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Minireview

ER chaperones in mammalian development and human diseases

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Abstract The field of endoplasmic reticulum (ER) stress in mammalian cells has expanded rapidly during the past decade, contributing to understanding of the molecular pathways that allow cells to adapt to perturbations in ER homeostasis. One major mechanism is mediated by molecular ER chaperones which are critical not only for quality control of proteins processed in the ER, but also for regulation of ER signaling in response to ER stress. Here, we summarized the properties and functions of GRP78/BiP, GRP94/gp96, GRP170/ORP150, GRP58/ERp57, PDI, ERp72, calnexin, calreticulin, EDEM, Herp and co-chaperones SIL1 and P58^{IPK} and their role in development and diseases. Many of the new insights are derived from recently constructed mouse models where the genes encoding the chaperones are genetically altered, providing invaluable tools for examining the physiological involvement of the ER chaperones in vivo.

Keywords: Endoplasmic reticulum; Chaperones; Mammalian development; Diseases

1. Introduction

The endoplasmic reticulum (ER) is an essential cellular compartment for protein synthesis and maturation. It also functions as a Ca^{2+} storage organelle and resource of calcium signals. The perturbation of ER functions, such as disruption of Ca^{2+} homeostasis, inhibition of protein glycosylation or disulfide bond formation, hypoxia and virus or bacteria infection, can result in accumulation of unfolded or misfolded proteins and the failure of the ER to cope with the excessive protein load. This leads to ER stress, which is defined as an imbalance between the cellular demand for ER function and ER capacity. To reduce the excessive protein loading, the cells trigger the unfolded protein response (UPR), which signals transient attenuation of protein translation, degradation of malfolded proteins and the induction of molecular chaperones and folding enzymes to augment the ER capacity of protein folding and degradation. However, if the ER stress cannot be relieved, apoptotic pathways are activated in the damaged cells. The ER contains a number of molecular chaperones physiologically involved in the post-translational modification, disulfide bond formation, folding, assembly and quality control of newly synthesized proteins to preserve cellular homeostasis. Upon ER stress, upregulation of ER chaperones is pivotal for cell survival by facilitating the correct folding and assembly of ER proteins and preventing their aggregation. Furthermore, specific chaperones are also involved in stress signaling regulation and protein degradation process to attenuate apoptotic stimuli. While the link between molecular chaperones and human diseases awaits direct proof in most cases, recent construction and characterization of novel mouse models where the gene encoding for the ER chaperone protein is deleted or genetically altered provide new insights on the physiological contribution of these proteins in vivo. This review highlights recent progress in understanding the role of ER chaperones in response to ER stress and their functional roles in mammalian development and human diseases.

2. Chaperoning function of ER proteins

The ER chaperones can be categorized into three groups: (a) chaperones of heat shock protein family including GRP78, GRP94 and the co-chaperones; (b) chaperone lectins like calnexin, calreticulin and EDEM; and (c) substrate-specific chaperones such as Hsp47. Additionally, there are at least two groups of folding catalysts, namely thiol oxidoreductases of the protein disulfide isomerase (PDI) family such as PDI and GRP58/ERp57 and peptidyl prolyl isomerases (PPIs). There are two known chaperone systems in the ER, calnexin/calreticulin and GRP78/GRP94 [1]. The properties and function of ER chaperones, co-chaperones and folding enzymes covered in this review are summarized in Tables 1 and 2.

2.1. Calnexin/calreticulin chaperone system

Calnexin is a 90 kDa type I ER membrane protein and calreticulin is a 60 kDa soluble ER lumen protein with a C-terminal KDEL signal [2,3]. When the newly synthesized polypeptides enter the ER, they are often modified by N-linked glycans ($Glc_3Man_9GlcNAc_2$) and the glucoses are rapidly removed by glucosidases I and II [4]. The nascent protein with monoglucosylated N-linked glycans ($Glc_1Man_9GlcNAc_2$) is recognized by the calnexin/calreticulin system for subsequent

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Abbreviations: ER, endoplasmic reticulum; ERAD, ER-associated degradation; HHcy, hyperhomocysteinemia; PDI, protein disulfide isomerase; PrP^{sc}, scrapie-associated PrP; STEC, Shiga toxigenic *Escherichia coli*; SubAB, AB5 subtilase cytotoxin; UPR, unfolded protein response

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Table 1
Summary of function and disease relevance of ER chaperones, co-chaperones and folding enzymes

