Published in final edited form as:
Semin Cell Dev Biol. 2010 July ; 21(5): 479–485. doi:10.1016/j.semcdb.2010.03.004.

GRP94 in ER Quality Control and Stress Responses

Davide Eletto, **Devin Dersh**, and **Yair Argon**
Division of Cell Pathology, Department of Pathology and Lab Medicine, The Children's Hospital of Philadelphia and the University of Pennsylvania, 3615 Civic Center Blvd., Philadelphia PA, 19104, USA

Abstract

A system of endoplasmic reticulum (ER) chaperones has evolved to optimize the output of properly folded secretory and membrane proteins. An important player in this network is Glucose Regulated Protein 94 (GRP94). Over the last decade, new structural and functional data have begun to delineate the unique characteristics of GRP94 and have solidified its importance in ER quality control pathways. This review describes our current understanding of GRP94 and the four ways in which it contributes to the ER quality control: 1) chaperoning the folding of proteins; 2) interacting with other components of the ER protein folding machinery; 3) storing calcium; and 4) assisting in the targeting of malfolded proteins to ER associated degradation (ERAD).

Keywords

ER chaperone; HSP90; IGF; calcium; ERAD

1. Introduction

The structural variety of secretory and membrane proteins dictates the need for multiple chaperone systems, and the ER has a resident member of the Heat Shock Protein (HSP) 90 family – GRP94. In addition to its induction upon glucose deprivation [1, 2] this protein was identified independently based on other activities, and thereby also named gp96, endoplasmin, Tra-1 or HSP108 (see refs. in [3]). These names all refer to the same product of the gene HSP90B1 (NCBI GeneID: 7184) and in every organism characterized so far GRP94 is the only HSP90-like protein that resides in the ER. GRP94 is a ubiquitously expressed chaperone, with especially high levels in secretory tissues (http://biogps.gnf.org). Though its up-regulation is often used as a hallmark of responses to ER stress, the functional significance of GRP94 has remained poorly characterized. Only the role of GRP94 in cellular immune responses has been studied intensely; for reviews, see [4, 5]. However, recent genetic, biochemical and cell biological studies have begun to shed light on the functions that GRP94 fulfils within the ER quality control system.

Address correspondence to: Dr. Yair Argon, The Children's Hospital of Philadelphia, Department of Pathology and Laboratory Medicine, 816 Abramson Research Center, Philadelphia, PA 19104. Tel: 267-426-5131. Fax: 267-426-5165, yargon@mail.med.upenn.edu.

2. Structure/Function

GRP94 is a soluble, obligate dimer both *in vitro* and *in vivo* [6, 7]. Its structure has been determined by X-ray crystallography [8, 9], electron microscopy and small angle X-ray scattering [10]. Like all members of the HSP90 family, GRP94 comprises 4 domains: an N-terminal domain (NTD), an acidic linker domain (LD), a middle domain (MD) and a C-terminal domain (CTD) (Fig. 1). GRP94 is an ATPase whose nucleotide-binding is mediated by the NTD, but whose hydrolytic activity requires cooperative action of the linker and middle domains.

The conformation of GRP94 has been investigated extensively in relation to its ATPase cycle. The protein crystallizes in a “twisted V” conformation with either ATP or ADP [8], but in solution it assumes a spectrum of conformations similar to those of its HSP90α and HSC82 homologs [10]. The vast majority of molecules have an extended, “chair-like” conformation (Fig. 1C). A second, minor population is in a less extended conformation whose CTD/NTD orientation is similar to that seen in the GRP94 crystal structure [8]. Yet a third, rare population is even more closed, a conformation represented by the known GRP94ADP/AMP-PNP crystal structures [9, 11]. The addition of nucleotide shifts the equilibrium toward the more closed states, but the extended conformation remains the most common [10]. This extended conformation is more active than others in an *in vitro* chaperone assay [12], perhaps because it allows for a larger surface of interaction with client proteins.

The NTD harbors most of the binding activities of the protein – it binds peptides through a curved β sheet [13](Fig. 1), at least two distinct receptors on dendritic cells [14-16], nucleotides and two small molecule inhibitors, geldanamycin and radicicol [17]. Co-crystallization and biochemical competition experiments [8, 9, 11, 17, 18] show that the inhibitors and nucleotides all bind to the same pocket, on the opposite face of the peptide-binding site (Fig. 1).

Binding of geldanamycin, radicicol or their derivatives has important functional consequences: when they occupy the nucleotide binding site, the activity of GRP94 towards clients is impaired [19, 20] and GRP94 is converted to a more compact conformation [21, 22]. The ability of geldanamycin and radicicol to inhibit GRP94 is medically important, as they are used in cancer treatment. Because they are pan-HSP90 inhibitors, however, these drugs inhibit not only HSP90 clients like kinases and transcription factors, but also the set of GRP94 clients. On the other hand, the nucleotide analog NECA binds specifically to GRP94, because the entry to the nucleotide-binding site is distinct from that of other HSP90s [22]. This shows that GRP94-specific compounds can be discovered or designed and therefore used to inhibit the clientele of GRP94 specifically.

While the nucleotide-binding pocket of GRP94 is highly homologous to that of HSP90, its hydrolytic activity has been controversial. At first, GRP94 was thought to bind, but not hydrolyze ATP [23]. However, recent work has shown that GRP94 is an active ATPase with an inherent rate of hydrolysis similar to that of other HSP90s [8, 24]. Importantly, the ATPase activity is essential *in vivo* for the chaperoning activity, at least towards the client insulin-like growth factor (IGF) [25].

As in other HSP90s, the NTD of GRP94 belongs to the Bergerat fold of ATP-binding proteins [26, 27], which have an unusual split ATPase structure: the nucleotide-binding site is in the NTD but hydrolysis requires additional residues from the MD. Furthermore, the two parts of the active site are not aligned in GRP94, and the MD needs to swing ~90° to orient the catalytic loop properly [8]. Besides the catalytic loop, no functions have yet been mapped to the MD, which in cytosolic HSP90s mediate contacts with co-chaperones [28].

The acidic linker domain that connects the NTD and MD is essential for the activity of GRP94, because it mediates conformational changes that are needed for ATP hydrolysis [17, 21]. This acidic domain also includes at least one important calcium-binding site, whose occupation transmits a conformational change to the NTD [29]. Because it is not visible in crystals, the structure of linker domain is not known. However, the vestige of this domain that is resolved in the structure contacts the face of the NTD away from the nucleotide-binding site, near the peptide-binding site (Fig. 1A,B), potentially explaining the regulatory effect of the acidic domain on the function of GRP94.

The C-terminal domain is responsible for the constitutive dimerization of GRP94. A stretch of 44 amino acids provides the necessary interface between the subunits [7]. It also contains signature features that are common to other HSP90s, like helix 21 (Fig. 1A), whose specific functions are not yet known. The CTD terminates with the ER retention/retrieval signal KDEL, which together with the N terminal signal sequence accounts for the predominant ER location of GRP94. Although in some situations GRP94 can be secreted [30] or found on the cell surface [31, 32], it is mostly restricted to the ER lumen.

