Hanson Lectures Review

9/30/17

Brittany Brumback
Protein Processing in the Endoplasmic Reticulum

Lecture 1

Phyllis Hanson
Cell Biology Dept., Cancer Res Bldg 4625
phanson22@wustl.edu
Protein Processing and Quality Control in the Endoplasmic Reticulum

- Unfolded
- Unfolded protein response
- ERAD: ER-associated degradation
- Posttranslational modifications
  - Protein folding
- Native
- Exit from the ER

ERAD: ER-associated degradation
Protein Modifications and Folding in the ER

- Folding challenging in setting of ~400 mg/ml protein concentration
- Folding facilitated and monitored by chaperones, both classical (Hsp70/Hsp90) and glycosylation dependent
- Folded structure can be stabilized by disulfide bonds, facilitated by protein disulfide isomerases (i.e. ERp57)
- Final folding can require assembly of multimeric protein complexes
Role of classical chaperones in ER protein folding

- ER contains abundant Hsp70 and Hsp90 chaperones
- Chaperones help other proteins acquire native conformation, but do not form stable complex
- Hsp70s & Hsp90s bind exposed hydrophobic segments
- Hsp70 in ER is BiP, interactions with client proteins regulated by ATP hydrolysis and exchange, large variety of cofactors control these
- GRP94 is main ER Hsp90, also regulated by ATP status
Role of glycosylation dependent chaperones in ER folding

N-linked glycosylation:

Asn - X - Ser/Thr

Oligosaccharide addition containing a total of 14 sugars

En bloc addition to protein; subsequent trimming and additions as protein progresses through the secretory pathway; five core residues are retained in all glycoproteins

Figure 13-16
Molecular Cell Biology, Sixth Edition
© 2008 W.H. Freeman and Company
Fate of newly synthesized glycoproteins in the ER I

- Path when nascent protein folds efficiently (green arrows)
- Players
  - OST = oligosaccharyl transferase
  - GI, GII = glucosidase I and II
  - Cnx/Crt = Calnexin and Calreticulin, lectin chaperones
  - ERp57 = oxidoreductase
  - ERMan1 = ER mannosidase 1
  - ERGIC53, ERGL, VIP36 = lectins that facilitate ER exit
Fate of newly synthesized glycoproteins in the ER II

- Path when nascent protein goes through folding intermediates (orange arrows)

- Players
  - UGT1 (a.k.a. UGGT) = UDP-glucose–glycoprotein glucosyltransferase, recognizes “nearly native” proteins, acting as conformational sensor
  - Reglucosylated protein goes through Cnx/Crt cycle for another round
  - GII removes glucose to try again and pass QC of UGT1
  - BiP = hsc70 chaperone that recognizes exposed hydrophobic sequences on misfolded proteins
Fate of newly synthesized glycoproteins in the ER III

- Folding-defective proteins need to be degraded - transported out of the ER for degradation

- How do proteins avoid futile cycles?
  - UGT1 does not recognize fatally misfolded proteins and won’t reglucosylate them for binding to Cnx/Crt
  - Resident mannosidases will trim mannose residues - protein can no longer be glucosylated and bind to Cnx/Crt
  - BiP binds hydrophobic regions
  - Mannosidase trimmed glycans recognized by OS9 associated with ubiquitination machinery

- Leads to kinetic competition between folding and degradation of newly synthesized glycoproteins
Protein Processing and Quality Control in the Endoplasmic Reticulum

Unfolded → Unfolded protein response → Posttranslational modifications → Protein folding → Native → Exit from the ER

ERAD: ER-associated degradation
Logical hierarchy of quality control and degradation. (a) General pyramidal scheme with many substrates, several adaptors, a handful of membrane complexes, and a commonly shared mechanism for substrate extraction and degradation in the cytosol. Substrates vary with regard to topology, post-translational modifications, and nature of the folding defect. These parameters influence the specific pathway(s) available to the substrate. Although not depicted, some substrates might engage a ubiquitin ligase complex directly. There may also be considerable overlap among pathways: substrates could access multiple adaptors, and adaptors might be capable of binding multiple ligase complexes. (b) Several examples of putative adaptors (many of which are chaperones) and ubiquitin ligase complex components are listed.
Protein Processing and Quality Control in the Endoplasmic Reticulum

Unfolded → Posttranslational modifications → Protein folding → Native

Unfolded protein response

ERAD: ER-associated degradation

Exit from the ER
Unfolded Protein Response (UPR)

