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2.3.8 Protein synthesis and degradation in the liver

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Introduction

The proper physiological function of various organs relies on the expression of biologically active molecules such as antibodies, hormones and neurotransmitters as well as various ligands and receptors, and the signalling and other molecules by which they act. Many of these molecules are proteins, encoded in genes. Some are non-protein molecules (e.g. lipids, sugars and other small molecules such as nitric oxide gas) whose existence requires the action of proteins (e.g. enzymes that are involved in their synthesis). The DNA of the genes encoding these

proteins must be transcribed and processed into mRNAs and exported out of the nucleus into the cytoplasm, as a prerequisite for gene expression. This occurs by complex mechanisms that are outside the scope of this review. Once in the cytoplasm, mRNAs can be either quiescent, as a result of binding proteins that prevent their translation into proteins, or actively engaged in making their encoded polypeptides.

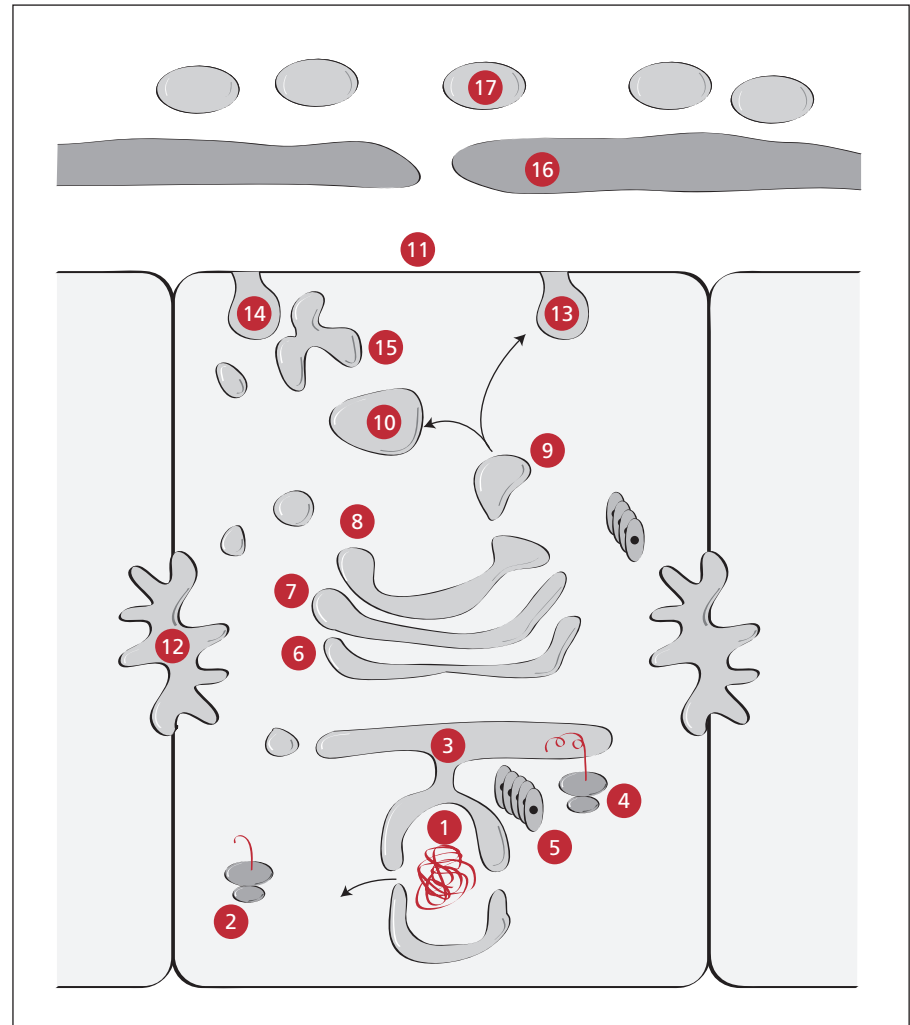
One important function of the liver is the synthesis and secretion of a wide range of proteins into the bloodstream, including blood clotting factors and transporter proteins such as lipoproteins and albumin. Another important function is the synthesis and correct localization of integral membrane proteins of the plasma membrane and other compartments. A common feature of these classes of proteins is that their fates are determined very early during translation of the mRNAs in which they are encoded. The nascent chains of these proteins are targeted to the membrane of the endoplasmic reticulum (ER), a specialized intracellular compartment that initiates their journey by subsequent vesicle fission and fusion through the secretory pathway (Fig. 1). Regulatory steps along the way ultimately determine the amount, function and/or localization of these proteins. Some of these steps, such as the initial event of translocation across (for a secretory protein) or integration into (for a membrane protein) the membrane of the ER, are, in their simplest manifestation, irreversible events. However, under certain conditions, these irreversible steps are overcome by cellular machineries that degrade undesired proteins, and thereby end their action. In this chapter, we describe first the fundamentals of early events in mRNA translation and degradation of newly synthesized secretory and membrane proteins. Subsequently, we discuss regulatory mechanisms of protein synthesis and degradation with emphasis on examples relevant to liver function and pathophysiology.

