Minireview

Protein processing:

a role in the pathophysiology of genetic disease

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Abstract Genetic diseases associated with an enzyme deficiency frequently have reduced intracellular levels of the mutant protein, despite apparently normal levels of message and protein synthesis. It has been suggested that the endoplasmic reticulum (ER) can recognise mutant protein as incorrectly folded and invoke 'quality control' processes which cause the retention and degradation of this protein. This process may occur, even for mutations which do not abrogate protein activity, contributing directly to pathophysiology. Genetic diseases associated with defects in ER and Golgi processing proteins have also been reported and generally result in impaired processing of multiple protein products. In this review the role of the ER and Golgi in the pathogenesis of genetic diseases relating to the vacuolar network are discussed.

Key words: Protein processing; Folding; Mutation; Molecular chaperone; Genetic disease; Endoplasmic reticulum; Protein degradation; Pathophysiology

1. Introduction and definitions

The endoplasmic reticulum (ER) has evolved as a separate cellular compartment to provide the selective environment required to mediate complex protein folding and modification reactions. Two components of the ER have been recognised: (1) the rough ER which is usually in close proximity to the nucleus and has a functional role in the synthesis, folding and post-translational modification of polypeptides [1]; (2) the smooth ER which may extend to the cell periphery, has a role in calcium sequestration and may be involved in protein degradation [2]. In this review the general term ER has been used as some of the processing proteins and events described have yet to be specifically localised to one or other of these compartments.

Within the lumen of the ER, a range of molecular chaperones (*a term used here to describe proteins which bind transiently to newly synthesised protein and may be involved in protein folding, oligomerisation and quality control reactions*) are involved in the folding and post-translational modification of newly synthesised polypeptide. These interactions serve not only to correctly fold and modify the nascent polypeptide to form its correct secondary (*hydrogen-bond interactions which form α-helices and β-sheets or covalent disulfide intrachain linkages*), tertiary (*domains and subunits*) and quaternary (*assembled subunits*) structure, but are also thought to anchor the incoming polypeptide chain within the ER lumen and may physically drive the translocation process via ATP dependent hydrolysis. Molecular chaperones also act to prevent the formation of incorrect intermediates or inappropriately folded protein products.

The Golgi also plays an important role in protein processing, modifying N-linked oligosaccharide structures attached in the ER, orchestrating O-glycosylation and generating the targeting signals which control protein traffic in the vacuolar network (*vacuolar network refers to membrane bound intracellular organelles, which include the ER, Golgi, carrier vesicles, endosomes and lysosomes*). The Golgi apparatus is centrally located in many cells and is compartmentalised into membranous stacks called cisternae, which allow the separation of specialised processing events. There is vesicular transport between Golgi cisternae and protein traffic generally proceeds from the *cis-* to the *trans*-Golgi where the control of protein targeting is mediated into either the secretory pathway or the distal elements of the vacuolar network.

2. Molecular chaperones of the ER

A brief description of some typical ER proteins involved in polypeptide folding follows (Table 1).

2.1. Immunoglobulin heavy chain binding protein (BiP)/Glucose regulated protein 78 (GRP78)

BiP/GRP78 is one of the most abundant and probably best characterised of the proteins in the ER lumen [3]. BiP/GRP78 is a member of the heat shock protein 70 (Hsp70) family of proteins, but is distinguished by an ER targeting signal peptide which allows translocation into the ER membrane and a KDEL ER localisation signal to prevent trafficking beyond the salvage compartment [4]. BiP/GRP78 has been postulated to interact transiently with a wide range of polypeptides by binding a hydrophobic motif, which is part of the globular core domain of many proteins [5]. BiP/GRP78 probably mediates folding by interacting with this motif and through its capacity to hydrolyse ATP. BiP/GRP78 has also been reported to bind selectively to incorrectly folded protein (Fig. 1 [6]).

2.2. Glucose regulated protein 94 (GRP94)

GRP94, also known as endoplasmin, is a member of the heat shock protein 90 (HSP90) family of proteins. Like BiP/GRP78, GRP94 is abundant, has a KDEL retention signal, and possesses Mg^{2+}-dependent ATPase activity [7]. The co-association and sequential action of BiP/GRP78 and GRP94

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have been reported during protein folding [8,9].

