Chapter 9
Co-chaperones of the Mammalian Endoplasmic Reticulum

Armin Melnyk, Heiko Rieger and Richard Zimmermann

Abstract In mammalian cells, the rough endoplasmic reticulum or ER plays a central role in the biogenesis of most extracellular plus many organellar proteins and in cellular calcium homeostasis. Therefore, this organelle comprises molecular chaperones that are involved in import, folding/assembly, export, and degradation of polypeptides in millimolar concentrations. In addition, there are calcium channels/pumps and signal transduction components present in the ER membrane that affect and are affected by these processes. The ER lumenal Hsp70, termed immunoglobulin-heavy chain binding protein or BiP, is the central player in all these activities and involves up to seven different co-chaperones, i.e. ER-membrane integrated as well as ER-lumenal Hsp40s, which are termed ERj or ERdj, and two nucleotide exchange factors.

Keywords Human endoplasmic reticulum · Cellular calcium hoemostasis · Protein transport · Protein folding · Protein degradation

Introduction

In all nucleated human cells the endoplasmic reticulum or ER forms a vast and dynamic membrane network (Palade 1975; English and Voeltz 2013). The rough ER is studded with 80S ribosomes. These ribosomes are engaged in the biosynthesis of most secretory and many organellar proteins by cotranslationally inserting nascent polypeptides into the membrane and lumen of the ER, thus defining one major function of the rough ER. The peripheral ER contacts the plasma membrane, the tubular ER contacts mitochondria (Kornmann et al. 2009; Hayashi et al. 2009; Bakowski

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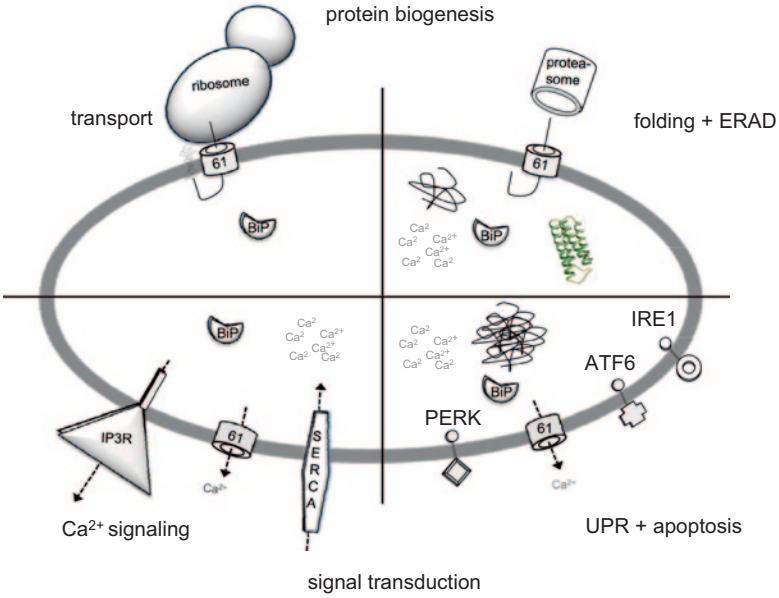

Fig. 9.1 Cross section through the ER, highlighting the central role of Sec61 complex and BiP in protein biogenesis and calcium homeostasis in human cells. *ERAD* ER-associated protein degradation, *SERCA* sarcoplasmic endoplasmic reticulum calcium ATPases, *UPR* unfolded protein response

et al. 2012). These contacts play important roles in cellular calcium homeostasis, thus defining another major function of the mammalian ER. In addition, the ER membrane forms a continuum with the outer nuclear envelope membrane.

Protein translocation into the ER is the first step in the biogenesis of many proteins of eukaryotic cells (such as proteins of the ER, ERGIC, Golgi apparatus, endosome, lysosome, nucleus, peroxisome, plasma membrane) as well as of most extracellular proteins (Fig. 9.1, "transport") (Blobel and Dobberstein 1975a, b). Typically, protein translocation into the ER involves amino-terminal signal peptides in the precursor polypeptides and a complex machinery of transport components, most notably the heterotrimeric Sec61 complex in the ER-membrane and the ER-lumenal Hsp70-type molecular chaperone BiP and its co-chaperones plus nucleotide exchange factors or NEFs.

Protein transport into the ER is followed by folding and assembly of the newly imported polypeptides (Fig. 9.1, "folding"). Typically, this folding and assembly of proteins involve some of the above-mentioned components, such as the calcium-dependent chaperone BiP and its co-chaperones plus NEFs (Haas and Wabl 1983; Bole et al. 1986; Weitzmann et al. 2007; Zahedi et al. 2009; Bulleid 2012). Except for resident proteins of the ER, the native proteins are delivered to their functional location by vesicular transport (Schekman 2004, 2005; Sambrook 1990; Pelham 1990).

In cases of mis-folding or mis-assembly of polypeptides in the ER membrane or lumen, the polypeptides are exported to the cytosol and degraded by the proteasome

(Fig. 9.1, "ERAD") (Smith et al. 2011; Bagola et al. 2011; Thibault and Ng 2012; Olzmann et al. 2012). Export of mis-folded polypeptides from the ER lumen to the cytosol can also involve some of the above-mentioned components, such as the Sec61 complex and BiP and its co-chaperones (Pilon et al. 1997; Plemper et al. 1997; Schäfer and Wolf 2009).