One main enigma about GRP94 is the identity of the sites for binding other proteins, both clients and co-factors. Peptide binding by GRP94, extensively characterized *in vitro* [4, 14], does not seem to be a major *in vivo* function of GRP94 [33]. Therefore, peptide binding is not a valid surrogate for client protein binding, like it is in many other chaperones, and there must be undiscovered protein interacting surfaces elsewhere in the protein.

3. Regulation of GRP94 activity

Most information about the regulation of GRP94 is derived from in vitro studies that attempt to describe its conformational changes and relate them to the ATPase activity. While the conformational equilibrium of GRP94 and its inherent ATPase activity resemble that of other HSP90s, the regulation of the conformational changes appears mechanistically different in GRP94 [12, 24, 34], perhaps reflecting the unique conditions in the ER. One major difference is the response to nucleotide binding: GRP94 binds ATP and ADP with K_D ~5 μM [35] and shows little discrimination between them. By contrast, other HSP90s bind ADP 5- to 10-fold better than ATP [35-37]. Additionally, ATP does not become kinetically trapped upon binding to GRP94; the “lid” formed by helices 1,4,5 and their connecting loops (Fig. 1B) opens upon binding of nucleotide [9, 34], rather than closing on the nucleotide as in HSP90 [38]. Finally, the lid in GRP94 is more sensitive to the identity of the nucleotide than in HSP90, illustrating variation in the molecular regulation within the HSP90 family.

A second aspect of GRP94 regulation is how the ATPase cycle is coupled to chaperone activity. An appealing model postulates that ATP binding promotes N-terminal dimerization, in addition to the CTD-mediated dimerization, to yield a molecular clamp for client proteins. Upon ATP binding, the 90° rotation around the NTD/MD interface aligns the nucleotide binding site in the NTD with the catalytic residues of the MD and transiently dimerizes the NTD. This “catalytically competent” ATP-bound closed dimer is also stabilized by cross-subunit interactions between the opposing NTDs [8, 9] and between the NTD of one subunit and the MD of its opposite subunit [39].

Thus, the emerging picture is of nucleotide-driven conformational changes in GRP94 that are limited to the NTD and occur in the transition from the unliganded state to the adenosine nucleotidebound state, rather than from the ATP to the ADP state. Such conformational changes shift the equilibrium from the extended solution conformation towards the more compact conformations seen in the crystal structures, and involve transient interactions

elsewhere in the GRP94 dimer. It remains to be shown how these conformational changes are related to the activity of the protein [10, 39].

Regulation of GRP94's chaperone function may not be solely based on nucleotide binding, however. HSP90 co-chaperones were recently discovered to help regulate conformational intermediates even without nucleotides [40], and similar mechanisms may be conserved in GRP94. Unlike HSP90, though, GRP94 has no known co-chaperones that would affect its ATPase cycle, assist in the loading of clients or affect the conformational equilibrium of the chaperone. The current absence of co-chaperones may suggest a unique conformational and functional cycle for GRP94 that may rely on its inherent activity. It is also possible that regulatory functions that in the cytosol are performed by co-chaperones are provided in the ER by interactions with other chaperones or even clients themselves. On the other hand, the ability to manipulate the levels of GRP94 expression together with proteomic tools may lead to the discovery of accessory proteins that are not homologous by sequence but are similar in function to those in the HSP90 machinery.

4. Function

4.1. General functional concepts

Our view of the physiological roles for GRP94 has evolved considerably over the past decade, in part due to studies that ablated its expression or activity. GRP94 is essential for organisms; it is necessary for the proper development of plants [41], nematodes (gene TO5.11.3; http://wormbase.org), fruit flies [42] and mice [43]. Significantly, in each organism GRP94 is required during a specific developmental stage. The requirement for GRP94 does not coincide with the onset of expression of the protein, which is made in virtually every cell from the zygote onward. Rather, it is likely that an essential function for GRP94 is exposed when it is needed for particular clients, which differ depending on cell and tissue type during the development of multi-cellular organisms. The relevant clients are only now beginning to emerge, with the Clavata complex in *Arabidopsis* and IGF in mice the first of GRP94 clients with known roles in development [20, 41, 43]. Whatever the function, it is essential only for metazoans, because GRP94 is not expressed in unicellular organisms and is also not essential for growth of individual mammalian cells in culture. GRP94-deficient cells can grow normally [44], albeit more slowly [20], do not have global deficiency in surface receptors expression [44], and are still capable of differentiation [43]. These observations are consistent with co-evolution of GRP94 with clients that are needed for cell-cell interactions.

4.2. Chaperoning folding of clients

The most important activity of GRP94 is as a chaperone that directs folding and/or assembly of secreted and membrane proteins. Unlike other ubiquitous luminal chaperones, GRP94 is rather selective, with a smaller client list than PDI, calreticulin or BiP. Many secretory proteins, like transferrin receptor or MHC class I, do not require GRP94's activity for proper folding. Even among secretory proteins that do associate with GRP94, only a few were also shown to require it for proper expression [45]. The selectivity of GRP94 is illustrated in the case of the integrin family [44]: while many integrins require chaperoning by GRP94, surface expression of β1 integrins is independent of GRP94 [46].

The first GRP94 clients discovered were from the immunoglobulin family: folding intermediates of either heavy or light chains (LC) were found complexed first with BiP, then with GRP94 [47]. This interaction is functionally important, because LC secretion is inhibited when GRP94 is ablated [19] and the LCs are targeted for ERAD. On the other hand, Li et al. showed that serum immunoglobulin levels are not depleted in mice whose GRP94 is deleted in B cells [48], suggesting that there is a bypass mechanism which enables

secretion without GRP94. Perhaps like in the integrin family, not all immunoglobulins require this chaperone.

In the case of another family of clients, insulin-like proteins, both the mammalian insulin-like growth factor (IGF)-I and -II strictly depend on GRP94 for their maturation [20, 49]. In its absence, the accumulated pro-IGF intermediates are targeted to ERAD [20] and signaling–competent hormones are not secreted [20, 43]. Studies with immunoglobulins, thyroglobulin, bile salt-dependent lipase and IGF suggest that GRP94 typically governs advanced folding intermediates [20, 47, 50]. However, the selectivity of GRP94 raises the question of the structural features that are being recognized. Unlike the lectin chaperones that recognize carbohydrates, or the HSP70s that bind short peptides, and even unlike HSP90, which binds many kinases and DNA binding domains, there is yet no common feature to the clients of GRP94 other than the presence of disulfide bonds (Table I). Whatever the molecular basis for client recognition, the *Drosophila* orthologue, gp93, with 74% homology to mammalian GRP94, can support surface expression of several mammalian integrins and TLRs in a GRP94-deficient cell line [46]. Such functional complementation by a non-authentic chaperone underscores that GRP94 functions with its clients in fundamentally the same manner.

