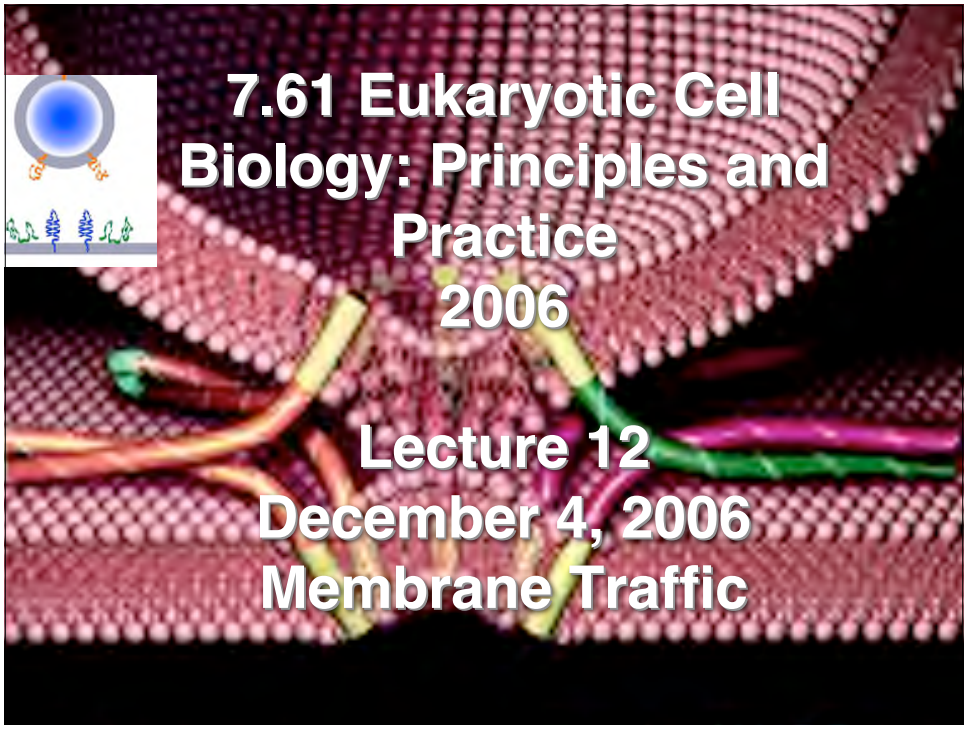
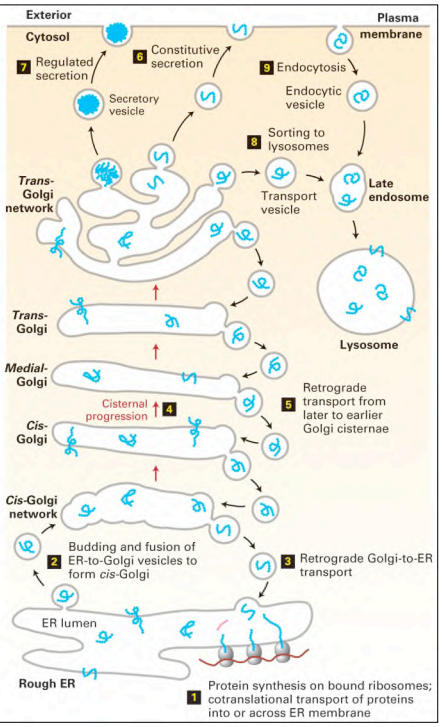
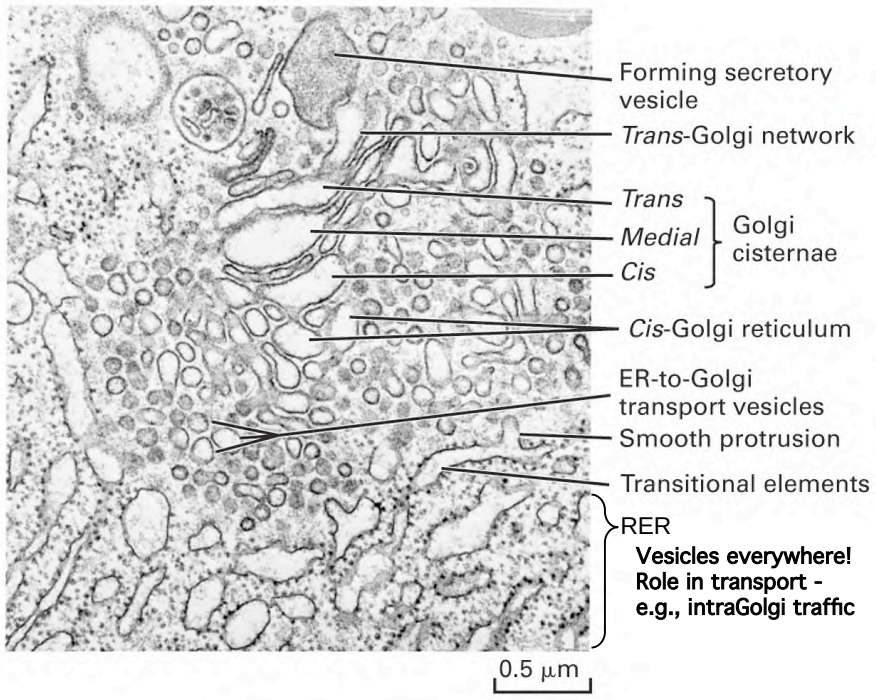


Secretory Pathway and Membrane Traffic

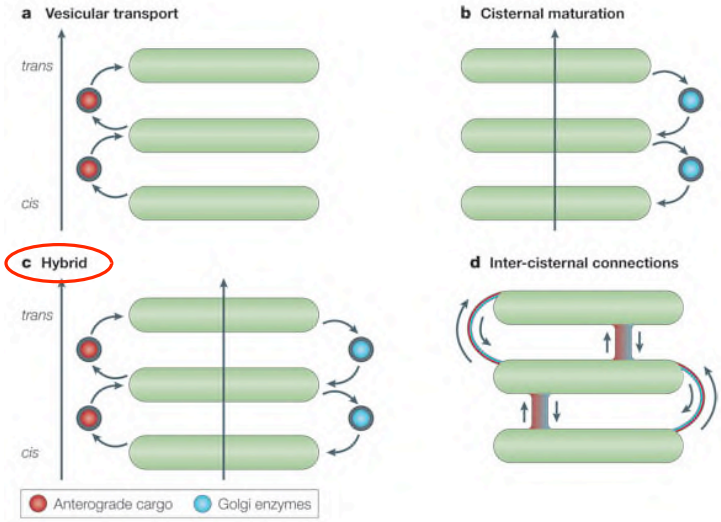
Questions arrising:

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 machinary 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?





Schematic representation of four different models of intra-Golgi transport.



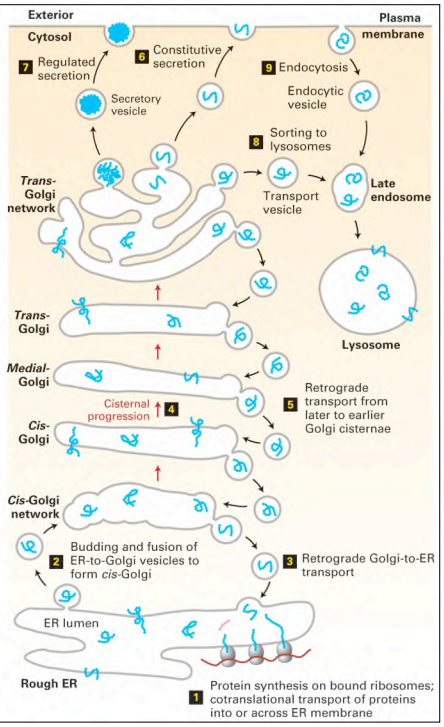
A schematic representation of four different models of intra-Golgi transport. **a** | In the vesicular transport model, coatomer protein complex-I (COPI) vesicles carry cargo and move in an anterograde fashion from one Golgi cisterna to the next. **b** | In the cisternal maturation model, the COPI vesicles 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 COPI 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 COPI 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.

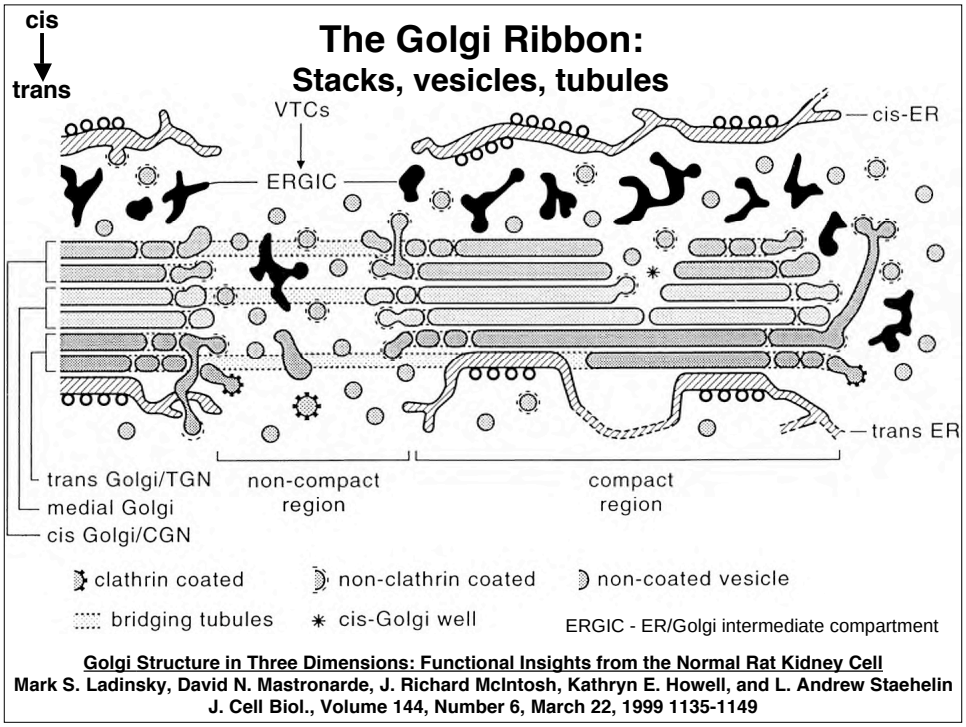
Rabouille C, Klumperman J. Opinion: The maturing role of COPI vesicles in intra-Golgi transport. Nat Rev Mol Cell Biol. 2005 Sep 15;

Structure of the Golgi

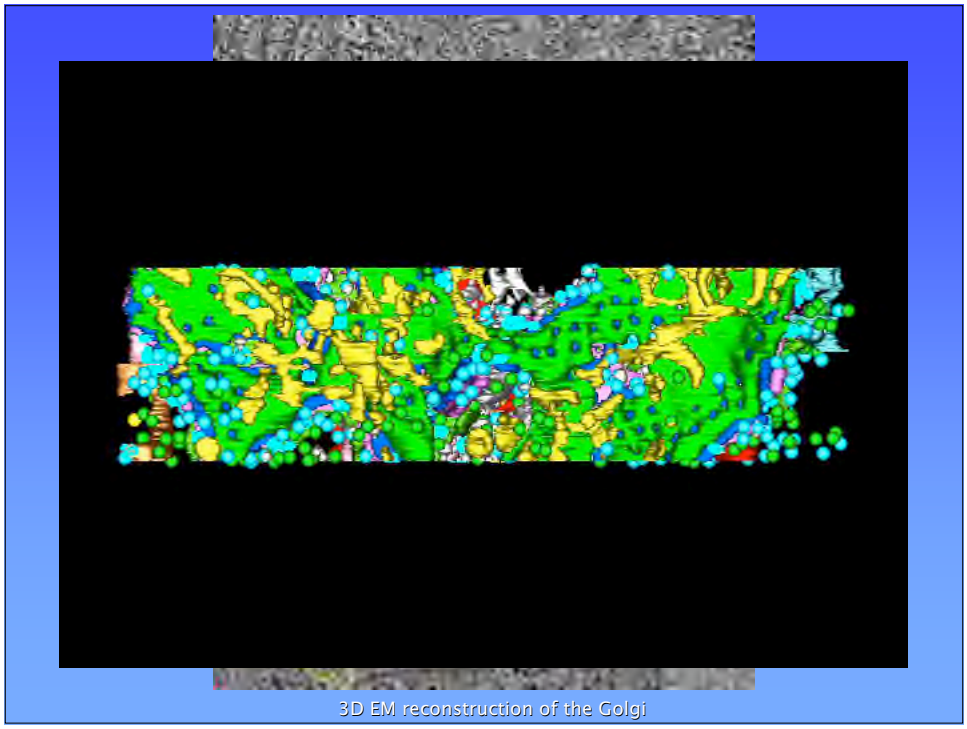
http://www.rkm.com.au/CELL/organelles/organelleimages/golgi.jpg

