Cell. Mol. Life Sci. 66 (2009) 1556 – 1569
1420-682X/09/091556-14
DOI 10.1007/s00018-009-8745-y

Cellular and Molecular Life Sciences

Review

Functions and pathologies of BiP and its interaction partners

J. Dudek, J. Benedix, S. Cappel, M. Greiner, C. Jalal, L. Müller and R. Zimmermann*

Medical Biochemistry and Molecular Biology, Saarland University, 66421 Homburg (Germany), Fax: +49 6841 1626288, e-mail: bcrzim@uks.eu

Received 20 November 2008; received after revision 09 December2008; accepted 12 December 2008
Online First 21 January 2009

Abstract. The endoplasmic reticulum (ER) is involved in a variety of essential and interconnected processes in human cells, including protein biogenesis, signal transduction, and calcium homeostasis. The central player in all these processes is the ER-lumenal polypeptide chain binding protein BiP that acts as a molecular chaperone. BiP belongs to the heat shock protein 70 (Hsp70) family and crucially depends on a number of interaction partners, including co-chaperones, nucleotide exchange factors, and signaling molecules. In the course of the last five years, several diseases have been linked to BiP and its interaction partners, such as a group of infectious diseases that are caused by Shigella toxin producing *E. coli*. Furthermore, the inherited diseases Marinesco-Sjögren syndrome, autosomal dominant polycystic liver disease, Wolcott-Rallison syndrome, and several cancer types can be considered BiP-related diseases. This review summarizes the physiological and pathophysiological characteristics of BiP and its interaction partners.

Keywords. BiP, ERj proteins, nucleotide exchange factors, unfolded protein response, haemolytic uraemic syndrome, Marinesco-Sjögren syndrome, polycystic liver disease, Wolcott-Rallison syndrome.

Introduction

The endoplasmic reticulum (ER) plays a crucial role in protein biogenesis [1, 2], signal transduction [3–6], and calcium homeostasis in human cells [5, 6]. The rough ER represents the intracellular site where many proteins are synthesized; nascent polypeptides are either translocated into the ER lumen or integrated into the ER membrane (Fig. 1) [1, 2]. ER processing is essential for most secreted proteins, for many resident proteins of eukaryotic cell organelles (ER, ER-Golgi intermediate compartment, Golgi, endosome, lysosome), and for other membrane proteins (plasma membrane, nuclear envelope, peroxisomal membrane). Proteins are folded and assembled in the ER and are subsequently delivered to their functional location by vesicular transport [7, 8]. In case of misfolding or misassembly, proteins are earmarked for retrograde transport into the cytosol and for degradation by the proteasome (ER-associated degradation, ERAD) [9, 10]. Several signal transduction pathways originate in the ER and are activated by protein misfolding, for example the unfolded protein response (UPR) [3, 4]. In addition, the ER represents the major calcium storage organelle and, therefore, is involved in various signal transduction pathways [5, 6]. Moreover, many ER proteins are calcium-binding proteins and their activity may depend on calcium [5]. Therefore, drastic changes in the calcium concentration within the ER may cause misfolding of polypeptides. The central player in all these processes is the ER-lumenal polypeptide chain binding protein BiP (also termed glucose regulated protein 78 kDa, Grp78 or HspA5) that acts as a molecular chaperone [11–13]. BiP belongs to the heat shock protein 70 kDa

* Corresponding author

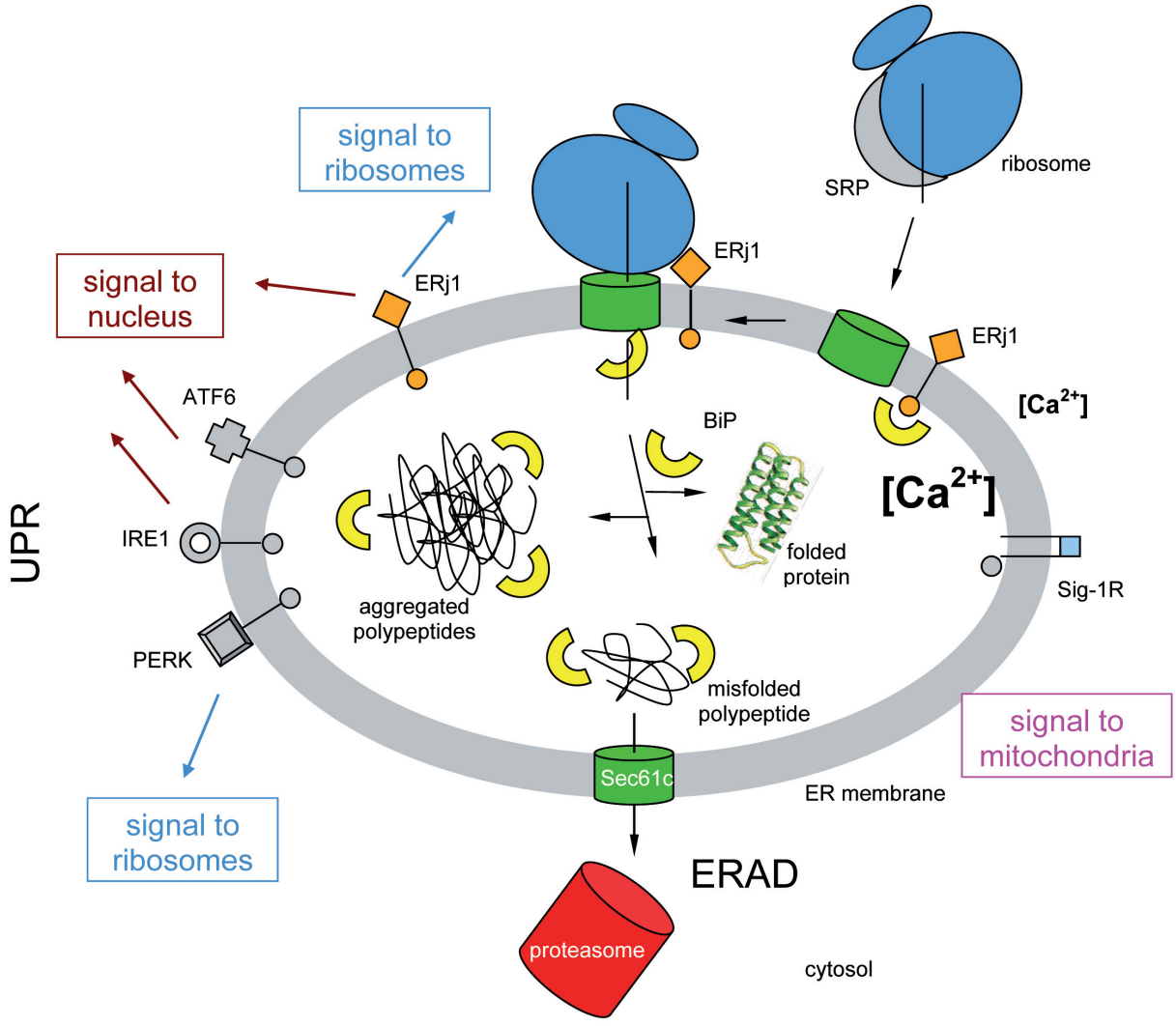

Figure 1. Functions of BiP in protein biogenesis, signal transduction, and calcium homeostasis. We note that the concentration of calcium ions is around 100 nM in the cytosol and about 1 mM in the ER lumen. Various ER-membrane proteins contain lumenal domains (depicted as filled circles) that interact with BiP. ERAD, ER-associated protein degradation; UPR, unfolded protein response.

(Hsp70) family and relies on a number of interaction partners, including Hsp40 co-chaperones, nucleotide exchange factors, and signal transducers for its various activities (Fig. 2, Table 1).

BiP and its interaction partners

BiP was originally termed immunoglobulin heavy chain binding protein for its role in the assembly of immunoglobulins [11]. BiP is the most abundant member of the Hsp70 protein family in the ER lumen, reaching a concentration in the millimolar range [14]. Another much less abundant member of the Hsp70 family in the ER is glucose-regulated protein 170 kDa (Grp170, also termed oxygen-regulated protein 150, ORP150, or hypoxia-upregulated protein 1, HYOU1) [15–18]. BiP and Grp170 are able to form a stable complex in the absence of ATP [19]. The ER lumen also harbors Stch, an Hsp70-related, truncated protein that only contains a nucleotide-binding domain (see below) [20].

In general, molecular chaperones of the Hsp70-type family reversibly bind to substrate polypeptides via the substrate binding domains (SBD) of the chaperones [21–28]. Typically, Hsp70 substrates are hydrophobic oligopeptides within more or less unfolded polypeptides [29, 30]. The binding of a substrate to the SBD inhibits unproductive interactions of the polypeptide and favors productive folding and assembly that occurs concomitant with release from Hsp70. In addition, Hsp70s can regulate the activities of folded polypeptides (e.g. the paradigm sigma^{32} factor in *E. coli*). The binding and release of substrates by Hsp70

Table 1. BiP and its interaction partners in human and murine cells. Alternative names are given in parentheses.