Principles of posttranscriptional gene expression

Translation of mRNA to protein

Translation of mRNA into protein starts by (i) binding of methionyl-tRNA (tRNA_{Met}) to the small ribosomal subunit (40S), (ii) recruitment of the complex to mRNA at the first AUG codon and (iii) joining of the large ribosomal subunit (60S) to the mRNA–40S complex. The eukaryotic initiation factors (eIF) are involved in all three steps of translation initiation [1]. Shortly before the start of translation, the eIF2 (composed of α , β and γ subunits) forms a complex with GTP and tRNA_{Met} to facilitate binding to the small ribosomal subunit. The binding of this preinitiation complex, known as the 43S complex, to mRNA is assisted both in *cis* and in *trans*. The *cis* element referred to is the ‘cap’, a modified guanine nucleotide at the 5′ end of mRNA, which in many cases is critical for the recruitment of 43S by the eIF4 complex [2]. The eIF4 complex is composed of several proteins including: eIF4E, which physically binds the

Fig. 1 Synthesis, maturation and degradation of a membrane glycoprotein. A schematic diagram of a hepatocyte is shown indicating numbered key compartments relevant to protein synthesis and degradation. Let us consider the pathway involved in integral membrane protein biogenesis and illustrate the roles of each compartment. First, the mRNA is transcribed, processed and exported from the nucleus (1) via the nuclear pores (see arrow). Translation is initiated in the cytoplasm on free ribosomes (2) in the cytosol. Secretory and integral membrane protein nascent chains are targeted to the ER (3) via the signal recognition particle (SRP), which binds the signal sequence and is released upon interaction with the SRP receptor at the cytoplasmic face of the ER membrane (4). This results in opening of the translocon in the ER membrane and translocation/integration of the nascent polypeptide into the ER membrane. Concurrently, the nascent chain interacts with luminal proteins (such as molecular chaperones) and undergoes posttranslational modifications (such as glycosylation and signal sequence cleavage). The maturation of polypeptides continues as they exit the ER and transit the Golgi complex, composed of *cis* (6), medial (7) and *trans* (8) stacks, by vesicle fission and fusion. Vesicles (9) leaving the trans-Golgi network can take a number of different paths depending on various determinants of trafficking pathways. Thus, the mature protein is transported to its final destination at the lysosome (10) or apical (11) or basolateral (12) plasma membrane. All these events involve vesicle fusion (13). Vesicles also form by internalization from the plasma membrane (14), leading to various endosome-related compartments (15) including the lysosome (10). Before exit from the ER, membrane proteins are scrutinized by proteins termed molecular chaperones for abnormal structural features and/or covalent modifications. If recognized as misfolded or unassembled, the protein is bound by the molecular chaperone, transported out of the ER through the translocon 'in reverse' and sent for degradation by the 26S proteasome (5). There are multiple forms of the proteasome in multiple locations within the cytoplasm. Also shown are the endothelial cells (16) and red blood cells (17) on the basolateral side of the hepatocyte. Note that the apical plasma membrane comprises the bile canaliculus in the hepatocyte and represents a very small surface area compared with the much larger basolateral surface on the bloodstream side.