3D-Reconstruction from EM serial sections

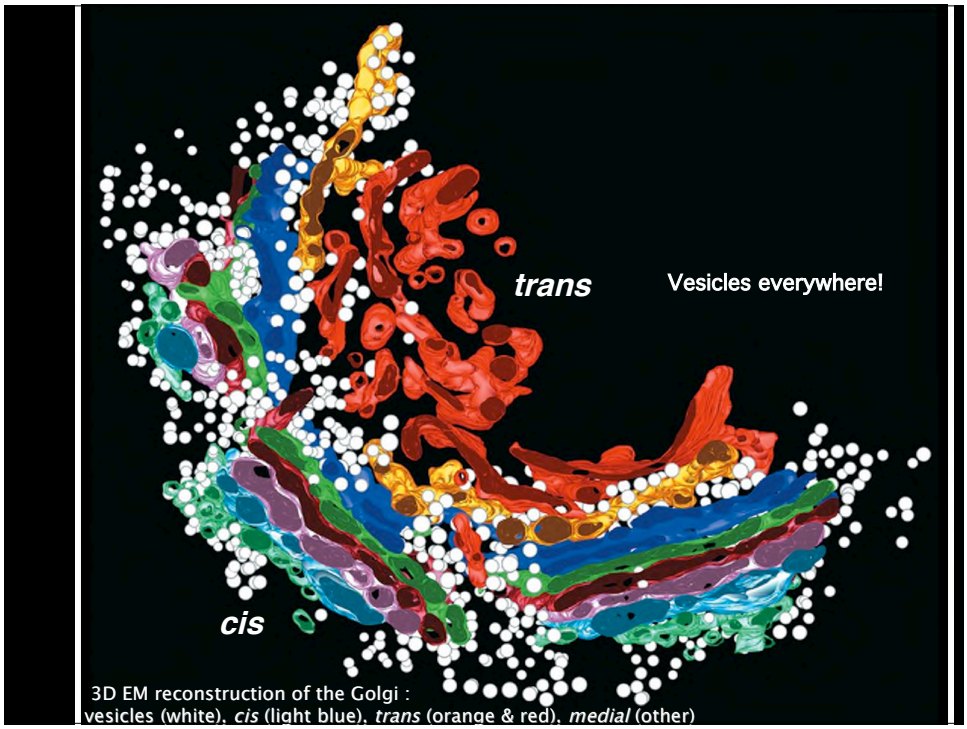




Golgi Structure in Three Dimensions: Functional Insights from the Normal Rat Kidney Cell
Mark S. Ladinsky, David N. Mastronarde, J. Richard McIntosh, Kathryn E. Howell, and L. Andrew Staehelin
J. Cell Biol., Volume 144, Number 6, March 22, 1999 1135-1149



3D EM reconstruction of the Golgi

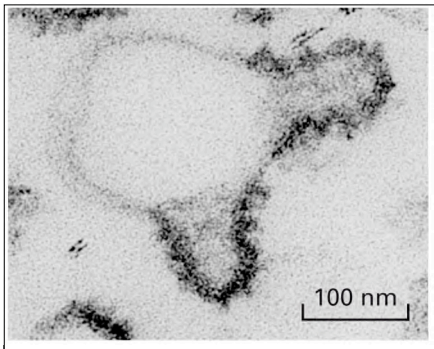


3D EM reconstruction of the Golgi :
vesicles (white), *cis* (light blue), *trans* (orange & red), *medial* (other)

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.

Marsh BJ. Lessons from tomographic studies of the mammalian Golgi. Biochim Biophys Acta. 2005 Jul 10;1744(3):273-92



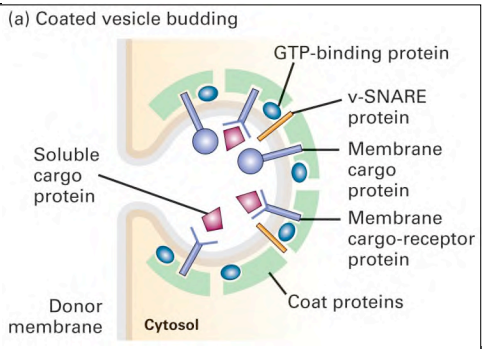
Coated Vesicle Budding

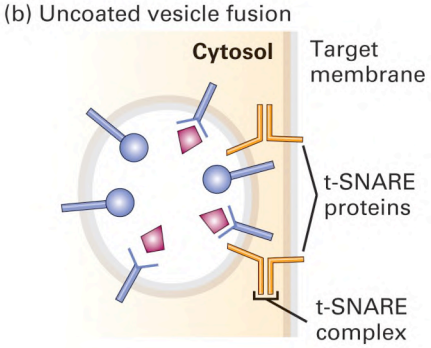
↓

Uncoating of vesicles

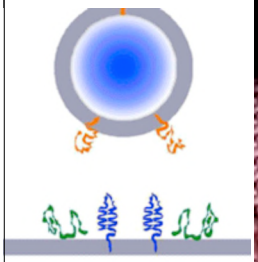
↓

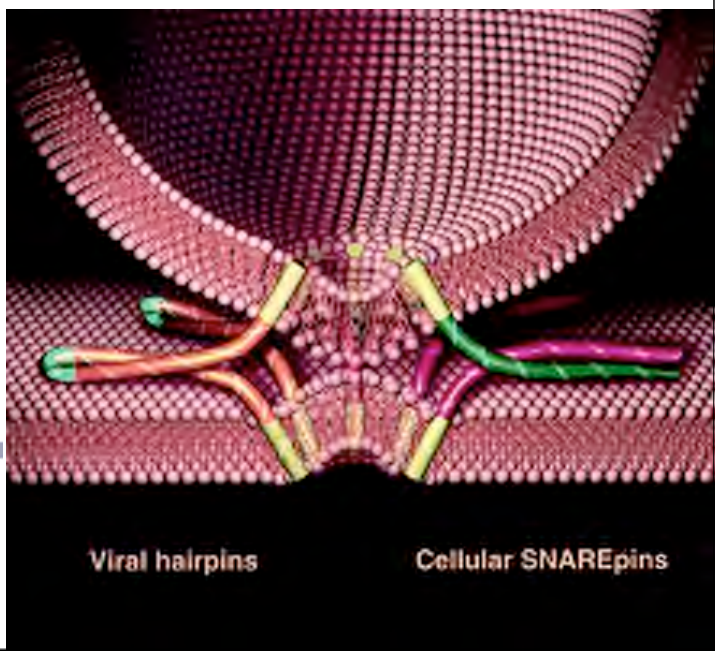
SNARE-mediated Fusion to target membrane





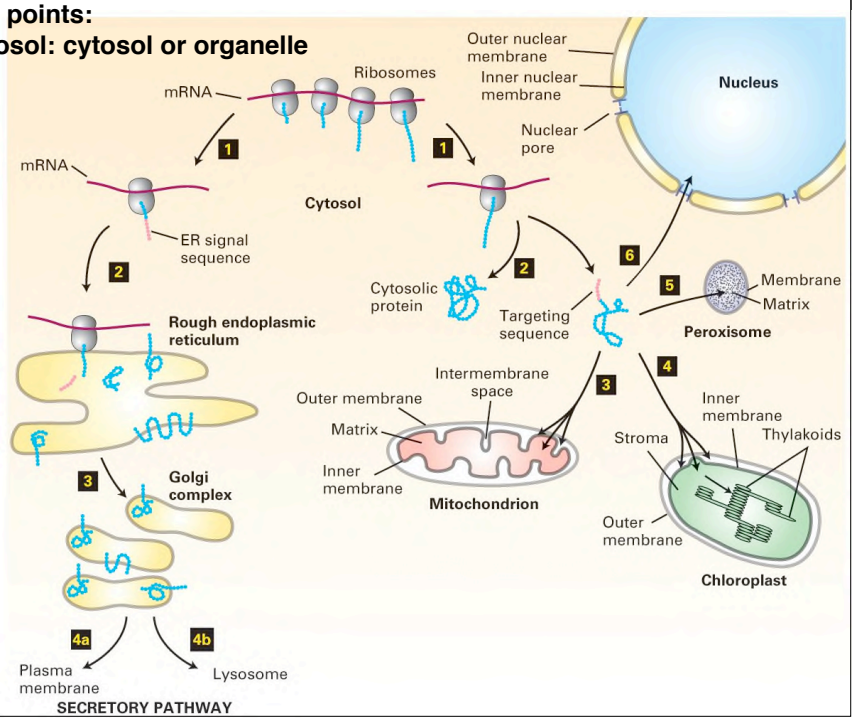
SNARE-mediated Membrane fusion

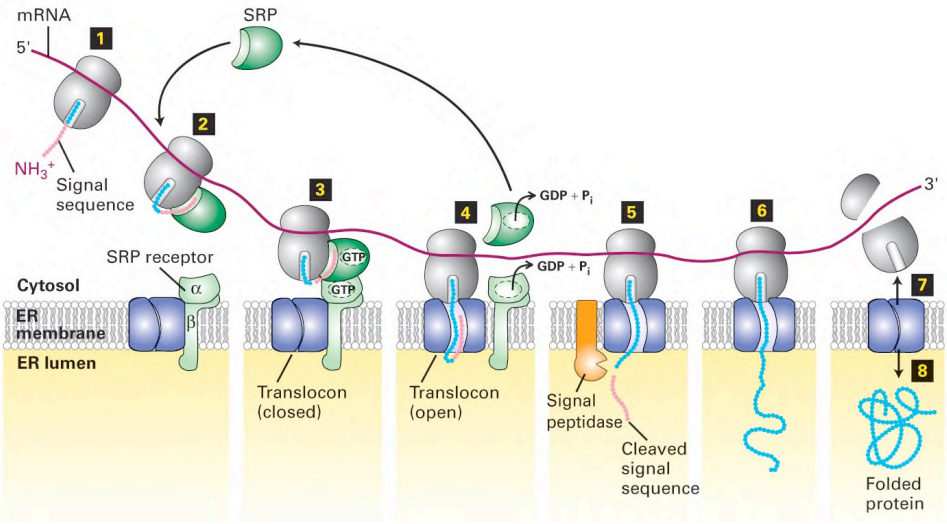




Decision points:

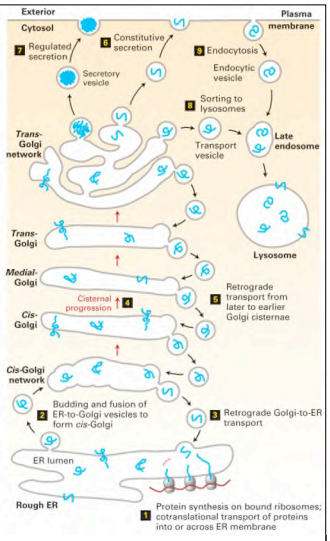
1. Cytosol: cytosol or organelle





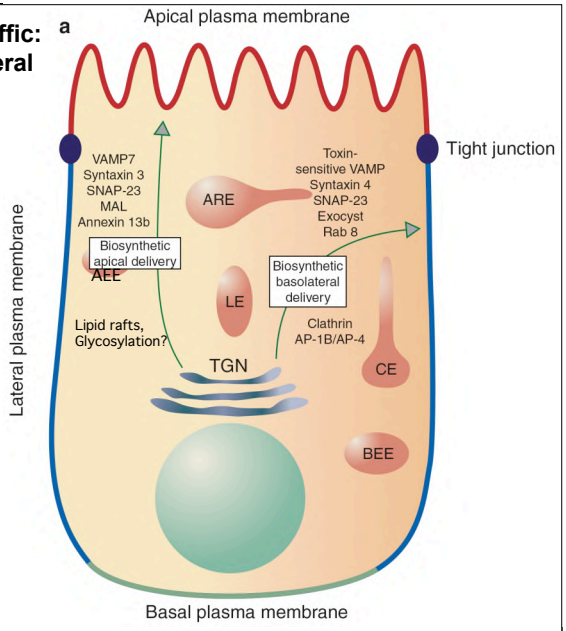
Decision points:

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**



Polarized epithelial membrane traffic: Biosynthetic Apical and Basolateral Sorting

AEE, apical early endosome
ARE, apical recycling endosome
LE, late endosome
CE, common endosome
BEE, basolateral early endosome

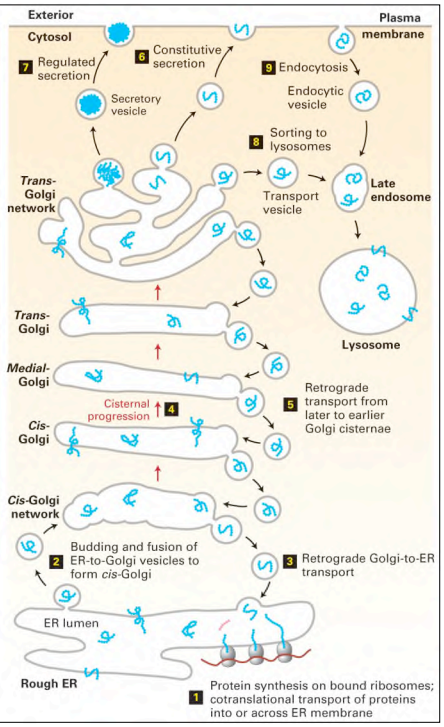


The apical surface is red,basal and lateral portions of the basolateral surface are green and blue, respectively. Known components of the trafficking machinery specific to each pathway are indicated. AEE, apical early endosome; ARE, apical recycling endosome; BEE, basolateral early endosome; CE, common endosome; LE, late endosome.