4.3. Calcium Buffering

The endoplasmic reticulum couples its quality control machinery to the storage and utilization of calcium [51]. Changes in the flux of calcium in and out of the ER are involved in many signaling pathways and physiological responses. Indeed, inhibiting the SERCA pumps with thapsigargin is a common experimental tool to stress the ER and induce a global unfolded protein response (UPR). Most calcium in the ER is stored bound to proteins, and GRP94 is one of a few major luminal calcium-binding proteins [52-54]. Each GRP94 molecule can bind between 16 and 28 Ca^{++} atoms [52, 53]. One or two high affinity sites are in the first acidic domain [29], but none of the other Ca^{++} binding sites of GRP94 have been mapped yet. GRP94 resembles calreticulin in these binding parameters [55] and considering their concentrations, we estimate that calreticulin and GRP94 each provides 30 μM of Ca^{++} storage capacity. Depletion of total ER Ca^{++} either with chaperone knockdown or thapsigargin can affect protein folding and chaperone selection in the ER. Examples include the misfolding of scavenger receptors [56] and the bias of thyroglobulin towards BiP and GRP94 after premature exit from the calnexin/calreticulin cycle [57].

GRP94 is not merely a Ca^{++} storage device, however: it is also affected by the levels of free Ca^{++}, since its peptide binding activity *in vitro* is augmented at physiological Ca^{++} levels [29]. It remains to be shown whether Ca^{++} also modulates GRP94 activity *in vivo* [25]. A specific function for GRP94 in Ca^{++} homeostasis may be inferred from the importance of GRP94 levels in muscle cells. Over-expression of GRP94 protects cardiomyocytes from the toxic effects of high free intracellular Ca^{++} [58], and reduced levels of GRP94 compromise the fusion competence of skeletal myoblasts [59].

4.4. GRP94 and ERAD

Quality control in the ER involves both protein folding as well as the detection of misfolded proteins for ER-associated degradation. How misfolded proteins are distinguished and targeted for degradation is an important and yet unsolved question, but recent work that defines components of the targeting machinery suggests a role for GRP94 in this process [60]. The degradation of !1-antitrypsin Null Hong Kong, a model ERAD substrate, is impaired in GRP94-depleted cells. This is likely due to the physiological interaction between GRP94 and a proposed ERAD sensor protein, OS-9 [60].

OS-9 is a soluble ER-resident protein that can distinguish between folded and misfolded conformation of proteins and is essential for the proper degradation of several examined ERAD substrates [61-65]. Although it is not currently known how OS-9 senses misfolded targets, it appears to use polypeptide-based signals to bind glycoproteins [60] and target them to ERAD pore complexes. The role of the GRP94/OS-9 interaction in this pathway is only beginning to be uncovered; further work will be needed to solidify the mechanistic details of ERAD sensing as well as how GRP94 affects the OS-9-mediated degradation of misfolded proteins.

4.5. IGF and ER stress

The obligate dependence of IGF on the chaperone activity of GRP94 highlights a novel connection between ubiquitous ER proteins and specific physiological responses like the UPR. The outcome of the response to ER stress can be either restoration of homeostasis or apoptotic cell death (Fig. 2). This is clearly dictated by a complex network of protein interactions, but one important factor is IGF signaling: IGFs are both UPR target genes as well as mediators that affect the initiation of the UPR.

The IGF signaling pathway, which is pro-growth and anti-apoptotic, requires bioavailability of either IGF-I or IGF-II. Both are targets of the UPR, based on a number of lines of evidence. First, secretion of mammalian IGF-II is induced by tunicamycin treatment [49]. Zebra fish IGF-I transcription is also activated by tunicamycin treatment, mediated by spliced XBP1 [66]. Moreover, exogenous expression of XBP1 in a cell line suffices to induce IGF signaling [66]. At least one UPR response element is also conserved in promoters of human, rodent and canine IGF-I genes (using the MATCH™ algorithm). Other components that regulate IGF bioavailability, like IGFBP1, are also UPR target genes [67]. Thus, in cells that produce IGF, like embryonic cells, hepatocytes or myocytes, this production is enhanced by UPR, when the physiology requires more anti-apoptotic signaling [43, 67-69]. The UPR also affects IGF availability post-translationally; since GRP94 controls production of mature IGFs [20], it also largely determines the level of subsequent signaling. This arms the cell with an autocrine/paracrine signaling mechanism, which in turn expands at least some pathways of UPR. The cellular outcome of this feedback is promotion of recovery from stress and suppression of apoptosis.

Conversely, IGF signaling also affects the response to ER stress. Administration of IGF-I together with thapsigargin significantly potentiates the UPR through both the IRE1 and PERK branches of the response [70]. Thus, IGF-I increases the chaperone reserves of the ER, perhaps to alleviate the stress signal (Fig. 2). There is also negative feedback between ER stress and IGF under conditions where metabolic needs activate the nutrient sensing apparatus. This leads, paradoxically, to inhibition of insulin/IGF signaling [71]. How the positive and negative feedback loops operate together is still unknown.

If parts of the IGF signaling machinery are induced during UPR, one would expect a similar induction during physiological UPR. Indeed, IGF-I mRNA is up-regulated during terminal differentiation of B cells, an IRE1-dependent process [72], in parallel with the production of ER chaperones and immunoglobulin [73]. Thus, both physiological and experimentally-induced stress responses involve GRP94, not only through its general involvement in protein folding, but specifically through its role in the production of IGF.

4.6. GRP94 and the ER chaperone network

Coordinate up-regulation of GRP94 with many other ER folding components was first discovered by using pharmacological treatments to induce UPR [74-76]. Later, this phenomenon was shown to be relevant for physiological processes, such as plasma cell

differentiation [77-79]. As the ER workload increases, GRP94 is transcriptionally co-regulated with other chaperones to increase the efficiency of folding and reduce the chance of misfolded proteins leaving the ER.

Does this co-regulation reflect a functional or physical network in the ER? Hendershot and others have described a large complex consisting of a subset of the ER proteins, with BiP and GRP94 being the most abundant components [80]. They proposed that this complex does not merely assemble in response to the folding needs of particular secretory proteins, but rather is a stable feature of the lumen of many cell types [80]. However, measurements of the diffusional mobilities of GFP-tagged GRP94, BiP and calreticulin strongly argue against a large fraction of these chaperones being engaged in stable complexes ([81] and E. Snapp, personal communication). An alternative scenario is that quality control components assemble as needed by the nature of the folding client. BiP and GRP94 are often found associated with itinerant proteins, and with consistent stoichiometry [47, 50, 80, 82, 83]. Furthermore, their actions are concerted, with GRP94 following BiP in folding immunoglobulins or thyroglobulin [47, 50]. These observations are consistent with the view that folding of some proteins requires one chaperone system to deal with early folding intermediates and another to orchestrate later stages of folding. Other clients, on the other hand, do not require both of these chaperones: many proteins associate with BiP but not GRP94, and at least one, IGF, interacts with GRP94 and apparently not with BiP [20, 25]. Thus, the cooperation with BiP is not an inherent property of the action cycle of GRP94. Interestingly, GRP94 has not been shown to interact with the lectin chaperones, suggesting functional separation between axes of chaperones, perhaps as proposed by Molinari and Helenius [84].