2.3. Protein disulphide-isomerase (PDI)/ERp59

A critical step in the generation of protein secondary structure is the formation of disulphide bonds between reactive cysteine residues in folding polypeptides. PDI appears to have two thioredoxin-like CXXC motifs which function as active sites (where C is cysteine and X is any amino acid [10]) and the action of PDI is highly dependent on the redox conditions in the ER lumen. Alkylation of these active site thiols abrogates PDI's isomerase activity but does not affect its molecular chaperone capacity [10]. Like BiP/GRP78 and GRP94, PDI can bind and hydrolyse ATP and has been shown to associate with mutant protein.

2.4. Calnexin (p88, IP90)

Calnexin and calreticulin are lectin-like ER molecular chaperones which interact with N-linked oligosaccharides on folding polypeptides [11,12]. Both calnexin and calreticulin react specifically with the mono-glucosylated N-linked high mannose oligosaccharides in the ER. Mono-glucosylated high mannose oligosaccharides can be re-glucosylated in the ER to produce alternating cycles of glucosylation and trimming [12]. This may be required for organising the attachment of calnexin/calreticulin and is one explanation of how processing steps requiring the ordered sequential interaction of molecular chaperones may occur. The sequential interaction of BiP/GRP78 and calnexin has been demonstrated [13].

3. Protein processing in the Golgi

In this review the discussion of Golgi processing has been restricted to the modification of N-linked oligosaccharides on glycoproteins.

Phosphorylation of high mannose oligosaccharides in the Golgi is an important modification as it determines the targeting of soluble lysosomal proteins in the vacuolar network actively avoiding the default secretory pathway. *N-acetylglucosamine-1-phosphotransferase* acts in the *cis*-Golgi to form the targeting structures which are the prerequisite for interaction with the *trans*-Golgi localised mannose-6-phosphate receptors, which direct protein into the endosome-lysosome pathway [14,15]. *N*-Acetylglucosamine-1-phosphotransferase attaches an *N*-acetylglucosamine-1-phosphate residue on to acceptor mannose residues in N-linked mannose oligosaccharide tree structures. The removal of the *N*-acetylglucosamine by α-*N*-acetylglucosminyl phosphodiesterase reveals the mannose-6-phosphate residues which are involved in lysosomal enzyme targeting. Inability to form mannose-6-phosphate residues or to interact with the mannose-6-phosphate receptor results in the extracellular secretion of lysosomal enzymes.

N-linked high mannose oligosaccharides attached in the ER can be processed down by α-*mannosidases* in the *cis*-Golgi [16]. Depending on the processing this allows the formation of either different types of high mannose oligosaccharides, or prerequisites for the formation of complex and hybrid oligosaccharides. To form complex and hybrid oligosaccharides *N-acetylglucosaminyltransferase* acts in the medial-Golgi to catalyse the attachment of *N*-acetylglucosamine to the mannoses on N-linked oligosaccharides. This structure may be further modified in the *trans*-Golgi by *galactosyltransferase* and *sialyltransferase* to attach terminal galactose and sialic acid residues.

4. The folding process and quality control

The cotranslation-translocation process proceeds at a rate of 4–5 amino acid residues per second, allowing complete chain elongation in the order of 0.5–2 min for most proteins. While the molten globular state can be achieved in the order of milliseconds for some proteins, the post translational modification and additional folding reactions can take several minutes. Thus, the extent of protein folding at the point of chain termination will depend on the size of the protein, the domain structure and its complexity, the presence of membrane spanning domains and the degree of post translational modification required.

