Secretory Pathway and Membrane Traffic

Questions arising:
0. How do you break the membrane barrier?
1. What happens to a protein as it travels thru the pathway?
2. Why so many different compartments? How are they maintained in spite of intercompartmental flow
3. What are the signals on the protein?
4. What cellular machinery is involved in decoding the signals?
5. How is specificity generated (specificity of cargo, specificity of targeting)?
6. How do you go about studying such complex pathways?
Vesicles everywhere!
Role in transport - e.g., intraGolgi traffic

A schematic representation of four different models of intra-Golgi transport.

a | In the vesicular transport model, coatamer protein complex-I (COP) vesicles carry cargo and move in an anterograde fashion from one Golgi cisterna to the next. 
b | In the cisternal maturation model, the COP vesicles move in a retrograde fashion and function as a retrieving device that is used by Golgi enzymes to maintain their specific and differential localization over the Golgi stack. 
c | The hybrid model proposes that COP vesicles mediate both the anterograde movement of cargo and the retrograde movement of Golgi-resident enzymes, and therefore possibly combines the vesicular transport and cisternal maturation models. 
d | The intercisternal connections model does not involve COP vesicles and proposes that cargo and Golgi-resident enzymes move forwards and backwards, respectively, through tubules that connect the rims and the core of heterologous cisternae in a given Golgi stack.

Structure of the Golgi


3D-Reconstruction from EM serial sections

The Golgi Ribbon: Stacks, vesicles, tubules

Golgi Structure in Three Dimensions: Functional Insights from the Normal Rat Kidney Cell
Mark S. Ladinsky, David N. Mastronarde, J. Richard McIntosh, Kathryn E. Howell, and L. Andrew Staehelin

J. Cell Biol., Volume 144, Number 6, March 22, 1999 1135-1149
Three-dimensional reconstructions of portions of the Golgi complex from cryofixed, freeze-substituted normal rat kidney cells have been made by dual-axis, high-voltage EM tomography at approximately 7-nm resolution. The reconstruction shown here (approximately 1 x 1 x 4 microm³) contains two stacks of seven cisternae separated by a noncompact region across which bridges connect some cisternae at equivalent levels, but none at nonequivalent levels. The rest of the noncompact region is filled with both vesicles and polymorphic membranous elements. All cisternae are fenestrated and display coated buds. They all have about the same surface area, but they differ in volume by as much as 50%. The trans-most cisterna produces exclusively clathrin-coated buds, whereas the others display only nonclathrin coated buds. This finding challenges traditional views of where sorting occurs within the Golgi complex. Tubules with budding profiles extend from the margins of both cis and trans cisternae. They pass beyond neighboring cisternae, suggesting that these tubules contribute to traffic to and/or from the Golgi. Vesicle-filled "wells" open to both the cis and lateral sides of the stacks. The stacks of cisternae are positioned between two types of ER, cis and trans. The cis ER lies adjacent to the ER-Golgi intermediate compartment, which consists of discrete polymorphic membranous elements layered in front of the cis-most Golgi cisterna. The extensive trans ER forms close contacts with the two trans-most cisternae; this apposition may permit direct transfer of lipids between ER and Golgi membranes. Within 0.2 microm of the cisternae studied, there are 394 vesicles (8 clathrin coated, 190 nonclathrin coated, and 196 noncoated), indicating considerable vesicular traffic in this Golgi region. Our data place structural constraints on models of traffic to, through, and from the Golgi complex.
Golgi region of the pancreatic beta cell line, HIT-T15, visualized by high-resolution electron tomography

The Golgi complex with the seven cisternae (cis–trans: C1–C7) is at the center. The color coding is as follows:
- C1, light blue;
- C2, pink;
- C3, cherry red;
- C4, green;
- C5, dark blue;
- C6, gold; and
- C7, bright red. The Golgi is displayed in the context of all surrounding organelles, vesicles, ribosomes, and microtubules: endoplasmic reticulum (ER), yellow; membrane-bound ribosomes, blue; free ribosomes, orange; microtubules, bright green; dense core vesicles, bright blue; clathrin-negative vesicles, white; clathrin-positive compartments and vesicles, bright red; clathrin-negative compartments and vesicles, purple; and mitochondria, dark green.