- Intracellular signal transduction pathways that mediate communication between ER and nucleus
- Activated by accumulation of unfolded proteins in the lumen of the ER
- First characterized in yeast
- Conserved and more complex in animals, with at least three pathways
The UPR in yeast

Ire1=inositol-requiring protein-1, ER-localized transmembrane kinase and site specific endoribonuclease

Ire1 is maintained in inactive state by binding to BiP. Removal of BiP (by binding to misfolded proteins) leads to Ire1 activation

Ire1 activation triggers splicing of intron in mRNA encoding Hac1, a dedicated UPR transcriptional activator

Hac1 then binds to UPRE elements to selectively upregulate gene expression of targets that will help alleviate the overload of misfolded proteins
Misfolded proteins, ER stress, and disease

- Cystic fibrosis transmembrane conductance regulator ΔF508 mutation is well studied example (among 100s known)
- Protein could be functional as chloride channel at PM, but does not pass ER QC
- Ameliorative strategies include use of chemical chaperones, efforts to modulate specific folding factors, and efforts to adjust overall “proteostasis”
Protein Processing and Quality Control in the Endoplasmic Reticulum

Posttranslational modifications
Protein folding

Unfolded

Unfolded protein response

ERAD: ER-associated degradation

Native

Exit from the ER
ER exit sites defined as sites of COPII vesicle formation

Fig. 1. ER exit sites. Members of both the inner (Sec24B) and outer (Sec31A) COPII coat are found concentrated at punctae on the ER membrane called ER exit sites. Image of Sec31A kindly provided by Soomin Shim (University of California at Berkeley, Berkeley, CA, USA).
Minimal COPII machinery

Five proteins added to liposomes or *in vitro* reactions form vesicles:

*Sar1p, Sec23p, Sec24p, Sec13p, Sec31p*
Overview of COPII vesicle biogenesis
Specific amino acid signals mediate selective transport:
Diacidic motifs are common theme in efficiently secreted proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>TM-aa</th>
<th>Signal Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSV-G</td>
<td>18</td>
<td>YTDIEMNRLGK</td>
</tr>
<tr>
<td>CFTR</td>
<td>212</td>
<td>YKDADLYLLLD-287aaTM</td>
</tr>
<tr>
<td>GLUT4</td>
<td>36</td>
<td>YLGPDEND</td>
</tr>
<tr>
<td>LDLR</td>
<td>17</td>
<td>YQKTTEDEVICH-20aa</td>
</tr>
<tr>
<td>CI-M6PR</td>
<td>26</td>
<td>YSKVSKEETDENE-127aa</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>95</td>
<td>YDSLLVFDYEGLGSGS-42aa</td>
</tr>
<tr>
<td>EGFR</td>
<td>58</td>
<td>YKGLWIPEGEKVKIP-467aa</td>
</tr>
<tr>
<td>ASGPR H1</td>
<td>1</td>
<td>MTKEYQDLQHLDNEES-24aaTM</td>
</tr>
<tr>
<td>NGFR</td>
<td>65</td>
<td>YSSLPPACREASEEVEKLLNG-74aa</td>
</tr>
<tr>
<td>TfR</td>
<td>19</td>
<td>YTRFSLARQVDGDNHSV-26aaTM</td>
</tr>
</tbody>
</table>
And what about large cargo?
Retrograde traffic from Golgi to ER

- Includes receptor-mediated mechanism for retrieving ER resident proteins
- HDEL receptor identified in yeast – ERD2; multispanning transmembrane protein
- KDEL receptor in higher euks.
- Dilysine motif in C-terminal tail of receptor binds to COPI coat; lumenal domain binds HDEL/KDEL motif in pH dependent manner
The Secretory Pathway
Phyllis Hanson

Lecture 2

9-12-17
Figure 14-1

Molecular Cell Biology, Sixth Edition

© 2008 W.H. Freeman and Company
Membrane trafficking requires that transport intermediates undergo **fission** from donor compartment and **fusion** with target compartment. None of this happens spontaneously, and is instead controlled by a highly regulated series of molecular reactions.
Genetic dissection of the secretory pathway in *Saccharomyces cerevisiae*

- Isolation of yeast mutants with temperature sensitive defects in secretion
- Mutant strains still used today to block transport at specific steps in the secretory pathway
- 2013 Nobel Prize
• **Approach:** Isolated yeast with temperature sensitive defects in secretion, eventually identified and studied molecules involved.