Partially folded protein intermediates may be continuously bound and released by different molecular chaperones until the polypeptide has reached the completed stage, involving the sequential action of molecular chaperones. The hydrolysis

Table 1
ER molecular chaperones

Molecular chaperone	Eponym	Molecular weight (kDa)	Er retention signal	Protein recognition site	Protein family	Purported function
Ig heavy chain binding protein/ Glucose regulated protein 78	BiP/GRP78	78	KDEL	Hydrophobic motif	HSP70	Chaperonerol Folding quality control
Glucose regulated protein 4/ Endoplasmin	GRP94	94	KDEL	Peptide?	HSP90	Chaperone Folding quality control
Protein disulphide isomerase	PDI	59/61	KDEL	Cysteine motif	Thioredoxin	Isomerase Chaperone Quality control
Peptidyl-prolyl *cis-trans* isomerase	PPIase/CyPB	23	VEKPFAIKE	Proline peptide bond	Cyclophilin	Folding Peptide bond isomerisation
Calnexin	p88/IP90	88, 90	type I integral membrane protein	Glucose on N-linked high mannose	Lectin	Chaperone Folding quality control

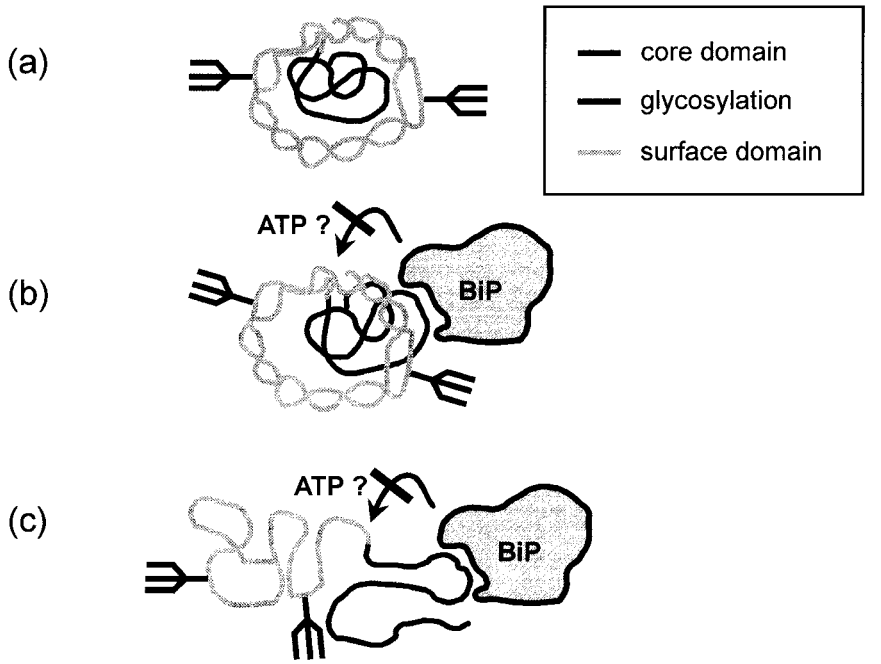

Fig. 1. Some possible scenarios for protein folding. (a) Normally folded protein with stable domain structure. (b) Mutant protein with exposed core domain. BiP/GRP78 interacts with the mutant protein which has achieved a stable but incorrect conformation. ATP hydrolysis and release of the polypeptide is not achieved. (c) Mutant protein which fails to fold. BiP/GRP78 interacts with and retains the mutant protein, which can not achieve a stable conformation and is not released by ATP hydrolysis.

of ATP may drive this process, pushing the polypeptide towards an appropriate conformation and in the process releasing the folding intermediate for further modification by other molecular chaperones.

The folding process is not 100% efficient and some polypeptides will not achieve correct folding, particularly where the

Table 2
Genetic diseases in which protein folding/processing have been implicated in pathophysiology