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Coated Vesicle Budding → Uncoating of vesicles → SNARE-mediated Fusion to target membrane
SNARE-mediated Membrane fusion

Decision points:

1. Cytosol: cytosol or organelle
Decision points:
1. Cytosol: cytosol or Organelle (focus on ER, also mitochondrion and peroxisome and nucleus)
2. ER lumen: modifications, proper folding and transfer to Golgi (conformation, assembly, Bip)
3. transitional ER or cis golgi: return to ER (KDEL sequence)
4. cis golgi: modify for lysosomal targeting
5. cis golgi: transfer to medial golgi
6. medial golgi: modifications and transfer to trans golgi
7. trans golgi: modifications and transfer to TGN
8. TGN: modifications, constitutive or regulated secretion
9. TGN: lysosomal or cell surface targeting (or retrograde)
10. TGN: differential sorting to polar surfaces (apical, basolateral)
11. surface: stable expression/recycling
12. endosomes: return to surface, transcytosis, return to Golgi, degradation

Selected topics in secretion:

Processing of newly synthesized proteins:
cotranslational (ER) & posttranslational (ER, Golgi, TGN, forming secretory vesicles)
A. glycosylation: N-linked (Asn), O-linked (Ser, Thr) and proteoglycans (Ser, Thr) (disaccharides (uronic acid+GalNAc or GlcNAc; sulfation), O-GlcNAc
[GlC: glucose, Gal: galactose, GlcNAc: N-acetylglucosamine, etc.]
B. fatty acylation & polyisoprenylation: palmitate (myristate on cytoplasmic), farnesylation
C. γ-carboxylation (requires: Vitamin K, molecular oxygen, CO₂), propeptide contains recognition element "γCRS", membrane bound carboxylase, γ-carboxyglutamic acid (Gla residues)-clotting

In the 1920s in the US, a bleeding disease in cattle was shown to be due to the consumption of improperly cured sweet clover hay (unspoiled fodder had no effect) and consequently a deficiency in the blood clotting factor prothrombin. At the same time, a prothrombin-deficiency based severe bleeding condition in hens fed a nutrient-depleted diet was discovered and subsequently led to the discovery of vitamin K, for which Doisy and Dam received the Nobel Prize in 1943. Dicumarol (3,3-methylenebis-9-[4-hydroxycoumarin]), the active component in the spoiled sweet clover is derived from oxidation of coumarin (responsible for the sweet smell of the clover) by the action of fungi in the moldy hay. Dicumarol proved to be relatively ineffective as a rodenticide (rat poison). A more potent compound discovered by Link and co-workers was 3-phenylacetyl ethyl 4-hydroxycoumarin. Link assigned patent rights to the Wisconsin Alumni Research Foundation, from which the name warfarin was derived. The first clinical study with warfarin to help prevent deleterious blood clot formation was reported in 1955. In the same year, President Eisenhower was treated with warfarin following a heart attack (blood clots often contribute to heart attacks). Mechanism...
D. hydroxylation: lysine & proline (collagen), asp and asn: 
erthro-ß-hydroxyaspartic acid and erythro-ß-
hydroxyasparagine, posttranslational hydroxylation of asp
and asn, domains homologous to EGF precursor, consensus
sequence: Cys-X-Asp/Asn-X-X-X-X- Phe/Try-X-Cys-X-Cys

E. SO₄, on tyrosine and sugar chains, unique N-link on
hormones from pituitary, also PO₄

F. Cleavage, insulin, preproopiomialocortin - dibasic aa

What are functions of these modifications?
Diverse- folding, targeting, activity

Where do these modifications take place along the pathway?

WHAT HAPPENS ONCE PROTEIN IN LUMEN OF ER?