Function	Protein	Related human disease	OMIM	UPR	Animal model	References
Hsp70-type chaperones	BiP (Grp78, HspA5)	haemolytic uraemic syndrome	235400	+	embryonic lethality	[112,118]
	Grp170 (ORP150, HYOU1)			+	embryonic lethality	[141]
Hsp40-type co-chaperones	ERj1 (Htj1, DNAJC1)			-		
	Sec63 (ERj2, ERdj2)	polycystic liver disease	174050	-		[119]
	ERj3 (ERdj3, DnaJB11)			+		
	ERj4 (ERdj4, DnaJB9)			+		
	ERj5 (ERdj5, DnaJC10)			+		
	$p58^{IPK}$ (ERj6, DnaJC3)			+	diabetic mouse	[142]
	ERj7					
Nucleotide exchange factors	Grp170 (ORP150, HYOU1)			+	embryonic lethality	[141]
	Sil1 (BAP)	Marinesco-Sjögren syndrome	248800	-	woozy mouse	[123,124,143]
Additional chaperones	Grp94 (CaBP4, ERp99, gp96)			+	embryonic lethality	[141]
	Calnexin (p90, p88)			+	postnatal lethality	[144]
	Calreticulin (CaBP3, ERp60)			+	embryonic lethality	[145]
	Sig-1R			+		
Signal transducers	IRE1α (ERN1)					
	IRE1β (ERN2)					
	IRE2					
	ATF6α			+		
	ATF6β					
	PERK (EIF2AK3, PEK)	Wolcott-Rallison syndrome	226980		diabetic mouse	[128,129,146]
	ERj1			-		
Ca^{2+} sensor	Sig-1R			+		

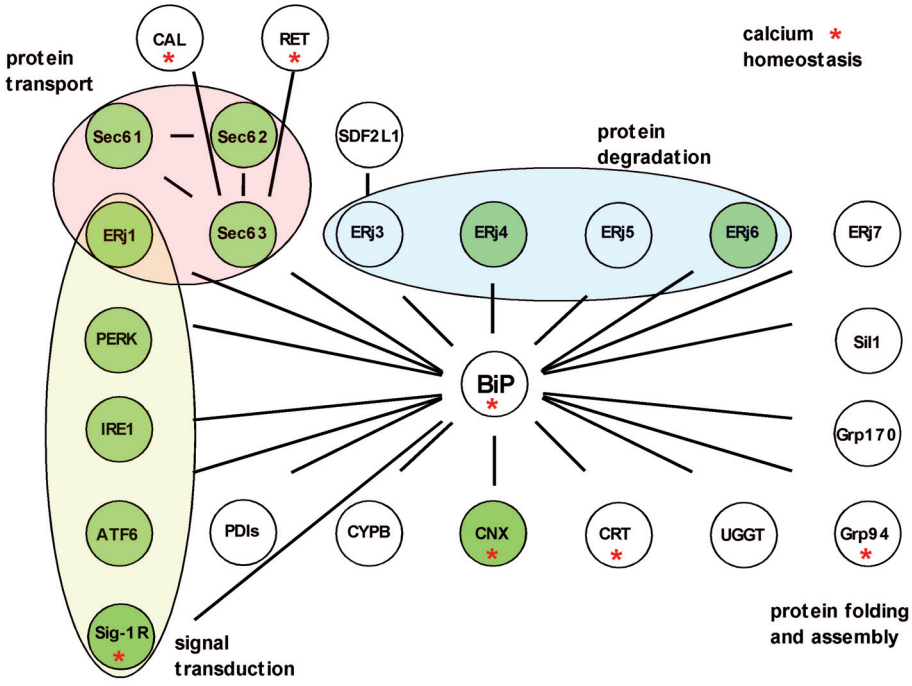

Figure 2. Interaction partners of BiP in human cells. Membrane proteins are depicted as green circles. CAL, calumenin; CNX, calnexin; CRT, calreticulin; CYPB, cyclophilin B; Grp94, glucose-regulated protein 94 kDa; PDIs, protein disulfide isomerases (such as PDIA1, PDIA4 and PDIA6); RET, reticulocalbin; SDF2L1, stromal-cell derived factor 2-like 1; UGGT, UDP-glucose-glycoprotein-glycosyltransferase.

are modulated by communication between its SBD and its nucleotide binding-domain (NBD). In turn, the NBD is controlled by the ATPase cycle and by different Hsp70 interaction partners [26–28]. The ATP-bound state of BiP has a low affinity for substrate polypeptides and the ADP-bound state has a high substrate affinity. Hsp40-type co-chaperones stimulate ATPase activity, that is, they favor substrate binding. Nucleotide exchange factors stimulate the exchange of ADP for ATP and, therefore, induce substrate release. Hsp40 proteins are characterized by J-domains that allow interaction with Hsp70 [31–42]. We note that some Hsp40 co-chaperones have the additional ability to bind substrate polypeptides and deliver them to Hsp70s. In order to be able to do so, they contain additional domains (i. e. in addition to the J-domains; see below).

There may be up to ten different Hsp40-type molecular chaperones present in the human ER, although not necessarily simultaneously in the same cell (Fig. 3) [43–67]. To date, seven of these have been characterized in some detail and were termed ERj1 through ERj7 (or ERdjs). The Hsp40-type co-chaperones can be subclassified into insoluble membrane proteins with a lumenal J-domain and soluble lumenal proteins (Fig. 3). Furthermore, they can be classified according to the domains they have in common with the archetypal Hsp40 bacterial DnaJ protein [31, 41, 42]. Type I Hsp40s contain four domains: an amino-terminal J-domain, a glycine-phenylalanine (G/F) rich domain, a Zn-finger- or cysteine repeat-domain, and a carboxy-terminal substrate binding domain. Type II Hsp40s contain three domains: an amino-terminal J-domain, a G/F-rich domain, and a carboxy-terminal substrate-binding domain. Type III Hsp40s contain only the J-domain and, in general, have more specialized functions compared to type I and II Hsp40s. Thus, only type I and II ER-lumenal Hsp40s (ERj3 and ERj4) are expected to have the ability to bind substrate polypeptides and deliver them to BiP, that is, to facilitate polypeptide folding, analogous to the paradigm of Hsp40, the DnaJ in *E. coli* [53–60]. However, the four thioredoxin domains within ERj5 and the tetratricopeptide repeat (TPR) domain in $p58^{IPK}$ (ERj6) may also play a role in substrate binding [60–67].

There are two different nucleotide exchange factors (NEFs) present in the ER lumen, termed Sil1 (or BAP) and Grp170 [68, 69]. Sil1 is closely related to HspBP1, one of the NEFs of cytosolic Hsc70 in eukaryotes (Fig. 3). Grp170 is the other member of the Hsp70 family that is present in the ER lumen and is closely related to Hsp110, an alternative NEF of cytosolic Hsc70 in eukaryotes. The structures of these two cytosolic paralogs were recently solved and revealed distinct interacting surfaces with the top of the NBD; thus, the NEF-binding sites on Hsp70 are different from the J-domain-binding site [70–73]. Based on the structural data, the two NEFs may be able to bind simultaneously on Hsp70.

Various components of the BiP functional cycle have been found in complexes with each other, with other

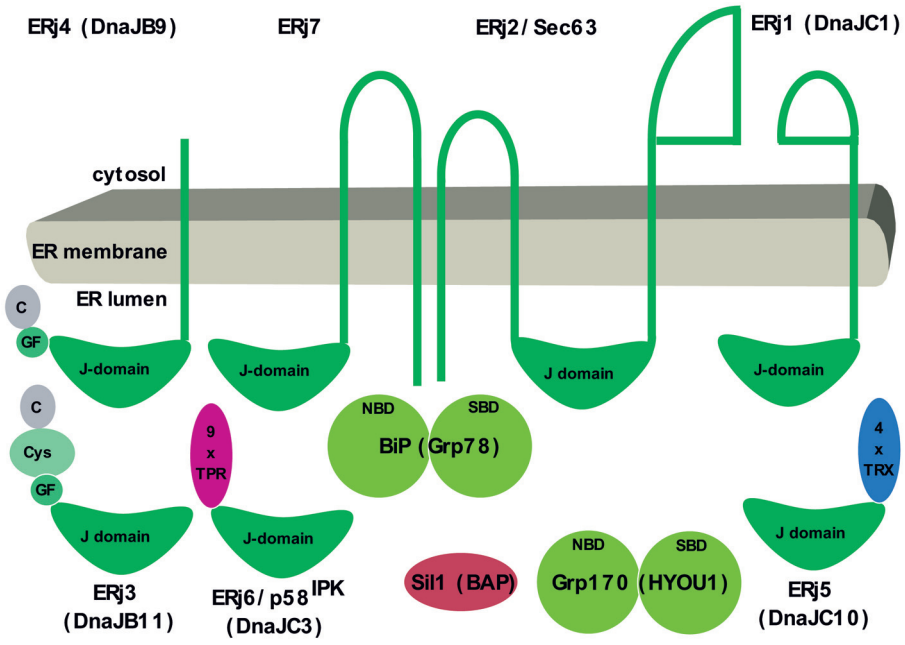

Figure 3. Topology and domain organization of BiP and its co-chaperones and nucleotide exchange factors in human cells. C, carboxy-terminal substrate binding domain; Cys, cysteine-repeat domain; GF, glycine-phenylalanine rich domain; NBD, nucleotide binding domain; SBD, substrate binding domain; TPR, tetratricopeptide repeat; TRX, thioredoxin domain.

chaperones (Grp94, calreticulin, calnexin) and with folding catalysts (protein disulfide isomerases (PDIs) and peptidylprolyl-*cis/trans*-isomerases (PPIases)). Some BiP interaction partners are complexed with other ER-resident proteins that are involved in protein transport (Sec61 complex), N- or O-glycosylation (UDP-glucose-glycoprotein-glycosyltransferase (UGGT), stromal cell-derived factor-2-like 1 (SDF2L1)) and calcium homeostasis (calumenin, reticulocalbin) (Fig. 2) [51, 52, 55, 74–77].

Other BiP interaction partners are involved in signal transduction from the ER to the cytosol or to the nucleus (unfolded protein response, or UPR) (Figs. 1 and 2, Table 1) [3, 4]. These proteins (IRE1, ATF6, PERK, Sig-1R) are similar to the membrane-integrated Hsp40s in that they are transmembrane proteins and have a lumenal domain that can interact with BiP [78–81]. In this case, however, the lumenal domains are not J-domains. In general, these signal transducers are inactive when BiP is bound to the lumenal domain. However, when BiP becomes sequestered by unfolded polypeptides, it is released and the signal transducers become activated (see below).

