidus (ADNDI) (OMIM entries 192 340, 125 700)

This is a rare disorder caused by defects (single or multiple base deletions, missense and nonsense mutations) in the gene encoding the antidiuretic hormone (adiuretin vasopressin, AVP; reviewed in [84]). Over 30 allelic variants have been reported; in all published kindreds, penetrance was 100%. In heterozygous individuals, polyuria and polydipsia develop months to years after birth and gradually progress to full DI due to complete AVP

Figure 3

Intracellular location of wild-type (A) or mutant (B) vasopressin hormone precursors. The proteins were expressed in COS cell culture and visualised by immunofluorescence two days after transfection. Wild-type prohormone localises to the ER, seen as a network structure throughout the cell, and the perinuclear Golgi apparatus (arrowhead), indicating the steady state distribution in the secretory pathway. Secretory granules are not seen because COS cells do not have regulated secretion. In cells expressing the mutant a strong ER staining is seen, while the Golgi staining is absent, indicating ER retention .
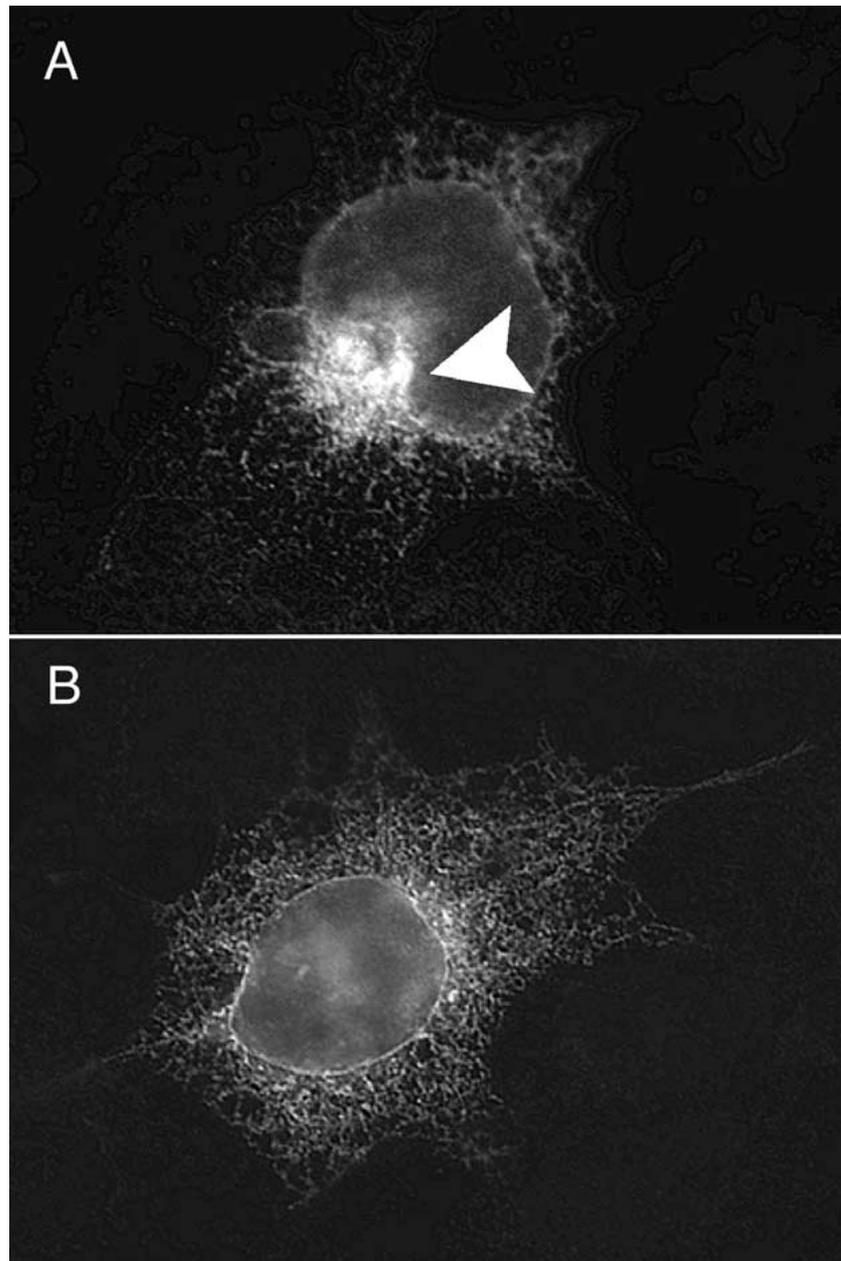
deficiency. Post-mortem findings in adult ADNDI patients demonstrated hypocellularity and gliosis of magnocellular vasopressinergic hypothalamic nuclei [85, 86]. There has been no study so far specifically linking a AVP gene mutation to such histological findings in a causative manner, but based on the genetic and post mortem data available, it is generally concluded that ADNDI represents a neurodegenerative disorder restricted to cells expressing mutant AVP precursor. This has been further substantiated by data from transfection studies demonstrating ER retention (see figure 3) and defective processing of mutant AVP precursors [87–89] as well as detrimental effects on neuronal cell viability elicited by stable expression of AVP mutants [90]. To date, there are no animal models of human ADNDI confirming this neurotoxicity hypothesis, and the mechanism of toxicity and the nature of the dominant-negative effect of mutant protein are not yet understood. Theoretically, transcription of AVP from the wild-type al-