Polarized epithelial membrane traffic: conservation and plasticity. Mostov K, Su T, ter Beest M. Nat Cell Biol. 2003 5(4):287-93.

Selected topics in secretion:

Processing of newly synthesized proteins:
cotranslational (ER) & posttranslational (ER, Golgi, TGN, forming secretory vesicles)

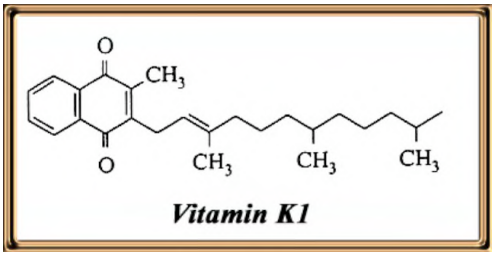


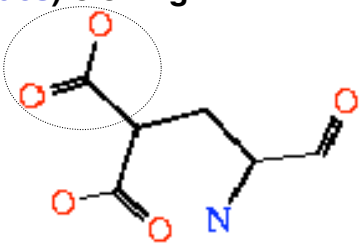
A. glycosylation: N-linked (Asn), O-linked (Ser,Thr) and proteoglycans (Ser,Thr) (disacharides (uronic acid+GalNAc or GlcNAc; sulfation), O-GlcNAc

[Glc: glucose, Gal: galactose, GlcNAc: N-acetylglucosamine, etc….]

B. fatty acylation & polyisoprenylation: palmitate (myristate on cytoplasmic), farnesylation

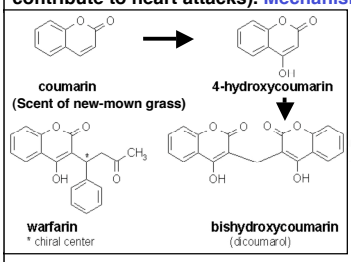
C. γ–carboxylation (requires: Vitamin K, molecular oxygen, CO_2), propeptide contains recognition element "γCRS", membrane bound carboxylase), γ-carboxyglutamic acid (Gla residues)-clotting

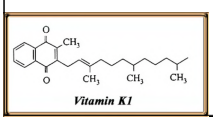


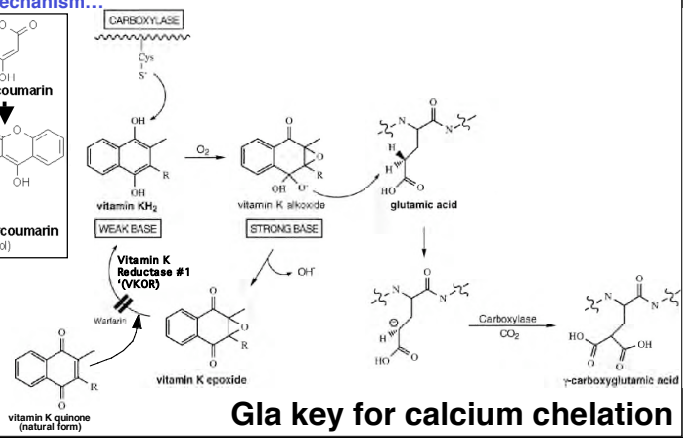


γ-carboxyglutamic acid (Gla)

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…







Gla key for calcium chelation

D. hydroxylation: lysine & proline (collagen), asp and asn: erythro-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. SO_4, on tyrosine and sugar chains, unique N-link on hormones from pituitary, also PO_4

F. Cleavage, insulin, preproopiomelanocortin - dibasic aa

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

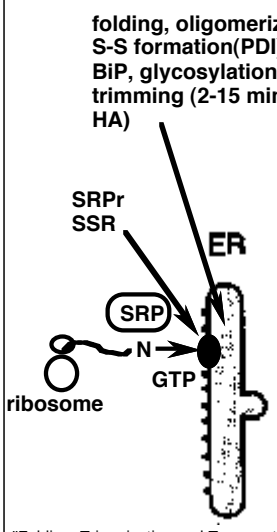
Where do these modifications take place along the pathway?

WHAT HAPPENS ONCE PROTEIN IN LUMEN OF ER?

Intrinsically important

Very useful tool

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



"Folding, Trimerization and Transport are Sequential Events in the Biogenesis of Influenza Virus Hemagglutinin" Copeland et. al. Cell 53:197-209. 1988

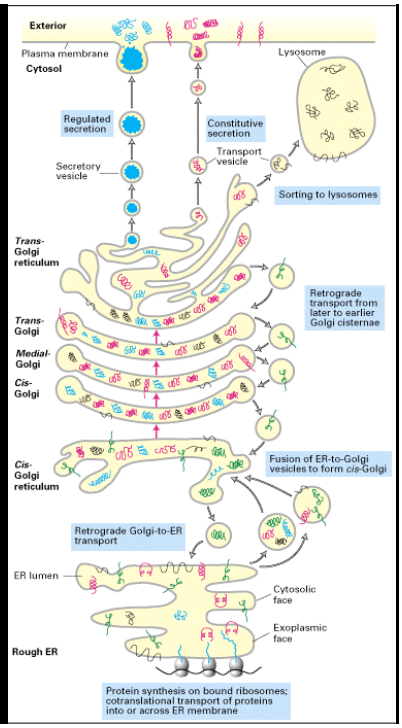
Membrane Trafficking and the Secretory Pathway:

ER->Golgi->out

Vesicular Stomatitis Virus (VSV) G-protein:

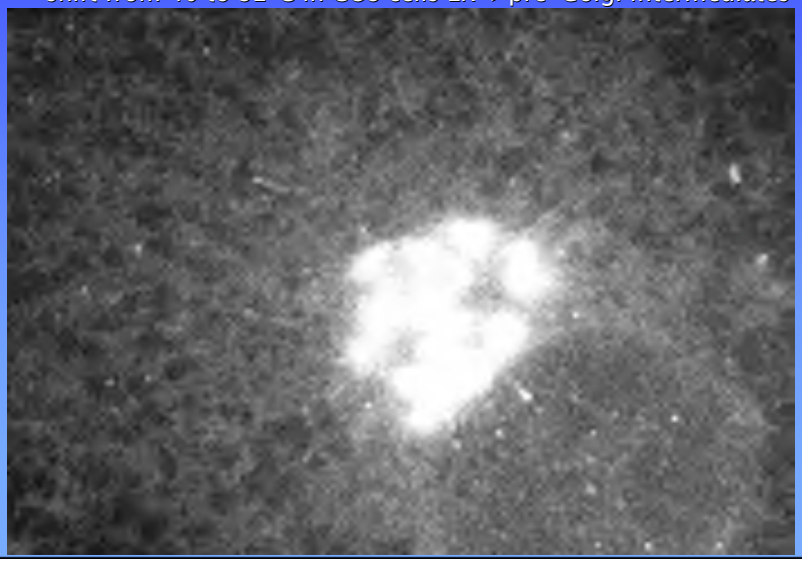
Trimeric Glycoprotein

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



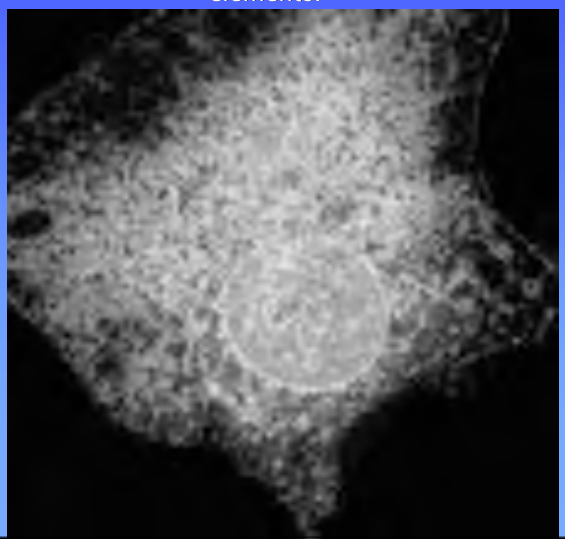
Formation and Life History of ER-to-Golgi Transport Intermediates

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



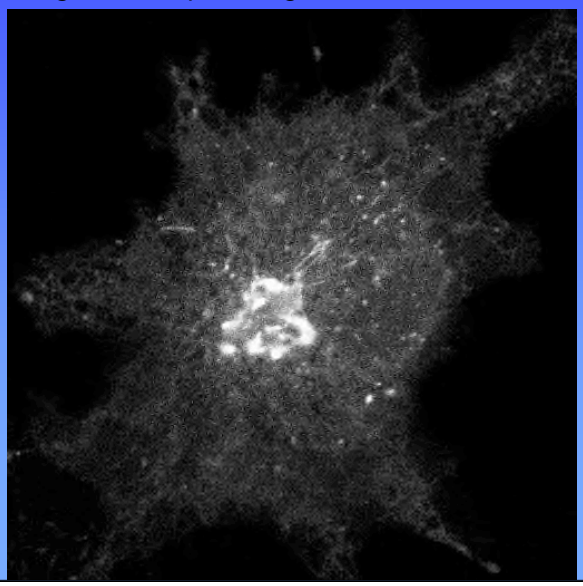
Role of Microtubules in ER-to-Golgi Traffic

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

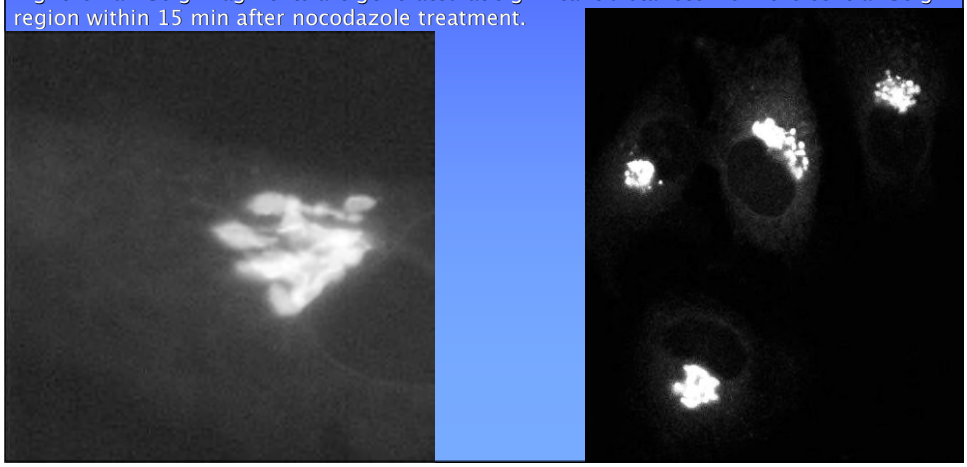
VSVG-GFP expressing COS cells whose Golgi-associated fluorescence was photobleached with high-intensity laser light at the time cells were warmed from 15 to 32°C.



