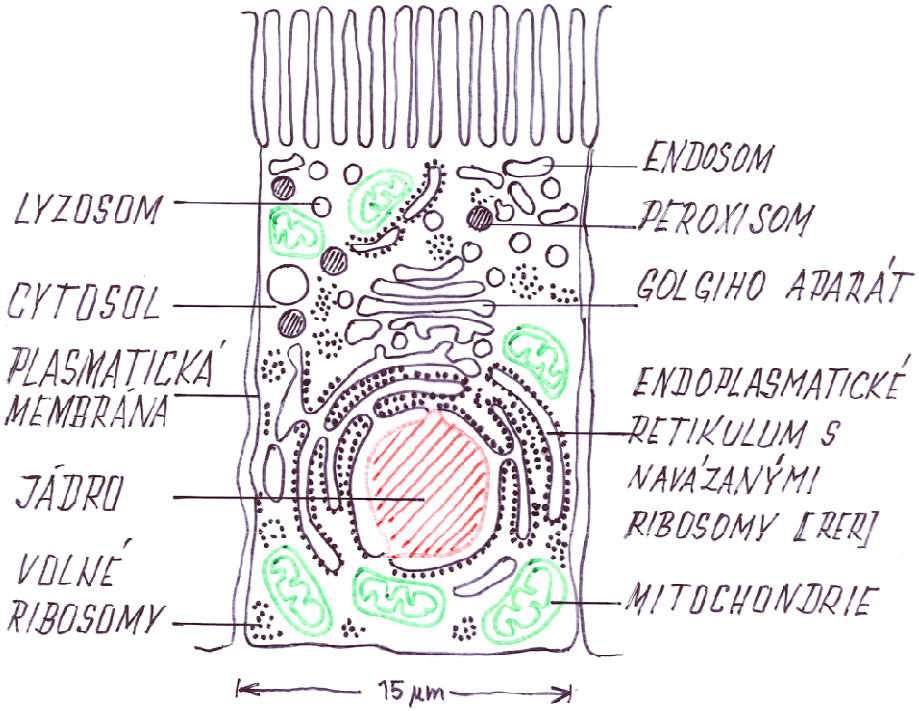
HOW MANY PROTEIN DESTINATIONS?



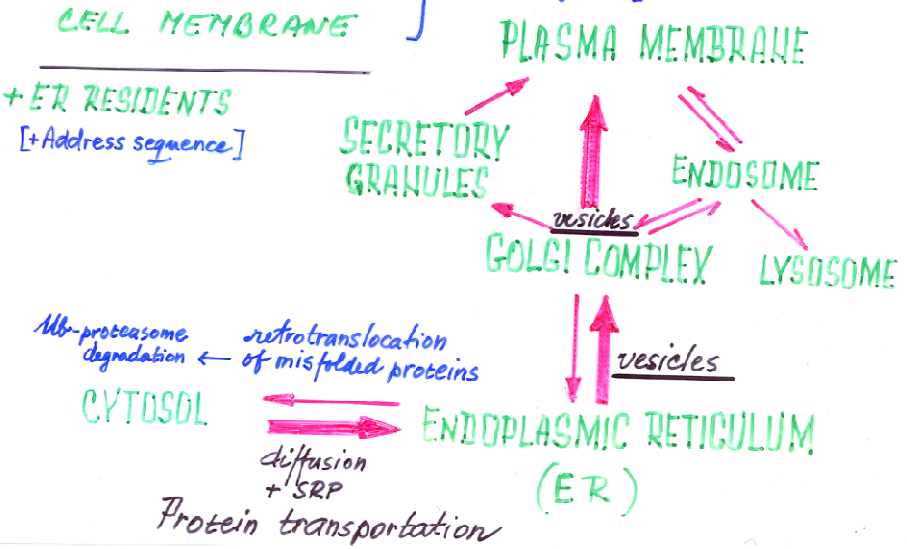
SMĚROVÁNÍ PROTEINŮ DO:	% objemu buňky	počet
CYTOSOL	54	1
MITOCHONDRIE	22	1700
ER	12	1
JÁDRO	6	1
GOLGIHO APARÁT	3	1
PEROXISOMY	1	400
LYZOSOMY	1	300
ENDOSOMY	1	200