lele should yield sufficient amounts of hormone for effective antidiuresis. Accordingly, destruction of vasopressinergic neurons in ADNDI offers a plausible explanation for the fact that heterozygous mutations suffice to produce the phenotype. We have shown that mutant AVP precursor retained in the ER forms large disulfide-linked intermolecular aggregates [88]. Whether such aggregates, the mutant protein *per se*, or both are toxic to cells remains unclear, but due to its features as ER storage and neurodegenerative disease, ADNDI serves as an experimental model to investigate the functional links between ER stress, ER-associated degradation, and cell death.

Inherited nephrogenic diabetes insipidus
(OMIM entries 304 800, 107 777)

Nephrogenic DI results from resistance to the action of AVP at the concentrating segment of the nephron. It is most commonly acquired but may also occur as an inherited disorder. The more

prevalent X-linked form is caused by loss-of-function mutations in the renal AVP receptor (AVPR2) [91]. The functional impediment may result from decreased binding affinity of the receptor to its ligand, from reduced binding capacity due to impaired insertion of the receptor in the plasma membrane, from decreased coupling to the receptor-associated G protein, or from a combination of these defects. Indeed, all of these functional consequences have been confirmed in transfection studies with multiple AQP-2 mutants [92–95]. Since the AVPR2 is also expressed in extrarenal tissues and mediates the AVP-induced increase in coagulation factor VIII (produced in the liver) and the von Willebrand factor (produced by the vascular epithelium), this response is blunted or absent in patients with X-linked nephrogenic DI. Conversely, it remains normal in patients with autosomally inherited forms. These patients carry mutations in the gene encoding aquaporin-2 (AQP-2), a water channel molecule expressed in the apical membrane of renal collecting duct cells [96]. As a consequence, water reabsorption in the kidney is impaired, causing the diabetic phenotype. A knock-in mutant mouse model has been generated which showed a severe phenotype with early mortality due to severe dehydration [97]. Expression studies in epithelial cells and *xenopus laevis* oocytes have shown that various AQP-2 mutants causing recessive nephrogenic DI are nonfunctional because they are misfolded and retained in the ER [98–101]. Notably and in analogy to the findings in mutant CFTR discussed above, AQP-2 mutants can function as water channels once their transport defect is corrected and plasma membrane insertion is achieved by treatment with a chemical chaperone, such as glycerol [99–101]. Thus, treatment of patients with the potentially lethal nephrogenic variety of DI with substances that correct the transport defect could be envisioned.