Intrinsically important

Very useful tool

folding, oligomerization
S-S formation(PDI),
BIP, glycosylation,
trimming (2-15 min for
HA)

How to assay folding, oligo-
ermerization, glycosylation, etc.?

*Folding, Trimerization and Transport are
Sequential Events in the Biogenesis of
Influenza Virus Hemagglutinin* Copeland et.
Membrane Trafficking and the Secretory Pathway:

ER->Golgi->out

Vesicular Stomatitis Virus (VSV) G-protein:
Trimeric Glycoprotein

Ts045: temperature-sensitive folding in the ER, VSVG-GFP: Green Fluorescent Protein (GFP) labeling, real-time microscopy

Formation and Life History of ER-to-Golgi Transport Intermediates

The ts045 VSVG-GFP confocal images acquired at 8.6-s time intervals after shift from 40 to 32°C in COS cells ER->pre-Golgi intermediates
Role of Microtubules in ER-to-Golgi Traffic

Images, acquired at 4-min intervals, of VSVG-GFP-expressing COS cells treated with nocodazole to depolymerize microtubules prior to temperature shift from 40 to 32°C. (Efficient export out of the ER into peripheral pre-Golgi elements.

Fate of Pre-Golgi Intermediates upon Reaching the Golgi Complex

VSVG-GFP expressing COS cells whose Golgi-associated fluorescence was photobleached with high-intensity laser light at the time cells were warmed from 15 to 32°C.
Vesicle Coat Disruption (BFA) or Microtubule Depolymerization (nocodazole) Effects on Golgi Morphology: HeLa cells expressing GalTase–GFP (resident Golgi oligosaccharide processing enzyme)

Left: Brefeldin A (BFA) induces the formation of a dynamic Golgi tubule network within 5–8 min of adding the drug. When one or more of the Golgi tubules fused with the ER, Golgi membranes redistributed rapidly into the ER, leaving no Golgi structure behind.

Right: Small Golgi fragments are generated at significant distances from the central Golgi region within 15 min after nocodazole treatment.

Glycosylation

Function:
Folding, Conformation, Activity, Stability, Sorting

N-linked

O-linked

Lipid-linked (glycolipids)

Proteoglycans (O-linked)
Initial N-glycosylated Protein, Oligosaccharide Structure

\[ \text{GlcNAc} = N\text{-Acetylglucosamine} \]
\[ \text{Man} = \text{Mannose} \]
\[ \text{Glc} = \text{Glucose} \]

\[ \text{Conserved} \]
\[ \text{Variable, often removed} \]

Gal = galactose; SA = sialic acid
GLYCOCONJUGATES:
Glycoproteins & Glycolipids

- Laminin
- Lassa fever
- Leprosy (micobac.)

PROTEOGLYCAN SUGARS (GAGS):
Nucleotide Sugar Precursors are the substrates of the Glycosyl Transferases that generate Glycoconjugates

UDP-GlcNAc (2-5% of all cellular glucose)

Scheme depicting the synthesis of nucleotide sugars (boxed). Main routes for further use of UDP-GlcNAc are indicated. Doubleheaded arrows represent several enzymatic steps not depicted in detail. Adapted from Schachter (1978).


UDP-GlcNAc-kinase

O-linked GlcNAc

GPI-linker

Golgi-glycosylation

1. GlcNAc-kinase

2. 4-epimerase
Levels of UDP-GlcNAc, and the subsequent addition of O-linked beta-N-acetylglucosamine (O-GlcNAc) to Ser/Thr residues, is involved in regulating nuclear and cytoplasmic proteins in a manner analogous to protein phosphorylation. O-GlcNAc protein modification is essential for life in mammalian & plant cells, highlighting the importance of this simple post-translational modification in basic cellular regulation. Recent research has highlighted key roles for O-GlcNAc serving as a nutrient sensor in regulating insulin signaling, the cell cycle, and calcium handling, as well as the cellular stress response.