	Defective protein	Chaperone/processing protein involved	Disease
(a)	Mutant processing proteins		
	Sulphatases [ER (1), L (8)]	ER, Not identified	Multiple sulphatase deficiency
	Steroids, estrogen [membrane, M]	ER, Steroid sulphatase	X-Linked ichthyosis
	Glycoproteins [M]	Cytoplasmic, Phosphomannomutase	Carbohydrate deficient glycoprotein syndrome I
	Glycoproteins [M]	Golgi, *N*-acetylglucosaminyltransferase II	Carbohydrate deficient glycoprotein syndrome II
	Lysosomal enzymes [L]	Golgi, *N*-acetylglucosamine-1-phosphotransferase	I-cell disease (Mucolipidosis II)
	Proteoglycan [EM]	Golgi, β-1,3-galactosyltransferase	Tn syndrome
	Proteoglycan [EM]	Golgi?	Polycystic kidney disease
	Glycoproteins [M]	Golgi, α-mannosidase II	HEMPAS, CDAII
	Phospholipid [M]	Golgi, phosphatidyl inositol 5-phosphatase	Lowe syndrome
	Proteoglycan [EM]	Golgi, β-1,4-galactosyltransferase	Progeroid syndrome
(b)	Mutant proteins interacting with the normal processing machinery		
	α-L-Iduronidase [L]	?	Mucopolysaccharidosis I (MPS I)
	N-Acetylgalactosamine-4-sulphatase [L]	? BiP/GRP78	Mucopolysaccharidosis VI (MPS VI)
	β-D-Glucuronidase [L]	?	Mucopolysaccharidosis VII (MPS VII)
	α-D-Glucosidase [L]	?	Pompe
	β-Hexosaminidase A [L]	? BiP/GRP78	Tay-Sachs
	β-Hexosaminidase B [L]	?	Sandhoff
	Glucocerebrosidase [L]	?	Gaucher
	Aspartylglucosaminidase [L]	?	Aspartylglucosamiuria
	LDL-receptor [CS]	?	Familial hypercholesterolemia
	CFTR [CS]	Calnexin, HSP 70	Cystic fibrosis
	Insulin receptor [CS]	?	Leprechaunism
	α-1-procollagen [EM]	BiP/GRP78	Osteogenesis imperfecta
	Fibrillin [EM]	?	Marfan syndrome
	α-1-Antitrypsin [S]	?	α-1-Antitrypsin deficiency
	Lipoprotein lipase [M]	?	Atherosclerosis

Normal protein localisations are shown in square brackets, and are ER, endoplasmic reticulum; L, lysosomal; M, multiple cellular and extracellular localisation; CS, cell surface and EM, extracellular matrix. ? indicates ER retention and or misfolding has been detected but the molecular chaperone involved has not been identified.

cell is under conditions of stress or where mutations have been introduced into the polypeptide. The ER has developed a 'quality control' process which actively retains and degrades incompletely folded intermediates, unassembled polypeptides, aggregated intermediates and misfolded polypeptides [17]. This is an important process as it is essential to avoid the export of protein aggregates into the cell and to prevent structurally altered and therefore potentially functionally altered proteins from entering the cellular machinery. The inability to achieve correct conformation may not allow the ATP-dependent release of the polypeptide from its molecular chaperone, effectively retaining it in the ER (Fig. 1). Calnexin [11], BiP/GRP78 [13], and PDI [18] have been implicated as part of the 'quality control' process in the ER. The mechanism for the degradation of misfolded protein appears to be non-lysosomal and may involve the cytoplasmic proteasomal degradation system, or a related system which is in close proximity to the ER [19,20].

5. Genetic diseases involving protein processing

To determine the importance of processing events as a mechanism contributing to the pathogenesis of genetic disease, examples of mutant proteins synthesised in and or trafficking via the vacuolar network, will be outlined (Table 2). Two different groups of gene mutations are described, the first involving mutant processing proteins and the second involving mutant protein interacting with the normal processing machinery.

5.1. Mutant processing proteins

5.1.1. Mutant processing proteins in the ER. Relatively few genetic diseases have been attributed to mutations in genes coding for molecular chaperones. This could be explained by molecular chaperones having domain structures which tend to resist the effects of mutation. However, in vitro mutagenesis studies indicate that mutations causing altered function in these molecules may be lethal [21,22]. Moreover, cell lines expressing BiP/GRP78 point mutations have been reported to result in the disruption of the structure of the ER, with marked vacuolation [23]. These observations are not unexpected, as many of the processing proteins in the ER exhibit multi-functionality and would be expected to affect the processing of a large number of proteins.