The role of BiP in protein transport into the ER

Protein transport into the ER is a first step in the biogenesis of most secreted proteins and many organellar proteins of eukaryotic cells [1, 2]. Similarly, integration into the ER membrane is the initial step in the biogenesis of many membrane proteins. Typically, protein transport into the ER involves amino terminal signal peptides in the precursor proteins and transport machinery (Fig. 4). Transport can be divided into three stages: targeting, membrane insertion, and completion of translocation. Precursor protein targeting or membrane association involves the ribosome, the signal recognition particle (SRP), and the SRP and ribosome receptors on the ER surface (SRP-receptor (SR) and Sec61 complex) [82, 83]. Alternatively, synthesis of the respective precursor proteins can be initiated at ribosomes that are permanently associated with the ER [84], thus eliminating the involvement of SRP and SR. Membrane insertion involves the Sec61 complex, comprised of α-, β-, and γ-subunits [85–88], and additional components, including the ER-lumenal chaperone BiP, its co-chaperones, and nucleotide exchange factors (see below). These same molecules are also involved in completion of translocation. Typically, following membrane insertion and prior to completion of translocation, the precursor proteins are covalently modified by signal peptidase (Spase) and/or oligosaccharyl transferase [89, 90].

BiP appears to have several functions in protein translocation: (i) it is involved in the insertion of precursor polypeptides into the Sec61 complex or opening of the Sec61 channel [18, 91]; (ii) it binds to the incoming precursor polypeptide and acts as a molecular ratchet, thereby facilitating completion of translocation [92, 93]; (iii) it is involved in closing the Sec61 channel, thus minimizing the uncontrolled efflux of calcium ions from the ER during protein translocation [94–96]. Grp170 is also involved in the insertion step [18] and a hitherto unidentified Hsp40 is required in channel closure [96]. At the completion of

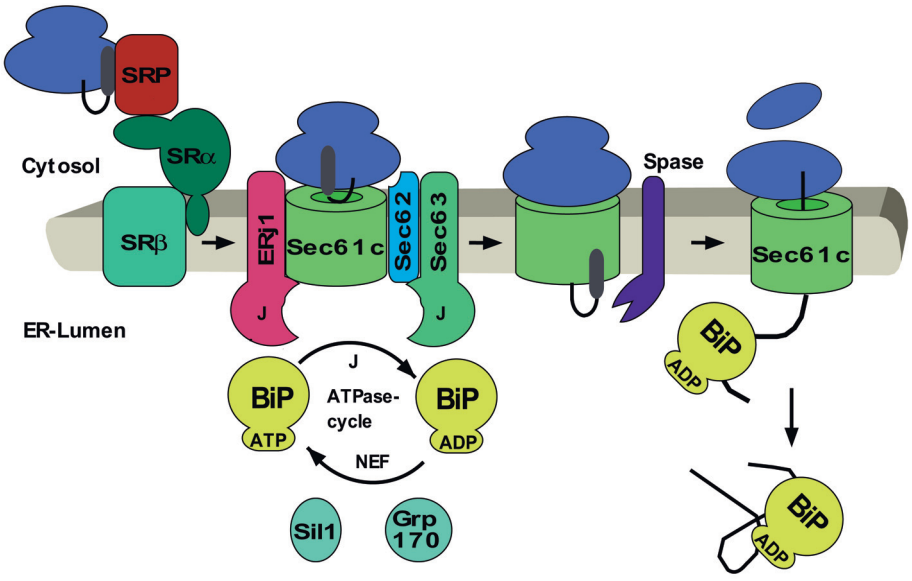

Figure 4. Protein transport into the endoplasmic reticulum. J, J-domain; Spase, signal peptidase; SRP, signal recognition particle; SR, SRP receptor. ADP, adenosine diphosphate; ATP, adenosine triphosphate; NEF, nucleotide exchange factor.

translocation, the precursor polypeptide in transit is the substrate of BiP. For the opening and closing of the Sec61 channel, the Sec61 complex is expected to represent the substrate of BiP. In the latter case, BiP regulates the activity of a folded protein. In analogy to the situation in *Saccharomyces cerevisiae* [97, 98], Sec63 (which forms a complex with the membrane protein Sec62 [51, 52]) may recruit BiP to the Sec61 complex and to translating ribosomes; Sec63 also activates BiP for interaction with its substrates (Fig. 4) [52]. In contrast to the situation in yeast, there is a second Hsp40 present in the membrane of the human ER (ERj1) which contacts translating ribosomes on the ER surface [46, 48, 49]; this may represent a functional alternative to Sec63. This view is supported by the fact that human ERj1 can complement a yeast Sec63p mutant strain [47]. Further support comes from the observation that Sec63 and ERj1 are not under the control of the UPR (see below). Sil1 and Grp170, the two alternatively acting nucleotide exchange factors of BiP, guarantee efficient regeneration of BiP in its ATP state [68, 69].

However, a word of caution is required here. At present it is not clear whether the observed actions of BiP are general; i.e. not every single precursor polypeptide may require the help of BiP at all three stages of protein translocation. Alternatively, BiP might only be involved in the translocation of some precursor proteins or might play a regulatory role in protein translocation [99].

The role of BiP in folding and assembly of proteins

Protein transport into the ER is followed by folding and assembly of the newly synthesized soluble and membrane proteins [7, 8]. BiP was first identified as a cofactor of immunoglobulin assembly, hence its name immunoglobulin heavy chain binding protein [11]. In particular the C_H1 domain of immunoglobulin depends on binding of BiP for productive interaction with the light chain during immunoglobulin assembly [12, 100–103]. Hsp40 co-chaperones (ERj3) and nucleotide exchange factors (Sil1) are also required for BiP function in immunoglobulin assembly [56, 68]. After folding and assembly, the native proteins are delivered to their functional location by vesicular transport.

The role of BiP in export and degradation of proteins

Misfolding or misassembly of polypeptides may be caused by mutation or by ER stress (e.g. glucose- or calcium-depletion); these non-native proteins are exported to the cytosol and delivered to the proteasome for degradation (quality control or ERAD) [9, 10]. In further analogy to the situation in *Saccharomyces cerevisiae* [104, 105], the Sec61 complex, Sec63 and BiP may be involved in the export of at least some misfolded polypeptides from the ER lumen to the cytosol (Fig. 1). Furthermore, ERj3 through ERj5 were implicated in ERAD in mammalian cells, thus directly demonstrating a role for BiP in ERAD [57, 60, 63]. This view is supported by the observation that ERj3 through ERj5 are under the control of the UPR (see below).

Again, a word of caution is required here. The Sec61 complex may be just one proteinaceous pore that serves in the export of misfolded polypeptides from the ER to the cytosol. Current alternative candidates are Derlin-1 through Derlin-3 (summarized in [106]).

The inhibitor of protein kinase ($p58^{IPK}$ or ERj6) represents an Hsp40 co-chaperone of BiP that appears to be involved in degradation of misfolding polypeptides [66, 67]. The terms "cotranslocational degradation" and "preemptive quality control" were coined in order to differentiate the respective process from ERAD [64, 65]. Again, this view is supported by the observation that $p58^{IPK}$ is under UPR control.

The role of BiP in signal transduction

When misfolded proteins accumulate in the ER under stress conditions, several signal transduction pathways are activated that initiate the unfolded protein response (UPR) [3, 4]. The result is an increase in the biosynthetic capacity and a decrease in the biosynthetic burden of the ER (Fig. 1). Two UPR pathways induce the transcription of a whole variety of ER proteins that play a role in protein folding, protein export and protein degradation (UPR target genes). These two transcriptional pathways are triggered when BiP is sequestered by misfolded polypeptides, causing the release of BiP from ER-lumenal domains of IRE1α and ATF6 [78, 80, 107]. This causes the dimerization of IRE1α and subsequent activation of the exonuclease activity, located in the IRE1α cytosolic domain. In turn, the exonuclease activates XBP-1 mRNA which encodes a selective transcription factor. In addition, BiP sequestration results in the dissociation of ATF6 homodimers, ATF6 transport to the Golgi, and the subsequent regulated intramembrane proteolysis (RIP) of ATF6 by two Golgi resident proteases (S1P and S2P). RIP leads to the liberation of the cytosolic domain of ATF6 from the membrane and triggers its action as a selective transcription factor. UPR also has an effect on translation. Double-stranded RNA-activated protein kinase (PKR)-like kinase or eukaryotic translation initiation factor 2-α kinase 3 (EIF2AK3, also termed PEK or PERK) phosphorylates eukaryotic initiation factor 2 (eIF-2) in response to protein misfolding in the ER [79, 108]. This results in global inhibition of protein synthesis and simultaneous selective translation. The release of BiP from the lumenal domain of PERK results in the dimerization and subsequent activation of PERK.

We note that there are different isoforms of IRE1 and ATF6, and that additional UPR-related signal transduction components have recently been described (CREBH, OASIS/CREB3L1, BBF2H7) [109–111]. In addition, one of the ER membrane-integrated Hsp40 co-chaperones (ERj1) may also be involved in UPR. The cytosolic domain of ERj1 has the ability to inhibit protein synthesis at the level of initiation and to enter the nucleus and bind to DNA [45, 48]. Therefore, ERj1 was classified as another ER-resident integral membrane transcription factor that is activated by RIP [45].

When UPR fails to relieve the biosynthetic burden of ER-stress in cells, apoptosis is induced in these cells in order to protect the whole organism [112]. This involves the transcription factor CHOP [113] as well as several other proteins (Bak, Bax, Bcl-2, Bim, caspase-12, c-Jun N-terminal kinase [JNK]).

There are also connections between the signaling pathways in UPR and inflammation through various mechanisms, including calcium release from the ER (for a recent review see [114]). At the molecular level, JNK and nuclear factor κB (NF-κB) were proposed to provide the link between UPR and inflammatory processes.

The role of BiP in calcium homeostasis

The ER plays a central role in calcium homeostasis and calcium-related signal transduction [5, 6]; thus, the ER membrane harbors ryanodine-receptors (RyR), inositol-1,4,5-trisphosphate-receptors (IP_3R), and sarcoplasmic ER calcium (SERCA) pumps. The receptors trigger the release of calcium ions from the ER upon plasma membrane receptor stimulation and the SERCA pumps refill the ER-resident calcium stores.