Zachara NE, Hart GW. Cell signaling, the essential role of O-GlcNAc! Biochim Biophys Acta. 2006 May-Jun;1761(5-6):599-617.
### Functional groups and proteins modified by O-GlcNAc

<table>
<thead>
<tr>
<th>Protein group</th>
<th>Example(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcription factors</td>
<td>Sp1, AP-1 (c-fos and c-jun), CTF, hepatocyte nuclear factor 1, pancreas-specific transcription factor, serum response factor, p53, β-catenin, ELF-1, NFκ B, PAX-6, Ocat1, c-myc, RB, V-erbB, and ER-β. FOXO</td>
</tr>
<tr>
<td>Polymerase(s)</td>
<td>Large subunit of RNA Pol II.</td>
</tr>
<tr>
<td>Phosphatases, kinases and adapter proteins</td>
<td>Nuclear tyrosine phosphatase p65, casein kinase II, AKT, insulin receptor substrate 1, 2, GSK-3β, and PI3-kinase.</td>
</tr>
<tr>
<td>Cytoskeletal proteins</td>
<td>Keratins 8, 13, 18, neurofilaments H, M, L, talin, vinculin, Band 4.1, ankryinG, E-cadherin, synapsin, myosin, collagen, α-tubulin, dynein LC1, MAP 2 and 4, Tau, β-actin, Pericolo, AP-3 and -180, β-APP, and adenovirus type 2 and 5 fiber proteins.</td>
</tr>
<tr>
<td>Chaperones</td>
<td>Hsp 27, HspC70, and Hsp90.</td>
</tr>
<tr>
<td>Enzymes</td>
<td>eNOS, GS, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, eNOS, pyruvate kinase, UDP-glucose pyrophosphorylase, and OGT.</td>
</tr>
</tbody>
</table>

### Proteins modified by O-GlcNAc

<table>
<thead>
<tr>
<th>Functional subgroup</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear pore proteins (NUP)</td>
<td>p54, p62, p153, p155, p180, p153, p214, p358</td>
</tr>
<tr>
<td>Chromatin</td>
<td>Sp1, AP-1 (c-fos and c-jun), CTF, hepatocyte nuclear factor 1, pancreas-specific transcription factor, serum response factor, c-Myc, p53, E2F, β-catenin, NFκ B, ELF-1, PAX-6, enhancer factor 2D, human C2, transcription factor KIAA0044, Ocat1, Fakoglobins, YY1, P63, CREB, RB, PKCδ</td>
</tr>
<tr>
<td>RNA binding proteins</td>
<td>hnRNP G (L-antigen), Ewing sarcoma RNA-binding protein, eukaryotic initiation factor 4A1, elongation factor 1-a, 40S ribosomal protein S24</td>
</tr>
<tr>
<td>Phosphatases, kinases and adapter proteins</td>
<td>Nuclear tyrosine phosphatase p65, casein kinase II, AKT, insulin receptor substrate 1, 2, GSK-3β, and PI3-kinase</td>
</tr>
<tr>
<td>Elements</td>
<td>Keratins 8, 13, 18, neurofilaments H, M, L, Band 4.1, Talin, Vinculin, AnkryinG, Synapsin, Myosin, E-cadherin, Cofilin, MAP 2 and 4, Tau, β-actin, Pericolo, AP-3 and -180, β-APP, and adenovirus type 2 and 5 fiber proteins</td>
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</tbody>
</table>

~100-500 proteins subject to O-GlcNAcylation

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Zachara NE, Hart GW. Cell signaling, the essential role of O-GlcNAc! Biochem Biophys Acta. 2006 May-Jun;1761(5-6):599-617.
Figure 3. The O-GlcNAc modification of several proteins involved in glucose metabolism in adipose tissue may lead to peripheral insulin resistance. Increased flux through the hexosamine biosynthetic pathway (HBP) results in greater UDP-GlcNAc levels upon which OGT is highly dependent. The increased O-GlcNAcylation of glycogen synthase (GS) reduces glycogen storage. Increased O-GlcNAcylation of IRS and PI3K leads to suppressed AKT activity and Glut4-mediated glucose uptake.