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

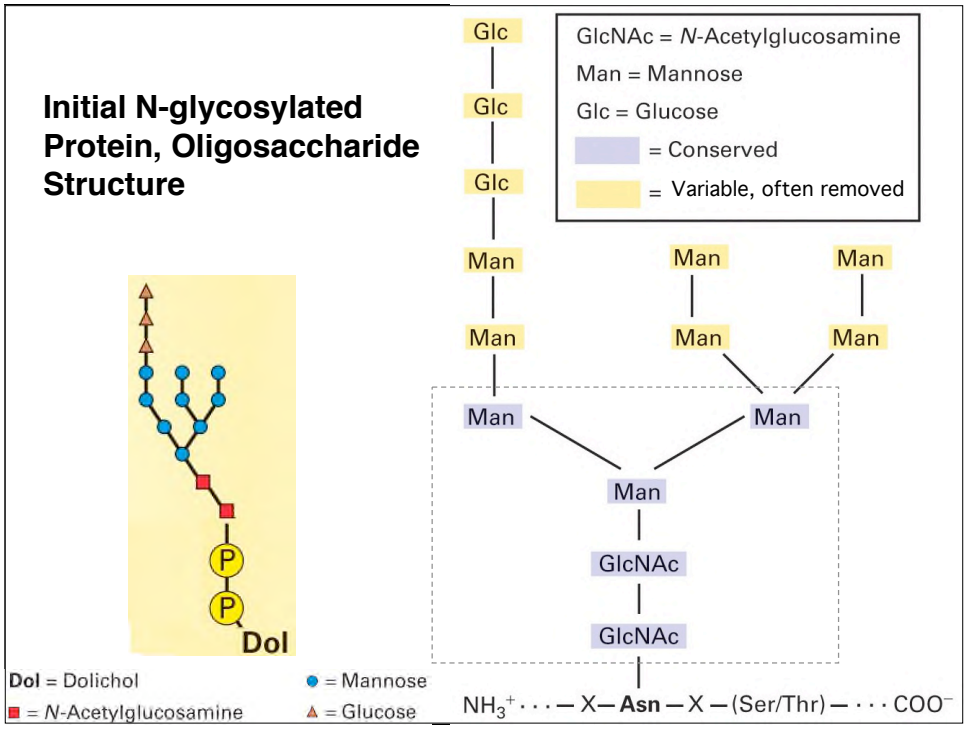
N-linked

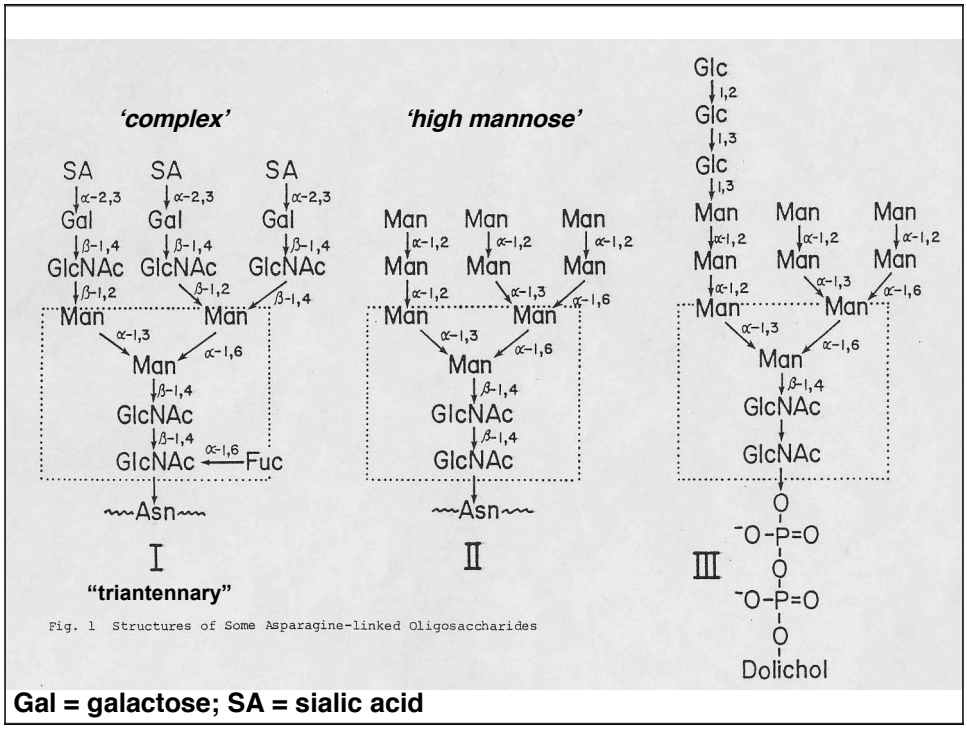
O-linked

Lipid-linked (glycolipids)

Proteoglycans (O-linked)

Initial N-glycosylated Protein, Oligosaccharide Structure





Gal = galactose; SA = sialic acid

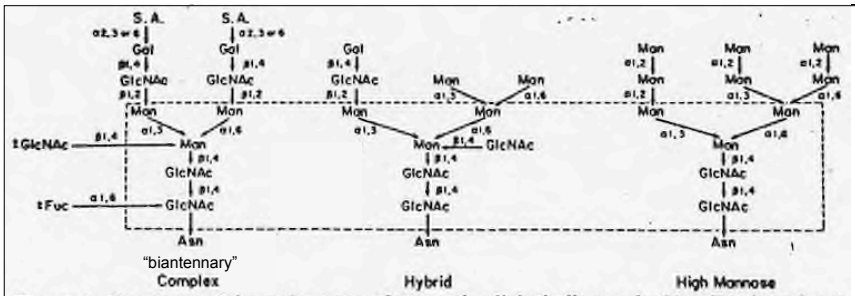


Figure 1 Structures of the major types of asparagine-linked oligosaccharides. The boxed area encloses the pentasaccharide core common to all N-linked structures.

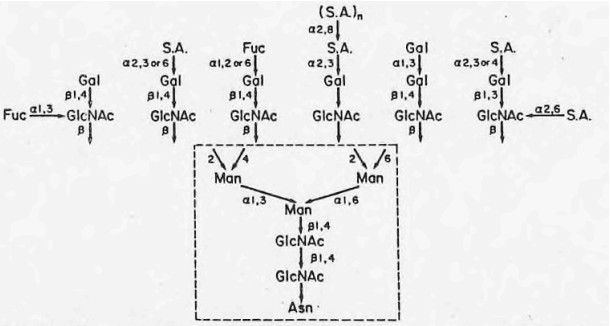
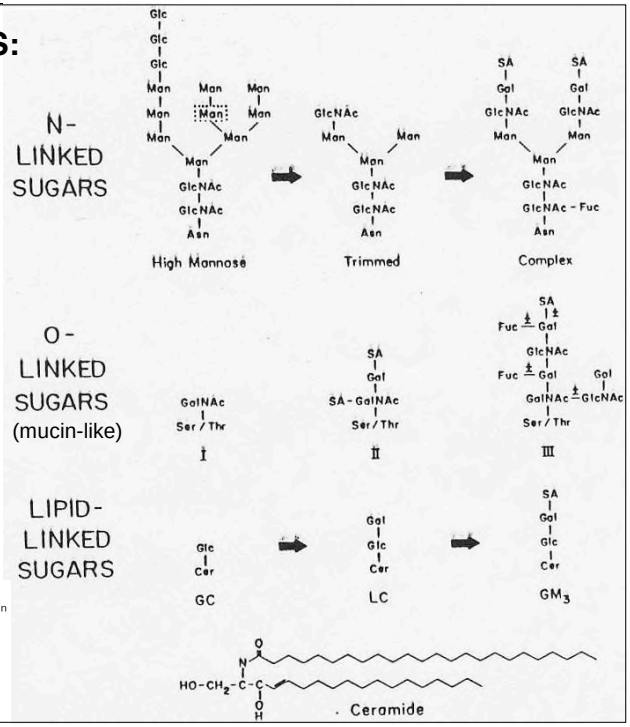


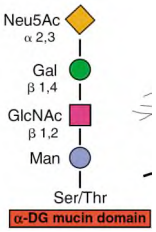
Figure 2 Various outer chain sequences found in complex-type oligosaccharides. The boxed area encloses the core region to which four outer chains may be attached.

Glc, Glucose; Fr-6-P, fructose 6-phosphate; Man, mannose; GlcNAc, N-acetylglucosamine; Gal, galactose; SA, sialic acid; Fuc, fucose; Dol-P, dolichol phosphate.

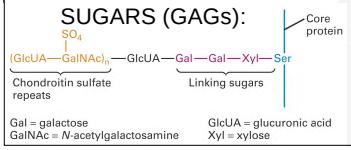
GLYCOCONJUGATES: Glycoproteins & Glycolipids



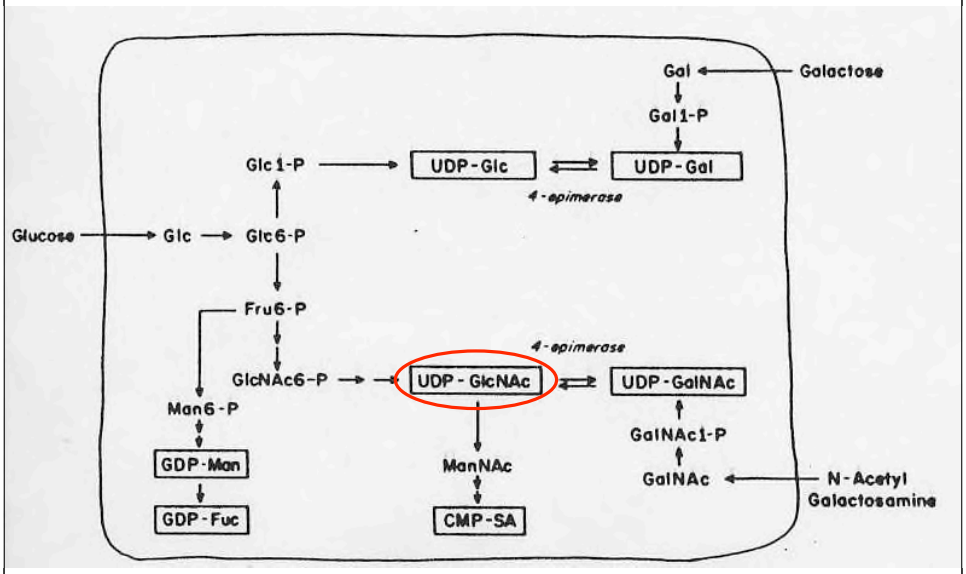
Laminin
Lassa fever
Leprosy
(micobac.)

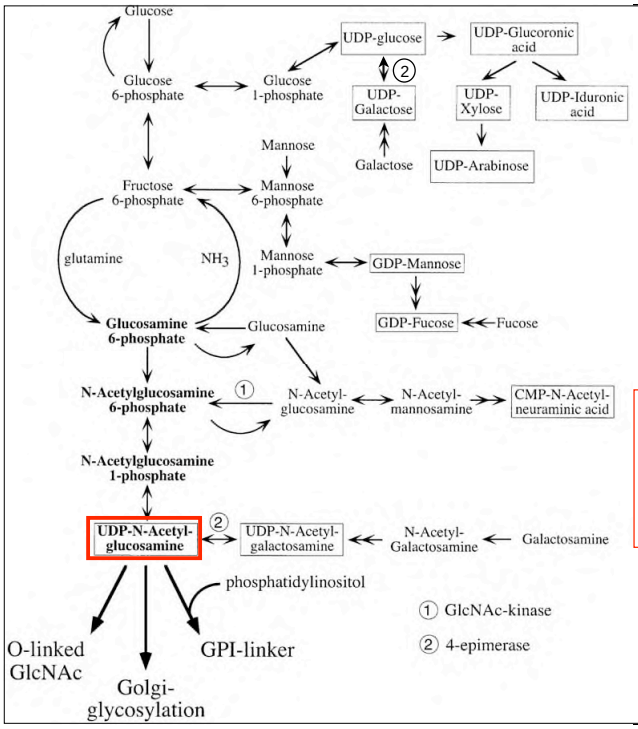


PROTEOGLYCAN SUGARS (GAGs):



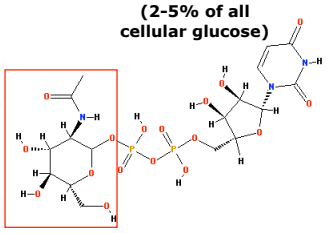
Nucleotide Sugar Precursors are the substrates of the Glycosyl Transferases that generate Glycoconjugates





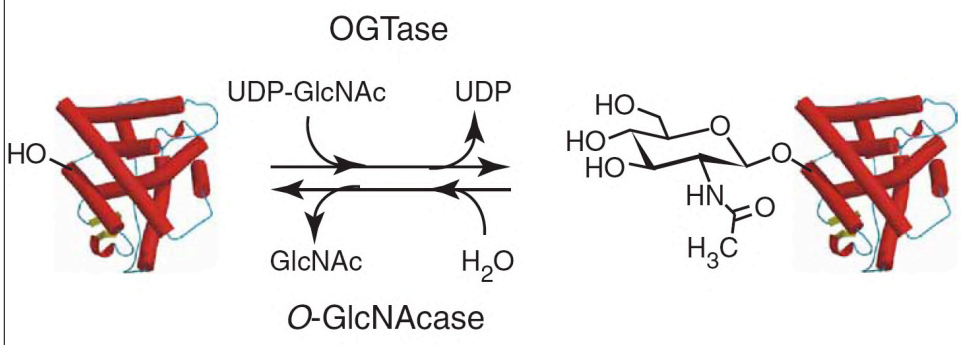
Scheme depicting the synthesis of nucleotide sugars (boxed)...Main routes for further use of UDP-GlcNAc are indicated. Doubleheaded arrows represent several enzymatic steps not depicted in detail. Adapted from Schachter (1978).