One prediction of the putative quality control network is that depletion of one chaperone would affect either the activity or expression of another. Indeed, using *C. elegans*, Link et al. described compensatory regulation among nine ER chaperones, including BiP and GRP94 [85]. The induction of BiP when GRP94 is silenced by RNAi involves the IRE1/XBP1 branch of the UPR [85]. BiP is also induced when the worm E3 ubiquitin ligase HRD-1 is depleted via RNAi [86], in line with the physical interactions between luminal proteins and the ERAD targeting machinery [60, 87]. A compensatory relationship among ER chaperones extends to mammalian cells: knockdown of BiP [88], its specific inactivation by cleavage with a bacterial subtilase [89], or even changing its subcellular localization by mutating the KDEL sequence [90, 91], induces the expression of GRP94, PDI, calreticulin and ERp57. Silencing of calreticulin stimulates the expression of GRP94 and PDI [88], and calreticulin-null embryonic fibroblasts also show constitutively higher levels of GRP94 and other ER proteins ([92] Eletto, unpublished data). On the other hand, this phenomenon is not universal: GRP94-null cells do not display increased expression of other chaperones [43]. In mice, partial loss of BiP due to heterozygosity leads to an adaptive, transcriptional response where a subset of chaperones, including GRP94 and PDI, are induced [93]. Again, the response is not reciprocal, because *grp94*+/- mice do not exhibit significant up-regulation of BiP, calnexin or calreticulin [43].

The above perturbations and responses show the existence of evolutionary conserved regulatory mechanisms that compensate for the loss of some quality control components by selectively increasing others. These responses appear to be selective and are not always mutual. A second conclusion is that some form of chronic UPR induction can be activated by deprivation of even a single chaperone. The reasons for the compensatory effects are not obvious at present; since the activity of GRP94 in protein folding is not redundant with BiP's, the functional gain from inducing GRP94 upon losing BiP it is not clear. Perhaps elucidating the mechanism underlying the selective compensation will explain the functional interactions between ER chaperone subsets.

5. Conclusions

The roles of GRP94 in protein folding, calcium binding and targeting to ERAD are only beginning to be understood. Its client selectivity and interactions with late folding intermediates suggest that GRP94 performs unique chaperone functions in the ER. Clients like IGFs position GRP94 in key pathways that are critical for cell growth, differentiation and responses to ER stress. Further work on the structure, function, and regulation of GRP94 *in vivo*, coupled with mutational analysis and genetic complementation tests, should help define how GRP94 binds clients, whether it interacts with co-chaperones and how its action cycle is regulated. Such knowledge will explain how the inherent activities of GRP94 are regulated in the cell, further define its unique roles in protein folding, and highlight its interaction with other components of the of ER quality control.

Acknowledgments

We thank Drs. D. Agard, J. Buchner and D. Gewirth for advice and discussion of data, and Dr. B. Barton for constructive critiques.

References

1. Shiu RP, Pouyssegur J, Pastan I. Glucose depletion accounts for the induction of two transformation-sensitive membrane proteins in Rous sarcoma virus-transformed chick embryo fibroblasts. Proc Natl Acad Sci USA. 1977; 74:3840–4. [PubMed: 198809]
2. Kang HS, Welch WJ. Characterization and purification of the 94-kDa glucose-regulated protein. J Biol Chem. 1991; 266:5643–9. [PubMed: 1900837]
3. Argon Y, Simen BB. GRP94, an ER chaperone with protein and peptide binding properties. Semin Cell Dev Biol. 1999; 10:495–505. [PubMed: 10597632]
4. Srivastava PK. Therapeutic cancer vaccines. Curr Opin Immunol. 2006; 18:201–5. [PubMed: 16464565]
5. Baker-LePain JC, Reed RC, Nicchitta CV. ISO: a critical evaluation of the role of peptides in heat shock/chaperone protein-mediated tumor rejection. Curr Opin Immunol. 2003; 15:89–94. [PubMed: 12495739]
6. Nemoto T, Matsusaka T, Ota M, Takagi T, Collinge DB, Walther-Larsen H. Dimerization characteristics of the 94-kDa glucose-regulated protein. J Biochem (Tokyo). 1996; 120:249–56. [PubMed: 8889807]
7. Wearsch PA, Nicchitta CV. Endoplasmic reticulum chaperone GRP94 subunit assembly is regulated through a defined oligomerization domain. Biochemistry. 1996; 35:16760–9. [PubMed: 8988013]
8. Dollins DE, Warren JJ, Immormino RM, Gewirth DT. Structures of GRP94-nucleotide complexes reveal mechanistic differences between the hsp90 chaperones. Mol Cell. 2007; 28:41–56. [PubMed: 17936703]
9. Immormino RM, Dollins DE, Shaffer PL, Soldano KL, Walker MA, Gewirth DT. Ligand-induced conformational shift in the N-terminal domain of GRP94, an Hsp90 chaperone. J Biol Chem. 2004; 279:46162–71. [PubMed: 15292259]
10. Krukenberg KA, Bottcher UM, Southworth DR, Agard DA. Grp94, the endoplasmic reticulum Hsp90, has a similar solution conformation to cytosolic Hsp90 in the absence of nucleotide. Protein Sci. 2009
11. Soldano KL, Jivan A, Nicchitta CV, Gewirth DT. Structure of the N-terminal domain of GRP94: Basis for ligand specificity and regulation. J Biol Chem. 2003; 278:48330–8. [PubMed: 12970348]
12. Krukenberg KA, Southworth DR, Street TO, Agard DA. pH-dependent conformational changes in bacterial Hsp90 reveal a Grp94-like conformation at pH 6 that is highly active in suppression of citrate synthase aggregation. J Mol Biol. 2009; 390:278–91. [PubMed: 19427321]
13. Gidalevitz T, Biswas C, Ding H, Schneidman-Duhovny D, Wolfson HJ, Stevens F, et al. Identification of the N-terminal peptide binding site of Glucose-regulated Protein 94. J Biol Chem. 2004; 279:16543–52. [PubMed: 14754890]