cap structure; eIF4A, which unwinds the secondary structures in the 5' untranslated region (UTR); and eIF4G, which plays a scaffolding role by interacting with other eIFs. Subsequent to the formation of the ribosome–mRNA complex, the ribosome starts translation at the AUG codon corresponding to the first methio-

nine. According to one proposal, the ribosome scans the mRNA molecule at the 5' UTR until it reaches the first AUG codon in a favourable context (known as the Kozak sequence) [3]. An alternative mechanism for translation initiation has also been proposed (described below).

Targeting to the secretory pathway

While the basic aspects of translation initiation and elongation of secretory and membrane proteins are identical to those of the cytoplasmic proteins, the biogenesis of these different classes of proteins diverges shortly after the start of translation. Sorting and targeting of membrane and secretory proteins to the ER starts when the signal sequence emerges from the ribosome. Despite very little sequence homology between them, signal sequences can be best characterized as an extension of 20–50 amino acids containing a stretch of hydrophobic residues (the H-domain) sandwiched between a charged (N-domain) and a polar (C-domain) region, typically predicted to have a beta bend in secondary structure [4]. Extensive biochemical analyses in cell-free systems have established that the H-domain of the signal sequence is recognized by a cytoplasmic *trans*-acting factor termed the signal recognition particle (SRP). Mammalian SRP is a ribonucleoprotein consisting of six polypeptides (SRP9, SRP14, SRP19, SRP54, SRP68 and SRP72, where the numbers correspond to their molecular weight) and an RNA (SRP-RNA) [5]. Signal sequence recognition and SRP-RNA binding is attained through SRP54, a GTPase that mediates ER targeting of the ribosome–nascent chain complex when bound to GTP. During targeting, GTP hydrolysis is inhibited by both the ribosome and the signal sequence. Thus, translation is transiently arrested until targeting is completed. Following targeting, the interaction between the SRP and SRP receptor (SR) stimulates GTP hydrolysis, SRP release and the resumption of protein synthesis. SR is also composed of two GTPase subunits. The α subunit (SR α) is closely related to the GTPase domain of SRP54. SR α interacts with SR β , which is an integral membrane protein with a GTPase domain related to Sar1 and the ARF family.

Translocation across the ER

Following ER targeting, translation resumes, with the chain entering the aqueous environment of the translocation channel, also termed the translocon. During translocation of the polypeptide chain, the ribosome is classically viewed as forming a tight seal with the translocon. This seal shields the nascent chain from the cytosolic environment [6]. At this point, many irreversible decisions are taken, and the nascent chain is folded, covalently modified and assembled into a functional unit through a dynamic network of protein–protein interactions [7]. Signal peptidase and oligosaccharyl transferase (OST) are responsible for cleavage of the signal sequence at the C-domain and addition of sugar residues to the consensus N-linked glycosylation sites respectively. The folding and assembly of the nascent chain is assisted by ER resident proteins such as calnexin, immunoglobulin heavy chain binding protein (BiP), protein disulphide isomerase (PDI) and others. In addition, in the course of translocation, whereas secretory proteins completely cross the ER membrane to enter the lumen, individual segments of topologically distinct membrane proteins are parti-