Many of the ER-resident chaperones are low affinity calcium binding proteins (CaBPs) and their activity may be modulated by calcium ions [115, 116]. Alternatively, proteins that eventually leave the cell, including secretory and plasma membrane proteins, may require high calcium concentrations during their folding and assembly (possibly due to the fact that they will encounter high calcium concentrations in their functional location). Thus, it is not surprising that the inhibition of SERCA pumps by thapsigargin leads to ER stress and UPR [3, 4]. Intriguingly, BiP is one of the CaBPs that is inhibited by high concentrations of calcium ions; i. e. it is activated in its ATPase activity by calcium depletion (see below) [117]. In addition, the ER lumen contains some professional chelators, EF-hand domain proteins that are dedicated to binding of calcium ions, including calumenin and reticulocalbin [77]. Interestingly, these proteins appear to interact with Sec63.

Furthermore, calcium ions are involved in communication between the ER and mitochondria; this is important for bioenergetics and cell survival, at least in certain cell types (Fig. 1). Normally, the ER membrane protein sigma-1 receptor (Sig-1R) is associated with BiP [81]. Upon calcium depletion from the ER via IP_3R, the calcium-sensitive Sig-1R dissociates from BiP and associates with IP_3R, thereby protecting the otherwise unstable IP_3R from ERAD and prolonging calcium signaling to the mitochondria. Thus, BiP is involved in calcium-mediated signaling between the ER and mitochondria. We note that the ER lumenal domain of Sig-1R also has chaperone activity. Thus, calcium depletion may also increase the general chaperoning activity in the ER lumen. This is supported by the observation that increasing the cellular Sig-1R concentration counteracts UPR, while decreasing its concentration enhances apoptosis. However, it is not clear whether Sig-1R plays a role in general protein folding; this would require a significant concentration of Sig-1R in normal cells.

In addition, BiP plays a direct role in controlling efflux of calcium ions from the ER by closing the Sec61 channel during protein translocation and in the absence of translocation (see above) [94–96].

Infectious diseases

Shiga toxigenic *Escherichia coli* (STEC) strains cause morbidity and mortality in developing countries [118]. Some of these pathogens produce Shiga toxin and AB_5 toxin (also termed subtilase AB), and are responsible for gastrointestinal diseases, including the life-threatening haemolytic uraemic syndrome (HUS) (OMIM 235400). SubAB is a particularly devastating AB_5 cytotoxin which was responsible for an outbreak of HUS in Australia in 1998. AB_5 toxin is comprised of pentameric B-subunits that bind to toxin receptors on the cell surface and an A-subunit that has subtilase-like serine protease activity. During infection, the bacterial cytotoxin enters human cells by endocytosis and retrograde transport to the ER. In the ER, BiP is the major target of the catalytic A-subunit, which inactivates BiP by limited proteolysis. Finally, all BiP functions are completely lost and the affected cells die (Fig. 2).

Inherited diseases

Autosomal dominant polycystic liver disease

Autosomal dominant polycystic liver disease (ADPCLD) (OMIM 174050) is a rare human inherited disease that is characterized by the progressive development of multiple biliary epithelial liver cysts [119, 120]. It usually remains asymptomatic at a young age and becomes manifest between the ages of 40 and 60 years. Liver function is usually preserved, even in advanced cases. However, the mass effect of the enlarged liver can give rise to serious symptoms, including abdominal pain and shortness of breath; therefore, it may require surgical intervention.

ADPCLD involves at least two different genes: *protein kinase C substrate 80K-H (PRKCSH)* is located on chromosome 19q13.2 and encodes the ß-subunit of glucosidase II (also termed hepatocystin); *SEC63* is located on chromosome 6q21. A loss of Sec63 function has been postulated in several genetic mutations, including two frame-shift mutations that cause premature chain termination and three nonsense mutations. In addition, a single amino acid substitution and a single amino acid deletion have been described and are of particular interest from the functional point of view.

Although no mechanism has been firmly established for ADPCLD, the disease can be explained by a two-step process (termed two-hit mechanism): patients with one inherited mutant allele and one wild-type allele may lose the wild-type allele in some liver cells through somatic mutation. A plausible scenario is that Sec63 is essential for the import and/or folding of a subset of non-essential secretory or plasma membrane proteins that are involved in the control of biliary cell growth and proliferation. Thus, without functional Sec63, these proteins do not reach the correct location at the cell surface. The result is uncontrolled proliferation of the respective liver cells and, thus, cyst formation. We note that this concept was recently challenged on the basis of novel data that were obtained for patients with a mutation in the *SEC63* gene [121], and additional new results may suggest a divergent mechanism for cystogenesis in patients with mutations in the *PRKCSH* gene versus the *SEC63* gene [122]. In the case of mutations in the *PRKCSH* gene, it was concluded that the lack of hepatocystin does not result in proliferation but in disrupted cell adhesion.

Marinesco-Sjögren syndrome

Marinesco-Sjögren syndrome (MSS) (OMIM 248800) is a rare autosomal recessively inherited neurodegenerative disease [123–127]. The hallmarks of MSS are cerebellar ataxia, cataracts, developmental and mental retardation, and progressive myopathy.

The cause of the disease in the majority of MSS patients has been characterized as a mutation in the *SIL1* gene on chromosome 5q31 that results in mutated or truncated Sil1. Sil1 is a nucleotide exchange factor for BiP, and its role is to provide

BiP with ATP. To date, three frame-shift mutations that cause premature chain termination and four nonsense mutations have been associated with a loss of Sil1 function. In addition, a missense mutation was described that is of particular interest from the functional point of view. This latter mutation (L457P) and one of the nonsense mutations (L456X) are expected to affect the ER-retention of Sil1, due to a missing or improperly folded putative retention motif (KELR) in the mutant protein.

A loss of Sil1 function results in a reduction of functional BiP. Several possible consequences of this include: (i) some precursor proteins may not be transported into the ER, causing precursor polypeptides to accumulate in the cytosol; (ii) some proteins that are successfully transported into the ER may not be folded correctly, leading to accumulation of misfolded polypeptides in the ER; or (iii), as a consequence of (i) or (ii), some essential secretory or plasma membrane proteins may not reach their functional location, thus, affecting cell and organ development. Alternatively, excessive protein accumulation in the cytosol or the ER lumen could result in cell death.

Wolcott-Rallison syndrome

Wolcott-Rallison syndrome (WRS) (OMIM 226980) is a rare autosomal recessive disorder characterized by permanent neonatal and early infant insulin-dependent diabetes associated with various multisystemic clinical manifestations [128, 129].

The cause of the disease has been characterized as a mutation in the *PERK* gene on chromosome 2p12 that results in a mutated or truncated PERK protein. Five *PERK* frame-shift mutations that cause premature chain termination, one splice site mutation and three nonsense mutations have been described, and all lead to truncated polypeptides. In addition, four single amino acid substitutions have been described that are within the coding region of the kinase domain. Based on the analysis of some of the mutant proteins, a loss of PERK function is expected in all of these cases.

PERK seems to be essential in postnatal pancreatic ß cells and may play a role in pancreatic development *in utero*. Because PERK is only one of four kinases that are known to phosphorylate eIF2A, it was argued that PERK may also have an important metabolic function and that the latter may be the essential function in ß cells.

Cancer

Due to poor vascularization and the resulting hypoxia and glucose starvation, tumor cells are prone to ER stress and UPR [130, 131]. In cultured cells, BiP is one of the proteins involved in protecting cancer cells against ER stress-induced apoptosis [132]. In addition to this general link between BiP and cancer, some of the above mentioned BiP interacting proteins have been connected to certain tumors.

Sec63 is mutated in microsatellite-unstable human cancers

Sec63 is an ER membrane-resident Hsp40 that may play a role in protein transport into the ER (see above). The *SEC63* gene was found among the most frequently mutated genes in cancers that had deficient DNA mismatch repair, including hereditary nonpolyposis colorectal cancer (HNPCC)-associated malignancies and sporadic cancers with frequent microsatellite instability [133, 134]. In small bowel cancer, *SEC63* exhibited frameshift mutations. In some cases of gastric and colorectal cancers, *SEC63* alterations were characterized as bi-allelic. Thus, these genetic alterations may be associated with a more or less pronounced loss of Sec63 function. This alone may contribute to tumorigenesis, or it may result in a non-physiological Sec62/Sec63 ratio. The latter may result in the extra-ER location of Sec62 and a gain of novel function (see below). This hypothesis is further supported by a study on the gene expression signatures of sporadic colorectal cancers which recognized the overexpression of *SEC62* as part of a 43-gene cDNA panel that was used for predicting the long-term outcome of colorectal cancer patients [135].

Sec62 is overproduced in prostate cancer

Sec62 forms complexes with Sec63 and Sec61 and may also be involved in protein transport into the ER. Gene amplification at chromosome 3q25-q26 commonly occurs in prostate cancer. Mapping the 3q25-q26 amplification and identifying candidate genes with quantitative real-time PCR revealed that the *SEC62* gene (chromosome 3q26.2) had the highest known amplification frequency (50%) in prostate cancer and was found to be up-regulated at the mRNA level in all tumors analyzed [136]. These findings suggested that *SEC62* was the best candidate within the 3q amplification unit for involvement in prostate cancer. Overproduction of Sec62 was confirmed by western blot analysis. Thus, *SEC62* over-expression appears to be associated with a proliferative advantage for prostate cancer cells. The most likely scenario is that upon overproduction, Sec62 gains a function that is not related to protein secretion, possibly at an extra-ER location. We proposed that in an organ such as the pancreas, that has stoichiometric amounts of the Sec61 complex, Sec63, and Sec62, the Sec62 protein (with no ER-retention motif) may be retained in the

ER by association with its partners, and may be involved in protein translocation. Upon overproduction, excess Sec62 may leave the ER by vesicular transport and gain a function at an unknown location along the secretory pathway that is unrelated to protein translocation. Indeed, in some data bases, Sec62 is listed as a putative non-selective cation channel. Therefore, Sec62 may act as a calcium channel, e.g. in the plasma membrane in prostate tumor cells, and may cause or at least contribute to a proliferative advantage for the cancer cells.

In summary, a Sec62-Sec63 imbalance is likely to contribute to the development of human malignancies by leading to the translocation of the uncomplexed Sec62 to a putative cellular location along the secretory pathway. Therefore, the overexpression of *SEC62* or the loss of Sec63 function may be associated with a proliferative advantage in cancer cells.