• **Strategy for isolating sec mutants:** At non-permissive temperature, cells with temperature sensitive mutations in the secretory pathway will stop secreting proteins. However, the cells will continue to make secretory proteins (needed among other things for cell surface growth). These proteins accumulate inside the cells, making the cells more dense than wild-type counterparts.
• Yeast sec screen:
  • Mutagenized cells and grew at permissive temperature (24 ºC).
  • Shifted cells to 37 ºC (non-permissive temperature) for several hours.
  • Separated cells by density gradient centrifugation.
  • Plated dense cells at 24 ºC.
  • Screened individual colonies from these plates for defects in secretion via EM.
Fate of secreted proteins

Normal secretion

Accumulation in the cytosol

Accumulation in rough ER

Defective function

Transport into the ER

Budding of vesicles from the rough ER
Look at phenotypes of double mutants to determine sequence of events.
Other coats responsible for vesicle biogenesis throughout the cell

<table>
<thead>
<tr>
<th>VESICLE TYPE</th>
<th>TRANSPORT STEP MEDIATED</th>
<th>COAT PROTEINS</th>
<th>ASSOCIATED GTPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPII</td>
<td>ER to <em>cis</em>-Golgi</td>
<td>Sec23/Sec24 and Sec13/Sec31 complexes, Sec16</td>
<td>Sar1</td>
</tr>
<tr>
<td>COPI</td>
<td><em>cis</em>-Golgi to ER Later to earlier Golgi cisternae</td>
<td>Coatomers containing seven different COP subunits</td>
<td>ARF</td>
</tr>
<tr>
<td>Clathrin and adapter proteins*</td>
<td><em>trans</em>-Golgi to endosome</td>
<td>Clathrin + AP1 complexes</td>
<td>ARF</td>
</tr>
<tr>
<td></td>
<td><em>trans</em>-Golgi to endosome</td>
<td>Clathrin + GGA</td>
<td>ARF</td>
</tr>
<tr>
<td></td>
<td>Plasma membrane to endosome</td>
<td>Clathrin + AP2 complexes</td>
<td>ARF</td>
</tr>
<tr>
<td></td>
<td><em>cis</em>-Golgi to lysosome, melanosome, or platelet vesicles</td>
<td>AP3 complexes</td>
<td>ARF</td>
</tr>
</tbody>
</table>

*Each type of AP complex consists of four different subunits. It is not known whether the coat of AP3 vesicles contains clathrin.

Table 14-1
*Molecular Cell Biology, Sixth Edition*
© 2008 W.H. Freeman and Company
Proteins are modified as they travel through the Golgi apparatus.

- Mannosidase II
- GlcNAc transferase I
- Galactosyltransferase
- Sialyltransferase

Localization of first SNARE proteins: Vesicle and target membrane in nerve terminal

Syntaxin

SNAP-25

t-SNAREs

Synaptobrevin/VAMP

v-SNARE
‘The master of a large ship crushed the index finger of his right hand with the anchor. Seven days later a somewhat foul discharge appeared; then trouble with his tongue -- he complained that he could not speak properly…his jaws became depressed together, his teeth were locked, then symptoms appeared in his neck; on the third day opisthotonous appeared with sweating. Six days after the diagnosis was made he died.’

Hippocrates
Discovery that clostridial toxins cleave SNAREs supports role in fusion

- Toxins found to be metalloproteases
- Toxins quickly inhibit synaptic vesicle exocytosis
- Therefore targets must be important for neurosecretion
- Comparison of treated vs. non-treated synaptosomes shows cleavage of VAMP/synaptobrevin
- Injecting peptides encompassing cleavage site in VAMP/synaptobrevin inhibit action of tetanus toxin
Structure of core SNARE complex leads to mechanism of membrane fusion

Sutton et al., 1998  Nature 395:347
**Fig. 2.** (A) The zippering model for SNARE-catalyzed membrane fusion. Three helices anchored in one membrane (the t-SNARE) assemble with the fourth helix anchored in the other membrane (v-SNARE) to form trans-SNARE complexes, or SNAREpins. Assembly proceeds progressively from the membrane-distal N termini toward the membrane-proximal C termini of the SNAREs. This generates an inward force vector (F) that pulls the bilayers together, forcing them to fuse. Complete zippering is sterically prevented until fusion occurs, so that fusion and the completion of zippering are thermodynamically coupled. (B) Therefore, when fusion has occurred, the force vanishes and the SNAREs are in the low-energy cis-SNARE complex.