TRANSPORTATION OF NEWLY SYNTHESIZED PROTEINS WITHIN THE CELL – THEIR DESTINATION IS DETERMINED GENETICALLY

1. DIRECT TRANSPORTATION FROM THE CYTOSOL, TARGETING TAKES PLACE ACCORDING TO ADDRESS SEQUENCE TO :
   - NUCLEUS [NLS]
   - CHLOROPLASTS
   - MITOCHONDRIA
   - PEROXISOMES

2. INDIRECT TRANSPORTATION VIA ENDOPLASMIC RETICULUM (ER). THE PROTEINS CARRY ADDRESS SEQUENCE CALLED SIGNAL SEQUENCE TO BE TARGETED TO ER. MEDIATED BY SRP, (SIGNAL RECOGNITION PARTICLE)
   TO:
   - GOLGI
   - LYSOSOMES [Man-6P]
   - ENDOSOMES
   - NUCLEAR MEMBRANE } [Address sequence]
   - CELL MEMBRANE

   + ER RESIDENTS [+Address sequence]



Protein transportation

PROTEIN TARGETING, SORTING AND PROCESSING

Site of protein synthesis : RIBOSOMES
- IN THE CYTOSOL
- WITHIN ORGANELLES

FREE RIBOSOMES : synthesize mostly soluble proteins,
→ CYTOSOL and mitochondrial (chloroplastal, peroxisomal) proteins, and nuclear proteins

MEMBRANE-BOUND RIBOSOMES : manufacture
→ RER transmembrane proteins destined for
- secretion
- operation within the ER
- incorporation into lysosomes

~ 40% of proteins that a cell synthesizes must be processed (modified):

COTRANSLATIONALLY (a secretory pathway)

POSTTRANSLATIONALLY (other protein targetting pathways; e.g. mt proteins)

(~ 30% of newly synthesized proteins are defective ribosomal products scheduled for degradation (after Ub addition))

SOME ADDRESS SEQUENCES

SIGNAL FUNCTION — TARGETING SEQUENCE / ADDRESS SEQUENCE(AS) (No gene specificity)

A. **Import to nucleus:** -Pro-Pro-Lys⁺-Lys⁺-Arg⁺-Lys⁺-Val- **NLS**

B. **Import to mitochondria matrix:** H_3^+N-Met-Leu-Ser-Leu-Arg⁺-Gln-Ser-Ile-Arg⁺-
(20-60 residues) -Phe-Phe-Lys⁺-Pro-Ala-Thr-Arg⁺-Thr-Leu-Cys-
-Ser-Ser-Arg⁺-Tyr-Leu-Leu-
basic + hydroxylated res.
[These presequences do not interact with SRP]

C. **Retention in ER:** -Lys-Asp-Glu-Leu-COO⁻ (+lack of acidic AAs) KDEL (HDEL yeast) at C-terminus

D. **Import into ER** ⇒ secretion, transmembr. pr.
= "SIGNAL SEQUENCE" (PRESEQUENCE) (SS)
(13-36 residues) = N-terminus
H_3^+N-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-
-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-
-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
hydrophobic res.

⇒ PREPROTEINS, PREPROPROTEINS (e.g. preproinsulin, preprocollagen): MALWMRLLPLLALLALWGPDPAAAFVN
Signal peptidase ↓