tioned between the cytosol, the lumen and the ER membrane. In order to accommodate these modifications and cope with the complexity of the topology of membrane proteins, the translocon must provide a dynamic environment for the growing polypeptide [8]. In eukaryotes, the translocon is formed from heteromeric membrane proteins referred to as the Sec61 complex (with α , β and γ subunits) [9]. This complex is necessary and, in some cases, sufficient for translocation. The translocation of the majority of proteins is facilitated by the translocating chain-associating membrane (TRAM), a multispinning glycoprotein that aids the formation of a tight ribosome–membrane junction through a signal sequence-dependent process [10]. In addition, the translocon complex interacts with the ribosome, the signal peptidase complex and the OST, as well as the translocon-associated protein (TRAP), in a manner that accommodates the enormous diversity of proteins that transit the secretory pathway. Therefore, the profile of secretory and membrane protein synthesis is dependent not only on signals encoded by the primary amino acid sequence (such as the signal sequence) but also on a dynamic interplay between cellular factors whose interactions with the nascent chain during translocation ultimately determine protein topology and folding. Mutations in the genes that encode components of the ER translocation and processing machinery have been identified in patients with autosomal-dominant polycystic liver disease [11]. The precise mechanism of integration into the ER membrane remains unclear. The simple-minded notion that hydrophobic domains are stabilized in the bilayer as they emerge from the ribosome seems untenable in the light of evidence that the translocation channel is aqueous [12,13], that multiple transmembrane loops are positioned in some cases before integration occurs [14,15] and the occurrence of discrete charged, extracytoplasmic sequences (termed stop transfer effector sequences) that are necessary for integration at subsequent hydrophobic domains, at least in some cases [16,17]. However, a specific integration receptor has yet to be identified for transmembrane protein biogenesis. Of particular interest is evidence of *trans*-acting factors that can alter the fate of a nascent chain, taking a polypeptide that would be destined to be an integral membrane protein and, instead, directing it to a different translocation/folding pathway by which it is secreted [18,19].

Beyond the ER

During and subsequent to translation and translocation, the newly synthesized proteins are either degraded (described in the next section) or further processed and matured in order to contribute to the protein's physiological function. Maturation involves non-covalent folding and covalent, post- and cotranslational modifications, as well as trafficking through the secretory pathway to the correct final destination. These events may be interconnected, for example some posttranslational modifications occur in specialized compartments (e.g. sialylation of proteins in the *trans*-Golgi network). In other cases, a posttranslational

modification is a requisite for a particular compartmental trafficking event (e.g. the role of mannose residue phosphorylation in directing lysosomal enzymes out of the secretory pathway and to those organelles; see [20]).

Proteins destined for posttranslational maturation leave the ER either by a signal-mediated event (i.e. receptor–ligand interaction) or by bulk flow through budding and fusion of vesicles with consecutive organelles [21]. Budding of ER vesicles is driven by polymerization of a protein called coat protein II (COPII) from specialized ER exit sites. These vesicles lose their coat and fuse to the so-called vesicular tubular clusters (VTCs), also known as the ER–Golgi intermediate compartment. The cargo is then taken from VTCs in vesicles to the Golgi complex. ER resident proteins, however, recycle back to the ER in vesicles coated with a different protein, termed COPI.

The processing and sorting of proteins continues at the Golgi complex by sequential progress through the *cis*-, medial- and *trans*-Golgi cisternae. Transport of the cargo from *trans*-Golgi to the plasma membrane, lysosome or to other endomembranes takes place by clathrin-coated vesicles. In addition to COP coatomers and clathrin, various adapter proteins are involved in the transport of cargo between secretory compartments. The lectin ERGIC-53, for example, is involved in the transport of various glycoprotein cargo including clotting factors V and VIII [22]. Mutations in ERGIC-53 or an associated protein (MCFD2) cause a bleeding disorder, probably by disrupting the secretion of blood clotting factors by the liver [23].

An important dimension of postsynthetic sorting that is particularly prominent in the liver, but will not be discussed in detail here, involves directing proteins to the apical vs. the basolateral aspects of the plasma membrane [24] (see Chapter 2.2.2). In the liver, the apical plasma membrane is limited to the bile canalicular membrane, while the much larger basolateral aspect is in contact with the bloodstream via the sinusoidal space (discussed in Chapter 2.2.2). The exocyst is a cytoplasmic particle comprising multiple proteins that is involved in the trafficking of vesicles during apical vs. basolateral sorting [24]. Recent evidence suggests a regulatory connection between protein synthesis and trafficking, by which, perhaps, cells are able to ‘keep count’ of proteins synthesized and targeted to one membrane face vs. another [25–27]. Thus, Sec61 β , a component of the translocon complex, has been associated with binding components of the exocyst, although the molecular mechanism of this interaction and the regulatory feedback loops involved remain unclear.