Regulatory Crosstalk

Fig. 3. The reciprocal relationship between O-GlcNAc and protein phosphorylation is indicated. In this model, O-GlcNAc and phosphorylation compete for the same Ser/Thr residues and thus regulate the levels of each other.
N-Glycosylation in the Secretory Pathway

**Step 2:** GlcNAc Transferase I; required for subsequent step 3 (Mannosidase II)

**Reversible. Why?**

Step 1: GlcNAc Transferase I; required for subsequent step 3 (Mannosidase II)
**Tools:**

**Inhibitors of glycosylation:**

- **tunicamycin**: UDP-GlcNAc:dolichyl-P GlcNAc-P transferase, ER, nucleoside antibiotic blocks all N-glycosylation, no chemical analogue for O-links (step 1); however, IdID cells have mutation (reversible block)

- **castanospermine**: plant alkaloid, glucosidase I (step 2)

- **1-deoxynojirymmetric**: glucosidases I & II, delays exit from ER (steps 2 & 3)

- **deoxymannojirimycin**: mannosidase I, -Man₅GlcNAc₂ chains (step 4)

- **swainsonine**: indolizidine alkaloid, mannosidase II in Golgi, gives hybrid structures
Tools used for marking probable site of glycoprotein in ER->Golgi pathway:

- Tunicamycin sensitive electrophoretic mobility: probably has N-linked chains, drug must be added during synthesis
- Endoglycosidase F or N-glycanase sensitive: protein has N-linked chains, Asn->Asp

- Endoglycosidase H sensitive - Endo H used with proteins whose fully mature forms have "complex-type" N-linked chains. Endo H sensitive protein (usually shift in electrophoretic mobility of metabolically labeled protein) has probably not been processed through the medial Golgi - during synthesis of complex N-linked chain, the precursor forms are Endo H sensitive, after addition of GlcNAc (by GlcNAc transferase I, step 2) and subsequent removal of a specific mannose in the medial Golgi (step 3), the chain becomes Endo H resistant. N-linked chains whose normal mature form is hybrid or high mannose chains are always Endo H sensitive. Cuts between GlcNAcs.
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• tunicamycin sensitive electrophoretic mobility: probably has N-linked chains, drug must be added during synthesis
• Endoglycosidase F or N-glycanase sensitive: protein has N-linked chains, Asn→Asp
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• Sialidase (neuraminidase) sensitive: protein N- and/or O-linked chains have been modified with sialic acid, usually occurs in the trans Golgi or TGN.
• O-glycanase: protein may have "mucin-type" (GalNAc-attached) O-linked chains, specific for O-GalNAc-Gal, usually must treat with sialidase first to remove any blocking sialic acid.
• Alkaline borohydride sensitive: protein may have "mucin-type" (GalNAc-attached) O-linked chains.
• Oligosaccharide metabolic labeling with [3H]glucosamine added to cells.

Pulse ([35S]met/cys) /Chase, Immunoprecipitation, SDS-PAGE Analysis of the Biosynthesis of the LDL Receptor in Cultured Cells: Several N-linked chains, many O-linked chains

| Temp (°C) | 34 |
| Pulse (h) | 0.5 |
| Chase (h) | 0 | 1 | 3 | 6 | 8 |

- LDL/complex N-linked chain
- O-linked cluster
- N-linked
Estimate ~6 chains, actually 11 (mutagenesis) [2 essential]

Pulse Chase Analysis
HDL Receptor (mSR-BI)

?????
11 N-linked chains, Why no shift?
WHY IS THERE SUCH COMPLEXITY IN GLYCOSYLATION?
E.G., N-LINKED PROCESSING IN THE ER, 3 Glucoses added,
only to be removed in the ER!!