Recently, an autosomal dominant form of nephrogenic DI due to a mutation in AQP-2 has been described [102]. Interestingly, this mutant can exit the ER but is retained in the Golgi apparatus. The mechanism of this inheritance pattern is unclear but may result from the retention of wild-type molecules by their mutant counterparts with which they form hetero-oligomers.

Miscellaneous ER storage diseases

Numerous other diseases have been demonstrated to result from impaired ER exit of secretory proteins; a detailed discussion of all of them is beyond the scope of this review. Table 1 gives an overview of established and putative ERSDs; in some instances ER retention has not been demonstrated by detailed experimental analysis but can be assumed due to the type of mutation involved. The table is not comprehensive; for more extensive discussion, see reference [1].

In *autosomal recessive protein C deficiency*, a thrombotic disorder, Protein C Nagoya is an allelic variant which fails to exit the ER [103].

Compound heterozygosity for two mutations in the prohormone convertase 1 gene, which encodes an endopeptidase, was found in a female suffering from *extreme childhood obesity* and showing elevated plasma levels of various prohormones which were not processed due to the lack of the enzyme [104]. The mutations caused the protein to be retained in the ER.

In *tyrosinase-deficient albinism*, mutations in tyrosinase cause enzyme misfolding, prolonged association with the ER chaperones calnexin and calreticulin, and ER retention in melanocytes [105].

ER exit defects also underlie a number of *disorders of lipid metabolism*. E.g., LDL-receptor defects cause autosomal dominant hypercholesterolaemia. Among the identified mutations in the LDL-receptor gene is one that results in the truncation of a significant portion of the C-terminus, which causes quantitative ER retention of the gene product [106]. This so-called "Lebanese allele" may be responsible for the high incidence of hypercholesterolaemia in Lebanon.

Osteogenesis imperfecta is a group of bone matrix disorders due to inborn errors of metabolism of collagen synthesis. Mutations in the two genes of type 1 procollagen cause various forms of the disease, which may be inherited in a recessive or dominant fashion and present with varying phenotypic severity. In a lethal form of osteogenesis imperfecta a point mutation was identified which directs the synthesis of misfolded procollagen [107]. In fibroblast cultures obtained from a deceased infant, ER transit of the mutant protein was retarded and it was partially degraded by ERAD. Interestingly, assembly and secretion into extracellular matrix of collagen triple helix containing mutant strands occurred, but collagen content of the matrix was greatly reduced.

In inherited forms of *Prion diseases* [108], e.g. familial Creutzfeldt-Jakob disease, the GPI-anchored transmembrane protein PrP is mutated. Recent data show that several mutant prion proteins (PrP^{sc}) associated with human disease are inefficiently transported to the cell surface and retained in the ER, albeit not quantitatively [109]. The significance of this finding with respect to the pathogenesis of prion diseases is currently under investigation.

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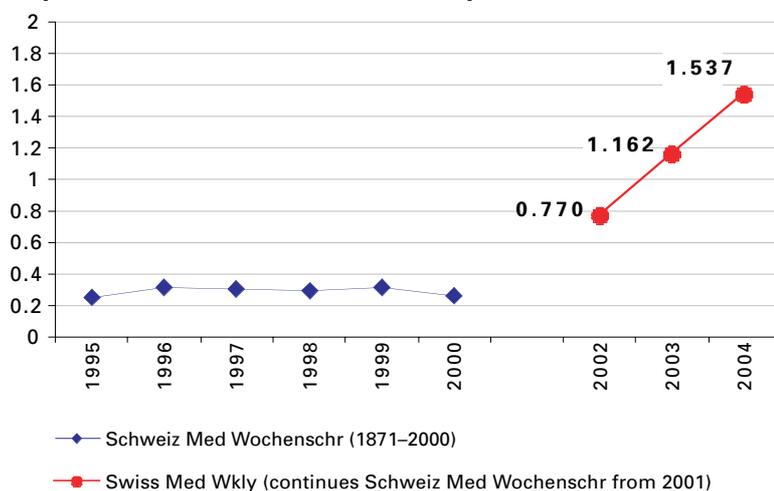- No page charges and attractive colour offprints at no extra cost

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