Degradation

Among a large number of proteases involved in protein degradation, the best understood are the lysosomal and the 26S proteasomal degradation pathways. The former takes place in lysosomes, a membrane-enclosed acidic environment filled with hydrolytic enzymes. The lysosomal degradation pathway is critical for bulk proteolysis by visceral tissues such as the liver and for clearance of unwanted proteins recycled from the plasma

membrane [28]. The proteasomal degradation pathway, on the other hand, is largely involved in processes that require fine control of protein degradation. The 26S proteasome consists of a central hollow cylinder (the 20S proteasome) and two caps on each end (the 19S caps). The 20S proteasome is composed of distinct proteases arranged with their active sites facing the inner chamber. The 19S cap includes protein subunits with ATPase activity, which unfold proteins and move them to the interior chamber. Perhaps the best understood function of proteasomes is in ER-associated degradation (ERAD) [29]. It is generally believed that the ER is equipped with machinery for the selection of misfolded or unassembled proteins that are recognized through exposure of structural tags (such as disulphide bonds) or posttranslational modifications (such as signal sequence cleavage and glycosylation) [7]. These proteins are generally believed to be misfolded forms recognized by the ER molecular chaperones that exercise a ‘quality control’ function over protein products before they enter the later stages of the secretory pathway. The journey of such misfolded molecules through the secretory pathway is blocked at the level of ER where they are, instead, targeted for return to the cytoplasm and destruction by the 26S proteasome complex. For example, the majority of mutations in the low-density lipoprotein receptor, which cause familial hypercholesterolaemia, result in its intracellular retention and, hence, the inability of the liver to maintain lipoprotein metabolism [30]. Other liver disorders that are caused by defective trafficking of secretory and membrane proteins include α -1-antitrypsin deficiency [31] and Laron syndrome (as a consequence of mutations in growth hormone receptor) [32].

The details of the ERAD machinery are best understood in the case of glycoproteins (Fig. 1) [33]. The lectin chaperones calnexin and calreticulin identify aberrant sugar residues on glycoproteins and retain these proteins in the ER through successive cycles of removal and readdition of glucose residues to the core sugars initially added during translocation across the ER membrane. These cycles offer the misfolded protein an opportunity to refold in a productive manner. If successful, the correctly refolded molecule is deglycosylated, released and exported to the vesicles destined for the Golgi apparatus. If refolding is unsuccessful, the consequence can be degradation by the 26S proteasome. Before degradation by the proteasome, however, these polypeptides must recross the ER membrane through a process referred to as retrotranslocation or dislocation [34]. Numerous genetic and biochemical studies have suggested that Sec61, in addition to its well-established role in translocation, is also critical for the disposal of unwanted proteins from the ER into the cytosol. Recently, two parallel reports identified Derline-1 as an ER membrane protein responsible for retrotranslocation of certain substrates [35,36]. In all cases, the driving force for extracting the polypeptide from the ER membrane is likely to be provided by the p97 ATPase complex [34]. During extraction from the ER membrane, unwanted proteins are covalently modified by the addition of a chain of small molecules referred to as ubiquitin (Ub). The polyubiquitylated proteins are targeted

to the 26S proteasome, which ultimately cleaves them into small peptides but spares the Ub moiety. In order to facilitate proteasomal degradation, N-glycans are removed prior to proteolysis by a ubiquitous cytosolic N-glycanase [37].

In some cases, unwanted proteins are selected for degradation at sites other than the ER, such as in the nucleus. A recent study has identified that the characteristics of nuclear degradation machinery resemble those of ERAD [38].

Translational control of gene expression

In order to cope with the environmental and physiological challenges constantly encountered by organs and cells, the production of proteins is subject to regulation at all levels of synthesis. Since protein synthesis requires both amino acids and metabolic energy, in some instances, such as in starvation, regulation of translation initiation is an important survival factor. Regulation of protein synthesis is also important in cases where variations of protein levels are essential for the progress of cells through specific stages, such as the steps of the cell cycle and development.