Perspectives

One of the open questions in this area of research is related to the mechanism of ATP transport into the ER. The recent identification of the first ER membrane-resident ADP/ATP carrier (ER-ANT1) in the plant *Arabidopsis thaliana,* represents a major breakthrough [137]. However, this carrier does not appear to have an ortholog in either yeast or mammals. Previous work in mammals implicated the mitochondrial outer membrane protein porin (VDAC1) as an ATP carrier in the sarcoplasmic reticulum [138]. In analogy to the situation in yeast, the Golgi membrane protein Mcd4 (also termed PIG-N) was proposed to provide the ER with ATP [139].

Another more or less unexplored area is related to the regulatory mechanisms that may be associated with the various interaction partners of BiP. It is currently clear that some components are not present in every cell type (e.g. PERK). This indicates developmental regulation of gene expression, at least in certain cases. In addition, there are various miRNAs listed in databases that may potentially be directed against some of the proteins that were discussed here; thus, this may potentially constitute another layer of regulatory mechanisms in gene expression. Furthermore, some proteins have already been described as substrates of various cytosolic kinases; also, various kinases have been found to be associated with the ER [140]. Thus the various processes that were discussed are also expected to be targets of allosteric regulatory mechanisms.

Furthermore, we note that there are many more medical aspects of BiP and its interaction partners than could possibly have been discussed here (for a recent review see [141]).

Acknowledgements. This review is dedicated to former Nobel laureate George Palade who started this area of research and died on the 8th of October 2008 at the age of 95. We apologize to those whose work could not be cited due to space limitations. We are grateful to Drs Roland Beckmann (Munich), Albert Sickmann (Dortmund), Erik Snapp (New York), Gerhard Unteregger (Homburg), Richard Wagner (Osnabrück) and Bernd Wullich (Erlangen) for fruitful collaborations. Our own work on this subject was supported by the Deutsche Forschungsgemeinschaft (DFG) and Deutsche Krebshilfe.

1 Palade, G. (1975) Intracellular aspects of protein synthesis. Science 189, 347–358.
2 Blobel, G. and Dobberstein, B. (1975) Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. J. Cell Biol. 67, 835–851.
3 Ma, Y. and Hendershot, L. M. (2001) The unfolding tale of the unfolded protein response. Cell 107, 827–830.
4 Zhang, K. and Kaufman, R. J. (2004) Signaling the unfolded protein response from the endoplasmic reticulum. J. Biol. Chem. 279, 25935–25938.
5 Meldolesi, J. and Pozzan, T. (1998) The endoplasmic reticulum Ca^{2+} store: a view from the lumen. Trends Biochem. Sci. 23, 10–14.
6 Clapham, D. E. (2007) Calcium Signaling. Cell 131, 1047–1058.
7 Helenius, A., Marquardt, T. and Braakman, I. (1992) The endoplasmic reticulum as a protein-folding compartment. Trends Cell Biol. 2, 227–231.
8 Buck, T. M., Wright, C. M. and Brodsky, J. L. (2007) The activities and function of molecular chaperones in the endoplasmic reticulum. Semin. Cell. Dev. Biol. 18, 751–761.
9 Ellgaard, L. and Helenius, A. (2003) Quality control in the endoplasmic reticulum. Nat. Rev. 4, 181–191.
10 Römisch, K. (2005) Endoplasmic reticulum-associated degradation. Annu. Rev. Cell Dev. Biol. 21, 435–456.
11 Haas, I. G. and Wabl, M. (1983) Immunoglobulin heavy chain binding protein. Nature 306, 387–389.
12 Bole, D. G., Hendershot, L. M. and Kearney, J. F. (1986) Posttranslational association of immunoglobulin heavy chain binding protein with nascent heavy chains in nonsecreting and secreting hybridomas. J. Cell Biol. 102, 1558–1566.
13 Munro, S. and Pelham, H. R. B. (1986) An Hsp70-like protein in the ER: identity with the 78 kd glucose-regulated protein and immunoglobulin heavy chain binding protein. Cell 46, 291–300.
14 Guth, S., Völzing, C., Müller, A., Lerner, M., Jung, M. and Zimmermann, R. (2004) Protein transport into canine pancreatic microsomes: a quantitative approach, Eur. J. Biochem. 271, 3200–3207.
15 Lin, H.-Y., Masso-Welch, P., Di, Y.-P., Cai, J.-W., Shen, J.-W. and Subjeck, J. R. (1993) The 170-kDa glucose- regulated stress protein is an endoplasmic reticulum protein that binds immunoglobulin. Mol. Biol. Cell 4, 1109–1119.
16 Lee-Yoon, D., Eastons, D., Murawski, M., Burt, R. and Subjeck, J. R. (1995) Identification of a major subfamily of large hsp70-like proteins through the cloning of the mammalian 110-kDa heat shock protein. J. Biol. Chem. 270, 15725–15733.
17 Chen, X., Easton, D., Oh, H.-J., Lee-Yoon, D.-S., Liu, X. and Subjeck, J. R. (1996) The 170 kDa glucose regulated stress protein is a large HSP70-, HSP110-like protein of the endoplasmic reticulum. FEBS Lett. 380, 68–72.

18 Dierks, T., Volkmer, J., Schlenstedt, G., Jung, C., Sandholzer, U., Zachmann, K., Schlotterhose, P., Neifer, K., Schmidt, B. and Zimmermann, R. (1996) A microsomal ATP-binding protein involved in efficient protein transport into the mammalian endoplasmic reticulum. EMBO J. 15, 6931–6942.
19 Weitzmann, A., Baldes, C., Dudek, J. and Zimmermann, R. (2007) The heat shock protein 70 molecular chaperone network in the pancreatic endoplasmic reticulum – a quantitative approach. FEBS J. 274, 5175–5187.
20 Otterson, G. A., Flynn, G. C., Kratzke, R. A., Coxon, A., Johnston, P. G. and Kaye, F. J. (1994) Stch encodes the 'ATPase core' of a microsomal stress 70 protein. EMBO J. 13, 1216–1225.
21 Hartl, F. U. (1996) Molecular chaperones in cellular protein folding. Nature 381, 571–579.
22 Bukau, B. and Horwich, A. L. (1998) The Hsp70 and Hsp60 chaperone machines. Cell 92, 351–366.
23 McCarty, J. S., Buchberger, A., Reinstein, J. and Bukau, B. (1995) The role of ATP in the functional cycle of the DnaK chaperone system. J. Mol. Biol. 249, 126–137.
24 Theyssen, H., Schuster, H. P., Packschies, L., Bukau, B. and Reinstein, J. (1996) The second step of ATP binding to DnaK induces peptide release. J. Mol. Biol. 263, 657–670.
25 Wang, T. F., Chang, J. H. and Wang, C. (1993) Identification of the peptide binding domain of hsc70. 18-Kilodalton fragment located immediately after ATPase domain is sufficient for high affinity binding. J. Biol. Chem. 268, 26049–26051.
26 Flaherty, K. M., DeLuca-Flaherty, C. and McKay, D. B. (1990) Three-dimensional structure of the ATPase fragment of a 70K heat-shock cognate protein. Nature 346, 623–628.
27 Zhu, X., Zhao, X., Burkholder, W. F., Gragerov, A., Ogata C. M., Gottesman, M. E. and Hendrickson, W. A. (1996) Structural analysis of substrate binding by the molecular chaperone DnaK. Science 272, 1606–1614.
28 Jiang, J., Prasad, K., Lafer, E. M. and Sousa, R. (2005) Structural basis on interdomain communication in the Hsc70 chaperone. Mol. Cell 20, 513–524.
29 Flynn, G. C., Pohl, J., Flocco, M. T. and Rothman, J. E. (1991) Peptide-binding specificity of the molecular chaperone BiP. Nature 353, 726–730.
30 Blond-Elguindi, S., Cwirla, S. E., Dower, W. J., Lipshutz, R. J., Sprang, S. R., Sambrook, J. F. and Gething, M.-J. (1993) Affinity panning of a library of peptides displayed on bacteriophages reveals the binding specificity of BiP. Cell 75, 717–728.
31 Cheetham, M. E. and Caplan, A. J. (1998) Structure, function and evolution of DnaJ: conservation and adaptation of chaperone function. Cell Stress Chaperones 3, 28–36.
32 Gassler, C. S., Buchberger, A. and Laufen, T. (1998) Mutations in the DnaK chaperone affecting interaction with the DnaJ cochaperone. Proc. Natl. Acad. Sci. USA 95, 15229–15234.
33 Suh, W. C., Burkholder, W. F., Lu, C. Z., Zhao, X., Gottesman, M. E. and Gross, C. A. (1998) Interaction of the Hsp70 molecular chaperone, DnaK, with its cochaperone DnaJ. Proc. Natl. Acad. Sci. USA 95, 15223–15228.
34 Liberek, K., Marszalek, J., Ang, D., Georgopoulos, C. and Zylicz, M. (1991) Escherichia coli DnaJ and GrpE heat shock proteins jointly stimulate ATPase activity of DnaK. Proc. Natl. Acad. Sci. USA 88, 2874–2878.
35 Silver, P. A. and Way, J. C. (1993) Eukaryotic DnaJ homologues and the specificity of Hsp70 activity. Cell 74, 5–6.
36 Wall, D., Zylicz, M. and Georgopoulos, C. (1994) The N-terminal 108 amino acids of the Escherichia coli DnaJ protein stimulate ATPase activity of DnaK and are sufficient for replication. J. Biol. Chem. 269, 5446–5451.
37 Szyperski, T., Pellecchia, M., Wall, D., Georgopoulos, C. and Wüthrich, K. (1994) NMR structure determination of the *Escherichia coli* DnaJ molecular chaperone: secondary structure and backbone fold of the N-terminal region (residues 2–108) containing the highly conserved J domain. Proc. Natl. Acad. Sci. USA 91, 11343–11347.
38 Karzai, A. W. and McMacken, R. (1996) A bipartite signaling mechanism involved in DnaJ-mediated activation of the Escherichia coli DnaK protein. J. Biol. Chem. 271, 11236–11246.
39 Laufen, T., Mayer, M. P., Beisel, C., Klostermeier, D., Mogk, A., Reinstein, J. and Bukau, B. (1999) Mechanism of regulation of hsp70 chaperones by DnaJ cochaperones. Proc. Natl. Acad. Sci. USA 96, 5452–5457.
40 Johnson, J. L. and Craig, E. A. (2001) An essential role for the substrate-binding region of Hsp40s in *Saccharomyces cerevisiae*. J. Cell Biol. 152, 851–856.
41 Hennessy, F., Nicoll, W. S., Zimmermann, R., Cheetham, M. E. and Blatch, G. L. (2005) Not all J domains are created equal: implications for the specificity of Hsp40-Hsp70 interactions. Protein Sci. 14, 1697–1709.
42 Nicoll, W. S., Botha, M., McNamara, C., Schlange, M., Pesce, E.-R., Boshoff, A., Ludewig, M. H., Zimmermann, R., Cheetham, M. E., Chapple, J. P.and Blatch, G. L. (2007) Cytosolic and ER J-domains of mammalian and parasitic origin can functionally interact with DnaK. Int. J. Biochem. Cell Biol. 39, 736–751.
43 Brightman, S. E., Blatch, G. L. and Zetter, B. R. (1995) Isolation of a mouse cDNA encoding MTJ1, a new murine member of the DnaJ family of proteins. Gene 153, 249–254.
44 Chevalier, M., King, S., Wang, C., Gething, M.-J., Elguindi, E. and Blond, S. Y. (1998) Substrate binding induces depolymerization of the C-terminal peptide binding domain of murine GRP78/BiP. J. Biol. Chem. 273, 26827–26835.
45 Zupicich, J., Brenner, S. E. and Skarnes, W. C. (2001) Computational prediction of membrane-tethered transcription factors. Genome Biol. 2, 50.1–50.6.
46 Dudek, J., Volkmer, J., Bies, C., Guth, S., Müller, A., Lerner, M., Feick, P., Schäfer, K. H., Morgenstern, E., Hennessy, F., Blatch, G. L. Janoscheck, K., Heim, N., Scholtes, P. Frien, M., Nastainczyk, W. and Zimmermann, R. (2002) A novel type of cochaperone mediates transmembrane recruitment of DnaK-like chaperones to ribosomes. EMBO J. 21, 2958–2967.
47 Kroczynska, B., Evangelista, C. M., Samant, S. S., Elguindi, E. C. and Blond, S. Y. (2004) The SANT2 domain of murine tumor cell DnaJ-like protein 1 human homologue interacts with α1-antichymotrypsin and kinetically interferes with its serpin inhibitory activity. J. Biol. Chem. 279, 11432–11443.
48 Dudek, J., Greiner, M., Müller, A., Hendershot, L. M., Kopsch, K., Nastainczyk, W. and Zimmermann, R. (2005) ERj1p plays a basic role in protein biogenesis at the endoplasmic reticulum. Nat. Struct. Mol. Biol. 12, 1008–1014.
49 Blau, M., Mullapudi, S., Becker, T., Dudek, J., Zimmermann, R., Penczek, P. A. and Beckmann, R. (2005) ERj1p uses a universal ribosomal adaptor site to coordinate the 80S ribosome at the membrane. Nat. Struct. Mol. Biol. 12, 1015–1016.
50 Skowronek, M. H., Rotter, M. and Haas, I. G. (1999) Molecular characterization of a novel mammalian DnaJ-like Sec63p homolog. Biol. Chem. 380, 1133–1138.
51 Meyer, H.-A., Grau, H., Kraft, R., Prehn, S., Kalies, K.-U. and Hartmann, E. (2000) Mammalian Sec61 is associated with Sec62 and Sec63. J. Biol. Chem. 275, 14550–14557.
52 Tyedmers, J., Lerner, M., Bies, C., Dudek, J., Skowronek, M. H., Haas, I. G., Heim, N., Nastainczyk, W., Volkmer, J. and Zimmermann, R. (2000) Homologs of the yeast Sec complex subunits Sec62p and Sec63p are abundant proteins in dog pancreas microsomes. Proc. Natl. Acad. Sci. USA 97, 7214–7219.
53 Bies, C., Guth, S., Janoschek, K., Nastainczyk, W., Volkmer, J. and Zimmermann, R. (1999) A Scj1p homolog and folding catalysts present in dog pancreas microsomes. Biol. Chem. 380, 1175–1182.
54 Yu, M., Haslam, R. H. A. and Haslam D. B. (2000) HEDJ, an Hsp40 Co-chaperone localized to the endoplasmic reticulum of human cells. J. Biol. Chem. 275, 24984–24992.
55. Bies, C., Blum, R., Dudek, J., Nastainczyk, W., Oberhauser, S., Jung, M. and Zimmermann, R. (2004) Characterization of

