The ability of cells to communicate with each other and their environment in specific ways is essential to survival in multi-cellular organisms.

Angiogenesis

One of the primary ways this is done is through proteins expressed on the cell surface or secreted from the cell.
Direct cell to cell communication
One of the primary ways this is done is through proteins expressed on the cell surface or secreted from the cell.

Secretion of effector molecules
One of the primary ways this is done is through proteins expressed on the cell surface or secreted from the cell.

Cell migration/homing
One of the primary ways this is done is through proteins expressed on the cell surface or secreted from the cell.
These cell surface and secreted proteins are synthesized in the Endoplasmic Reticulum.

Folding can begin before translation is complete, which adds more complexity to the process.

Protein folding is guided by properties of the individual amino acids and their interactions with other amino acids within the polypeptide chain.

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- But in the ER the molar concentration of unfolded proteins is very high and proteins can begin to fold co-translationally (before the entire polypeptide chain is synthesized).
- Thus, a family of proteins known as molecular chaperones are required to prevent inappropriate interactions and foster correct ones!

- Reactive cysteines
- Hydrophobic regions
- N-linked glycans

Molecular chaperone families of the mammalian ER

<table>
<thead>
<tr>
<th>Chaperone</th>
<th>Activity/Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>BiP/GRP78</td>
<td>Hsp70</td>
</tr>
<tr>
<td>BiP/ERDJ1-7</td>
<td>Hsp70</td>
</tr>
<tr>
<td>S100BAP</td>
<td>Hsp70</td>
</tr>
<tr>
<td>GRP78/CRT</td>
<td>Hsp70</td>
</tr>
<tr>
<td>GRP94</td>
<td>Hsp70</td>
</tr>
<tr>
<td>Cyclophilin-B</td>
<td>Hsp70</td>
</tr>
<tr>
<td>PDI family</td>
<td>Hsp70</td>
</tr>
<tr>
<td>Calnexin/CRT</td>
<td>Hsp70</td>
</tr>
<tr>
<td>UDP-GT</td>
<td>Hsp70</td>
</tr>
<tr>
<td>ERp57</td>
<td>Hsp70</td>
</tr>
<tr>
<td>ERp29</td>
<td>Hsp70</td>
</tr>
</tbody>
</table>

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BiP was the first ER chaperone to be identified and was found to associate with unassembled Ig heavy chains

Pre-B cells
Plasma cells
BiP
IgM HC
IgG HC
Ig LC

Pre-B, Ig LC
No secretion
Plasma cell, Ig LC

BiP is a soluble Hsp70 protein of the ER!

BiP
GRP70
GRP94
PDI
Calreticulin
KDEL Receptor
ERp72

ER
Golgi

Pre-B cells
Intracellular retention; eventual degradation
Proteasome

B and plasma cells
Assembly with LC; transport to Golgi
Exit to Golgi

Bole et al., JCB, 1986
Hendershot et al., JCB, 1987

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Crystal structure of Hsc70 ATPase domain and DnaK peptide binding domain

- Nucleotide binding domain
- Polypeptide binding domain

Wisniewska et al., PLoS One, 2010
Mayer, M. P. and Bukau, B. CMLS, 2005

Characterization of peptide sequences that bind to BiP

- 7-8mers with alternating hydrophobics bind best

Blond-Elguindi et al., Cell, 1993

Light chains cover a hydrophobic patch on the folded C,1 domain, which could be a BiP binding site

- Light chain
- Heavy chain
- Hydrophobic patch on C,1 domain

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One way to determine the folding status of ER proteins in cells is to examine the formation of disulfide bonds.

Unlike other domains of unassembled heavy chains, CH1 domain remains unfolded.

CH1 domain is intrinsically disordered in the absence of association with CH2.

LC association induces its folding.
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ATPase cycle of BiP

ATPase activity of BiP is required for substrate folding, assembly, and secretion

Disruption of BiP/GRP78 gene in mice results in very early embryonic lethal phenotype
- Cells matured till embryonic day 3.5 but could develop no further
- 24 hrs later cells had stopped dividing

H2H2L

H2L

HC + LC

ATPase mutant BiP

HC + LC

Chase (hr): 0 1.5 3 3m 0 1.5 3 3m

WT BiP

WT BiP

Luo et al., MCB, 2006

Vanhove et al., Immunity, 2001

Vanhove et al., Immunity, 2001

BiP

BiP

BiP
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Highly virulent AB5 subtilase toxin of Shiga toxigenic E. coli exerts its pathogenic effects by cleaving BiP

Expression of the BiP mutant, SubA272B, caused the toxin to no longer be able to cleave BiP and kill cells

May et al., Inf Immun, 2010

72 kDa
44 kDa
28 kDa

1. Maintaining ER permeability barrier during early stages of translocation
2. Assisting proper folding and assembly of nascent proteins
3. Targeting incompletely assembled or improperly folded proteins for degradation
4. Housing intracellular Ca²⁺ stores
5. Regulating UPR signal transducers

Recruitment of BiP to substrates by ERdj proteins

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Bip forms a complex with other ER chaperones that can bind directly to unfolded substrates. But the ERdj proteins are not part of the complex.