Regulation of translation initiation in *cis* and *trans*

Control of translation initiation is widespread, relying on both the structural features of individual mRNAs (*cis*-regulatory factors) and the molecules involved in the translation initiation process (*trans*-regulatory factors). Alternative models for the recruitment of ribosomes to mRNA have been proposed that are independent of the cap, such as use of an internal RNA structure known as the internal ribosomal entry site (IRES) [39]. These structures were first found in picornavirus mRNA [40] and later in hepatitis C virus and related pestiviruses [41]. These viruses manipulate cellular eIFs, either by phosphorylation or through degradation, in a manner that blocks the cap-dependent translation without compromising IRES-mediated translation [42]. By doing so, the essential eIFs, which are normally engaged by the cap-dependent translation of host mRNA, are now hijacked for the translation of viral mRNA through IRES-dependent initiation. IRES-dependent translation initiation has also been described for a number of cellular mRNAs including BiP, fibroblast growth factor 2, vascular endothelial growth factor and so on [2]. Some cellular mRNAs contain a 5' cap-dependent initiation site in addition to an IRES element located in their coding region [43]. This enables proteins of distinct functions to be generated by alternative translation initiation of a single mRNA molecule. In an extreme case, the translation of the fibroblast growth factor 2 can generate five different products through four IRES sites in addition to cap-dependent translation.

In eukaryotes, IRES-dependent translation initiation is mainly involved in the expression of a selective number of genes under conditions in which cap-dependent protein synthesis is blocked, such as during stress, some stages of the cell cycle and apoptosis

[42]. At least four kinases have been identified that are activated in response to viral infection, low levels of haem in red blood cells, amino acid starvation and during the ER stress response. These kinases typically inhibit translation initiation through phosphorylation of eIF2 α . In addition, degradation of eIF4G, which prevents cap-dependent translation, has been reported in cells undergoing apoptosis or infected with certain viruses [43]. Thus, the rate and the level of translation, as well as the site of translation initiation, are governed by structural elements of mRNA and modification of eIFs.

Regulation of protein degradation

Examples of cellular processes regulated by degradation include apoptosis, development, cell cycle, antigen processing and lipid metabolism by the liver [44]. In addition, the half-life of normally short-lived proteins is thought to be determined by degradation through recognition of signals inherent in their amino acid sequence. Among these signals, the best characterized is the N-degron of the N-end rule pathway, in which the half-life of a protein is determined by the identity of its N-terminal amino acid [45].

Lipid metabolism by the liver is a prime example of regulated degradation [46]. The liver enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) is an integral membrane protein of the ER, and it functions as a rate-limiting enzyme for the synthesis of cholesterol. HMGR degradation is accelerated in the presence of sterol precursors of cholesterol when production is unnecessary. This process, which is similar to ERAD, is inhibited when the flux through the sterol pathway is low and HMGR activity is essential for cholesterol production. In addition to HMGR, sterol regulation by the liver is subject to regulated degradation at other levels. For example, apolipoprotein 100 (ApoB) is also degraded by the ERAD if not assembled into very-low-density lipoprotein particles in the liver secretory pathway [47,48]. Therefore, proteolysis directs many cellular processes, primarily by controlling the levels of intracellular proteins, a function that was originally solely assigned to transcription and translational control.

Future prospects: regulation of translocation and targeting

Relative to translation initiation and degradation, regulatory events at the level of targeting and translocation have primarily been limited to examples involving proofreading (accuracy) functions. However, because many important and irreversible events govern targeting and translocation steps, regulation at this stage may influence key features of proteins including localization, structure and function. In the following section, we will discuss the stages of translocation and targeting that could be points of regulation. We will also present a new view, in which these regulatory stages could control protein conformation, a concept that may provide a rationale for many phenomenological

observations of protein function that cannot be explained by conventional views [49].