pancreatic ERj3p, a homolog of yeast DnaJ-like protein Scj1p. Biol. Chem. 385, 389–395.
56 Shen, Y. and Hendershot, L. M. (2004) ERdj3p, a stress-inducible endoplasmic reticulum DnaJ homologue, serves as a cofactor for BiP′s interactions with unfolded substrates. Mol. Biol. Cell 16, 40–50.
57 Jin, Y., Awad, W., Petrova, K. and Hendershot, L. M. (2008) Regulated release of ERdj3 from unfolded proteins by BiP. EMBO J. 27, 2873–2882
58 Shen, Y., Meunier, L. and Hendershot, L. M. (2002) Identification and characterization of a novel endoplasmic reticulum (ER) DnaJ homologue, which stimulates ATPase activity of BiP in vitro and is induced by ER stress. J. Biol. Chem. 277, 15947–15956.
59 Kurisu, J., Honma, A., Miyajima, H., Kondo, S. Okumura, M. and Imaizumi, K. (2003) MDG1/ERdj4, an ER-resident DnaJ family member, suppresses cell death induced by ER stress. Genes Cells 8, 189–202.
60 Dong, M., Bridges, J. P., Apsley, K., Xu, Y. and Weaveret T. E. (2008) ERdj4 and ERdj5 are required for endoplasmic reticulum-associated protein degradation of misfolded surfactant protein C. Mol. Biol. Cell 19, 2620–2630.
61 Cunnea, P. M., Miranda-Vizuete, A., Bertoli, G., Simmen, T., Damdimopoulos, A. E., Hermann, S., Leinonen, S., Huikko, M. P., Gustafsson J.-A., Sitia, R. and Spyrou, G. (2003) ERdj5, an endoplasmic reticulum (ER)-resident protein containing DnaJ and thioredoxin domains, is expressed in secretory cells or following ER stress. J. Biol. Chem. 278, 1059–1066.
62 Hosoda, A., Kimata, Y., Tsuru, A. and Kohno, K. (2003) JPDI, a novel endoplasmic reticulum-resident protein containing both a BiP-interacting J-domain and thioredoxin-like motifs. J. Biol. Chem. 278, 2669–2676.
63 Ushioda, R., Hoseki, J., Araki, K., Jansen, G., Thomas, D. Y. and Nagata, K. (2008) ERdj5 is required as a disulfide reductase for degradation of misfolded proteins in the ER. Science 321, 569–572.
64 Oyadomari, S., Yun, C., Fisher, E. A., Kreglinger, N., Kreibich, G., Oyadomari, M., Harding, H. P., Goodman, A. G., Harant, H., Garrison, J. L., Taunton, J, Katze, M. G. and Ron, D. (2006) Cotranslocational degradation protects the stressed endoplasmic reticulum from protein overload. Cell 126, 727–739.
65 Kang, S. W., Rane, N. S., Kim, S. J., Garrison, J. L., Taunton, J. and Hegde, R. S. (2006) Substrate-specific translocational attenuation during ER stress defines a pre-emptive quality control pathway. Cell 127, 999–1013.
66 Rutkowski, D. T., Kang, S. W., Goodman, A. G., Garrison, J. L., Taunton, J., Katze, M. G., Kaufman, R. J. and Hedge, R. S. (2007) The role of p58IPK in protecting the stressed endoplasmic reticulum. Mol. Biol. Cell 18, 3681–3691.
67 Petrova, K., Oyadomari, S., Hendershot, L. M. and Ron, D. (2008) Regulated association of misfolded endoplasmic reticulum lumenal proteins with P58/DNAJc3. EMBO J. 27, 2862–2872.
68 Chung, K. T., Shen, Y. and Hendershot, L. M. (2002) BAP, a mammalian BiP associated protein, is a nucleotide exchange factor that regulates the ATPase activity of BiP. J. Biol. Chem. 277, 47557–47563.
69 Weitzmann, A., Volkmer, J. and Zimmermann, R. (2006). The nucleotide exchange factor activity of Grp170 may explain the non-lethal phenotype of loss of Sil1 function in man and mouse. FEBS Lett. 580, 5237–5240.
70 Shomura, Y., Dragovic, Z., Chang, H. C., Tzvetkov, N., Young, J. C., Brodsky, J. L., Guerriero, V., Hartl, F. U. and Bracher, A. (2005) Regulation of Hsp70 function by HspBP1: structural analysis reveals an alternate mechanism for Hsp70 nucleotide exchange. Mol. Cell 17, 367–379.
71 Liu, Q. and Hendrickson, W. A. (2007) Insights into Hsp70 chaperone activity from a crystal structure of the yeast Hsp110 Sse1. Cell 131, 106–120.
72 Polier, S., Dragovic, Z., Hartl, F. U. and Bracher, A. (2008) Structural basis for the cooperation of Hsp70 and Hsp110 chaperones in protein folding. Cell 133, 1068–1079.
73 Schuermann, J. P., Jiang, J., Cuellar, J., Llorca, O., Wang, L., Gimenez, L. E., Jin, S., Taylor, A. B., Demeler, B., Morano, K. A., Hart, P. J., Valpuesta, J. M., Lafe, E. M. and Sousa, R. (2008) Structure of the Hsp110:Hsc70 nucleotide exchange machine. Mol. Cell 31, 232–243.
74 Tatu, U. and Helenius, A. (1997) Interactions between newly synthesized glycoproteins, calnexin and a network of resident chaperones in the endoplasmic reticulum. J. Cell Biol. 136, 555–565.
75 Meunier, L., Usherwood, Y.– K., Chung, K. T. and Hendershot, L. M. (2002) A subset of chaperones and folding enzymes form multiprotein complexes in endoplasmic reticulum to bind nascent proteins. Mol. Biol. Cell 13, 4456–4469.
76 Fukuda, S., Sumii, M., Masuda, Y., Takahashi, M., Koike, N., Teishima, J., Yasumoto, H., Itamoto, T., Asahara, T., Dohi, K. and Kamiya, K. (2001) Murine and human SDF2L1 is an endoplasmic reticulum stress-inducible gene that encodes a new member of the Pmt/rt protein family. Biochem. Biophys. Res. Commun. 280, 407–414.
77 Tyedmers, J., Lerner, M., Nastainczyk, W. and Zimmermann, R. (2005) Calumenin and reticulocalbin are associated with the protein translocase of the mammalian endoplasmic reticulum. J. Biol. Sci. 5, 70–75.
78 Kozutsumi, Y., Segal, M., Normington, K., Gething, M.-J. and Sambrook, J. (1988) The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. Nature 332, 462–464.
79 Shi, Y., Vattem, K. M., Sood, R., An, J., Liang, J, Stramm, L. and Wek, R. C. (1998) Identification and characterization of pancreatic eukaryotic initiation factor 2 alpha-subunit kinase, PEK, involved in translational control. Mol. Cell. Biol. 18, 7499–7509.
80 Yoshida, H., Matsui, T., Yamamoto, A., Okada, T. and Mori, K. (2001) XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell 107, 881–891.
81 Hayashi, T. and Su, T.-P. (2007) Sigma-1 receptor chaperones at the ER- mitochondrion interface regulate Ca (2+) signaling and cell survival. Cell 131, 596–610.
82 Walter, P. and Blobel, G. (1981) Translocation of proteins across the endoplasmic reticulum. II. Signal recognition protein, SRP, mediates the selective binding to microsomal membranes of *in-vitro*-assembled polysomes synthesizing secretory protein. J. Cell Biol.. 91, 551–556.
83 Meyer, D. I. and Dobberstein, B. (1980) A membrane component essential for vectorial translocation of nascent proteins across the endoplasmic reticulum: requirements for its extraction and reassociation with the membrane. J. Cell Biol. 87, 498–502.
84 Potter, M. D., Seiser, R. M. and Nicchitta, C. V. (2001) Ribosome exchange revisited: a mechanism for translation-coupled ribosome detachment from the ER membrane. Trends Cell Biol. 11, 112–115.
85 Görlich, D., Prehn, S., Hartmann, E., Kalies, K.-U. and Rapoport, T. A. (1992) A mammalian homolog of SEC61p and SECYp is associated with ribosomes and nascent polypeptides during translocation. Cell 71, 489–503.
86 Hartmann, E., Sommer, T., Prehn, S., Görlich, D., Jentsch, S. and Rapoport, T. A. (1994) Evolutionary conservation of components of the protein translocation complex. Nature 367, 654–657.
87 Wirth, A., Jung, M., Bies, C., Frien, M., Tyedmers, J., Zimmermann, R. and Wagner, R. (2003) The Sec61p complex is a dynamic precursor activated channel. Mol. Cell 12, 261–268
88 van den Berg, B., Clemons, W. M., Collinson, I., Modis, Y., Hartmann, E., Harrison, S. C. and Rapoport, T. A. (2004). X-ray structure of a protein-conducting channel. Nature 427, 36–44.