DnaJ family members can be subdivided into 3 groups:

Type I - DnaJ
Type II
Type III

Identification and characterization of mammalian ER DnaJ proteins:

<table>
<thead>
<tr>
<th>ERdj protein</th>
<th>M.W. Yeast homolog</th>
<th>Topology</th>
<th>UPR induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERdj1/Mgj1</td>
<td>63kDa Sec63</td>
<td>Cytosol</td>
<td>No</td>
</tr>
<tr>
<td>ERdj2/Mgj2</td>
<td>85kDa Sec63</td>
<td>Lumen</td>
<td>No</td>
</tr>
<tr>
<td>ERdj3/Mgj3</td>
<td>43kDa Sec63</td>
<td>Lumen</td>
<td>Yes</td>
</tr>
<tr>
<td>ERdj4/Mgj4</td>
<td>25kDa none</td>
<td>Lumen</td>
<td>Yes</td>
</tr>
<tr>
<td>ERdj5/Sec65</td>
<td>96kDa none</td>
<td>Lumen</td>
<td>Yes</td>
</tr>
<tr>
<td>ERdj6/p58IPK</td>
<td>58kDa none</td>
<td>Lumen</td>
<td>Yes</td>
</tr>
<tr>
<td>ERdj7</td>
<td>42kDa none</td>
<td>Lumen</td>
<td>No</td>
</tr>
</tbody>
</table>

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1. Maintaining ER permeability barrier and aiding early stages of translocation
2. Assisting proper folding and assembly of nascent proteins
3. Targeting incompletely assembled or improperly folded proteins for degradation
4. Preventing ER stress and activation of the unfolded protein response (UPR)
5. Regulating UPR signal transducers

Mutations in Sec63 (ERdj2) cause an autosomal dominant form of Polycystic Liver Disease

BIP must release for substrates to fold

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Gal-4-BiP ATPase domain fusion protein used in a yeast 2-hybrid screen identified BAP/Sil1p

BAP/Sil1 serves as a nucleotide releasing factor for BiP

BiP must release for substrates to fold
Potential consequences of BAP/Sil1 loss

1. Failure to deliver critical proteins to cell surface or to secrete them
2. Accumulation of unfolded proteins in the ER, which could activate the unfolded protein response and apoptotic programs
3. Decreased availability of free BiP for newly synthesized proteins leading to their aggregation

Mutations in BAP/Sil1 cause Marinesco-Sjögren syndrome, a genetic multi-system disorder characterized by cerebellar ataxia with cataract and myopathy

Sil1 mutations associated with Marinesco-Sjögren's Syndrome are predicted to affect BiP interactions
3 disease causing mutations affect only the C-terminal amino acids: Do they lead to loss of Sil1 from the cell?

WT

Δ1366

1367 T → A

1370 T → C

Δ1366

Δ1366 Sil1

Sil1

ER60 Overlay

Hr:

Why isn't Sil1 loss lethal if BiP loss is?
Identification of the family of large Hsp70 proteins

- Hsp110 showed great sequence similarity to Hsp70
- GRP170 showed great homology at N-terminal regions to BiP

The yeast ER member of this family, Lhs1, has nucleotide exchange activity for yeast BiP (Kar2)

... so does GRP170, the mammalian large Hsp70!

The Sil1 knock out mouse phenocopies aspects of Marinesco-Sjogren Syndrome

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And gives clues as to why loss of Sil1 is not embryonic lethal like BiP

UPR is activated in cerebellum of Sil1 null mouse.

GRP170 appears to have two functions:
1) Molecular chaperone
2) Nucleotide exchange factor for BiP
   - Unlike Sil1, GRP170 is an essential protein
   - Which function(s) is critical for survival?

Like BiP and other regulators, Sil1 is highly expressed in secretory tissues... but unlike other regulators, Sil1 is not induced by ER stress; GRP170 is
BiP forms a complex with other ER chaperones that can bind directly to unfolded substrates

Ag8(8) cells (1, LC)
BiP
ERdj3
HC
PDI
UGP-47
UDP-GT
CaBP1
Cyclophilin B (47k)

Ag8.653 cell (2q)
BiP
P.A.

GRP94 is one of the most abundant resident ER proteins, but its function is poorly understood

- It is related in sequence and structure to Hsp90, and similarly it exists as a dimer and has ATPase activity
- But unlike Hsp90 it does not appear to have co-factors to regulate its function and its clients are largely unknown

http://ssrl.slac.stanford.edu/research/highlights_archive/grp94-nucleotide.html

GRP94 is required for Toll-like receptor transport

Cont. WT Mut.
r-TLR (1411)
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---

**GRP94 is an essential gene**

Null embryos fail to gastrulate and die at day 7.5

- **WT**
- **E5.5**
- **E6.5**
- **E7.5**

Wanderling et al., MBC, 2007

---

**ES cells derived from GRP94 null cells will differentiate into a number of tissue types (Neurons, hepatocytes, adipocytes): But not into various muscle lineages**

- **Myosin : DAPI**

Wanderling et al., MBC, 2007

---

**IGF-II secreted**

(pg/ml)

- **WT**
- **KO**

IGF-II is an essential client of GRP94

Wanderling et al., MBC, 2007

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**GRP94 is required for the degradation of some glycoproteins**

---

**The ER has two resident Immunophils: FKBP13 and Cyclophilin B**

---

**Cyclophilin B accelerates proline isomerization that is required for complete folding of C₈₁ domain in vitro**

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...which is necessary for Ig assembly and secretion in vivo

BIP forms a complex with other ER chaperones that can bind directly to unfolded substrates

pERp1 is a lymphoid specific chaperone/oxidoreductase

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Feige et al., Mol Cell, 2009

BiP forms a complex with other ER chaperones that can bind directly to unfolded substrates

Meunier et al., MBC, 2002

pERp1 is a lymphoid specific chaperone/oxidoreductase

Shimizu et al., PNAS, 2009
van Anken et al., PNAS, 2009
It takes a team to fold and assemble an antibody molecule.
Sequential action of molecular chaperones.