The opportunity to regulate targeting begins as soon as the signal sequence emerges from the ribosome. Recognition of the signal sequence by the SRP results in a transient arrest in translation [50]. At this point, the time window of translation arrest and, hence, the rate of synthesis could be regulated by signalling events. The strength of interaction between SRP and the signal sequence could also be modified in a manner preferable for some signal sequences over the others. In extreme cases, dissociation of SRP from the signal sequence may prevent targeting and generate a cytoplasmic protein that serves a different function, the same function at a distinct cellular location or that may be rapidly degraded. The last possibility has been described recently for PrP as a result of the inefficiency of signal sequence function, which generates proteasomally degraded cytosolic protein [51]. Alternatively, in the absence of bound SRP, polypeptide chains may be synthesized in the cytoplasm and, hence, available for signalling molecules and posttranslational modifications that would otherwise be inaccessible during cotranslational translocation. These modified molecules may then be translocated across the ER through posttranslational translocation [52]. Posttranslational modifications will not necessarily provide steric hindrance for translocation across the Sec61 channel, as N-linked glycosylation moieties are well tolerated for retrotranslocation through the same channel.

The next opportunity for regulation comes after targeting when the nascent chain–ribosome complex is docked at the translocon. According to the dogma, the growing chain is protected from cytosolic factors by virtue of a tight seal formed between the ribosome and the ER membrane. However, it has been shown recently that the length of the polypeptide chain accessible to cytosolic proteases varies depending on the heterogenous signal sequence [53]. Although a physiological significance for this phenomenon remains to be demonstrated, it can promote the exposure of an otherwise shielded mature domain to cytosolic factors. Indeed, pause transfer sequences have been identified in ApoB that mediate sufficient temporal and spatial opening of the ribosome membrane junction to allow for interaction with cytoplasmically applied macromolecules [54].

Each step in protein biogenesis represents a unique opportunity for regulation. Translation initiation, for example, is the last step at which a cell can decide whether to express a particular gene or not. Degradation, on the other hand, is the only way to diminish the amounts of an already existing protein. What could the regulatory process at the level of translocation accomplish that is unique and inaccessible during translation initiation or degradation? During translocation, a nascent polypeptide must acquire its proper structure through an array of interactions and modifications by ER resident proteins and cytosolically accessible molecules, as well as the ribosome. At this stage, a nascent chain is directed to multiple folded states, some of which are thermodynamically unfavourable. With reference to most developing

processes, plasticity is conceivable for early translocational events that shape the structure of a protein. For instance, *trans*-acting factors (such as TRAM) could influence the energetics of protein folding by changing the environment of the translocon in favour of one structure over the others [55,18]. By doing so, for any unique amino acid sequence, multiple structures and, hence, functions could be assigned. Functionally and conformationally distinct proteins could be selected either by signalling molecules that impinge on *trans*-acting factors during translocation or by selective degradation during or after synthesis. There is precedence for both these mechanisms. First, in the case of PrP, at least three conformationally distinct forms have been identified arising from a single amino acid sequence. Two of these forms span the membrane once in the opposite direction, and the third form is fully translocated across the membrane [56]. An ER membrane protein (TRAP alpha) has been identified that favours the expression of secretory PrP over the membrane forms [18,19]. Consistent with the existence of different structures, multiple functions have been identified for PrP as both a proapoptotic and an antiapoptotic factor [57]. Second, degradation is another way in which functionally desired proteins can be selected. Cotranslational degradation is commonly observed for a large fraction of newly synthesized proteins [58,59]. In the case of the cystic fibrosis transmembrane conductance regulatory (CFTR) gene, less than 30% of newly synthesized molecules transit to post-ER compartments, with the majority of the wild-type molecules retained in the ER [60]. Whereas the ER-retained fraction degrades rapidly (half-life = 20–40 min), the post-ER fraction is remarkably stable with a half-life of hours. This quantitative heterogeneity persists even after inhibiting ER-to-Golgi traffic, indicating that differential stability is not a mere consequence of distinct localization. Instead, differential stability reflects different structural states, each with a unique folding thermodynamic. Although in the case of CFTR, the energetically unfavourable structural variant is retained in the ER, energetically unfavourable mutant proteins have been identified that exit the ER with an efficiency similar to wild type in a tissue-specific manner [61]. Together, these findings imply that the commonly observed cotranslational degradation is not merely a way to rid the cell of non-functional molecules that are structurally unstable. ERAD and other degradative machinery may serve to prune gene expression and eliminate *functional* protein conformations that are not desired at that precise time or by certain cell types. More studies are needed to see whether this and other alternative notions of regulated gene expression are generally valid or represent unusual variations observed for a small number of specialized proteins.