However, there are some reports of diseases arising from gene defects which produce mutant ER processing proteins. Recently, multiple sulphatase deficiency (clinical presentation similar to the lysosomal storage disorders described below) in which a range of lysosomal sulphatases and a microsomal sulphatase (steroid sulphatase) have reduced activity, a common processing event has been implicated in the disease process. The modification of a critical cysteine residue, which is in a motif conserved in sulphatases, has been postulated to occur in the ER and is required to generate active sulphatases [24].

X-linked ichthyosis, which is one of a group of cornification disorders results from mutations in the gene coding for the microsomal enzyme steroid sulphatase [25]. Clinically X-linked ichthyosis patients present with generalised scaling and histologically by hyperkeratosis. Steroid sulphatase is a membrane associated microsomal protein and is an important enzyme in the processing of steroid sulphate precursors (e.g. estrogen synthesis).

Both of the latter ER proteins affect the processing of multiple protein products. Genetic diseases arising from mutations in genes coding for molecular chaperones like BiP/GRP78 and PDI have yet to be identified, but this may be because they are incompatible with life.

5.1.2. Mutant processing proteins in the Golgi. A deficiency in *N*-acetylglucosamine-1-phosphotransferase causes the disorder known as I-cell disease [26], which affects the targeting of lysosomal proteins, resulting in their extracellular secretion and causing the lysosomal accumulation of glycosaminoglycan, which can not be degraded. Clinical symptoms of this disorder resemble that of the lysosomal storage disorders which are described below. A number of lysosomal proteins are inappropriately processed in I-cell disease, which seems to be typical of defects in processing proteins. This is also observed for several other genetic diseases affecting the assembly and Golgi processing of N-linked oligosaccharide structures (Table 2).

5.2. Mutant protein interacting with the normal processing machinery

5.2.1. Mutant lysosomal proteins. The lysosome is a specialised organelle involved in the degradation of complex macromolecules. A genetic disease affecting one of the specific enzymes involved in catalysing degradation in the lysosome, results in a lysosomal storage disorder by causing the lysosomal accumulation of the partially degraded substrate, which is normally catabolised by the specific lysosomal enzyme. Lysosomal storage disorders occur with a combined frequency of approximately 1:5000 live births and usually result in very severe clinical presentation, which may include dwarfism, skeletal malformation, hepatosplenomegaly, stiff joints, corneal clouding, mental retardation and early death.

In the lysosomal storage disease Mucopolysaccharidosis type I (MPS I), most patient fibroblast cell lines only contain low levels of α-L-iduronidase protein [27]. In general, the level of enzyme catalytic capacity (*ability to turn over substrate*) correlates with both the lowered intracellular level of protein and the patients clinical phenotype, suggesting that if normal levels of protein were present then the disease state would be averted. The targeted disruption of GRP94 mRNA resulted in reduced induction and synthesis of both GRP94 and BiP/GRP78 and caused a six fold reduction in the synthesis of recombinant human α-L-iduronidase [28], suggesting that these molecular chaperones are involved in α-L-iduronidase protein processing.

In fibroblasts from Mucopolysaccharidosis type VI (MPS VI) patients, another lysosomal storage disorder, the lysosomal enzyme *N*-acetylgalactosamine 4-sulphatase (4S) is reduced in activity to less than 5% of normal controls [27,29]. This correlates with a reduced level of 4S protein, which appears to be conformationally altered when studied by a panel of monoclonal antibodies [29]. The correlation of residual 4S protein, to 4S activity, to patient clinical phenotype, implies that the major reason for the onset of pathology in MPS VI is the reduced protein level. An individual with 5% of normal control catalytic capacity presented with no obvious clinical signs of MPS VI, suggesting that there is a threshold for 4S activity, above which the onset of pathology will be averted [29].