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



Boehmelt G, Wakeham A, Elia A, Sasaki T, Plyte S, Potter J, Yang Y, Tsang E, Ruland J, Iscove NN, Dennis JW, Mak TW. Decreased UDP-GlcNAc levels abrogate proliferation control in EMeg32-deficient cells. EMBO J. 2000 Oct 2;19(19):5092-104.

Reversible O-GlcNAcylation: Cytoplasm and Nucleus

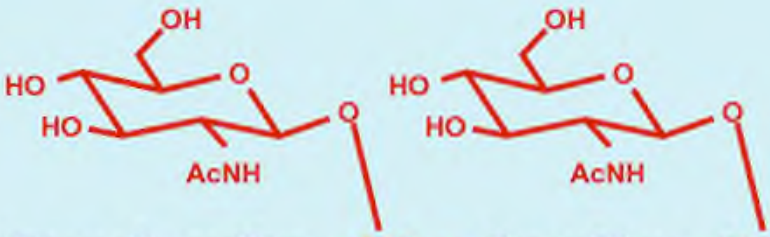


Levels of UDP-GlcNAc, and the subsequent addition of O-linked beta-N-acetylglucosamine (O-GlcNAc) to Ser/Thr residues, is involved in regulating nuclear and cytoplasmic proteins in a manner analogous to protein phosphorylation. **O-GlcNAc protein modification is essential for life in mammalian & plant 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.

Nature Structural & Molecular Biology 13, 365 - 371 (2006)

GlycoWord

O-linked N-acetylglucosamine (O-GlcNAc)



Tyr-Ser-Pro-Thr-Ser-Pro-Ser

http://glycoforum.gr.jp/science/word/glycoprotein/GPA09_images/fig_01.gif

Functional groups and proteins modified by O-GlcNAc

Protein group	Example(s)
Nuclear pore proteins	Nuclear pore proteins p54, p62, p153, p155, p180, p153, p214 and p358.
Transcription factors	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, Oct1, c-myc, RB, V-*erb*A, and ER-β. FOXO
Polymerase(s)	Large subunit of RNA Pol II.
RNA binding proteins	hnRNP G (La-antigen), Ewing sarcoma RNA-binding protein, eukaryotic initiation factor 4A1, elongation factor 1-α and 40S ribosomal protein s24.
Phosphatases, kinases and adapter proteins	Nuclear tyrosine phosphatase p65, casein kinase II, AKT, insulin receptor substrate 1,2, GSK-3β, and PI3-kinase.
Cytoskeletal proteins	Keratins 8, 13, 18, neurofilaments H, M, L, talin, vinculin, Band 4.1, ankyrinG, E-cadherin, synapsin 1, myosin, cofilin, α-tubulin, dynein LC1, MAP 2 and 4, Tau, β-synuclein, Piccolo, AP-3 and -180, β-APP, and adenovirus type 2 and 5 fiber proteins.
Chaperones	HsP 27, HsC70, and HsP90.
Enzymes	eNOS, GS, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, enolase, pyruvate kinase, UDP-glucose pyrophosphorylase, and OGT.
Also: Proteasome, Stress, Cell Cycle & Viral Proteins	

~100-500 proteins subject to O-GlcNAcylation

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

Proteins modified by *O*-GlcNAc

Functional subgroup	Protein
Nuclear pore proteins (NUP)	p62
	Nup180
	Nup153, 214, 358
	Nup 54, 155
Chromatin	chromatin-associated proteins
Transcription factors	Sp1
	AP-1 (c-*fos* and c-*jun*)[a]
	CTF[a]
	hepatocyte nuclear factor 1
	V-*erb*A
	pancreas-specific transcription factor[a]
	serum response factor
	c-Myc
	p53
	ER-α,β
	β-catenin
	NF-κB
	ELF-1
	PAX-6
	enhancer factor 2D[a]
	human C1 transcription factor[a]
	KIAA0144, Oct1[a]
	Plakoglobin
	YY1
	PDX-1
	CREB
	RB
	P107
RNA binding proteins	HnRNP G (La-antigen)
	Ewing-sarcoma RNA-binding protein
	eukaryotic initiation factor 4A1[a]
	elongation factor 1-α[a]
	40S ribosomal protein s24[a]
Phosphatases	nuclear tyrosine phosphatase p65
Kinases and adapter proteins	CK II
	insulin receptor substrate 1,2
	GSK-3β
	PI3-kinase
Cytoskeletal proteins	
Intermediate filaments	keratins 8, 13, 18
	neurofilaments H, M, L
Actin-based	Band 4.1
	Talin
	Vinculin
	Ankyrin$_G$
	Synapsin 1
	Myosin[a]
	E-cadherin[a]
	Cofilin[a]
Microtubule-based	Tau
	MAP 2 and 4
	Dynein LC1[a]
	α-tubulin[a]
Other	adenovirus type 2 and 5 fiber proteins
	AP-3 and -180
	β-APP
Chaperones	β-synuclein
	Piccolo[a]
	HsP 27
	HsC70
	HsP90[a]
	HsP70[a]
Metabolic enzymes	eNOS
	enolase[a]
	glyceraldehyde-3-phosphate dehydrogenase[a]
	phosphoglycerate kinase[a]
	pyruvate kinase[a]
	UDP-glucose pyrophosphorylase[a]
	glycogen synthase
Other	eukaryotic peptide chain initiation factor -2 αp67
	OGT
	CRMP-2
	ubiquitin carboxy hydrolase (UCH) L1
	Glut-1
	Annexin 1[a]
	nucleophosmin[a]
	proteosome component C2 Q04323, UCH homolog[a]
	Sec23, human homolog
	Ran[a]
	peptidyl prolylisomersae[a]
	Rho GDP-dissociation inhibitor 1[a]
	phosphatase-2a inhibitor[a]
	Gaba-receptor interacting protein-1 splice variants
Viral proteins	SV-40 large T-antigen
	virion basic phosphoprotein
	NS26 rotavirus protein
	baculovirus gp41
	tegument protein
	HCMV UL32 (BPP) tegument protein

[a] *O*-GlcNAc modification of these proteins is considered putative as supporting structural work has not been published.

Zachara NE, Hart GW. Cell signaling, the essential role of O-GlcNAc! Biochim Biophys Acta. 2006;1761(5-6):599-617.

O-GlcNAc: *Potential* Role in Glucose Metabolism & Insulin Resistance

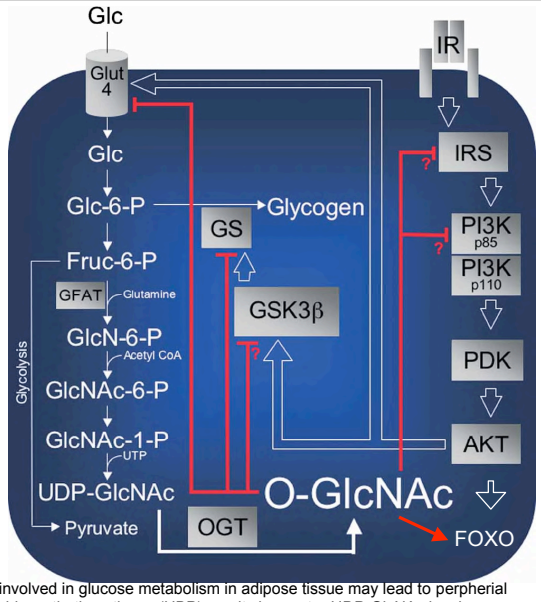


Figure 3. The O-GlcNAc modification of several proteins involved in glucose metabolism in adipose tissue may lead to perpherial insulin resistance. Increased flux through the hexosamine biosynthetic pathway (HBP) results in greater UDP-GlcNAc levels upon which OGT is highly dependent. The increased O-GlcNAcylation of glycogen synthase (GS) reduces glycogen storage. Increased O-GlcNAcylation of IRS and PI3K leads to suppressed AKT activity and Glut4-mediated glucose uptake. glutamine: fructose-6-phosphate transferase (GFAT), phosphoinositide-dependent kinase (PDK)

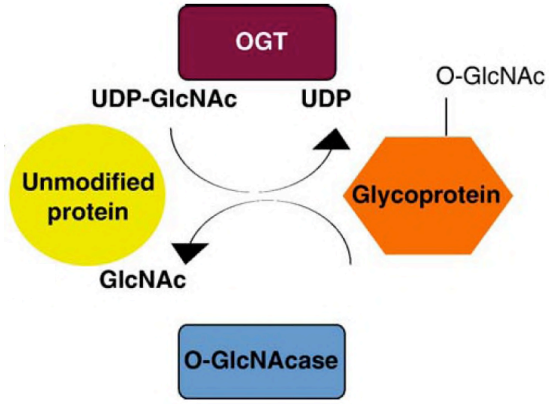
Slawson C, Housley MP, Hart GW. O-GlcNAc cycling: how a single sugar post-translational modification is changing the way we think about signaling networks. J Cell Biochem. 2006 Jan 1;97(1):71-83. http://www3.interscience.wiley.com/cgi-bin/fulltext/112101836/HTMLSTART

Pathways altered by changes in glucose/glucosamine metabolism

Tumor growth	Nutrient sensing	Calmodulin Kinase kI/ATPase	NF-κB
Cell cycle	Leptin levels	Tau and neurodegenerative diseases	Metabolic enzymes
Neutrophil activation	Diabetes-associated apoptosis	Nuclear localization	eNOS
Stress response pathways	Glycogen synthase eNOS	Connexin36 ID2, transcriptional repressor	PAI-1 expression Osteoporin
Capacitive Ca^{2+} entry	P38 Map Kinase	Transcription	Transforming growth factor-α
Insulin resistance	PKC	Insulin responsive transcription	Transforming growth factor-β
		Fibronectin	Angiotensin gene expression
			Calmodulin
			Cox-2 expression
			Haptoglobin

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

Regulatory Crosstalk



Regulatory Crosstalk

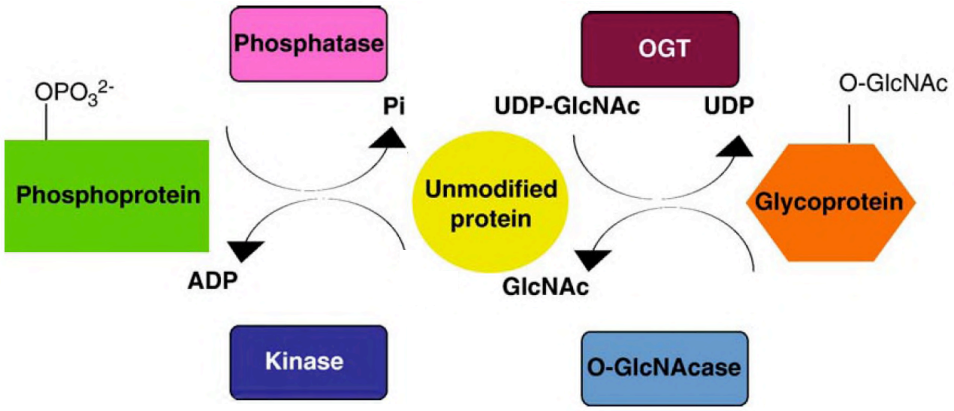
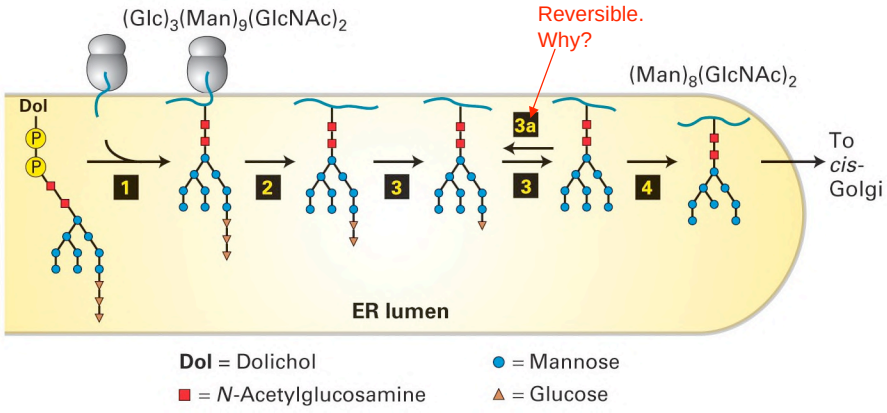
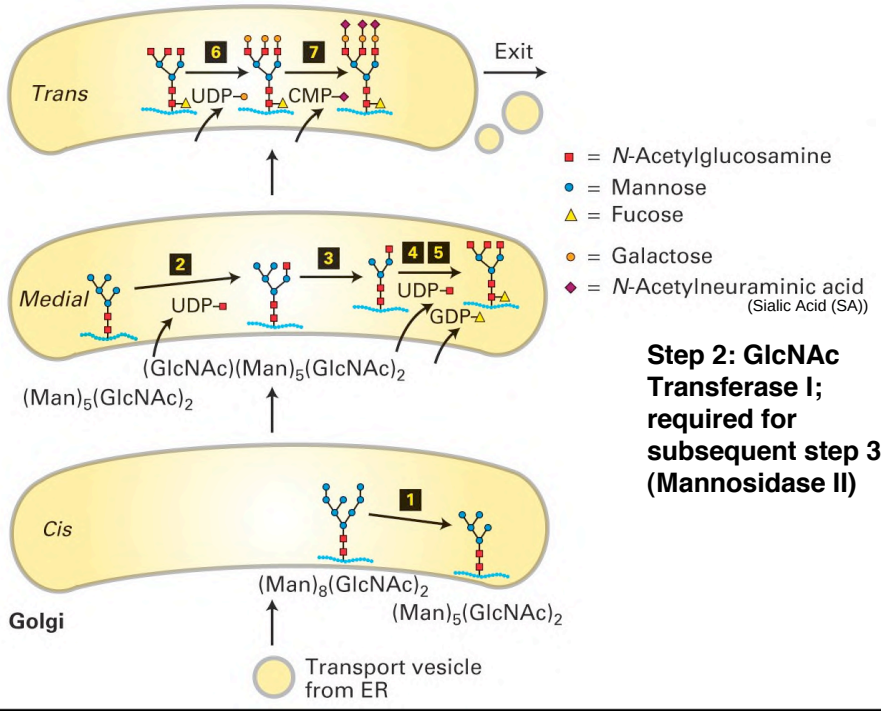