ER Quality Control:
Proper protein folding
Glucose removal and addition (3a)
Molecular Chaperones in the ER:
Glucose is ‘unfolded’ signal
UDP-Glucose:Glycoprotein Glucosyltransferase
is the folding ‘sensor’

The calnexin/calreticulin cycle. Immediately after addition of the core glycan to a growing polypeptide chain by oligosaccharyl transferase (OST), the outermost of the three glucose residues (n) is removed by glucosidase I. Soon thereafter, glucosidase II removes the middle glucose (m). Via the monoglycosylated core glycans thus generated, the glycoprotein binds to calnexin (CNX) and calreticulin (CRT). These sequester the nascent or newly synthesized chains and expose them to ERp57, a thiol-disulfide oxidoreductase that provides assistance during disulfide bond formation. When glucosidase II removes the remaining glucose (l), the glycoprotein dissociates from calnexin and calreticulin. The protein now encounters one of three possible fates. If properly folded, it is free to leave the ER. Exit may be assisted by mannose lectins, such as ERGIC-53, VIP36, and VIPL. If it is incompletely folded, UDP-Glc:glycoprotein glucosyltransferase uses UDP-glucose transported by a UDP-glucose/UMP exchanger from the cytosol to reglucosylate the high-mannose glycans located in improperly folded regions. Through these glycans, the glycoprotein rebinds to calnexin and calreticulin. The third fate is ER-associated degradation (ERAD) after retrotranslocation of the misfolded glycoprotein to the ER most likely through the translocon complex. ERAD of glycoproteins occurs when they have stayed in the ER lumen for some time and when they are recognized by a putative lectin (EDEM) because they have lost a mannose (i) through the action of ER mannosidase I. Red triangles are glucose residues. Abbreviations used are EDEM, ER degradation-enhancing mannosidase-like protein; VIP36, vesicular integral protein 36; VIPL, VIP36-like protein; ERAD, ER-associated protein degradation; ERGIC, ER-Golgi intermediate compartment; and ERp57, ER protein 57.

OTHER SIGNALS: SORTING

<table>
<thead>
<tr>
<th>Signal Sequence†</th>
<th>Proteins with Signal</th>
<th>Signal Receptor</th>
<th>Vesicles That Incorporate Signal-Bearing Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys-Asp-Glu-Leu (KDEL)</td>
<td>ER-resident luminal proteins</td>
<td>KDEL receptor in cis-Golgi membrane</td>
<td>COP1</td>
</tr>
<tr>
<td>Lys-Lys-X-X (KKXX)</td>
<td>ER-resident membrane proteins (cytosolic domain)</td>
<td>COPα and β subunits</td>
<td>COP1</td>
</tr>
<tr>
<td>Di-acidic (e.g., Asp-X-Glu)</td>
<td>Cargo membrane proteins in ER (cytosolic domain)</td>
<td>COPII / subunit</td>
<td>COPII</td>
</tr>
<tr>
<td>Mannose 6-phosphate (M6P)</td>
<td>Soluble lysosomal enzymes after processing in cis-Golgi</td>
<td>M6P receptor in trans-Golgi membrane</td>
<td>Clathrin/AP1</td>
</tr>
<tr>
<td></td>
<td>Secreted lysosomal enzymes</td>
<td>M6P receptor in plasma membrane</td>
<td>Clathrin/AP2</td>
</tr>
<tr>
<td>Asn-Pro-X-Tyr (NPXY)</td>
<td>LDL receptor in the plasma membrane (cytosolic domain)</td>
<td>AP2 complex</td>
<td>Clathrin/AP2</td>
</tr>
<tr>
<td>Tyr-X-X-Φ (YXXΦ)</td>
<td>Membrane proteins in trans-Golgi (cytosolic domain)</td>
<td>AP1 (μ1 subunit)</td>
<td>Clathrin/AP1</td>
</tr>
<tr>
<td></td>
<td>Plasma membrane proteins (cytosolic domain)</td>
<td>AP2 (μ2 subunit)</td>
<td>Clathrin/AP2</td>
</tr>
<tr>
<td>Leu-Leu (LL)</td>
<td>Plasma membrane proteins (cytosolic domain)</td>
<td>AP2 complexes</td>
<td>Clathrin/AP2</td>
</tr>
</tbody>
</table>