When protein mis-folding or mis-assembly prevail, a complex signal transduction pathway is activated and leads to an increase of the folding and degradation capacity of the ER and to a decrease of global protein synthesis (Fig. 9.1, "UPR") (Gardner et al. 2013; Ron and Harding 2012; Ma and Hendershot 2001; Schröder and Kaufman 2005). In mammals, UPR involves the three ER membrane proteins PERK, ATF6 and IRE1, respectively. These proteins comprise lumenal domains, which are not structurally related to J-domains, that interact with BiP and cytosolic domains that attenuate global translation (PERK) or induce selective transcription (ATF6, IRE1) in the absence of BiP.

When the protein mis-folding problem persists, however, the programmed cell death pathway or apoptosis is activated in the respective cell to protect the organism (Fig. 9.1, "apoptosis) (Madeo and Kroemer 2009; Tabas and Ron 2011). This switch involves efflux of calcium ions (Ca^{2+}) from the ER. Indirect evidence from various laboratories has first suggested that the Sec61 complex may transiently contribute to the ER Ca^{2+}leak after completion of protein translocation (Lomax et al. 2002; van Coppenolle et al. 2004; Flourakis et al. 2006; Giunti et al. 2007; Ong et al. 2007; Lang et al. 2011). Recently, this concept was confirmed by the observations that the open Sec61 complex is indeed Ca^{2+}permeable and that silencing the *SEC61A1* gene in HeLa cells prevents the Ca^{2+}leakage linked to completion of protein translocation (Lang et al. 2011; Erdmann et al. 2011; Schäuble et al. 2012). Under physiological conditions, BiP and its co-chaperones are involved in limiting Sec61 complex-mediated Ca^{2+}leakage or passive Ca^{2+}efflux. Therefore, it is tempting to speculate that the intrinsic Ca^{2+}permeability of the Sec61 complex and its regulation by BiP play an important role at the interface between protein biogenesis and Ca^{2+}homeostasis in mammalian cells (summarized in Fig. 9.1). Since the more than thousand-fold Ca^{2+}gradient between ER lumen and cytosol allows $Ca^{2+}$to play its central role as a second messenger in cellular signaling (Berridge 2002; Rizzuto and Pozzan 2006), it is the function of the sarcoplasmic endoplasmic reticulum calcium ATPase (SERCA) to counteract both the receptor-mediated Ca^{2+}release and the Ca^{2+}leakage from the ER in order to maintain the Ca^{2+}gradient of the resting cell (Wuytack et al. 2002).

The Chaperone Network of the ER

Both the yeast and the mammalian ER contain molecular chaperones and folding catalysts in millimolar concentrations (Van et al. 1989; Bies et al. 1999; Weitzmann et al. 2007). Many of these molecular chaperones belong to the classical Hsp40, Hsp70, and Hsp90 protein families (Table 9.1, Fig. 9.2). However, the ER also

Table 9.1 BiP and its interaction partners in the mammalian ER

Function	Protein (synonym)	Related human disease	OMIM	Animal model	First reference
Hsp70-type chaperone	BiP (Grp78, HspA5)	Haemolytic uraemic syndrome	235400	Embryonic lethality or surfactant deficiency	Haas and Wabl (1983)
Hsp40-type co-chaperones	ERj1 (Htj1, DNAJC1)				Brightman et al. (1995)
	ERj2 (Sec63, ERdj2)	Polycystic liver disease colorectal cancer	174050	Embryonic lethality	Skowronek et al. (1999)
	ERj3 (ERdj3, DnaJB11, HEDJ, Dj9)				Bies et al. (1999)
	ERj4 (ERdj4, DnaJB9, MDG1)			Postnatal lethality (surfactant deficiency)	Shen et al. (2002)
	ERj5 (ERdj5, DnaJC10, JPDI)			No phenotype	Hosoda et al. (2003); Cunnea et al. (2003)
	ERj6 ($p58^{IPK}$, DnaJC3, ERdj6)			Diabetic mouse	Rutkowski et al. (2007)
	ERj7 (Gng10, DnaJC25, ERdj7)				Zahedi et al. (2009)
Nucleotide exchange factors	Grp170 (ORP150, HYOU1)			Embryonic lethality	Lin et al. (1993)
	Sil1 (BAP)	Marinesco-Sjögren syndrome	248800	Woozy mouse	Chung et al. (2002)
Additional co-chaperones	Sig-1R (sigma-1 receptor)				Hayashi and Su 2007
	HspA5BP1 (GBP)				Oh-hashi et al. (2003)
Additional chaperones	Grp94 (CaBP4, ERp99, gp96, endoplasmin)			Embryonic lethality	Shiu et al. (1977)
	Calnexin (IP90, p88)			Postnatal lethality	Degen and Williams (1991)
	Calreticulin (CaBP3, ERp60)			Embryonic lethality	Burns et al. (1992)

Table 9.1 (continued)