Südhof & Rothman 2009 Science 323:474
Role for NSF?
Exocytosis at the synapse is regulated by Ca$^{2+}$

- Calcium influx via voltage gated channels triggers SV exocytosis within less than 1 msec
- Occurs at synapse active zone
- Steep calcium dependence, suggests 3-4 Ca$^{2+}$ ions are required
Ca$^{2+}$ sensor: synaptotagmin
Transport through the Golgi: anterograde, retrograde or both?

- **Two models** to debated over many years
  - Stable Compartments connected by vesicle traffic: secretory cargo (large and small) moves through these by a vesicle-mediated anterograde process
  - Cisternal Maturation: VTCs fuse into an ERGIC (ER-Golgi intermediate compartment). This matures into the cis-Golgi by gain of Golgi proteins and removal of VTC proteins via COPI vesicles that move in a retrograde direction. Cis-Golgi then matures to medial Golgi by similar mechanism, etc.
Figure 1. Anterograde vesicular transport between stable compartments. Secretery cargoes travel from the ER to the intermediate compartment (IC) and cis-Golgi in dissociative carriers. Golgi compartments are stable and biochemically distinct. Secretery cargoes move across the stack by means of COPI vesicles that bud from one compartment and fuse with the next, whereas resident Golgi proteins stay in place by being excluded from budding vesicles. This model does not provide a mechanism for transporting secretory cargoes that are too large to fit within COPI vesicles.
Figure 2. Cisternal progression/maturation. Secretory cargoes exit the ER in dissociative carriers, which coalesce with one another and with COPI vesicles derived from the cis-Golgi to form the intermediate compartment, which coalesces in turn to form a new cis-cisterna. In subsequent rounds of COPI-mediated recycling, the new cisterna matures by receiving medial and then trans-Golgi proteins from older cisternae while exporting cis and then medial-Golgi proteins to younger cisternae. Meanwhile, the cisterna progresses through the stack, carrying forward both small and large secretory cargoes. In the final stage of maturation, the cisterna is a TGN element that breaks down into anterograde and retrograde transport carriers.
Lysosomes and endocytic pathways

Phyllis Hanson

Lecture 3

General principles
Properties of lysosomes, delivery of enzymes to lysosomes
Endocytic uptake – clathrin, others
Endocytic pathways – recycling vs. degradation
• Vesicle formation & composition controlled by coat proteins and interacting factors
• Vesicle targeting and fusion controlled by rab proteins, docking/tethering factors, and specific SNARE combinations
• Anterograde and retrograde pathways
Characteristics of Lysosomes

- Major site of intracellular degradation; contain many hydrolytic enzymes
- Acidic (pH 5); maintained by a proton pump
- Semi-permeable membranes due to presence of multiple transporters
- Protease resistant membranes that contain special membrane proteins

How are newly synthesized enzymes targeted to lysosomes?

- Mannose-6-phosphate is sorting signal for lysosomal hydrolases
- Modification of N-linked oligosaccharide in cis-Golgi
- M-6-P is recognized by a receptor in trans-Golgi network

Figure 13–36. Molecular Biology of the Cell, 4th Edition.
Problems with lysosomal function: I-cell disease

- Caused by deficiency in GlcNAc-phosphotransferase, lysosomal enzymes therefore lack M6PR tag
- Leads to secretion of multiple lysosomal enzymes, cells become vacuolated and contain dense inclusion bodies
- Clinical manifestations: severe skeletal and neurological problems, retardation of grow and development, death by 5 yrs
Pathways for internalization at the cell surface

**Figure 1** Multiple portals of entry into the mammalian cell. The endocytic pathways differ with regard to the size of the endocytic vesicle, the nature of the cargo (ligands, receptors and lipids) and the mechanism of vesicle formation.