Paradoxically, processing studies indicate that mutant 4S polypeptide is synthesised at comparable levels to that found

in normal control cells [30] and that the residual protein detected in MPS VI cells has relatively normal enzyme activity. Several studies have provided evidence that an early biosynthetic compartment may be removing the mutant 4S protein, presumably due to the observed altered protein conformation [30–32]. Brooks et al. [31] demonstrated that a conservative C91S site-directed point mutation caused protein inactivation, but there was no evidence of a conformation change in the protein when assayed using conformation dependent monoclonal antibodies and this mutation resulted in normal targeting and normal intracellular levels of protein. In contrast, a C91T point mutation caused a detectable conformation change and resulted in low intracellular levels of 4S protein which was detected mainly in microsomes. Similarly, a stop codon mutation *534Q, which resulted in the synthesis of 4S containing an additional 51 amino acid tail sequence and a nine fold increase in 4S specific activity, caused a detectable conformation change and low levels of 4S protein in cells [30]. In processing studies, this protein was synthesised normally but not transported to the *trans*-Golgi network and appeared to be retained and degraded, in the ER. Thus, despite being synthesised normally and being catalytically active, this mutant protein is recognised as conformationally altered by the ER 'quality control' system and is degraded, contributing directly to the development of MPS VI pathology.

In patients with the lysosomal storage disorder Tay-Sachs disease, it has been postulated that only low amounts of β-hexosaminidase A (α-subunit) protein are able to leave the ER, contributing to the residual enzyme activity and associated clinical phenotype [33]. Site-directed mutagenesis of the α-subunit of β-hexosaminidase caused BiP/GRP78 association, with inappropriate disulphide bond and protein aggregate formation [34]. Zokaeem et al. [35] demonstrated that mutant β-hexosaminidase A was not post-translationally modified to form mannose-6-phosphate residues, was not proteolytically converted to the mature form and appeared to be retained in an early biosynthetic compartment. For β-hexosaminidase A deficiencies (β-subunit, Sandhoff disease), Dlott et al. [36] demonstrated that residual protein was synthesised and reacted with a specific polyclonal antibody against unfolded polypeptide. This polypeptide was not mannose-6-phosphorylated, indicating it had not reached the Golgi, and was rapidly degraded, presumably in the ER.

5.2.2. Mutant cell surface membrane proteins. Cystic Fibrosis (CF) is an autosomal recessive genetic disease with an incidence of approximately 1 in 2500 live births in whites. CF patients present mainly with respiratory and gastrointestinal tract symptoms which are caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, that codes for a plasma membrane localised chloride channel. It has been suggested that most cases of CF are caused by mutations which interfere with the biosynthetic folding of the CFTR protein. For example the common ΔF508 mutation results in CFTR protein synthesis, but the polypeptide does not leave the ER [37]. However, functional studies demonstrate that although retained in the ER the ΔF508-CFTR protein still forms a functional chloride channel [38]. ER retention mechanisms can therefore recognise and retain structurally altered molecules which still have functional capacity, contributing directly to the development of a pathological state. This is consistent with the findings for the lysosomal storage disorders described above.

The involvement of protein conformation in the ER retention of ΔF508-CFTR protein has been supported by the observation that glycerol, a protein stabiliser, reverses the misfolding phenotype [39]. Similarly, the trafficking of ΔF508-CFTR protein has been shown to be temperature sensitive [40]. Both of these treatments are presumed to stabilise the mutant CFTR protein, allowing what approaches correct folding and subsequent traffic of functional ΔF508-CFTR protein to the cell surface. The ER quality control mechanism which retains ΔF508-CFTR protein has been shown to involve calnexin binding [41]. While normal CFTR protein dissociates from calnexin (transient association), the ΔF508-CFTR protein remains associated with calnexin and is presumed to contribute to its intracellular mislocation in the ER. The degradation of mutant CFTR protein appears to be by a proteasome mediated pathway [19,20].

5.2.3. Mutant extracellular matrix and secretory proteins. Mutations in the type I collagen gene result in the lethal genetic disease osteogenesis imperfecta (OI). OI presents as a soft connective tissue disorder with clinically recognisable facial disproportions and concurrent presence of osteoporosis and deafness. Mutant procollagen from osteogenesis imperfecta cells specifically associates with BiP/GRP78 [42]. Procollagen has been shown to co-immunoprecipitate with BiP/GRP78 and appears to be aberrantly folded and localised in the ER [42]. However, unlike the other genetic diseases discussed, considering the autosomal dominant nature of the disease and the structural effect of mutant collagen, it may actually be beneficial to enhance the ER retention and degradation of mutant collagen. In the case of structural proteins where mutations in a single allele result in a disease process, the ER may actually reduce the severity of the disease by reducing the number of structurally aberrant molecules being incorporated into the extracellular matrix.