14. Biswas C, Sriram U, Ciric B, Ostrovsky O, Gallucci S, Argon Y. The N-terminal fragment of GRP94 is sufficient for peptide presentation via professional APCs. Int Immunol. 2006; 18:1147–57. [PubMed: 16772370]
15. Berwin B, Hart JP, Pizzo SV, Nicchitta CV. CD91-Independent Cross-Presentation of GRP94(gp96)-Associated Peptides. J Immunol. 2002; 168:4282–6. [PubMed: 11970968]
16. Berwin B, Hart JP, Rice S, Gass C, Pizzo SV, Post SR, et al. Scavenger receptor-A mediates gp96/GRP94 and calreticulin internalization by antigen-presenting cells. Embo J. 2003; 22:6127–36. [PubMed: 14609958]
17. Schulte TW, Akinaga S, Murakata T, Agatsuma T, Sugimoto S, Nakano H, et al. Interaction of radicicol with members of the heat shock protein 90 family of molecular chaperones. Mol Endocrinol. 1999; 13:1435–48. [PubMed: 10478836]
18. Schulte TW, Akinaga S, Soga S, Sullivan W, Stensgard B, Toft D, et al. Antibiotic radicicol binds to the N-terminal domain of Hsp90 and shares important biologic activities with geldanamycin. Cell Stress Chaperones. 1998; 3:100–8. [PubMed: 9672245]
19. Gidalevitz T, Simen BB, Ostrovsky O, Vogen SM, Dul JL, Argon Y. GRP94 activity is necessary for completion of immunoglobulin light chain folding. In Revision. 2009
20. Ostrovsky O, Ahmed NT, Argon Y. The chaperone activity of GRP94 towards of insulin-like growth factor II is necessary for the stress response to serum deprivation. Mol Biol Cell. 2009; 20:1855–64. [PubMed: 19158397]
21. Vogen SM, Gidalevitz T, Biswas C, Simen BS, Stein E, Gulmen F, et al. Radicicol-sensitive peptide binding to the N-terminal portion of GRP94. J Biol Chem. 2002; 277:40742–50. [PubMed: 12189140]
22. Rosser MFN, Nicchitta CV. Ligand interactions in the adenosine nucleotide binding domain of the Hsp90 chaperone, GRP94. I. Evidence for allosteric regulation of ligand binding. J Biol Chem. 2000; 275:22798–805. [PubMed: 10816561]
23. Wearsch PA, Nicchitta CV. Interaction of endoplasmic reticulum chaperone GRP94 with peptide substrates is adenine nucleotide-independent. J Biol Chem. 1997; 272:5152–6. [PubMed: 9030582]
24. Frey S, Leskovar A, Reinstein J, Buchner J. The ATPase cycle of the endoplasmic chaperone Grp94. J Biol Chem. 2007; 282:35612–20. [PubMed: 17925398]
25. Ostrovsky O, Makarewich C, Snapp EL, Argon Y. An essential role for ATP binding and hydrolysis in the chaperone activity of GRP94 in cells. Proc Natl Acad Sci U S A. 2009; 106:11600–5. [PubMed: 19553200]
26. Bergerat A, de Massy B, Gadelle D, Varoutas PC, Nicolas A, Forterre P. An atypical topoisomerase II from Archaea with implications for meiotic recombination. Nature. 1997; 386:414–7. [PubMed: 9121560]
27. Dutta R, Inouye M. GHKL, an emergent ATPase/kinase superfamily. Trends Biochem Sci. 2000; 25:24–8. [PubMed: 10637609]
28. Richter K, Buchner J. hsp90: twist and fold. Cell. 2006; 127:251–3. [PubMed: 17055424]
29. Biswas C, Ostrovsky O, Makarewich CA, Wanderling S, Gidalevitz T, Argon Y. The peptide binding activity of GRP94 is regulated by Calcium. Biochem J. 2007; 405:233–41. [PubMed: 17411420]
30. Bruneau N, Lombardo D, Bendayan M. Participation of GRP94-related protein in secretion of pancreatic bile salt-dependent lipase and in its internalization by the intestinal epithelium. J Cell Sci. 1998; 111:2665–79. [PubMed: 9701565]
31. Altmeyer A, Maki RG, Feldweg AM, Heike M, Protopopov VP, Masur SK, et al. Tumor-specific cell surface expression of the-KDEL containing, endoplasmic reticular heat shock protein gp96. Int J Cancer. 1996; 69:340–9. [PubMed: 8797880]
32. Koo BH, Apte SS. Cell-surface processing of the metalloprotease pro-ADAMTS9 is influenced by the chaperone GRP94/gp96. J Biol Chem. 285:197–205. [PubMed: 19875450]
33. Lev A, Dimberu P, Das SR, Maynard JC, Nicchitta CV, Bennink JR, et al. Efficient cross-priming of antiviral CD8+ T cells by antigen donor cells is GRP94 independent. J Immunol. 2009; 183:4205–10. [PubMed: 19752220]

34. Dollins DE, Immormino RM, Gewirth DT. Structure of unliganded GRP94, the endoplasmic reticulum Hsp90. Basis for nucleotide-induced conformational change. J Biol Chem. 2005; 280:30438–47. [PubMed: 15951571]
35. Ge J, Normant E, Porter JR, Ali JA, Dembski MS, Gao Y, et al. Design, synthesis, and biological evaluation of hydroquinone derivatives of 17-amino-17-demethoxygeldanamycin as potent, water-soluble inhibitors of Hsp90. J Med Chem. 2006; 49:4606–15. [PubMed: 16854066]
36. Prodromou C, Roe SM, O'Brien R, Ladbury JE, Piper PW, Pearl LH. Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. Cell. 1997; 90:65–75. [PubMed: 9230303]
37. McLaughlin SH, Ventouras LA, Lobbezoo B, Jackson SE. Independent ATPase activity of Hsp90 subunits creates a flexible assembly platform. J Mol Biol. 2004; 344:813–26. [PubMed: 15533447]
38. Pearl LH, Prodromou C. Structure and mechanism of the Hsp90 molecular chaperone machinery. Annu Rev Biochem. 2006; 75:271–94. [PubMed: 16756493]
39. Chu F, Maynard JC, Chiosis G, Nicchitta CV, Burlingame AL. Identification of novel quaternary domain interactions in the Hsp90 chaperone, GRP94. Protein Sci. 2006; 15:1260–9. [PubMed: 16731965]
40. Hessling M, Richter K, Buchner J. Dissection of the ATP-induced conformational cycle of the molecular chaperone Hsp90. Nat Struct Mol Biol. 2009; 16:287–93. [PubMed: 19234467]
41. Ishiguro S, Watanabe Y, Ito N, Nonaka H, Takeda N, Sakai T, et al. SHEPHERD is the Arabidopsis GRP94 responsible for the formation of functional CLAVATA proteins. Embo J. 2002; 21:898–908. [PubMed: 11867518]
42. Maynard JC, Pham T, Zheng T, Jockheck-Clark A, Rankin HB, Newgard CB, et al. Gp93, the Drosophila GRP94 ortholog, is required for gut epithelial homeostasis and nutrient assimilation-coupled growth control. Dev Biol. 2010
43. Wanderling S, Simen BB, Ostrovsky O, Ahmed NT, Vogen S, Gidalevitz T, et al. GRP94 is essential for mesoderm induction and muscle development because it regulates IGF secretion. Mol Biol Cell. 2007; 18:3764–75. [PubMed: 17634284]
44. Randow F, Seed B. Endoplasmic reticulum chaperone gp96 is required for innate immunity but not cell viability. Nat Cell Biol. 2001; 3:891–6. [PubMed: 11584270]
45. Yang Y, Li Z. Roles of heat shock protein gp96 in the ER quality control: redundant or unique function? Mol Cells. 2005; 20:173–82. [PubMed: 16267390]
46. Morales C, Wu S, Yang Y, Hao B, Li Z. Drosophila glycoprotein 93 Is an ortholog of mammalian heat shock protein gp96 (grp94, HSP90b1, HSPC4) and retains disulfide bond-independent chaperone function for TLRs and integrins. J Immunol. 2009; 183:5121–8. [PubMed: 19786553]
47. Melnick J, Dul JL, Argon Y. Sequential interaction of the chaperones BiP and GRP94 with immunoglobulin chains in the endoplasmic reticulum. Nature. 1994; 370:373–5. [PubMed: 7913987]
48. Liu B, Li Z. Endoplasmic reticulum HSP90b1 (gp96, grp94) optimizes B-cell function via chaperoning integrin and TLR but not immunoglobulin. Blood. 2008; 112:1223–30. [PubMed: 18509083]
49. Ostrovsky O, Eletto D, Makarewich C, Argon Y. Glucose Regulated Protein 94 is required for muscle differentiation through its control of the autocrine production of insulin-like growth factors. BBA-Molec Cell. 2009 in press.
50. Muresan Z, Arvan P. Thyroglobulin transport along the secretory pathway. Investigation of the role of molecular chaperone, GRP94, in protein export from the endoplasmic reticulum. J Biol Chem. 1997; 272:26095–102. published erratum appears in J Biol Chem 1997 Nov 28;272(48):30590. [PubMed: 9334173]
51. Meldolesi J, Pozzan T. The endoplasmic reticulum Ca2+ store: a view from the lumen. Trends Biochem Sci. 1998; 23:10–4. [PubMed: 9478128]
52. Macer DPJ, Koch GLE. Identification of a set of calcium-binding protein in reticuloplasm, the luminal content of the endoplasmic reticulum. J Cell Sci. 1988; 91:61–70. [PubMed: 3253304]
53. Van PN, Peter F, Soling HD. Four intracisternal calcium-binding glycoproteins from rat liver microsomes with high affinity for calcium. No indication for calsequestrin-like proteins in inositol