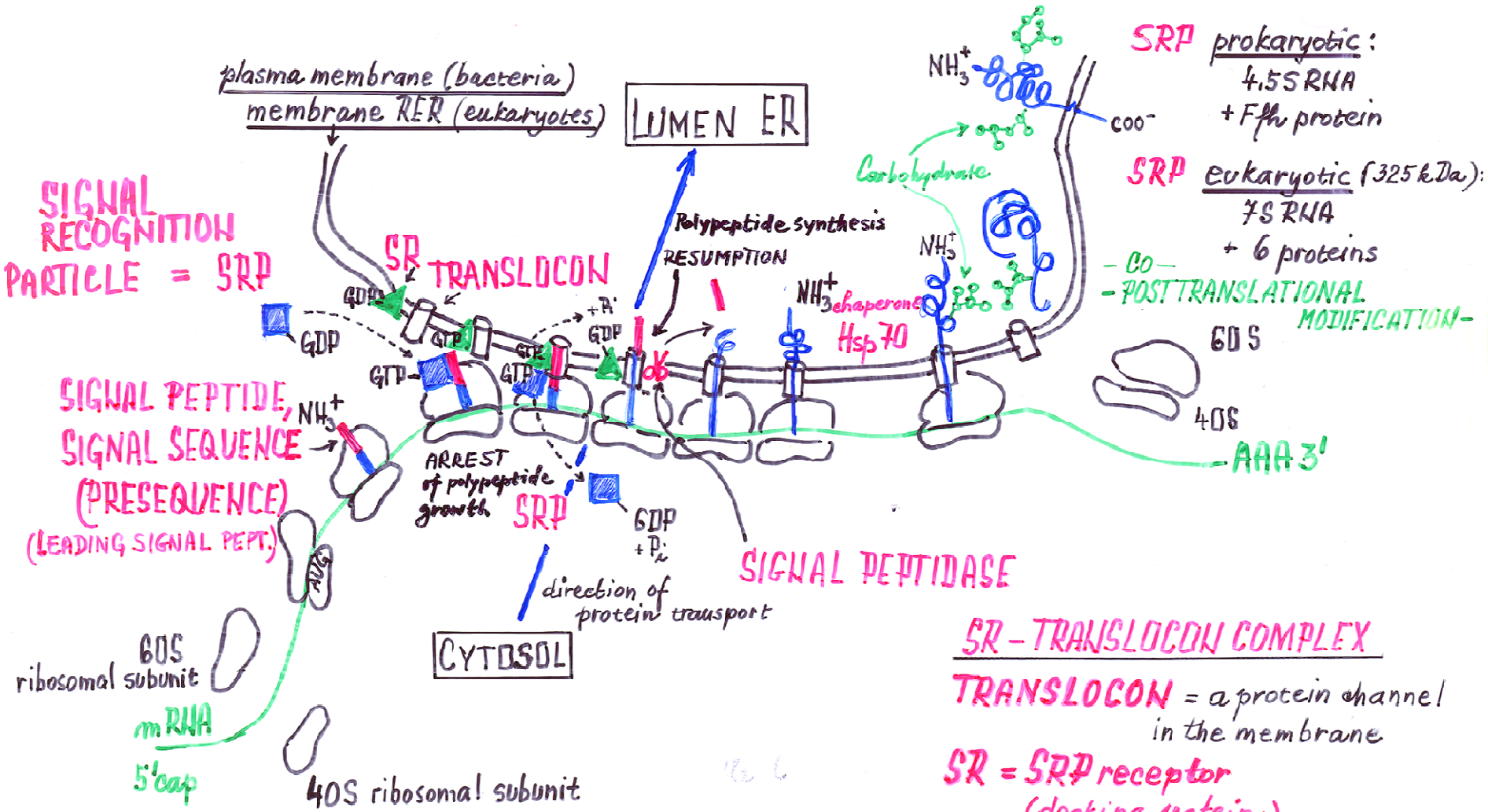
Membrane width: 8-9 AA in an elongated form
20 AA in an α-helix

E. **Import to peroxisomes:** -Ser-Lys-Leu- ; at the C-Terminus
(Transmembrane receptors for some hormones: 7x; more times)

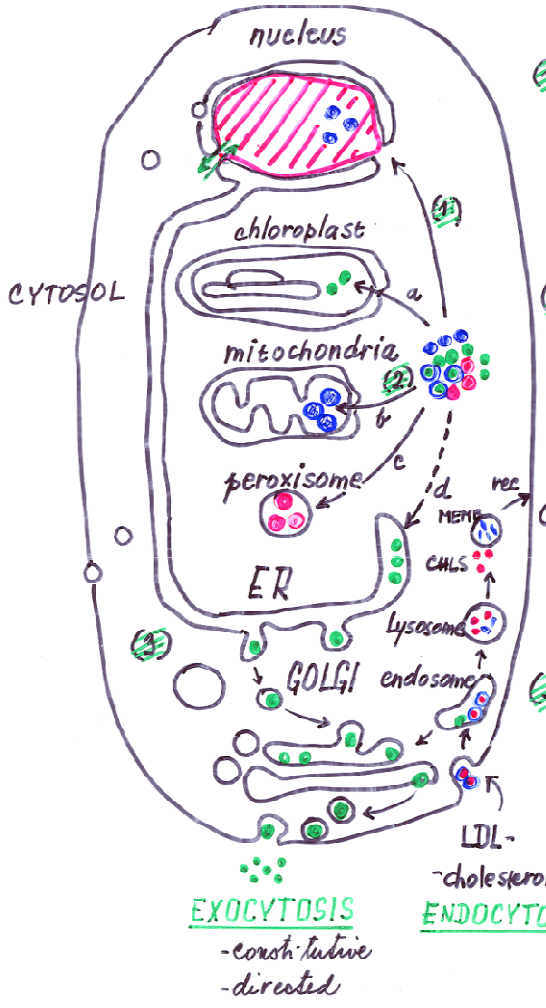
Modulation of Targeting:
- alternative translation initiation = with or without AS: → to mt / → to cytosol
- rat liver fumarase