Mutations in the gene coding for the complement (C1) inhibitor (*which is a serpin or serine protease inhibitor*) appear to affect the intracellular transport and secretion of the protein, resulting in type I hereditary angioedema. Patients present clinically with episodes of local swelling, affecting the face, extremities, upper airways and gastrointestinal tract. Biochemical and immunofluorescence studies indicate that in affected cells, the mutant protein is localised in the ER and is subsequently degraded [43]. Mutations in the carboxyl terminal domain of the C1 inhibitor and other serpins appear to be important in determining correct folding, which is necessary to avoid ER retention and ensure correct protein folding and trafficking [44].

6. Mechanism of action of molecular chaperones

The co-induction in the synthesis of molecular chaperones processing polypeptides [45], the identification of complexes which contain polypeptide folding intermediates associated with different molecular chaperones [46], and the sequential action of molecular chaperones on a common substrate [47], all suggest that ER processing is a coordinated process. This apparent concerted action may function to hold a polypeptide in a specific conformation so that it can be appropriately modified/processed or simply drive the folding process towards the formation of a particular conformation. Molecular chaperones have a role in preventing the aggregation of folding intermediates and may even be involved in protein disag-

gregation. It is important to prevent folding domains of different polypeptides from interacting during assembly, but it may be equally important to keep separate domains within a protein from interacting. A protein with a complex multi-domain structure may therefore have multiple chaperones acting upon it simultaneously, coordinating the sequential formation of subdomains and eventually condensing the polypeptide into a final compact tertiary/quaternary structure [48].

The folding process must be continually monitored for misfolded polypeptide, a process termed 'quality control' which is probably integrally linked to the action of molecular chaperones. The exact molecular mechanisms responsible for the ER 'quality control' processes are yet to be fully recognised. However, retention alone is not sufficient to induce the degradation of protein by the ER 'quality control' system. The attachment of the KDEL ER targeting signal to polypeptides which fold correctly therefore results in retention but not degradation of the protein. Exposure of buried internal domains of polypeptides has been postulated to signal incorrect folding, which is detected by molecular chaperones, targeting the protein for degradation (Fig. 1, [6]). While exposure of internal domains may be a prerequisite signal for retention and degradation, the fact that normal folding intermediates may have exposed internal domains during folding, would suggest that this is not the only component for the recognition of incorrect folding.

7. The role of protein processing in genetic disease

Gene mutations which cause genetic disease, must result in a structural change in the associated protein. Protein folding studies indicate that mutations frequently hinder the final stages of protein folding, arresting the process by the formation of incomplete folding intermediates [49]. In some cases these intermediates will have reduced structural stability while others may form stable structures which exhibit functional properties. In many genetic diseases residual levels of protein with at least partial function are detected. It is postulated that the majority of polypeptide is retained and or degraded in the ER by the 'quality control' system and that the residual protein levels and function seen in many patients represents the small amount of polypeptide which escapes this recognition process. This may indicate either inability of the ER to detect all intermediates, or the ability of some molecules to achieve near normal conformation and thereby evade the ER detection system. The examples described here (Table 2) and recently by Bychkova and Ptitsyn [49] and Thomas et al. [50] support the hypothesis that ER retention and degradation of mutant protein contributes to pathophysiology in genetic disease, but it remains to be determined how wide spread is this proposed phenomenon. For diseases like MPS VI the ER retention of mutant but partially functional polypeptides may be a major factor in the development of patient pathology, but this would obviously not be the case for diseases where there are a large number of deletion mutations, which tend to abrogate protein function. Where ER retention and degradation of either partially or fully active mutant protein occurs the ER 'quality control' process will contribute to the onset of pathology in the patient. Mutations in processing proteins can be expected to affect the function of multiple protein products and will also result in pathogenesis, but in some cases mutations in the processing machinery may be incompatible with life.

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