Function	Protein (synonym)	Related human disease	OMIM	Animal model	First reference
UPR signal transducers	IRE1α/β (ERN1/2)				Tirasophon et al. (1998)
	IRE2				Wang et al. (1998)
	ATF6α/β				Yoshida et al. (1998)
	PERK (EIF2AK3, PEK)	Wolcott-Rallison syndrome breast cancer	226980	Diabetic mouse	Shi et al. (1998); Harding et al. (1999)
Sec proteins	Sec61α1			Diabetic mouse	Görlich et al. (1992)
	Sec61β				Hartmann et al. (1994)
	Sec61γ	Glioblastoma			
	Sec62 (TLOC1)	Prostate/lung/thyroid cancer			Mayer et al. (2000); Tyedmers et al. (2000)

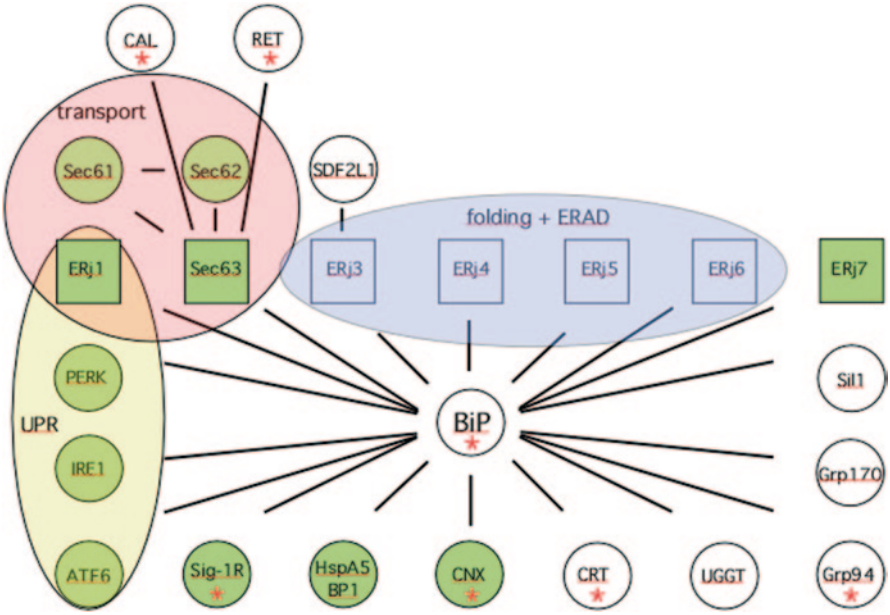

Fig. 9.2 Interaction partners of BiP that are involved in protein biogenesis and calcium homeostasis. The proteins that are involved in protein transport, folding, *ERAD*, and *UPR* are indicated, all other proteins are involved in protein folding or calcium homeostasis (*red asterisk*). Membrane proteins are depicted in green; ER-lumenal Hsp40s are represented as *squares*, all other proteins as *circles*

comprises a special class of molecular chaperones or lectins that are dedicated to the folding of glycoproteins. The mammalian ER, contains a soluble (calreticulin or CRT) as well as a membrane integrated (calnexin or CNX) lectin (Degen and Williams 1991; Burns et al. 1992). The folding catalysts of the ER deal with either the formation of disulfide bonds (protein disulfide isomerases or PDI) or the isomerization of proline-containing peptide bonds (peptidyprolyl-*cis/trans*-isomerases or PPIase). The PPIases belong to either the cyclosporin A- or the FK506-sensitive protein family (cylophilin or FK506-binding protein). All these chaperones and folding catalysts have been observed to be present in larger complexes in various combinations (Tatu and Helenius 1997; Meunier et al. 2002).

The Hsp70/Hsp40 Network of the ER

Just like the bacterial cytosol or the mitochondrial matrix, the ER contains the typical Hsp70 triad, comprising the Hsp70 itself (BiP in mammals) as well as a Hsp40-type co-chaperone, which stimulates the ATPase activity of BiP, and a NEF, which catalyzes the exchange of ADP for ATP (Tables 9.1 and 9.2, Fig. 9.3). These proteins have also been shown to be able to perform the classical Hsp70 reaction cycle, thereby mediating the folding and assembly of newly-synthesized and –imported polypeptides. Similarly to the two above-mentioned cellular compartments, there are two Hsp70-type chaperones in both the yeast as well as the mammalian ER (Haas and Wabl 1983; Bole et al. 1986; Munro and Pelham 1986; Weitzmann et al. 2007; Mimura et al. 2007; Luo et al. 2006). One of these, however, may also be referred to as a Hsp110 protein family member (Grp170 in mammals) and serves as a NEF for BiP (Lin et al. 1993; Kitao et al. 2004; Weitzmann et al. 2006). There also seems to be a *bona fide* functional homolog to bacterial GrpE in the ER lumen (BAP or Sil1 in mammals) (Chung et al. 2002; Zhao et al. 2005, 2010), i.e. there is redundancy at the level of the NEFs, which may explain the non-lethal phenotype of loss of Sil1 function that is associated with the neurodegenerative disease, Marinesco-Sjögren syndrome (Table 9.1, see below). The structures of the two cytosolic paralogs of the two NEFs were recently solved and revealed distinct interacting surfaces with the top of the nucleotide-binding domain (NBD) of BiP (Shomura et al. 2005; Polier et al. 2008); thus, the NEF binding sites on Hsp70 are different from the J-domain binding site, which resides at the NBD bottom. Based on these structural data, the two NEFs may even be able to bind simultaneously to BiP.