MEMBRANE, SECRETED AND LYSOSOMAL PROTEINS AND PROTEINS OF ENDOPLASMIC RETICULUM

are synthesized with a LEADING (N-terminal) SIGNAL SEQUENCE



Synthesis of all proteins starts in the cytoplasm and further targetting + sorting depends on the address segrence + post translational modification (glycosylation)



(1) TRANSPORT THROUGH NUCLEAR PORES
NLS (ADDRESS SEQ) + GTP + IMPORT RECEPTORS
PROTEINS FOLDED

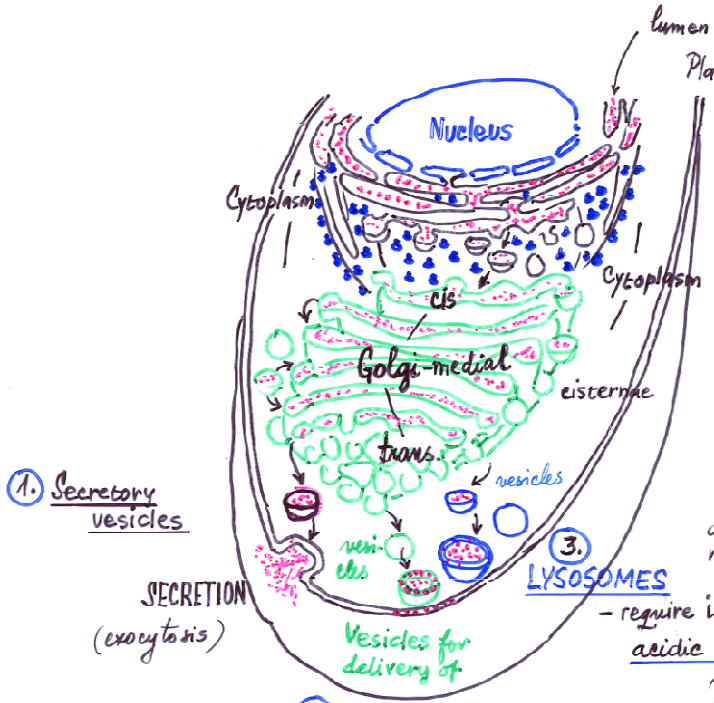
(2) TRANSPORT THROUGH MEMBRANE CHANNELS
(a-c) -TRANSLOCONS-
PROTEINS LINEARIZED
ADDRESS SEQUENCES

(d) TO ER
SIGNAL SEQUENCE
SRP, SR, TRANSLOCON

(3) TRANSPORT VIA COATED VESICLES
PROTEINS FOLDED
SORTING MARKERS
(glycosylation)

MOLECULAR MOTORS
dynein, kinesin
GTP
MICROTUBULES

PATHWAYS OF NEWLY SYNTESIZED SECRETORY, MEMBRANE AND LYSOSOMAL PROTEINS (Glykoproteins)



: ribosomes bound to RER

: proteins

1. Secretory vesicles

2. MEMBRANE PROTEINS

3. LYSOSOMES

degradation and recycling of cell components:
- require imported acidic hydrolases (pH 5):
  - nucleases
  - proteases
  - lipases
  - phosphatases
  - glycosidases
  - phospholipases
  - sulfatases

On reaching the TRANS GOLGI network, the now mature proteins are sorted and sent to their final cell. destinations. Membrane, secretory and lysosomal proteins are transported in vehicles known as COATED VESICLES (3 types). Coated vesicles originate by budding off from ER membrane. They transport proteins also between RER and GOLGI and subsequently fuse with cis-GOLGI.

MARKER FOR THE DELIVERY TO LYSOSOMES:
mannose 6-phosphate
(in cis-GOLGI)

PROTEIN MISLOCATION:

The lysosomes in the connective tissues of the victims of mucolipidosis contain large inclusions of glycosaminoglycans and glycolipids as a result of the absence of several lysosomal hydrolases.