1,4,5-trisphosphate-sensitive calcium sequestering rat liver vesicles. J Biol Chem. 1989; 264:17494–501. [PubMed: 2793869]

54. Cala SE, Jones LR. GRP94 resides within cardiac sarcoplasmic reticulum vesicles and is phosphorylated by casein kinase II. J Biol Chem. 1994; 269:5926–31. [PubMed: 8119936]
55. Baksh S, Michalak M. Expression of calreticulin in Escherichia coli and identification of its Ca2+ binding domains. J Biol Chem. 1991; 266:21458–65. [PubMed: 1939178]
56. Obermoeller LM, Chen Z, Schwartz AL, Bu G. Ca2+ and receptor-associated protein are independently required for proper folding and disulfide bond formation of the low density lipoprotein receptor-related protein. J Biol Chem. 1998; 273:22374–81. [PubMed: 9712858]
57. Di Jeso B, Ulianich L, Pacifico F, Leonardi A, Vito P, Consiglio E, et al. Folding of thyroglobulin in the calnexin/calreticulin pathway and its alteration by loss of Ca2+ from the endoplasmic reticulum. Biochem J. 2003; 370:449–58. [PubMed: 12401114]
58. Vitadello M, Penzo D, Petronilli V, Michieli G, Gomirato S, Menabo R, et al. Overexpression of the stress protein Grp94 reduces cardiomyocyte necrosis due to calcium overload and simulated ischemia. Faseb J. 2003; 17:923–5. [PubMed: 12670879]
59. Gorza L, Vitadello M. Reduced amount of the glucose-regulated protein GRP94 in skeletal myoblasts results in loss of fusion competence. FASEB J. 2000; 14:461–75. [PubMed: 10698961]
60. Christianson JC, Shaler TA, Tyler RE, Kopito RR. OS-9 and GRP94 deliver mutant alpha1-antitrypsin to the Hrd1-SEL1L ubiquitin ligase complex for ERAD. Nat Cell Biol. 2008; 10:272–82. [PubMed: 18264092]
61. Szathmary R, Bielmann R, Nita-Lazar M, Burda P, Jakob CA. Yos9 protein is essential for degradation of misfolded glycoproteins and may function as lectin in ERAD. Mol Cell. 2005; 19:765–75. [PubMed: 16168372]
62. Kim W, Spear ED, Ng DT. Yos9p detects and targets misfolded glycoproteins for ER-associated degradation. Mol Cell. 2005; 19:753–64. [PubMed: 16168371]
63. Alcock F, Swanton E. Mammalian OS-9 is upregulated in response to endoplasmic reticulum stress and facilitates ubiquitination of misfolded glycoproteins. J Mol Biol. 2009; 385:1032–42. [PubMed: 19084021]
64. Bhamidipati A, Denic V, Quan EM, Weissman JS. Exploration of the topological requirements of ERAD identifies Yos9p as a lectin sensor of misfolded glycoproteins in the ER lumen. Mol Cell. 2005; 19:741–51. [PubMed: 16168370]
65. Bernasconi R, Pertel T, Luban J, Molinari M. A dual task for the Xbp1-responsive OS-9 variants in the mammalian endoplasmic reticulum: inhibiting secretion of misfolded protein conformers and enhancing their disposal. J Biol Chem. 2008; 283:16446–54. [PubMed: 18417469]
66. Hu MC, Gong HY, Lin GH, Hu SY, Chen MH, Huang SJ, et al. XBP-1, a key regulator of unfolded protein response, activates transcription of IGF1 and Akt phosphorylation in zebrafish embryonic cell line. Biochem Biophys Res Commun. 2007; 359:778–83. [PubMed: 17560942]
67. Marchand A, Tomkiewicz C, Magne L, Barouki R, Garlatti M. Endoplasmic reticulum stress induction of insulin-like growth factor-binding protein-1 involves ATF4. J Biol Chem. 2006; 281:19124–33. [PubMed: 16687408]
68. Wilson EM, Rotwein P. Control of MyoD function during initiation of muscle differentiation by an autocrine signaling pathway activated by insulin-like growth factor-II. J Biol Chem. 2006; 281:29962–71. [PubMed: 16901893]
69. Nakanishi K, Sudo T, Morishima N. Endoplasmic reticulum stress signaling transmitted by ATF6 mediates apoptosis during muscle development. J Cell Biol. 2005; 169:555–60. [PubMed: 15897261]
70. Novosyadlyy R, Kurshan N, Lann D, Vijayakumar A, Yakar S, LeRoith D. Insulin-like growth factor-I protects cells from ER stress-induced apoptosis via enhancement of the adaptive capacity of endoplasmic reticulum. Cell Death Differ. 2008; 15:1304–17. [PubMed: 18437163]
71. Ozcan U, Ozcan L, Yilmaz E, Duvel K, Sahin M, Manning BD, et al. Loss of the tuberous sclerosis complex tumor suppressors triggers the unfolded protein response to regulate insulin signaling and apoptosis. Mol Cell. 2008; 29:541–51. [PubMed: 18342602]