There may be up to nine different Hsp40 type molecular chaperones present in the human ER, although not necessarily simultaneously in the same cell (Tables 9.1 and 9.2, Fig. 9.3). To date, seven of these have been characterized in some detail and were termed ERj1 through ERj7 (or ERdj). The two additional candidates for ERj proteins are DnaJC14 or HDJ3 and DnaJC16, the latter also containing two thioredoxin domains. The Hsp40-type co-chaperones in the ER can be divided into membrane proteins with a lumenal J-domain and into lumenal proteins (Fig. 9.3). Furthermore, they can be classified according to the domains they have in common

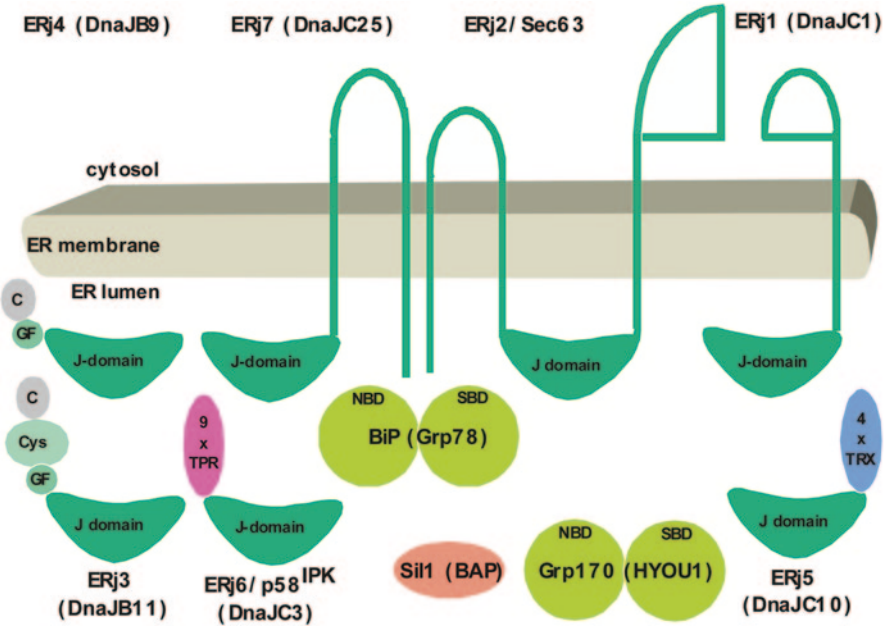

Fig. 9.3 Topology and domain organisation of BiP and its co-chaperones and nucleotide exchange factors. *C*, carboxy-terminal substrate binding domain, *Cys* cysteine-repeat domain, *GF* glycine-phenylalanine rich domain, *NBD* nucleotide binding domain, *SBD* substrate binding domain, *TPR* tetratricopeptide repeat, *TRX* thioredoxin domain. We note that ERj1 and Sec63 both comprise large cytosolic domains that are structurally un-related. In the case of ERj1, this domain is involved in ribosome binding; (Blau et al. 2005; Dudek et al. 2005) (Fig. 9.6), the cytoslic domain of Sec63 is structurally related to certain helicases (Pena et al. 2009) and is involved in interaction with Sec62 (Müller et al. 2010) (Fig. 9.5)

with the bacterial DnaJ protein (i.e. besides the actual J-domain) (Hennessy et al. 2005). 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 glycine-phenylalanine (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 the type I and II ER-lumenal Hsp40s, ERj3 (Bies et al. 1999, 2004; Yu et al. 2000; Shen and Hendershot 2005; Jin et al. 2008, 2009) and ERj4 (Shen et al. 2002 Kurisu et al. 2003; Dong et al. 2008; Lai et al. 2012; Fritz et al. 2014), 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*. However, the four thioredoxin domains within ERj5 (Cunnea et al. 2003; Hosoda et al. 2003; Dong et al. 2008; Ushioda et al. 2008; Ladiges et al. 2005; Hagiwara et al. 2011; Oka et al. 2013) and the tetratricopeptide repeat (TPR) domains in ERj6 ($p58^{IPK}$) (Kang et al. 2006; Rutkowski et al. 2007; Petrova et al. 2008;

Table 9.2 Properties of BiP and its co-chaperones and NEFs. We note that the given concentrations refer to a suspension of rough microsomes, which was isolated from canine pancreas and adjusted to a concentration of 1 equivalent/µl. In the ER lumen, the concentrations are approximately thousand-fold higher. The data were taken from Weitzmann et al. 2007; Zahedi et al. 2009). *GST* glutathione-S-transferase

Protein	UPR controlled	Cellular function(s)	Concentration in suspension of RM (µM)	Recombinant protein (amino acid residues)	Rate constants for interaction with BiP in the presence of ATP	
					k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})
BiP	+	ERAD, folding, Sec61-gating, transport, UPR	5.00	BiP-Hexahis (20-655)	–	–
ERj1	–	Unknown	0.36	GST-J-domain (44-140)	6.00×10^3	2.60×10^{-3}
ERj2	–	Transport	1.98	GST-J-domain (91-189)	0.81×10^3	2.60×10^{-3}
ERj3	+	ERAD, folding	0.29	GST-ERj3 (18-336)	1.25×10^3	3.60×10^{-3}
ERj4	+++	ERAD, folding	Not detectable	GST-ERj4 (23-222)		
ERj5	+	ERAD, folding	2.00	GST-ERj5 (26-793)	6.20×10^3	2.80×10^{-3}
ERj6	+	ERAD, folding	Not determined	GST-ERj6 (32-504)	64.4	3.97×10^{-3}
ERj7	+	Unknown	2.30	GST-J-domain (39-149)	5.07×10^3	5.70×10^{-3}
Grp170	+	Folding, NEF	0.60	–	Not determined	
Sil1	–	NEF	0.005	GST-39-461	Not detectable	