†X = any amino acid; Φ = hydrophobic amino acid. Single-letter amino acid abbreviations are in parentheses.
Caveat Emptor:
If it can be more complex than we have proposed, it probably is!
For Example
Coated Vesicle Transport Mechanisms

Roles of Coats and Coat Associated Proteins:
Endocytosis and AP2

![Diagram of coated vesicle budding](image)

TABLE 17-1  Coated Vesicles Involved in Protein Trafficking

<table>
<thead>
<tr>
<th>Vesicle Type</th>
<th>Coat Proteins</th>
<th>Associated GTPase</th>
<th>Transport Step Mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPII</td>
<td>Sec23/Sec24 and Sec13/Sec31 complexes, Sec16</td>
<td>Sar1</td>
<td>ER to cis-Golgi</td>
</tr>
<tr>
<td>COPI</td>
<td>Coatomers containing seven different COP subunits</td>
<td>ARF</td>
<td>cis-Golgi to ER, Later to earlier Golgi cisternae</td>
</tr>
<tr>
<td>Clathrin and adapter proteins*</td>
<td>Clathrin + AP1 complexes</td>
<td>ARF</td>
<td>trans-Golgi to endosome</td>
</tr>
<tr>
<td></td>
<td>Clathrin + GGA</td>
<td>ARF</td>
<td>trans-Golgi to endosome</td>
</tr>
<tr>
<td></td>
<td>Clathrin + AP2 complexes</td>
<td>ARF</td>
<td>Plasma membrane to endosome</td>
</tr>
<tr>
<td></td>
<td>AP3 complexes</td>
<td>ARF</td>
<td>Golgi to lysosome, melanosome or platelet vesicles</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.

Caveolae  Caveolin  From Plasma Membrane & others
Coated Vesicle-Mediated Transport

Clathrin Coated Pit/Vesicle-Mediated Endocytosis
Clathrin Coats on surface endocytic vesicles (fast freeze, deep etch)

Figure 2 Image reconstruction of a clathrin hexagonal barrel (heavy chains only) at 7.9 Å resolution. There are 36 clathrin triskelions in the structure, which has D6 symmetry. Thus, there are three symmetry-independent triskelions (or nine symmetry-independent legs). The coloured triskelions show one choice of the three independent triskelions. Noisy central density, from spatially disordered and substoichiometric AP-2 complexes, has been flattened.

Clathrin Assembly Movie (T. Kirchhausen)

http://www.cell.com/content/article/abstract?uid=PIIS0092867402007353
Direct siRNA depletion of clathrin blocks transferrin receptor and EGFR (YXXφ-type sorting signals) endocytosis, as well as LDLR (NPXY-type sorting signals) endocytosis

Depletion of either of 2 AP2 subunits (μ2 or α) dramatically reduces surface clathrin coats (AP2), but not Golgi (AP1), coats and blocks transferrin endocytosis

BUT did not block EGFR or LDLR (chimera) endocytosis!!!!

Inactivation of AP-2 by overexpression of the adaptor-associated kinase (AAK1) led to similar results

Reviewed in:

end
Autosomal Recessive Hypercholesterolemia:
Similar to LDLR negative Familial Hypercholesterolemia
High Plasma LDL, atherosclerosis, CHD, early death

Wild type  LDLR -/-  ARH -/-

αARH

αLDLR

LDLR

Extracellular space

Coated pit

CLATHRIN

AP-2

Cytosol
Current Hypothesis: Coiled-Coil long Golgins (GM130, giantin) tether vesicles to target membranes prior to SNARE-mediated fusion


Proposed Membrane Tethering Systems at the Golgi

Short B, Haas A, Barr FA. Golgins and GTPases, giving identity and structure to the Golgi apparatus. Biochim Biophys Acta. 2005 Jul 10;1744(3):383-95