72. Calfon M, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP, et al. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. Nature. 2002; 415:92–6. [PubMed: 11780124]
73. van Anken E, Romijn EP, Maggioni C, Mezghrani A, Sitia R, Braakman I, et al. Sequential waves of functionally related proteins are expressed when B cells prepare for antibody secretion. Immunity. 2003; 18:243–53. [PubMed: 12594951]
74. Chang SC, Erwin AE, Lee AS. Glucose-regulated protein (GRP94 and GRP78) genes share common regulatory domains and are coordinately regulated by common trans- acting factors. Mol Cell Biol. 1989; 9:2153–62. [PubMed: 2546060]
75. Drummond IA, Lee AS, Resendez E Jr, Steinhardt RA. Depletion of intracellular calcium stores by calcium ionophore A23187 induces the genes for glucose-regulated proteins in hamster fibroblasts. J Biol Chem. 1987; 262:12801–5. [PubMed: 3114264]
76. Lee AS. Coordinated regulation of a set of genes by glucose and calcium ionophores in mammalian cells. Trends Biochem Sci. 1987; 12:20–3.
77. Wiest DL, Burkhardt JK, Hester S, Hortsch M, Meyer DI, Argon Y. Membrane biogenesis during B cell differentiation: most endoplasmic reticulum proteins are expressed coordinately. J Cell Biol. 1990; 110:1501–11. [PubMed: 2335560]
78. Iwakoshi NN, Lee AH, Vallabhajosyula P, Otipoby KL, Rajewsky K, Glimcher LH. Plasma cell differentiation and the unfolded protein response intersect at the transcription factor XBP-1. Nat Immunol. 2003; 4:321–9. [PubMed: 12612580]
79. Lee AH, Iwakoshi NN, Glimcher LH. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. Mol Cell Biol. 2003; 23:7448–59. [PubMed: 14559994]
80. Meunier L, Usherwood YK, Chung KT, Hendershot LM. A subset of chaperones and folding enzymes form multiprotein complexes in endoplasmic reticulum to bind nascent proteins. Mol Biol Cell. 2002; 13:4456–69. [PubMed: 12475965]
81. Snapp EL, Sharma A, Lippincott-Schwartz J, Hegde RS. Monitoring chaperone engagement of substrates in the endoplasmic reticulum of live cells. Proc Natl Acad Sci U S A. 2006; 103:6536–41. [PubMed: 16617114]
82. Xu A, Bellamy AR, Taylor JA. BiP (GRP78) and endoplasmin (GRP94) are induced following rotavirus infection and bind transiently to an endoplasmic reticulum-localized virion component. J Virol. 1998; 72:9865–72. [PubMed: 9811722]
83. Linnik KM, Herscovitz H. Multiple molecular chaperones interact with apolipoprotein B during its maturation. The network of endoplasmic reticulum-resident chaperones (ERp72, GRP94, calreticulin, and BiP) interacts with apolipoprotein b regardless of its lipidation state. J Biol Chem. 1998; 273:21368–73. [PubMed: 9694898]
84. Molinari M, Helenius A. Chaperone selection during glycoprotein translocation into the endoplasmic reticulum. Science. 2000; 288:331–3. [PubMed: 10764645]
85. Kapulkin V, Hiester BG, Link CD. Compensatory regulation among ER chaperones in C. elegans. FEBS Lett. 2005; 579:3063–8. [PubMed: 15907843]
86. Sasagawa Y, Yamanaka K, Ogura T. ER E3 ubiquitin ligase HRD-1 and its specific partner chaperone BiP play important roles in ERAD and developmental growth in Caenorhabditis elegans. Genes Cells. 2007; 12:1063–73. [PubMed: 17825049]
87. Bernasconi R, Galli C, Calanca V, Nakajima T, Molinari M. Stringent requirement for HRD1, SEL1L, and OS-9/XTP3-B for disposal of ERAD-LS substrates. J Cell Biol. 2010; 188:223–35. [PubMed: 20100910]
88. Maruri-Avidal L, Lopez S, Arias CF. Endoplasmic reticulum chaperones are involved in the morphogenesis of rotavirus infectious particles. J Virol. 2008; 82:5368–80. [PubMed: 18385250]
89. Wolfson JJ, May KL, Thorpe CM, Jandhyala DM, Paton JC, Paton AW. Subtilase cytotoxin activates PERK, IRE1 and ATF6 endoplasmic reticulum stress-signalling pathways. Cell Microbiol. 2008; 10:1775–86. [PubMed: 18433465]
90. Mimura N, Yuasa S, Soma M, Jin H, Kimura K, Goto S, et al. Altered quality control in the endoplasmic reticulum causes cortical dysplasia in knock-in mice expressing a mutant BiP. Mol Cell Biol. 2008; 28:293–301. [PubMed: 17954555]

91. Mimura N, Hamada H, Kashio M, Jin H, Toyama Y, Kimura K, et al. Aberrant quality control in the endoplasmic reticulum impairs the biosynthesis of pulmonary surfactant in mice expressing mutant BiP. Cell Death Differ. 2007; 14:1475–85. [PubMed: 17464327]

92. Mesaeli N, Nakamura K, Zvaritch E, Dickie P, Dziak E, Krause KH, et al. Calreticulin is essential for cardiac development. J Cell Biol. 1999; 144:857–68. [PubMed: 10085286]

93. Ye R, Jung DY, Jun JY, Li J, Luo S, Ko HJ, et al. Grp78 Heterozygosity Promotes Adaptive Unfolded Protein Response and Attenuates Diet-Induced Obesity and Insulin Resistance. Diabetes. 2010; 59:6–16. [PubMed: 19808896]