Dong et al. 2008; Svard et al. 2011) may also play a role in substrate binding. Thus, ERj3 through ERj6 are involved in protein folding under physiological as well as stress conditions and in ERAD (Table 9.2, Fig. 9.2). This is consistent with the fact that these four BiP co-chaperones are over-produced together with BiP under stress conditions, i.e. when there is an increased demand for chaperone and degradation activity towards mis-folded polypeptides (Table 9.2). Therfore, it is not surprising that these members of the resident ER Hsp70-cycle have been found in large complexes with each other, with other chaperones and folding catalysts, and with other resident ER proteins that are involved in N- or O-glycosylation (UDP-glucose-glycoprotein-glycosyltransferase or UGGT, SDF2L1) and calcium homeostasis (calumenin, reticulocalbin), respectively (Fig. 9.2).

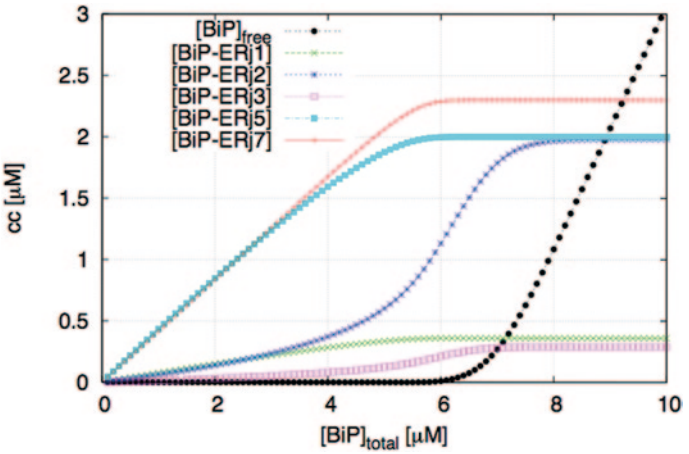

Fig. 9.4 Equilibrium concentrations for (free) BiP and reaction products BiP-ERjX (X = 1,2,3,5,7) as a function of the initial concentration of BiP as calculated numerically with the reaction equations, shown below, and using the experimentally determined rate constants k_a and k_d and initial concentrations [ERjX] in rough microsomes from canine pancreas (Table 9.2). The time evolution of the concentrations is then given by a coupled set of ordinary differential equations:

$$\frac{d}{dt}[BiP] = \sum_{X=1}^{7}\left\{k_d^{(X)}\,[BiP-ERjX] - k_a^{(X)}[BiP]\cdot[ERjX]\right\},$$

and

$$\frac{d}{dt}[ERjX] = k_d^{(X)}[BiP-ERjX] - k_a^{(X)}[BiP]\cdot[ERjX],$$

$$\frac{d}{dt}[BiP-ERjX] = -k_d^{(X)}[BiP-ERjX] + k_a^{(X)}[BiP]\cdot[ERjX],$$

where [BiP], [ERjX], and [BiP–ERjX] denote the concentrations of BiP, ERjX (X = 1,2,…,7), and [BiP–ERjX], respectively. Due to the lack of data we set [ERj6] and [BiP-ERj6] constant to zero. Using the measured values for the initial concentrations [ERjX](t = 0) and the rate constants k_a and k_d from Table 9.1 we solved the above differential equations numerically for various initial concentrations [BiP](t = 0) and zero initial concentrations of the reaction products [BiP-ERjX](t = 0). In Fig. 9.1 we show the results of the stationary (equilibrium) concentrations of BiP and the reaction products, $[BiP]_{eq}$ and $[BiP\text{-}ERjX]_{eq}$, respectively, as a function of the initial BiP concentration [BiP](t = 0)—which is equal to the total BiP concentration $[BiP]_{total}$, since [BiP–ERjX](t = 0) is zero for X = 1,…7

In Fig. 9.4, we have modelled the equlibrium concentrations of free BiP and complexes of BiP with its co-chaperones for canine pancreatic microsomes, based on the determined concentrations of the various proteins and the rate constants for their interacttion with BiP (Table 9.2). The complexes are formed transiently in order to stimulate the ATPase activity of BiP, thus creating the form of BiP with high substrate affinity. Typically, the ER lumenal concentrations of BiP are in the millimolar range and similar to the total concentration of ERjs (Weitzmann et al. 2007). The model illustrates that under normal conditions there is enough BiP available for interaction with all ERjs and that under conditions of UPR induction, where BiP and ERj3 through ERj6 are over-produced, BiP becomes limiting for ERj2, thus, selectively preventing import of additional precursor polypeptides. This can be deduced

from the observation that complex formation between BiP and ERj2 requires much higher concentrations of BiP as compared to complex formation between BiP and e.g. ERj5 or ERj7.