Protein	Localization	Function	Knockout mouse model	Diseases	Reference
GRP78/BiP	ER lumen ER transmembrane Cell surface Nucleus	Chaperone, Ca^{2+}-binding, ER stress sensor UPR regulator Anti-apoptosis	Embryonic lethality at E3.5 due to failure of embryo peri-implantation	Cancer Alzheimer's disease Parkinson's disease Prion diseases Atherosclerosis	[34,57,65,71,75–78,107]
SIL1	ER lumen	Co-chaperone, nucleotide exchange factor for GRP78	Woozy mouse associated with cerebellar Purkinje cell degeneration and ataxia	Marinesco-Sjögren syndrome	[58–60]
GRP94/gp96	ER lumen Cell surface transmembrane	Chaperone, Ca^{2+}-binding, Anti-apoptosis Tumor immunity	Embryonic lethality	Cancer Prion diseases Autoimmune disease	[65,71,86,87,89]
GRP170/ORP150	ER lumen	Chaperone, potential nucleotide exchange factor for GRP78	Embryonic lethality	Alzheimer's disease	[57,61,129]
GRP58/ERp57	ER lumen Nucleus Cytosol	Thio-oxidoreductase to catalyze disulfide bond formation of glycoprotein	Embryonic lethality (traditional knockout); *Grp58*$^{-/-}$ B cells are defective in antigen presentation (conditional knockout in B cells)	Prion diseases Alzheimer's disease	[35,62,92,130,131]
PDI	ER lumen Cell surface	Thio-oxidoreductase to catalyze disulfide bond formation	ND	Alzheimer's disease Parkinson's disease	[55,92,132]

folding and assembly steps. This process can be co-translational and release of the completely folded glycoprotein from this cycle is usually coupled with transportation from the ER to the Golgi complex. GRP58/ERp57, a member of the PDI family, is also an important component involved in the calnexin/calreticulin system. It contains two thioredoxin motifs and acts as a thiol oxidoreductase to catalyze the disulfide bond formations of the loaded glycoproteins [5]. If the glycoprotein cannot be correctly folded, another ER protein UGGT (UDP-glucose glycoprotein-glucosyltransferase) recognizes the unfolded or misfolded glycoprotein and catalyzes the transfer of a glucose unit from UDP-glucose to a specific mannose residue within the *N*-glycan chain of the glycoprotein [6]. Reglucosylation generates the monoglucosylated *N*-linked glycan that provides a new binding site of calnexin/calreticulin for re-entry of the glycoprotein into the cycle, until the correct folding is achieved [7]. Studies with hydrophobic peptides and glycoproteins have shown that UGGT recognizes surface-exposed hydrophobic regions [8,9]. While it was reported that UGGT can sense a local subtle structural alteration generated by a single point mutation and monoglucosylate glycans distant from misfolded determinants [10], another study using RNaseA/B as the substrate found that UGGT only selectively modifies the *N*-glycans close to or within the unfolded sites [11].

2.2. GRP78/GRP94 chaperone system

GRP78, also known as BiP, is the ER homologue of HSP70 proteins with a conserved ATPase domain and a peptide-binding domain [12,13]. As a chaperone, GRP78 recognizes and binds to the proteins with hydrophobic residues in the unfolded regions [14]. Therefore, some calnexin/calreticulin substrates can bind to GRP78 if the *N*-glycosylation is blocked. GRP78 is in a large multi-protein complex with a set of ER molecular chaperones, GRP94, PDI, ERp72, GRP170/ORP150, UGGT, CaBP1 (calcium binding protein), cyclophilin B and SDF2-L1, which forms an ER chaperoning network processing the unfolded protein substrates [15]. In this complex, GRP94, an ER homologue of HSP90 protein, often functions as a dimer providing a platform for the assembly or oligomerization of loaded protein cargo [16].

3. Regulation of stress signaling by ER chaperones

Cells developed an evolutionarily conserved integrated intracellular signaling cascade, referred to as the UPR, to reduce the unfolded protein load and increase folding capacity. For survival, the UPR signals pathways attenuating protein synthesis, upregulating the transcription of chaperone genes that increase ER capacity of folding and degradation, and retro-translocating misfolded proteins to the cytosol for degradation. There are three major UPR pathways with the ER-resident transmembrane proteins PERK, ATF6 and IRE1 as proximal signal sensors. Molecular chaperones play regulatory roles in UPR signaling pathway. The best characterized is the ER chaperone GRP78 which directly interacts with all three ER stress sensors, PERK, ATF6 and IRE1, and maintains them in inactive forms in non-stressed cells [17]. When accumulation of misfolded proteins occurs, GRP78 is titrated away. Release from GRP78 allows the activation and transduction of the unfolded protein signals across the ER membrane to the cytosol and the nucleus. Further characterization of post-translational modification of the ATF6 reveals