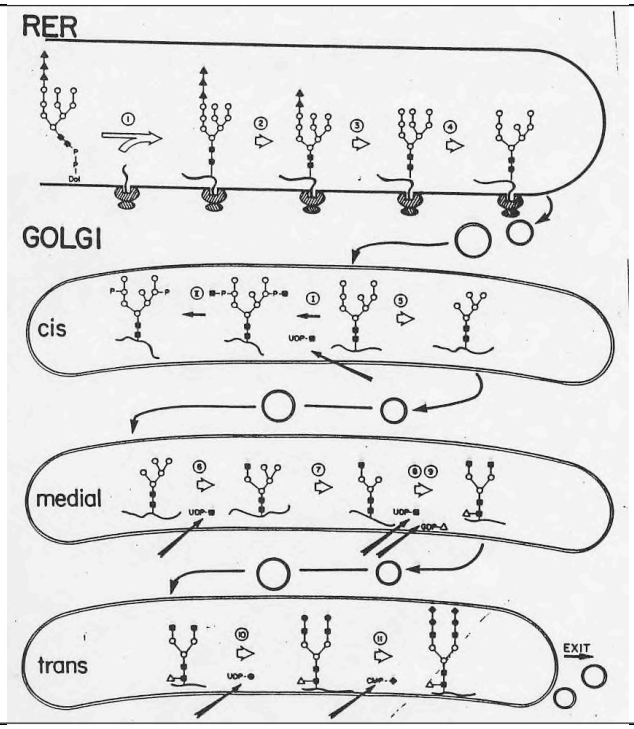
Fig. 3. The reciprocal relationship between O-GlcNAc and protein phosphorylation is indicated. In this model, O-GlcNAc and phosphorylation compete for the same Ser/Thr residues and thus regulate the levels of each other.

N-Glycosylation in the Secretory Pathway





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



Tools:
Inhibitors of glycosylation:

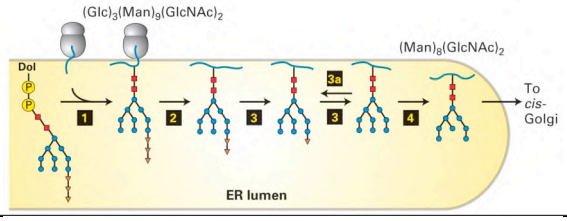
tunicamycin: UDP-GlcNAc:dolichyl-P GlcNAc-P transferase, ER, nucleoside antibiotic blocks all N-glycosylation, no chemical analogue for O-links (step 1); however, ldlD cells have mutation (reversible block)

castanospermine: plant alkaloid, glucosidase I (step 2)

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

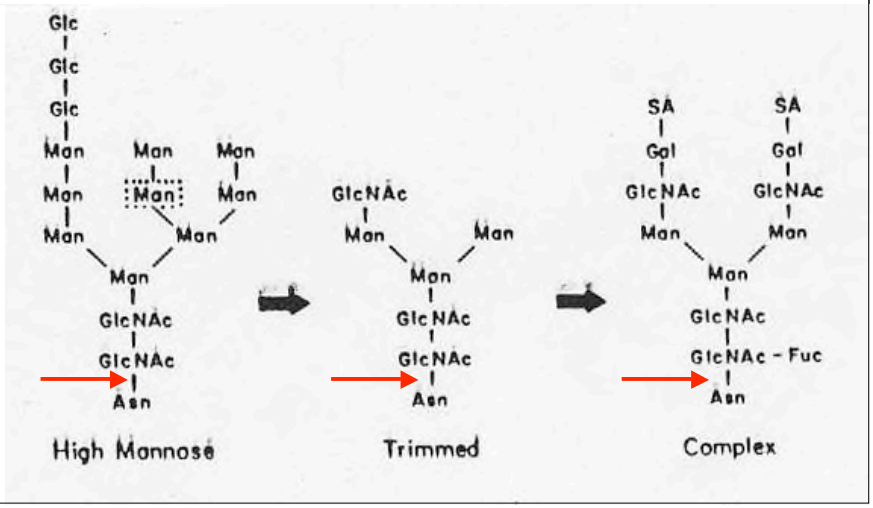
deoxymannojirimycin: mannosidase I -$Man_9GlcNAc_2$ chains (step 4)

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



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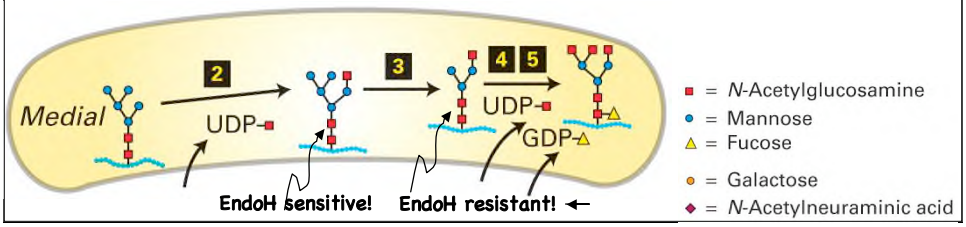
- Endoglycosidase H sensitive - Endo H used with proteins whose fully mature forms have "complex-type" N-linked chains. Endo H sensitive protein (usually shift in electrophoretic mobility of metabolically labeled protein) has probably not been processed through the medial Golgi - during synthesis of 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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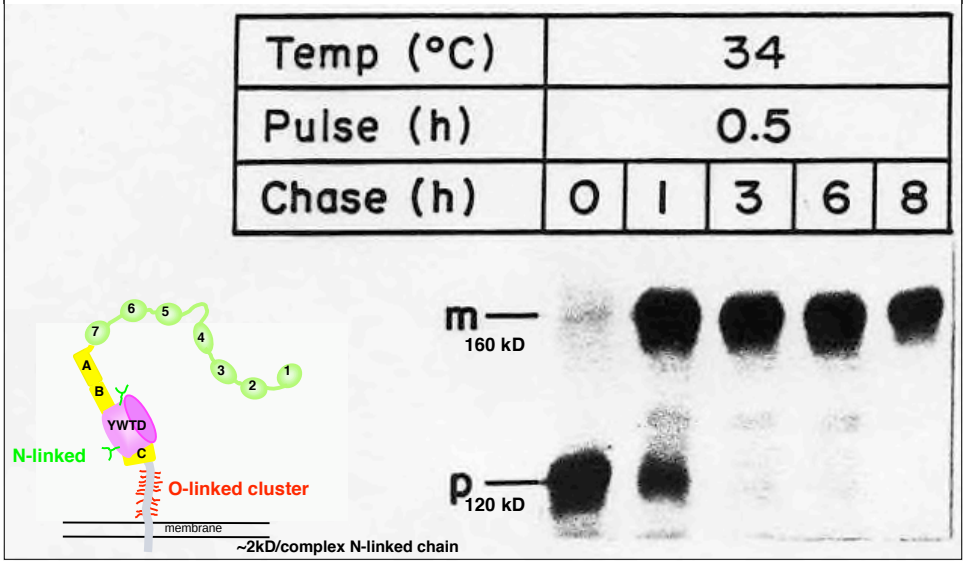
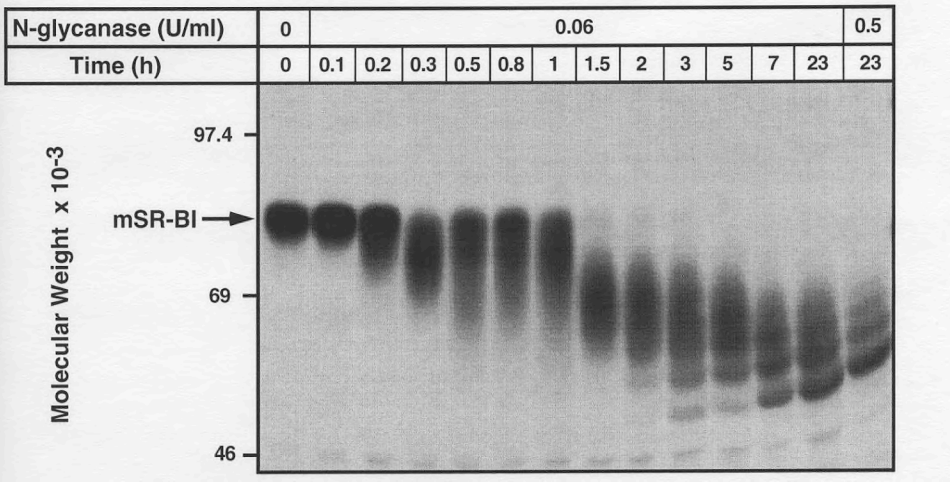
- Sialidase (neuraminidase) sensitive: protein N- and/or O-linked chains have been modified with sialic acid, usually occurs in the trans Golgi or TGN.
- O-glycanase: protein may have "mucin-type" (GalNAc-attached) O-linked chains, specific for O-GalNAc-Gal, usually must treat with sialidase first to remove any blocking sialic acid.
- Alkaline borohydride sensitive: protein may have "mucin-type" (GalNAc-attached) O-linked chains.
- Oligosaccharide metabolic labeling with [^{3}H]glucosamine added to cells.

Pulse ([^{35}S]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



Counting N-linked chains with time course of N-glycanase treatment.

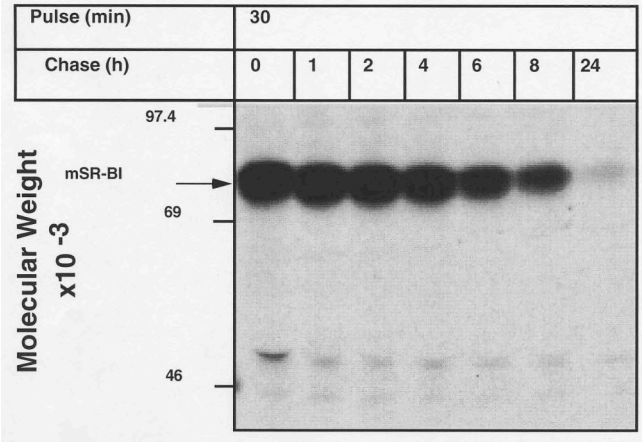
HDL Receptor (mSR-BI)



Estimate ~≥6 chains, actually 11 (mutagenesis) [2 essential]

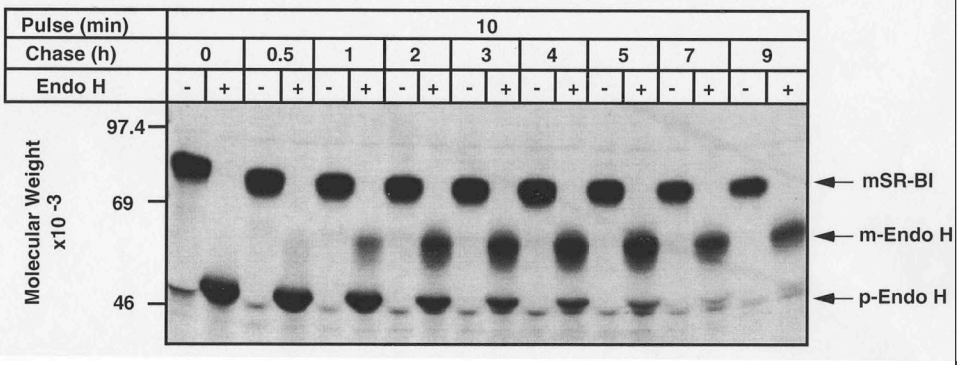
Pulse Chase Analysis

HDL Receptor (mSR-BI)



?????
11 N-linked chains,
Why no shift?