The Putative Role of BiP and its Co-chaperones in Protein Transport into the ER as an Example of Chaperone/Co-chaperone Action in the Mammalian ER

The structure of the Sec61 complex suggests a potential mechanism for BiP-mediated gating, i.e. opening and closing, of the Sec61 channel (Figs. 9.1 and 9.5) (Pfeffer et al. 2012, 2014; Zimmermann et al. 2011). We suggest that the ribosome

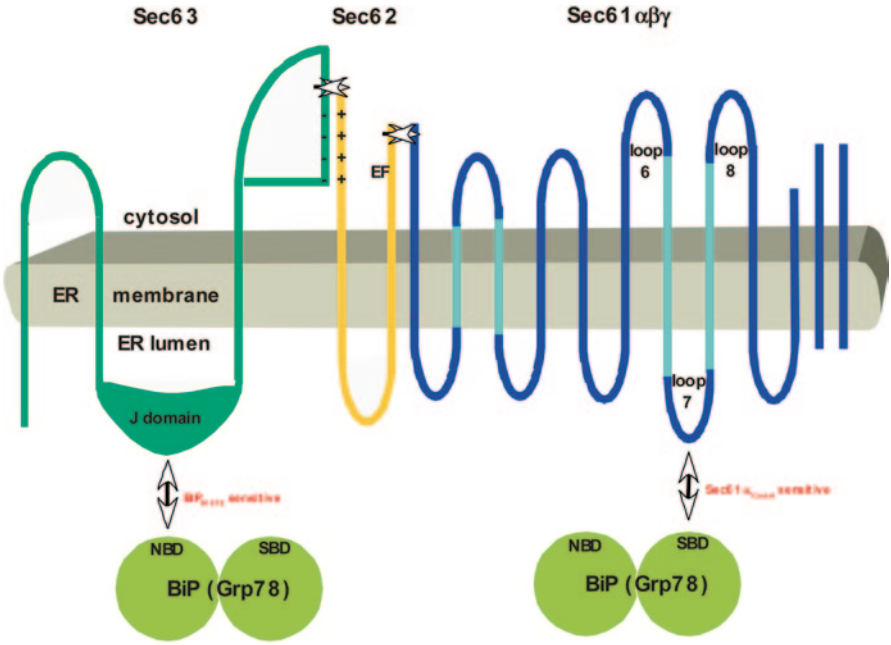

Fig. 9.5 Protein-protein interactions that are involved in gating of the Sec61-complex in the human ER membrane. The shown interactions of BiP with Sec61α (Schäuble et al. 2012), Sec62 with Sec61α (Linxweiler et al. 2013) and Sec62 with Sec63 (Müller et al. 2010) as well as their sensitivities to mutations were previously described. The BiP-Sec63 interaction was described by Tyedmers et al. (2000) and the effect of the R197E mutation by Awad et al. (2008). So far, the latter interaction as well as the Sec62-Sec63 interaction were found to be relevant only for protein transport into the ER, i.e. gating of the Sec61 complex from the closed to the open conformation; in contrast, the BiP co-chaperone for gating to the closed state is still elusive. Interactions are indicated by *arrows*, the transmembrane helices that form the lateral gate are shown in *light blue*, the cytosolic and ER luminal loops, which form the binding sites for ribosomes and BiP, respectively, are indicated. *NBD* nucleotide binding domain, *SBD* substrate binding domain

in cotranslational transport and the Sec62/Sec63 complex in posttranslational transport can prime the closed Sec61 complex for opening (Lang et al. 2012). The current view on opening of the Sec61 complex in protein translocation, i.e. channel gating from the closed to the open conformation, is that signal peptides of nascent presecretory polypeptides intercalate between the Sec61α transmembrane (tm) helices 2 and 7, opening the lateral gate of the Sec61 complex that these two tm helices form (van den Berg et al. 2004; Gumbart and Schulten 2007). It has been proposed that the minihelix within loop 7 plays a role in gating of the Sec61 complex from closed to open and that BiP binding to this minihelix may be required for gating from the closed state to the open state in the case of some precursor polypeptides, while others may be able to trigger gating on their own (Schäuble et al. 2012). Here, BiP binds the native Sec61α as a substrate and facilitates its conformational change. At this point of translocation, the nascent precursor polypeptide chain can be fully inserted into the Sec61 complex and initiate translocation. Next, BiP binds to the precursor polypeptide in transit and acts as a molecular ratchet, thus mediating completion of translocation (Nicchitta and Blobel 1993; Tyedmers et al. 2003; Shaffer et al. 2005). Here, BiP binds the non-native precursor polypeptide as a substrate and prevents it from sliding back into the cytosol. Subsequently, i.e. in the absence of a precursor polypeptide in transit, binding of BiP to loop 7 can facilitate closing of the Sec61 channel to limit ion efflux from the ER (Schäuble et al. 2012). We find this view attractive, because loop 7 connects tm helices 7 and 8, and is thus close enough to the lateral gate to influence gate movements. Interestingly, mutation of tyrosine 344 to histidine within the minihelix of loop 7 leads to diabetes in mice (Lloyd et al. 2010).