Conner & Schmid, 2003
Clathrin mediated endocytosis

* predominant endocytic pathway

* membrane and fluid uptake, responsible for most receptor mediated endocytosis

* 2-3% of cell surface occupied by clathrin coated pits

* lifetime of coated pit estimated to be ~1 minute before pinching off as coated vesicle
Clathrin coated vesicle cycle

- Adaptor protein(s) bind to membranes and cargo
- Adaptor protein(s) recruit clathrin, create nascent vesicle
- Clathrin and adaptor proteins are sufficient to form lattices and buds on liposomes, but cooperate with other “accessory” proteins \textit{in vivo}
- “Accessory” proteins regulate coat assembly, membrane fission, and clathrin coat disassembly
Dynamin: GTPase essential for membrane fission

- Self-assembles into tubular polymers at vesicle neck, these undergo conformational change to promote scission
- Also involved in localized actin dynamics
Other pathways for internalization from cell surface

- Caveolae
- Macropinocytosis
- Phagocytosis
- Other clathrin-independent mechanisms
Caveolae (= “little caves”) on the plasma membrane
Caveolar endocytosis

- Minor pathway compared to clathrin mediated endocytosis
- Internalizes membranes enriched in lipid rafts
- Pathway used by GPI-anchored proteins, toxins, viruses
- Internalized caveolae travel to caveosome, ER, Golgi, endosome
- Pathway requires dynamin, actin, and probably others
- Key difference from clathrin pathway is that caveolar “coat” does not disassemble, instead contents diffuse out or dissociate
Macropinocytosis

- Associated with membrane ruffling

- Induced in cells following growth factor stimulation

- Role for Rho-family GTPase and actin-driven formation of membrane protrusions

- Formation of macropinosome follows fusion of membrane protrusions with plasma membrane

**FIGURE 1**
Phase-contrast microscopy of macropinocytosis in mouse macrophages, taken from a series of video images. Minutes elapsed after the left-most panel are indicated at the bottom of each panel. A circular, phase-dense ruffle closes to form a phase-bright, intracellular vesicle that migrates away from the cell margin and towards the nucleus, shrinking *en route*. Bar, 3 μm.
Phagocytosis

- particle triggered engulfment of large structures such as bacteria, yeast, remnants of dead cells, arterial fat deposits
- best in professional “phagocytes”, e.g. macrophages, etc.
Overview of the endocytic pathway

Figure 2 | The early endosome. The figure shows an early endosome containing low-density lipoprotein (LDL)-gold particles endocytosed for 5 minutes (gold particles are visualized as white spots, as contrast was reversed). After internalization, cells were homogenized, crude fractions prepared and deposited on mica plates. Samples were analysed by freeze-etch electron microscopy. (Courtesy of J. Hauser, Washington University, Missouri, USA).
Intracellular itineraries of receptors

• Different receptors follow different pathways once inside cell
  • recycling to plasma membrane is default
  • delivery into lysosome requires signal

• Examples
  • LDL receptor
  • Transferrin receptor
  • EGF receptor
pH controls receptor-ligand interactions

Figure 23-13 Progressive decrease in luminal pH facilitates protein sorting in the endosomal compartment. Interactions of many cargo molecules with their receptors are pH dependent; dissociation places ligands in the luminal space, whereas receptors remain associated with membrane. Geometric considerations, as well as sorting motifs on the receptors, facilitate sorting of membrane from internal volume. Unoccupied receptors whose ligands, such as LDL, have dissociated under the relatively mild acidic conditions encountered in early endosomes are efficiently recycled back to the cell surface. Iron carried by transferrin (Tfn) dissociates at a pH of approximately 6, but apoTfn remains bound to and recycles with its receptor. Mannose-6-phosphate receptors (MPRs) carry their ligands to late endosomes before dissociation at lower pH and recycling back to the TGN. EGF remains bound, and both ligand and receptor (EGFR) are delivered to and degraded in lysosomes. PM, plasma membrane.
LDL (low density lipoprotein particle) receptor: receptor recycles, cargo is degraded

Cell gets amino acids, cholesterol, fatty acids from degraded LDL

Figure 13–46. Molecular Biology of the Cell, 4th Edition.
Transferrin receptor: receptor and transferrin recycle, Fe$^{3+}$ internalized

* Prototypical recycling receptor

* $t_{1/2}$ for recycling $\sim 16$ min

* similar to kinetics of bulk lipid recycling

* receptor recycles 100+ x during lifetime
EGF receptor: receptor, EGF degraded

- Accumulates in coated pits only after ligand binding
- Internalization requires active kinase domain
- Receptor and ligand both delivered to lysosomes & degraded
- Results in receptor down-regulation
Rab conversion to define early vs. late endosomes

Rab5
Rab7
cargo

Rink, Zerial et al., 2005