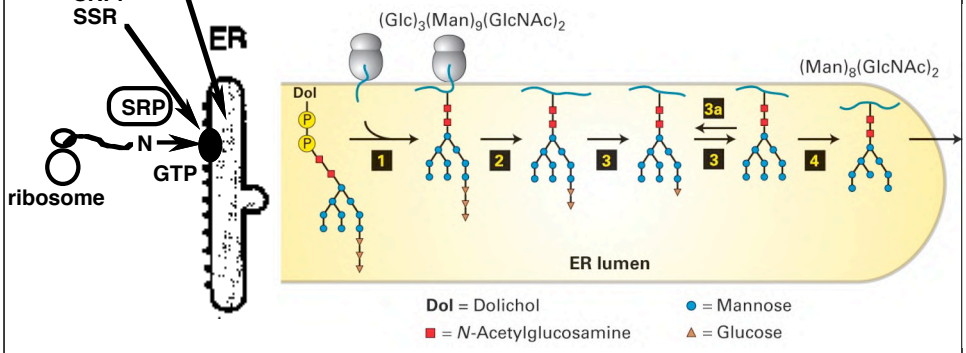
Endo H Analysis of Processing



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!!

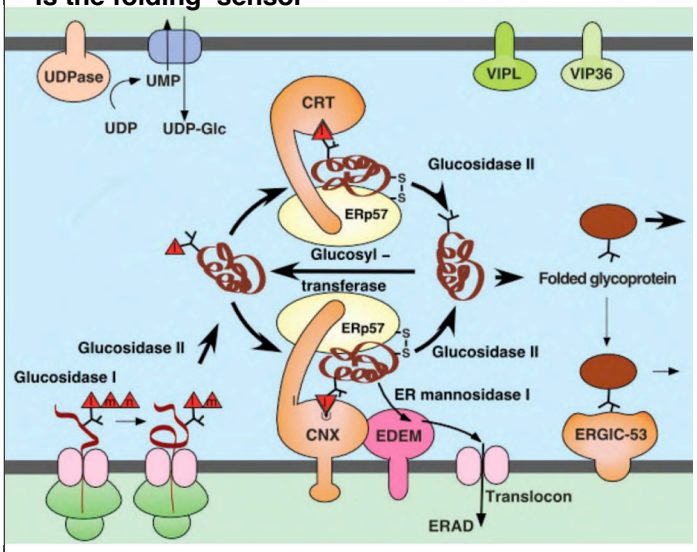
folding, oligomerization
S-S formation(PDI),
BiP, glycosylation,
trimming (2-15 min for HA)

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 monoglucosylated 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.

from: Helenius A, Aebi M. Roles of N-linked glycans in the endoplasmic reticulum. Annu Rev Biochem. 2004;73:1019-49.

OTHER SIGNALS: SORTING

TABLE 17-2 Known Sorting Signals That Direct Proteins to Specific Transport Vesicles

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

*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

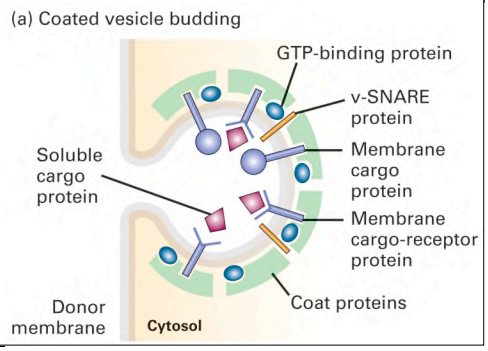


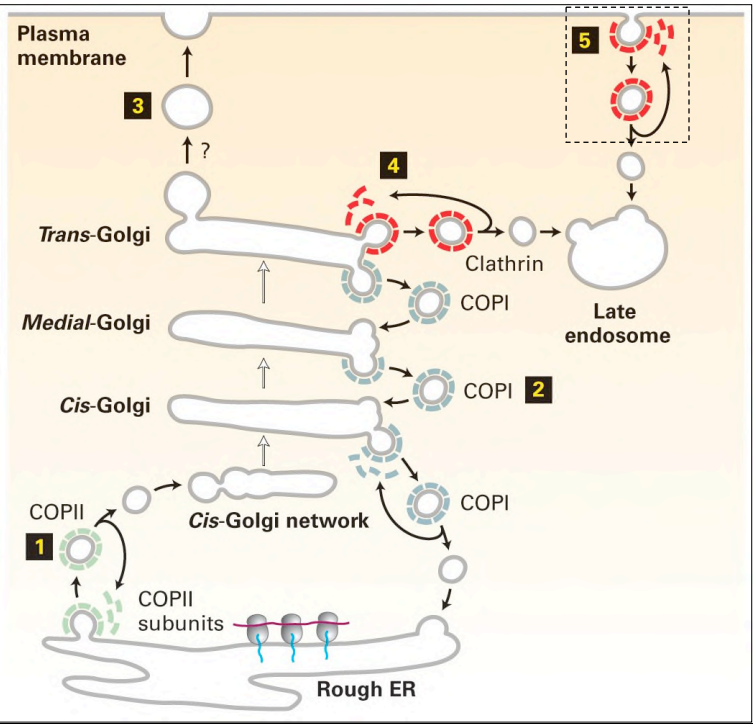
TABLE 17-1 Coated Vesicles Involved in Protein Trafficking

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

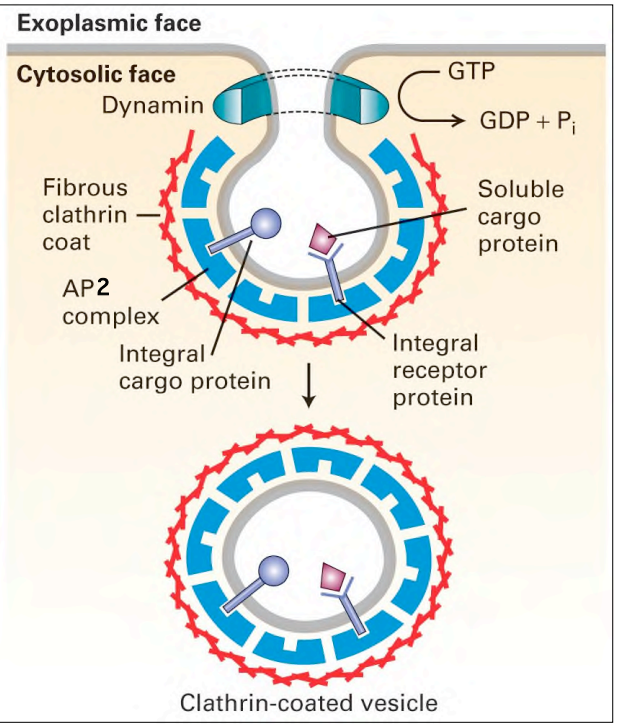
*Each type of AP complex consists of four different subunits. It is not known whether the coat of AP3 vesicles contains clathrin.

Caveolae **Caveolin** **From Plasma Membrane & others**

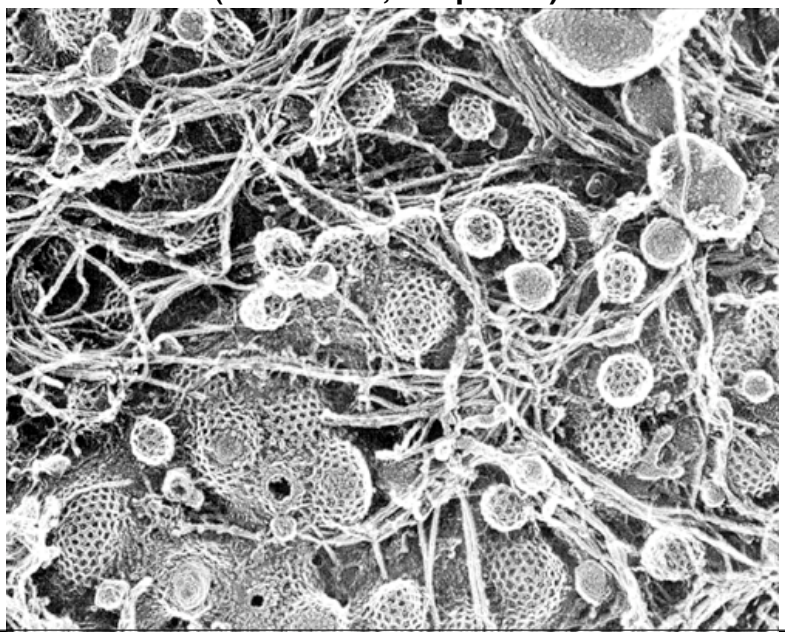
Coated Vesicle-Mediated Transport



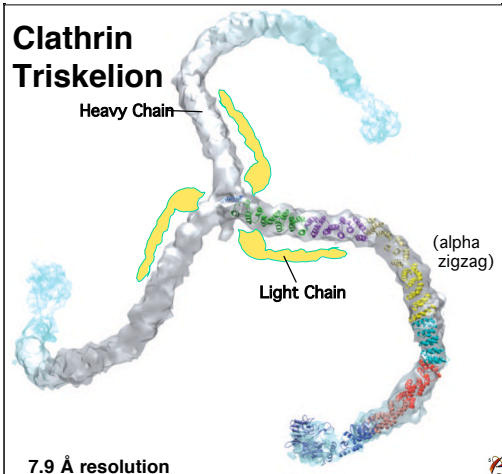
Clathrin Coated Pit/Vesicle-Mediated Endocytosis



Clathrin Coats on surface endocytic vesicles (fast freeze, deep etch)



Clathrin Triskelion



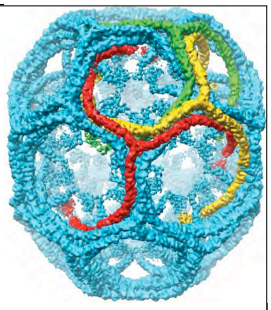
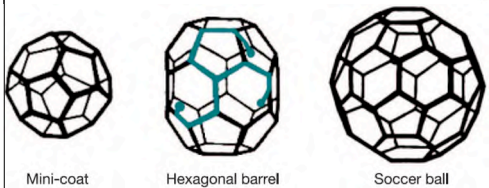
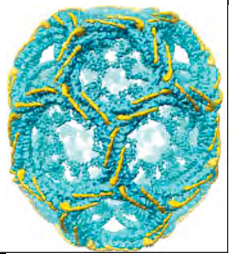


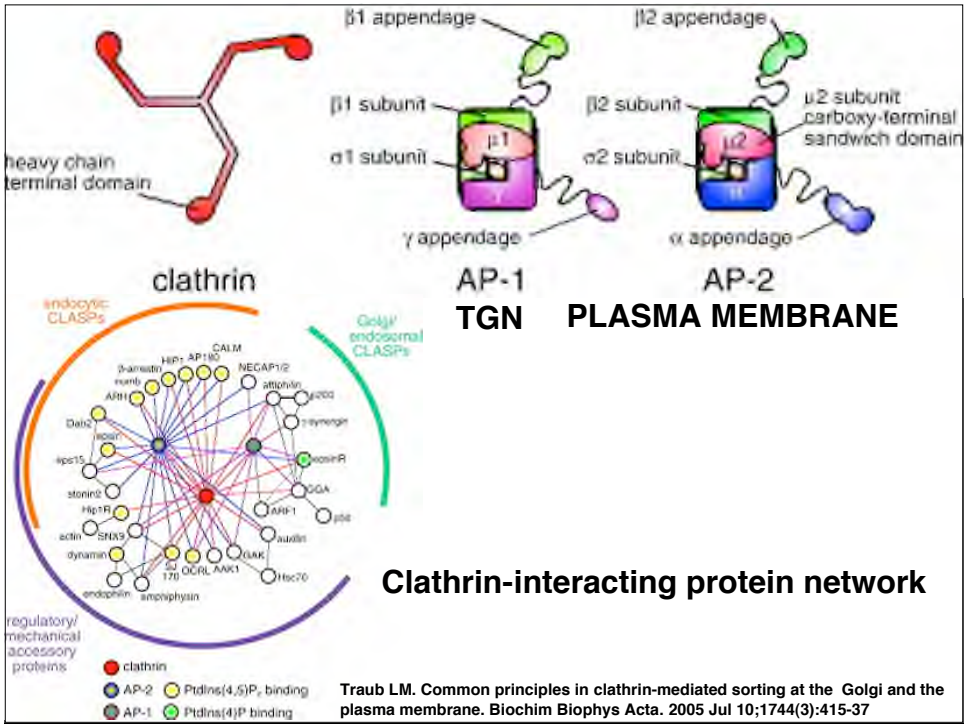
Figure 2 Image reconstruction of a clathrin hexagonal barrel (heavy chains only) at 7.9 Å resolution. There are 36 clathrin triskelions in the structure, which has D6 symmetry. Thus, there are three symmetry-independent triskelions (or nine symmetry-independent legs). The coloured triskelions show one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