There is no doubt that the physical and mechanistic link between the Sec61- and the BiP-reaction cycles is most efficiently provided by a membrane integrated Hsp40 with a lumenal J-domain. Indeed in yeast, Sec63p has been shown to provide the lumenal J-domain that allows Kar2p (BiP in yeast) to play its roles in insertion of precursors into the Sec61 complex as well as in completion of translocation (Lyman and Schekman 1995, 1997). Since in pancreatic microsomes Sec63 or ERj2 was found in association with the Sec61 complex and to be present in approximately stoichiometric amounts as compared to heterotrimeric Sec61 complexes, we expect mammalian Sec63 to play a similar role, i.e. recruit BiP to the Sec61 complex and stimulate ATPase activity of BiP for conversion to the high substrate affinity (Mayer et al. 2000; Tyedmers et al. 2000; Pena et al. 2009; Lang et al. 2012). However, it remains open, whether or not a single BiP molecule can first bind loop 7 of Sec61α and, subsequently, the incoming precursor polypeptide within one functional cycle (Schlecht et al. 2011). Interestingly, it has been shown that human ERj1 can complement the otherwise lethal deletion of Sec63p in yeast (Kroczynska et al. 2004). Therefore, ERj1 may play a similar role as Sec63 in the mammalian ER, thereby providing at least partial redundancy for this essential function that may explain the non-lethal phenotype of loss of Sec63 function, associated with polycystic liver disease (Table 9.1, see below). ERj1 was observed in association with translating ribosomes (Fig. 9.6; Dudek et al. 2002, 2005; Blau et al. 2005; Benedix et al. 2010). Therefore, we propose that in the mammalian ER two different membrane

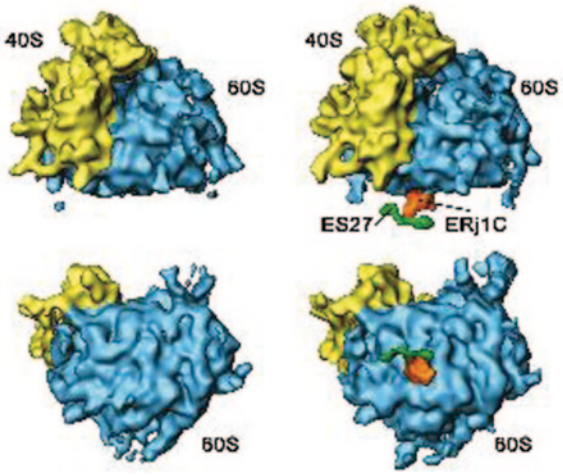

Fig. 9.6 ERj1's ribosomal contacts, overall position and conformational changes. Cryo-EM map of the dog pancreas 80S ribosome at a resolution of 23 Å. Left side: Yellow indicates the small (40S) ribosomal subunit, blue indicates the large (60S) subunit (Blau et al. 2005; Dudek et al. 2005). Top, side view; bottom, rotated 90° backwards, exposing the membrane attachment side of the ribosome. Right side: Cryo-EM map of the 80S ribosome- ERj1C complex at a resolution of 20 Å. ERj1C refers to the cytoslic domain of ERj1. Orange and green indicate the densities for ERj1C and the expansion segment 27 or ES27, respectively

proteins provide J-domains in the neighborhood of translating ribosomes and Sec61 complexes and allow BiP to play its roles in protein import. In addition, ERj1 appears to have regulatory roles that are related to transcription as well as to translation. The cytosolic domain of ERj1 has the ability to allosterically inhibit translation at the stage of initiation when it is not bound to BiP (Fig. 9.6). Thus, ERj1 would be ideally suited to allow initiation of synthesis of precursor polypeptides on ER bound ribosomes only when BiP is available on the other side of the membrane. Furthermore, ERj1 has all the features of a membrane-tethered transcription factor that can be activated by regulated intra-membrane proteolysis (Zupicich et al. 2001). The cytosolic domain has actually been shown to be able to enter the nucleus (Zupicich et al. 2001, Dudek et al. 2005). Last but not least, it was observed that a resident ER protein with a lumenal J-domain is also involved in sealing of the Sec61 complex in the mammalian system (Schäuble et al. 2012). At present, we only can exclude ERj1 as the co-chaperone for this BiP activity (Lang et al. 2011).

Regulatory Mechanisms

It has been known for some time that the genes of many of the protein transport components of the mammalian ER are under control of the unfolded protein response (see Table 9.2 for examples). In addition, various miRNAs apparently target

some of these same genes and there may be splice variants for some of these genes according to the respective data bases. But there apparently also is regulation on the protein level. In the case of mammalian BiP, ADP ribosylation was shown to be a mechanism for reversible inactivation of BiP when the concentration of unfolded polypeptides is low (Chambers et al. 2012). Various modifications have been observed for mammalian as well as yeast protein transport components, most notably phosphorylation. Phosphorylation of mammalian proteins ERj1 and Sec63 by CK2 was reported, but the functional consequences of these phosphorylations was not addressed (Götz et al. 2009; Ampofo et al. 2013). A first hint for the importance of CK2-dependent phosphorylation of components of the transport machinery may come from studies in yeast (Wang and Johnsson 2005). The essential Sec63p is phosphorylated by the protein kinase CK2 and non-phosphorylatable Sec63p causes a protein translocation defect. Taken together, these findings suggest a general role of phosphorylation for a network of transport factors in regulation of protein translocation across the ER-membrane.