89 Nilsson, I. M. von Heijne, G. (1993) Determination of the distance between the oligosaccharyltransferase active site and the endoplasmic reticulum membrane. J. Biol. Chem. 268, 5798–5801.
90 Whitley, P., Nilsson, I. M. and von Heijne, G. (1996) A nascent secretory protein may traverse the ribosome/endoplasmic reticulum translocase complex as an extended chain. J. Biol. Chem. 271, 6241–6244.
91 Klappa, P., Mayinger, P., Pipkorn, R., Zimmermann, M. and Zimmermann, R. (1991) A microsomal protein is involved in ATP-dependent transport of presecretory proteins into mammalian microsomes. EMBO J. 10, 2795–2803.
92 Tyedmers, J., Lerner, M., Wiedmann, M., Volkmer, J. and Zimmermann, R. (2003) Polypeptide chain binding proteins mediate completion of cotranslational protein translocation into the mammalian endoplasmic reticulum. EMBO rep. 4, 505–510.
93 Shaffer, K. L., Sharma, A., Snapp, E. L. and Hegde, R. S. (2005) Regulation of protein compartmentalization expands the diversity of protein function. Dev. Cell 9, 545–554.
94 Hamman, B. D., Hendershot, L. M. and Johnson A. E. (1998) BiP maintains the permeability barrier of the ER membrane by sealing the lumenal end of the translocon pore before and early in translocation. Cell 92, 747–758.
95 Haigh, N. G. and Johnson, A. E. (2002) A new role for BiP: closing the aqueous translocon pore during protein integration into the ER membrane. J. Cell Biol. 156, 261–270.
96 Alder, N. N., Shen, Y., Brodsky, J. L., Hendershot, L. M. and Johnson A. E. (2005) The molecular mechanism underlying BiP-mediated gating of the Sec61 translocon of the endoplasmic reticulum. J. Cell Biol. 168, 389–399.
97 Brodsky, J. L., Goeckeler, R. and Schekman, R. (1995) BiP and Sec68p are required for both co- and posttranslational protein translocation into the yeast endoplasmic reticulum. Proc. Natl. Acad. Sci. USA 92, 9643–9646.
98 Young, B. P., Craven, R. A., Reid, P. J., Willer, M. and Stirling, C. J. (2001) Sec63p and Kar2p are required for the translocation of SRP-dependent precursors into the yeast endoplasmic reticulum *in vivo*. EMBO J. 20, 262–271.
99 Hegde, R. S. and Kang, S.-W. (2008) The concept of translocational regulation. J. Cell Biol. 182, 225–232.
100 Knittler, M. R. and Haas, I. G. (1992) Interaction of BiP with newly synthesized immunoglobulin light chain molecules: cycles of sequential binding and release. EMBO J. 4, 1573–1581.
101 Skowronek, M. H., Hendershot, L. M. and Haas, I. G. (1998) The variable domain of non-assembled Ig light chains determines both their half-life and binding to the chaperone BiP. Proc. Natl. Acad. Sci. USA 95, 1574–1578.
102 Lee , Y. K., Brewer, J. W., Hellman, R. and Hendershot, L. M. (1999) BiP and immuno-globulin light chain cooperate to control the folding of heavy chain and ensure the fidelity of immunoglobulin assembly. Mol. Biol. Cell 10, 2209–2219.
103 Vanhove, M., Usherwood, Y. K. and Hendershot, L. M. (2001) Unassembled Ig heavy chains do not cycle from BiP in vivo but require light chains to trigger their release. Immunity 15, 105–114.
104 Plemper, R. K., Böhmler, S., Bordallo, J., Sommer, T. and Wolf, D. H. (1997) Mutant analysis links the translocon and BiP to retrograde protein transport for ER degradation. Nature 388, 891–895.
105 Brodsky, J. L., Werner, E. D., Dubas, M. E., Goeckler, J. L., Kruse, K. B. and McCracken, A. A. (1999) The requirement for molecular chaperones during endoplasmic reticulum-associated protein degradation demonstrates that protein export and import are mechanistically distinct. J. Biol. Chem. 274, 3453–3460.
106 Kawaguchi, S. and Ng, D. T. W. (2007) SnapShot: ER-associated protein degradation pathways. Cell 129, 1230.
107 Bertolotti, A., Zhang, Y., Hendershot L. M., Harding, H. P. and Ron, D (2000) Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat. Cell Biol. 2, 326–332.
108 Harding, H. P., Zhang, Y. and Ron, D. (1999) Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature 397, 271–274.
109 Zhang, K., Shen, X., Wu, J., Sakaki, K., Saunders, T., Rutkowski, D. T., Back, S. H. and Kaufman, R. J. (2006) Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. Cell 124, 587–599.
110 Omori, Y., Imai, J., Suzuki, Y., Watanabe, S,. Tanigami, A. and Sugano, S. (2002) OASIS is a transcriptional activator of CREB/ATF family with a transmembrane domain. Biochem. Biophys. Res. Commun. 293, 470–477.
111 Kondo, S., Saito, A., Hino, S., Murakami, T., Ogata, M., Kanemoto, S., Nara, S., Yamashita, A., Yoshinaga, K., Hara, H. and Imaizumi, K. (2007) BBF2H7, a novel transmembrane bZIP transcription factor, is a new type of endoplasmic reticulum stress transducer. Mol. Cell. Biol. 27, 1716–1729.
112 Luo, S., Mao, C., Lee, B. and Lee, A. S. (2006) GRP78/BiP is required for cell proliferation and protecting the inner cell mass from apoptosis during early mouse embryonic development. Mol. Cell. Biol. 15, 5688–5697.
113 Nakagawa, T., Zhu, H., Morishima, N., Li, E., Xu, J., Yankner, B. A. and Yuan, J. (2000) Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. Nature 403, 98–103.
114 Zhang, K. and Kaufman, R. J. (2008) From endoplasmic-reticulum stress to the inflammatory response. Nature 454, 455–462.
115 Lievremont, J. P., Rizzuto, R., Hendershot, L. M. and Meldolesi, J. (1997) BiP, a major chaperone protein of the endoplasmic reticulum lumen, plays a direct and important role in the storage of the rapidly exchanging pool of Ca^{2+}. J. Biol. Chem. 272, 30873–30879.
116 Van, P. N., Peter, F. and Söling, H.-D. (1989) Four intracisternal calcium-binding glycoproteins from rat liver microsomes with high affinity for calcium. J. Biol. Chem. 264, 17494–17501
117 Kassenbrock, C. K. and Kelly, R. B. (1989) Interaction of heavy chain binding protein (BiP/GRP78) with adenine nucleotides. EMBO J. 81, 1461–1467.
118 Paton, A. W., Beddoe, T., Thorpe, C. M., Whisstock, J. C., Wilche, M. C., Rossjohn, J., Talbot, U. M. and Paton, J. C. (2006) AB5 subtilase cytotoxin inactivates the endoplasmic reticulum chaperone BiP. Nature 443, 548–552.
119 Davila, S., Furu, L., Gharavi, A. G., Tian, X., Onoe, T., Qian, Q., Li, A., Cai, Y., Kamath, P. S., King, B. F., Azurmendi, P. J., Tahvanainen, P., Kääriäinen, H., Höckerstedt, Devuyst, O., Pirson, Y., Martin, R. S., Lifton, R. P., Tahvanainen, E., Tores, V. E. and Somlo, S. (2004) Mutations in SEC63 cause autosomal dominant polycystic liver disease. Nat. Gen. 36, 575–577.
120 Waanders, E., te Morsche, R. H. M., de Man, R. A., Jansen, J. B. M. J. and Drenth, J. P. H. (2006) Extensive mutational analysis of PRKCSH and SEC63 broadens the spectrum of polycystic liver disease. Hum. Mutat. 27, 830–839.
121 Waanders, E., Croes, H. J., Maass, C. N., Te Morsche, R. H., van Geffen, H. J,. van Krieken, J. H., Fransen, J. A. and Drenth, J. P. (2008) Cysts of PRKCSH mutated polycystic liver disease patients lack hepatocystin but express Sec63p. Histochem. Cell Biol. 129, 301–310.
122 Waanders, E., Van Krieken, J. H., Lameris, A. L. and Drenth, J. P. (2008) Disrupted cell adhesion but not proliferation mediates cyst formation in polycystic liver disease. Mod. Pathol. 2008.115.
123 Anttonen, A.-K., Mahjneh, I., Hämäläinen, R. H.,Lagier-Tourenne, C., Kopra, O., Waris, L., Anttonen, M., Joensuu, T., Kalimo, H., Paetau, A., Tranebjaerg, L., Chaigne, D., Koenig, M., Eeg-Olofsson, O., Udd, B., Somer, M., Somer, H. and Lehesjoki, A.-E. (2005) The gene disrupted in Marinesco-