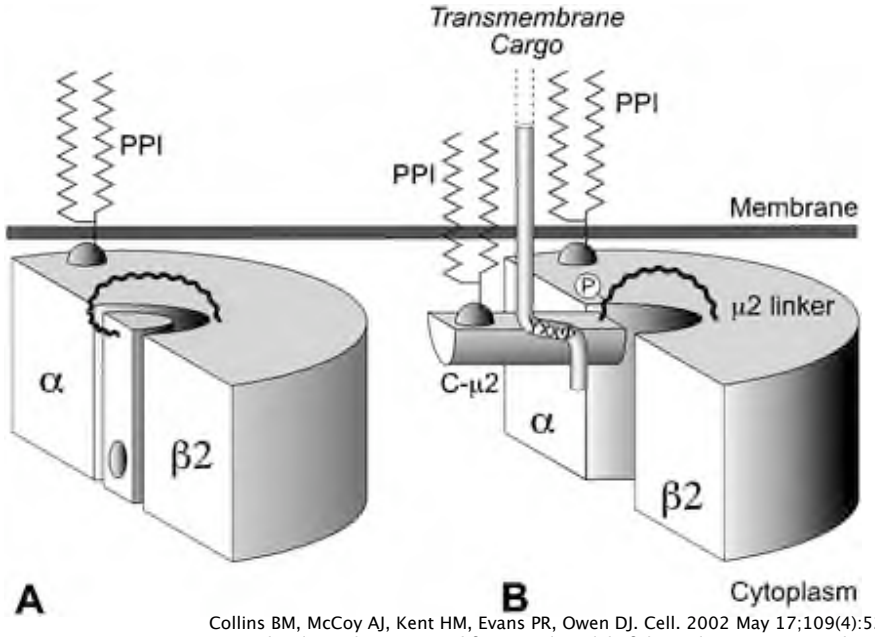
Fotin et al. Molecular model for a complete clathrin lattice from electron cryomicroscopy. Nature. 2004 Oct 24

Clathrin Assembly Movie (T. Kirchhausen)

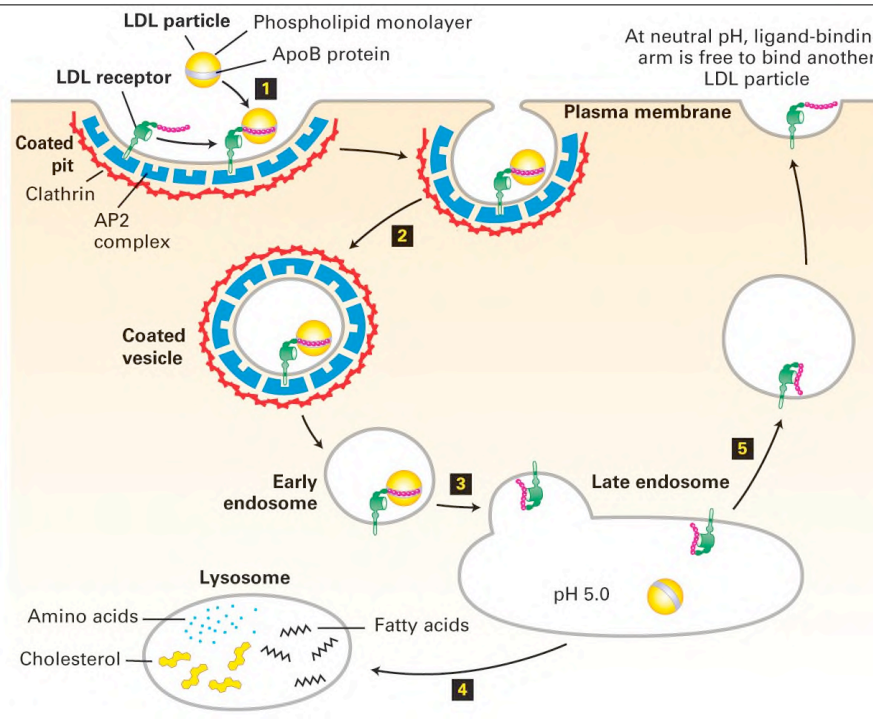


Traub LM. Common principles in clathrin-mediated sorting at the Golgi and the plasma membrane. Biochim Biophys Acta. 2005 Jul 10;1744(3):415-37

AP2 binding to membranes and Cargo



Collins BM, McCoy AJ, Kent HM, Evans PR, Owen DJ. Cell. 2002 May 17;109(4):523-35. Molecular architecture and functional model of the endocytic AP2 complex. 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

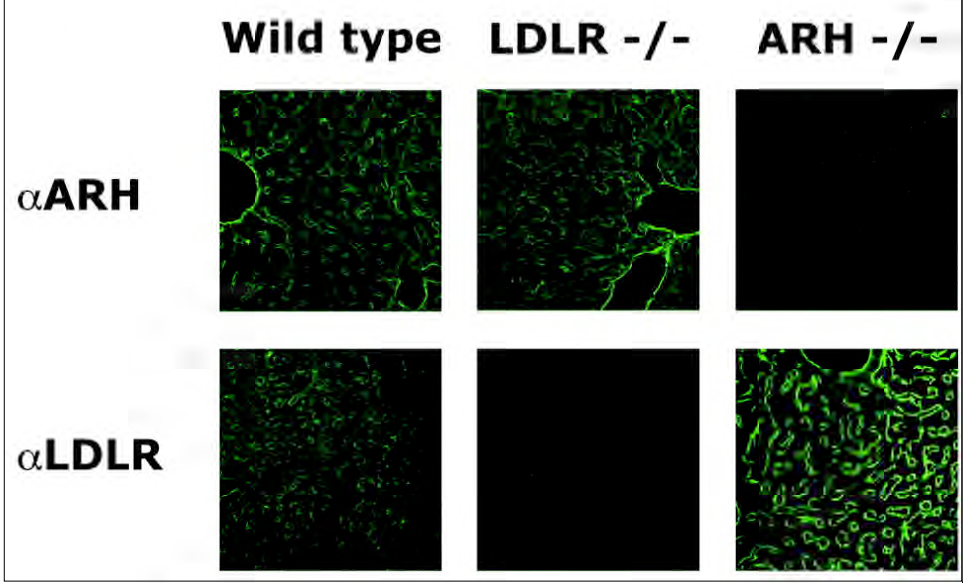
BUT did not block EGFR or LDLR (chimera) endocytosis!!!!

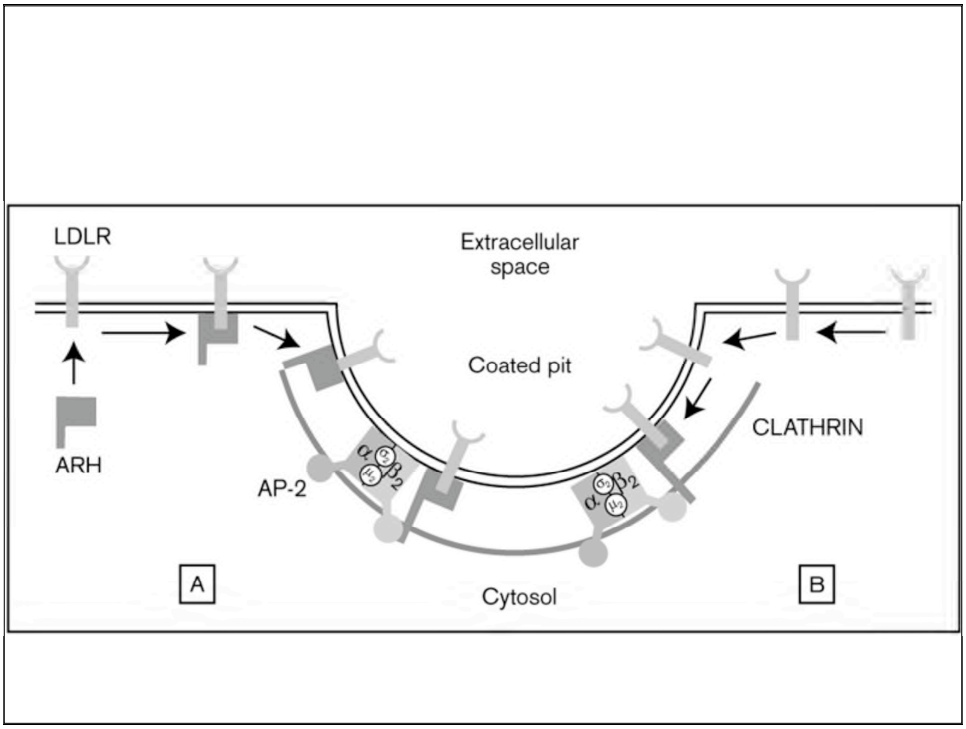
Inactivation of AP-2 by overexpression of the adaptor-associated kinase (AAK1) led to similar results

Motley, A., Bright, N.A., Seaman, M.N., and Robinson, M.S. (2003). J. Cell Biol. 162, 909-918.
Hinrichsen, L., Harborth, J., Andrees, L., Weber, K., and Ungewickell, E.J. (2003). J. Biol. Chem., in press.
Conner, S.D. and Schmid, S.L. (2003). J. Cell Biol. 162, 773-780.
Reviewed in:
Sever S. Ap-2 makes room for rivals. Dev Cell. 2003 Oct;5(4):530-2.

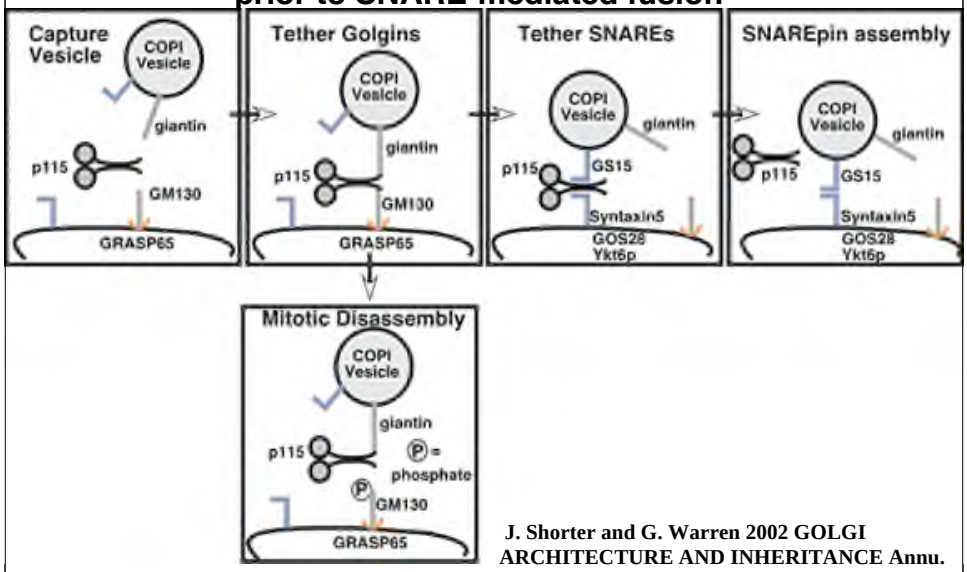
end

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



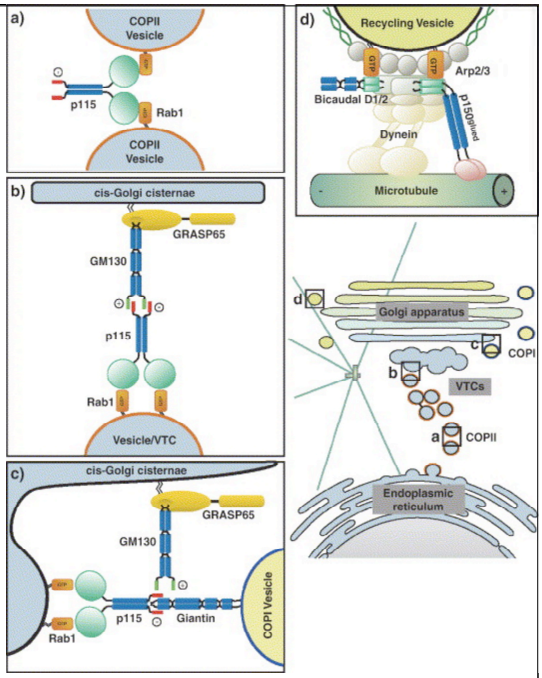


Current Hypothesis: Coiled-Coil long Golgins (GM130, giantin) tether vesicles to target membranes prior to SNARE-mediated fusion



J. Shorter and G. Warren 2002 GOLGI ARCHITECTURE AND INHERITANCE Annu. Rev. Cell. Dev. Biol., January 1,; 18(1): 379 - 420.

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