Medical Aspects

Shiga toxigenic *Escherichia coli* (STEC) strains cause morbidity and mortality in developing countries (Paton et al. 2006). Some of these pathogens produce AB_5 toxin or subtilase AB and are responsible for gastrointestinal diseases, including the life-threatening haemolytic uraemic syndrome (HUS) (OMIM 235400). During an 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 subunit, which inactivates BiP by limited proteolysis. Eventually, all BiP functions are lost, and the affected cells die.

Autosomal dominant polycystic liver disease (PLD) (OMIM 174050) is a rare human inherited disease that is characterized by the progressive development of multiple biliary epithelial liver cysts (Davila et al. 2004). It usually remains asymptomatic at young ages and manifests between the ages of 40 and 60 years. Liver function is usually preserved. A loss of Sec63 function has been postulated in several genetic mutations. Although no mechanism has been firmly established for PLD, the disease can be explained by a 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 ER import of a subset of non-essential secretory or plasma membrane proteins that are involved in the control of biliary cell growth or cell polarity. Thus, without functional Sec63, these proteins do not reach the correct location at the cell surface. This view was confirmed by recent results and it was concluded that the secondary lack of polycystins 1 and 2 results in disrupted cell adhesion and, therefore, cyst formation (Fedeles et al. 2011; Lang et al. 2012).

Marinesco-Sjögren syndrome (MSS) (OMIM 248800) is a rare autosomal recessively inherited neurodegenerative disease (Anttonen et al. 2005; Senderek et al.

2005). The hallmarks of MSS are cerebellar ataxia, cataracts, developmental and mental retardation, and progressive myopathy (Roos et al. 2014). The cause of the disease in the majority of MSS patients has been characterized as a mutation in the *SIL1* gene that results in mutated or truncated Sil1. Sil1 is a nucleotide exchange factor for BiP, and its role is to provide BiP with ATP (Weitzmann et al. 2006). Thus, the loss of Sil1 function results in a reduction of functional BiP. Several possible consequences are: (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 mis-folded polypeptides in the ER; (iii) some essential secretory or plasma membrane proteins may not reach their functional location, leading to secondary loss of functions; or (iv) Sec61 channel gating to the closed state may be compromised, thus, leading to apoptosis.

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 (Brickwood et al. 2003). The cause of the disease has been characterized as a mutation in the *PERK* gene that results in a mutated or truncated PERK protein. 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 β 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 β cells.

Due to poor vascularization and the resulting hypoxia and glucose starvation, tumor cells are prone to ER stress and UPR (Macario and Conway de Macario 2007; Aridor 2007). In cultured cells, BiP is one of the proteins involved in protecting cancer cells against ER stress-induced apoptosis (Fu et al. 2007). 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 an ER-membrane resident Hsp40 that, together with BiP, plays a role in gating of the Sec61 complex (Lang et al. 2012; Schäuble et al. 2012). The *SEC63* gene was found among the most frequently mutated genes in cancers that had deficient DNA mismatch repair, such as hereditary nonpolyposis colorectal cancer (HNPCC)-associated malignancies and sporadic cancers with frequent microsatellite instability (Mori et al. 2002; Schulmann et al. 2005). 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. This hypothesis is supported by a study on the gene expression signatures of sporadic colorectal cancers; they recognized the over-expression of *SEC62* as part of a 43-gene cDNA panel that was used for predicting the long-term outcome of colorectal cancer patients (Eschrich et al. 2005). Sec62 forms a complex with Sec63 and Sec61 and is also involved in Sec61 channel gating (Linxweiler et al. 2013). Gene amplification at chromosome 3q25-q26 commonly occurs in prostate- as well as several other cancers. Mapping the 3q25-q26 amplification and identifying candidate genes with

quantitative real-time PCR revealed that the *SEC62* gene had the highest known amplification frequency (50 %) in prostate cancer and was found to be up-regulated at the mRNA and protein level in all tumors analyzed (Jung et al. 2006). Recently, this was also observed for cancers of the lung and thyroid (Greiner et al. 2011a, 2011b; Linxweiler et al. 2012, 2013) and *SEC62* (*TLOC1*) was characterized as a cancer driver gene (Hagenstrand et al. 2013). Thus, *SEC62* over-expression appears to be associated with a proliferative advantage for various cancer cells, which appears to be due to the role of Sec62 in cellular calcium homeostasis. In summary, a Sec62-Sec63 imbalance is likely to contribute to the development of various human malignancies.

A common theme seems to emerge from some of the described patho-physiological situations in mice and men (summarized in Table 9.1): Mammalian cells, which are highly active in protein secretion, may be particularly sensitive towards problems in Sec61 channel closure and, therefore, constantly on the verge to apoptosis, e.g. seen in the β-cells of the mouse with the Sec61αY344H mutation. On the other hand, the secretory active cells may be particularly sensitive to imbalances in the Sec62 to Sec63 ratio, which result in over-efficient Sec61 channel closure and, thus, a proliferative advantage that can lead to cancer, e.g. seen after over-epression of *SEC62* in prostate or lung cancer. However, it remains to be seen to what extent the other diseases that are listed in Table 9.1 fit into this scheme.

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