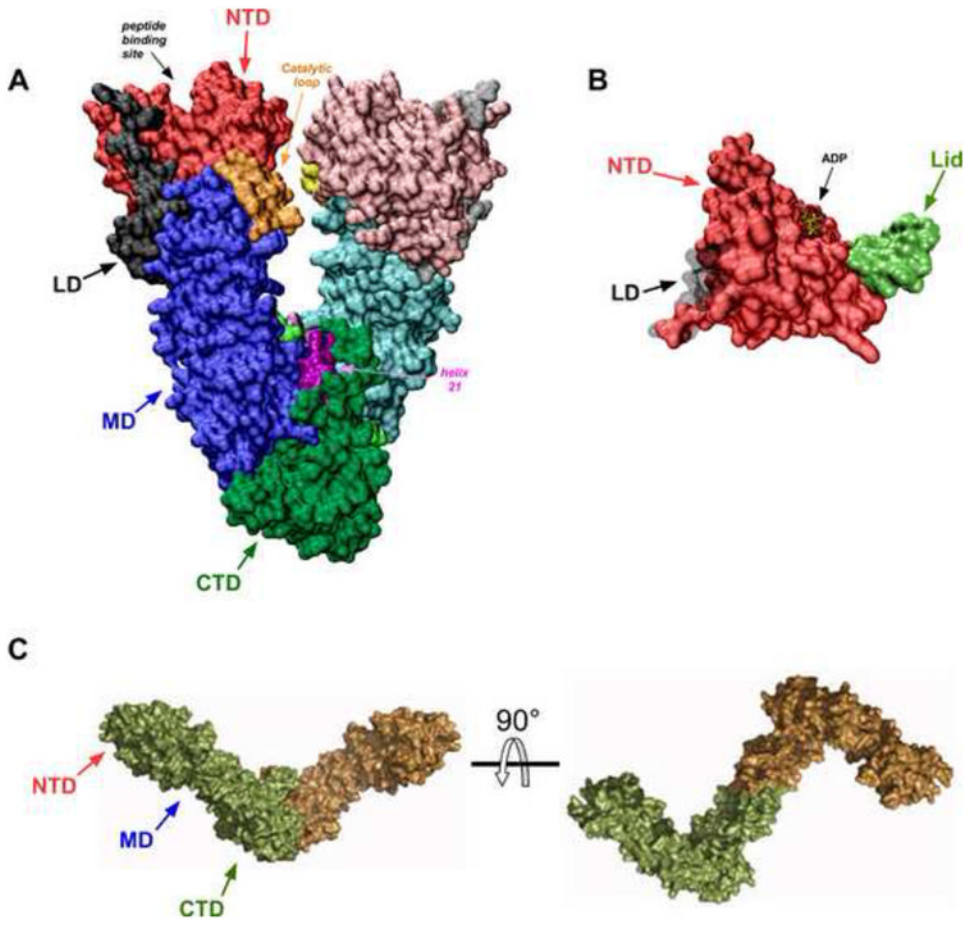

Fig. 1.

Structural features of GRP94.

(A) A molecular surface view of the GRP94 dimer (PDB entry 2O1V). The amino-terminal domain (NTD) is colored red, the linker domain (LD) - black, the middle domain (MD) - blue and the carboxy terminal domain (CTD) – green. The corresponding domains in the opposite monomer are colored in lighter shades. The peptide binding site in the NTD, the catalytic loop (orange) in the MD required for ATP hydrolysis and the amphipathic helix 21 (purple) in the CTD are indicated. Helix 21 projects into a large intra-dimer cleft, just above the hydrophobic surface that mediates the dimerization of the protein.

(B) Surface view of the NTD bound to ADP (PDB entry 1TC6). The NTD in this view is rotated relative to A to visualize the nucleotide binding pocket (ADP) and the lid (light green).

(C) The chair-like conformation of GRP94 in solution, with one monomer in green and the other in brown. Adapted, with permission, from [10].

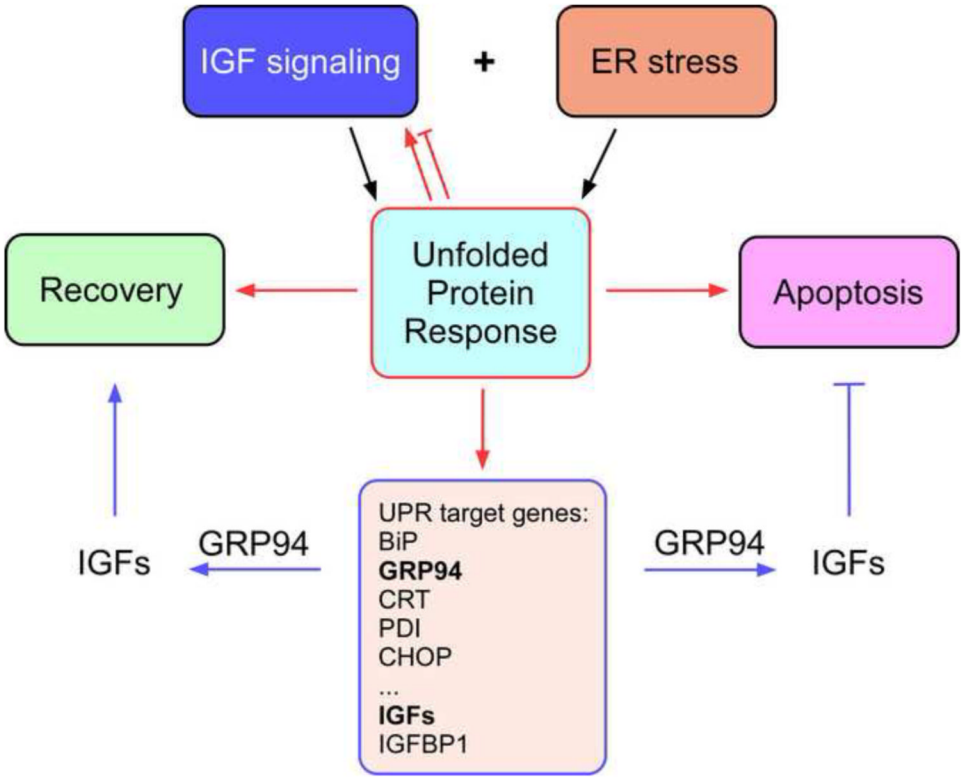

Fig. 2.

IGF is both a causative agent and a target of UPR.

The scheme illustrates that ER stress activates the UPR, leading either to resolution of the stress (recovery) or to apoptosis. In addition to the 'conventional' UPR target genes, (BiP, PDI, CHOP, etc.) IGF, IGFBP1 and possibly other members of the insulin/IGF signaling pathway are UPR targets. GRP94 and IGFs play a specific role in alleviating ER-stress: GRP94 is essential for production of IGF. Autocrine/paracrine IGF signaling can delivers both anti-apoptotic and pro-recovery signals. In addition, IGF signaling also potentiates an existing UPR, which in turn can control IGF signaling either positively or negatively.

Table 1

Structural features of known GRP94 Clients.

Client	Domain types[a]	Disulfides	Glyc[b]	Topology	Oligomer
Integrins	PSI, EGF, β- propeller	Yes	Yes	Membrane	Heterodimer
Immunoglobulins	Ig fold				Hetero-oligomer
VpreB		Yes	No	Luminal	
Light chain		Yes	No	Luminal	
Heavy chain		Yes	Yes	Membrane & luminal	
BSSL	Esterase	Yes	Yes	Luminal	Monomer + Dimer
IGF	insulin			Luminal	Monomer
IGF-I		Yes	Yes		
IGF-II		Yes	No		
TLR	LRR, TIR			Membrane	
TLR4		Yes	Yes		Monomer
TLR9		unknown	Yes		Monomer
Thyroglobulin	CRR Thioredoxin	Yes	Yes	Luminal	Dimer

[a]Domain types: CRR, Cysteine-rich repeats; EGF, epidermal growth factor; LRR, Leucine-rich repeats; TIR, Toll and Interleukin receptor;

[b]Glyc, N-linked glycosylation