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2.3.9 Glutathione

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Introduction

The tripeptide glutathione (GSH) is one of the most intensively studied intracellular non-protein thiols on account of the critical role that it plays in cell biochemistry and physiology. GSH is a tripeptide found in high concentrations in all cells. It is synthesized from glutamate, cysteine and glycine in the cytosol in two steps, each requiring adenosine triphosphate (ATP) hydrolysis. GSH has certain structural features responsible for its stability and biological functions. For instance, the peptide bond linking the amino-terminal glutamate and the cysteine residue of GSH is through the γ -carboxyl group of glutamate rather than

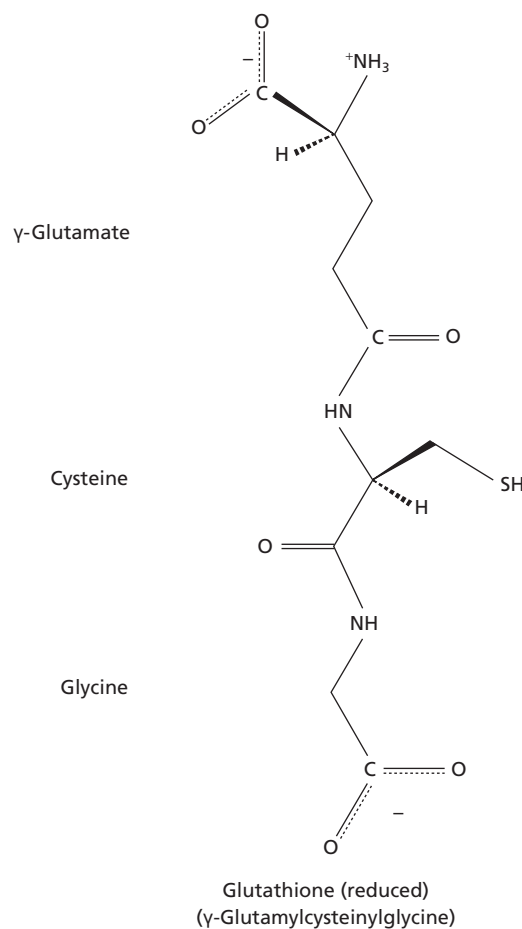


Fig. 1 Structure of GSH and its constituent amino acids. The bond between glutamate and cysteine determines the stability and resistance of GSH to hydrolysis, while its functions are mainly determined by the SH group of cysteine in its reduced form.

the conventional α -carboxyl group (Fig. 1). This unusual arrangement resists degradation by intracellular peptidases and is subject to hydrolysis by only one known enzyme, γ -glutamyltranspeptidase (GGT). Furthermore, the carboxyl-terminal glycine moiety of GSH protects the molecule against cleavage by intracellular γ -glutamylcyclotransferase. As a consequence, GSH is only metabolized extracellularly by GGT, which acts on the external face of certain cell types. Moreover, the sulphhydryl group of cysteine is required for GSH's functions, in particular the regulation of disulphide bonds of proteins and the disposal of electrophiles and oxidants.

Despite its exclusive synthesis in the cytosol, GSH is distributed in intracellular organelles, including endoplasmic reticulum (ER) and mitochondria. GSH is found predominantly in its reduced form except in the ER, where it exists mainly as oxidized glutathione (GSSG) to provide the adequate environment necessary for protein metabolism. A tight connection between ER and cytosol is necessary to ensure an appropriate secretory function of the ER, as GSH in the cytosol reduces protein