Sjögren syndrome encodes SIL1, an HSPA5 cochaperone. Nat. Gen. 37, 1309–1311.
124 Senderek, J., Krieger, M., Stendel, C., Bergmann, C., Moser, M., Breitbach-Faller, N., Rudinik-Schoneborn, S., Blaschek, A., Wolf, N., Harting, I., North, K., Smith, J., Muntoni F., Brockington, M., Quijano-Roy, S., Renault, F., Herrmann, R., Hendeshot, L.M., Schröder, J. M., Lochmüller, H., Topaloglu, H., Voit, T., Weis, J., Ebinger, F. and Zerres, K. (2005) Mutations in Sil1 cause Marinesco-Sjögren syndrome, a cerebellar ataxia with cataract and myopathy. Nat. Gen. 37, 1312–1314.
125 Karim, M. A., Parsian, A. J., Cleves, M. A., Cleves, M. A., Bracey, J., Elsayed, M. S., Elsobky, E. and Parsian, A. (2006) A novel mutation in BAP/SIL1 gene causes Marinesco-Sjögren syndrome in an extended pedigree. Clin. Genet. 70, 420–423.
126 Eriguchi, M., Mizuta, H., Kurohara, K., Fujitake, J. and Kuroda, Y. (2008). Identification of a new homozygous frameshift insertion mutation in the Sil1 gene in 3 Japanese patients with Marinesco-Sjögren syndrome. J. Neurol. Sci. 270, 197–200.
127 Anttonen, A.-K., Siintola, E., Tranebjaerg, L., Iwata, N. K., Bijlsma, E. K., Meguro, H., Ichikawa, Y., Goto, J., Kopra, O. and Lehesjoki, A.-E. (2008) Novel SIL1 mutations and exclusion of functional candidate genes in Marinesco-Sjögren syndrome. Eur. J. Hum. Genet. 16, 961–969.
128 Senée, V., Vattem, K. M., Delépine, M., Rainbow, L. A., Haton, C., Lecoq, A., Shaw, N. J., Robert, J. J., Rooman, R., Diatloff-Zito, C., Michaud, J. L., Bin-Abbas, B., Taha, D., Zabel, B., Franceschini, P., Topaloglu, A. K., Lathrop, G. M., Barrett, T. G., Nicolino, M., Wek, R. C. and Julier, C. (2004) Wolcott-Rallison Syndrome: clinical, genetic, and functional study of EIF2AK3 mutations and suggestion of genetic heterogeneity. Diabetes 53, 1876–1883.
129 Brickwood, S., Bonthron, D. T., Al-Gazali, L. I., Piper, K., Hearn, T., Wilson, D. I. and Hanley, N. A. (2003) Wolcott-Rallison syndrome: pathogenic insights into neonatal diabetes from new mutation and expression studies of EIF2AK3. J. Med. Genet. 40, 685–689.
130 Macario, A. J. L and Conway de Macario, E. (2007) Molecular chaperones: multiple functions, pathologies, and potential applications. Front. Biosci. 12, 2588–2600.
131 Aridor M. (2007) Visiting the ER: the endoplasmic reticulum as a target for therapeutics in traffic related diseases. Adv. Drug Deliv. Rev. 59, 759–781.
132 Fu, Y., Li, J. and Lee, A. S. (2007) GRP78/BiP inhibits endoplasmic reticulum BIK and protects human breast cancer cells against estrogen starvation-induced apoptosis. Cancer Res. 67, 3734–3740.
133 Schulmann, K., Brasch, F. E., Kunstmann, E., Engel, C., Pagenstecher, C., Vogelsang, H., Krüger, S., Vogel, T., Knaebel, H.-P., Rüschoff, J., Hahn, S. A., von Knebel-Doeberitz, M., Moeslein, G., Meltzer, S. J., Schackert, H. K., Tympner, C., Mangold, E. and Schmiegel, W. for the German HNPCC consortium (2005) HNPCC-associated small bowel cancer: Clinical and molecular characteristics. Gastroenterol. 128, 590–599.
134 Mori, Y., Sato, F., Selaru, F.M., Olaru, A., Perry, K., Kimos, M.C., Tamura G., Matsubara, N., Wang, S., Xu, Y., Yin, J., Zou, T.-T., Leggett, B., Young, J., Nukiwa, T., Stine, O. C., Abraham, J. M., Shibata, D. and Meltzer S. J. (2002) Instabilotyping reveals unique mutational spectra in microsatellite-unstable gastric cancers. Cancer Res. 62, 3641–3645.
135 Eschrich, S., Yang, I., Bloom, G., Kwong, K. Y., Boulware, D., Cantor, A., Coppola, D., Kruhoffer, M., Aaltonen, L., Orntoft, T. F., Quackenbush, J. and Yeatman, T. J. (2005) Molecular staging or surival prediction of colorectal cancer patients. J. Clin. Oncol. 23, 3526–3535.
136 Jung, V., Kamradt, J., Kindich, R., Jung, M., Mueller, M., Schulz, W. A., Engers, R., Stoeckle, M., Zimmermann, R. and Wullich, B. (2006) Genomic and expression analysis of the 3q25-q26 amplicon reveals TLOC1/SEC62 as a probable target gene in prostate cancer. Mol. Cancer Res. 4, 169–176.
137 Leroch, M., Neuhaus, H. E., Kirchberger, S., Zimmermann, S., Melzer, M., Gerhold, J. and Tjaden, J. (2008) Identification of a novel adenine nucleotide transporter in the endoplasmic reticulum of Arabidopsis. Plant Cell 20, 438–451.
138 Shoshan-Barmatz, V., Hadad, N., Feng, W., Shafir, I., Orr, I,. Varsanyi, M. and Heilmeyer, L. M. (1996) VDAC/porin is present in sarcoplasmic reticulum from skeletal muscle. FEBS Lett. 386, 205–210.
139 Zhong, X., Malhotra, R. and Guidotti, G. (2003) ATP uptake in the Golgi and extracellular release require Mcd4 protein and the vacuolar H+-ATPase. J. Biol. Chem. 278, 33436–33444.
140 Gruss, O. J., Feick, P., Frank, R. and Dobberstein, B. (1999) Phosphorylation of components of the ER translocation site. Eur. J. Biochem. 260, 785–793.
141 Ni, M. and Lee, A. S., (2007) ER chaperones in mammalian development and human diseases. FEBS Lett. 581, 3641–3651.
142 Ladiges, W. C., Knoblaugh, S. E., Morton, J. F., Korth, M. J., Sopher, B. L., Baskin, C. R., MacAuley, A., Goodman, A. G., LeBoeuf, R. C. and Katze, M. G. (2005) Pancreatic beta-cell failure and diabetes in mice with a deletion mutation of the endoplasmic reticulum molecular chaperone gene P58IPK. Diabetes 54, 1074–1081.
143 Zhao, L., Longo-Guess, C., Harris, B. S., Lee, J. W. and Ackerman, S. L. (2005) Protein accumulation and neurodegeneration in the woozy mutant mouse is caused by disruption of SIL1, a cochaperone of BiP. Nat. Genet. 37, 974–979.
144 Denzel, A., Molinari, M., Trigueros, C., Martin, J. E., Velmurgan, S., Brown, S., Stamp, G. and Owen, M. J. (2002) Early postnatal death and motor disorders in mice congenitally deficient in calnexin expression. Mol. Cell. Biol. 22, 7398–7404.
145 Mesaeli, N., Nakamura, K., Zvaritch, E., Dickie, P., Dziak, E., Krause, K. H., Opas, M., MacLennan, D. H. and Michalak, M. (1999) Calreticulin is essential for cardiac development. J. Cell Biol. 144, 857–868.
146 Harding, H. P., Zeng, H., Zhang, Y., Jungries, R., Chung, P., Plesken, H., Sabatini, D. D. and Ron, D. (2001) Diabetes mellitus and exocrine pancreatic dysfunction in perk-/- mice reveals a role for translational control in secretory cell survival. Mol. Cell 7, 1153–1163.