Progressive psychomotoric retardation!
Skeletal deformities
Death by age 10

=

lack of an enzyme catalysing mannose phosphorylation results in incomplete labeling of enzymes: NO TRANSPORT TO LYSOSOMES → deficient degradative activity → genetic disease

MUCOLIPIDOSIS
I-cell disease

MITOCHONDRIAL PROTEIN IMPORT: THROUGH ONE OR TWO MEMBRANES

(CHLOROPLASTS, PEROXISOMES)

98% of mt proteins are synthesized by cytosolic ribosomes (encoded by nuclear genes) (= 10-20% of all intracellular proteins)

Post-translational

Proteins are in unfolded states maintained by CHAPERONES (+ ATP)

(Hsp 70, MSF families)

N-terminal targeting sequence (presequence): 20-60 res. for matrix destin. (rich in basic + hydroxylated amino acids)

Internal targeting sequence (poorly characterized) for directing to IMM, inter-membranous space); cytochrome c is in IMM (outer surf.)

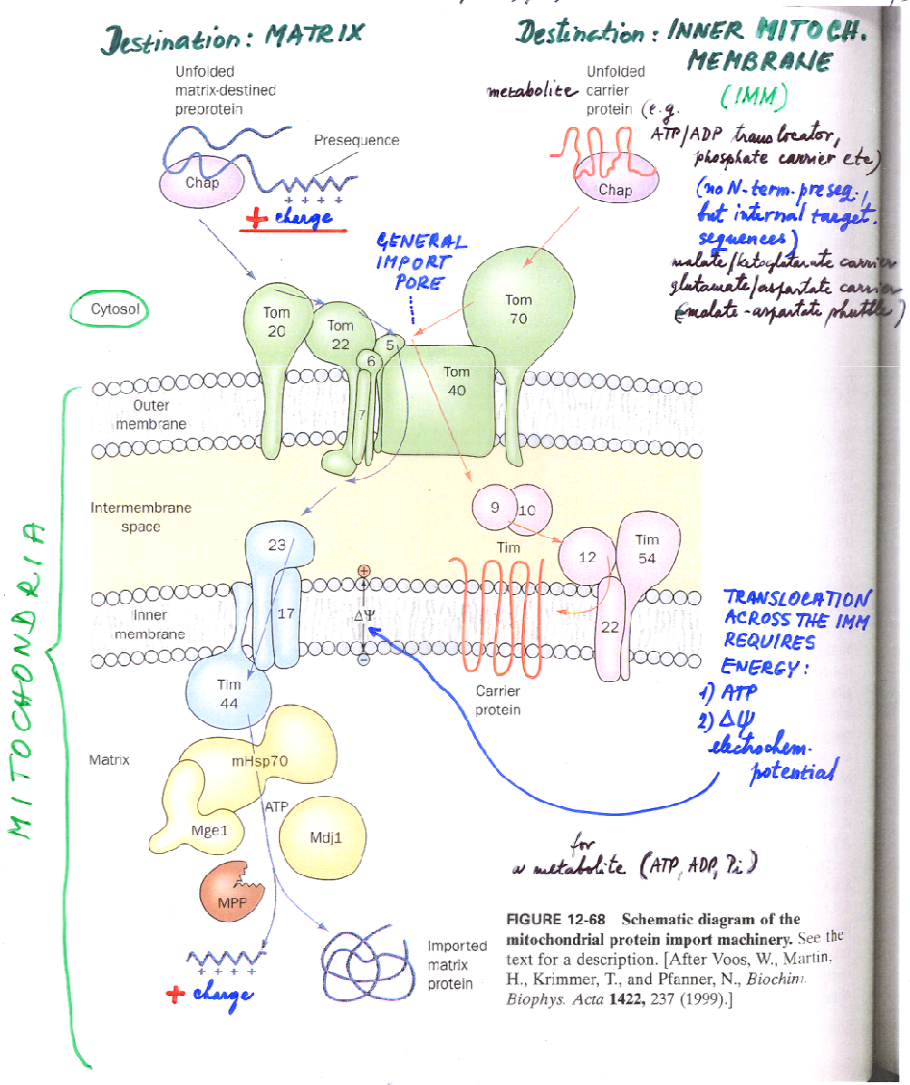
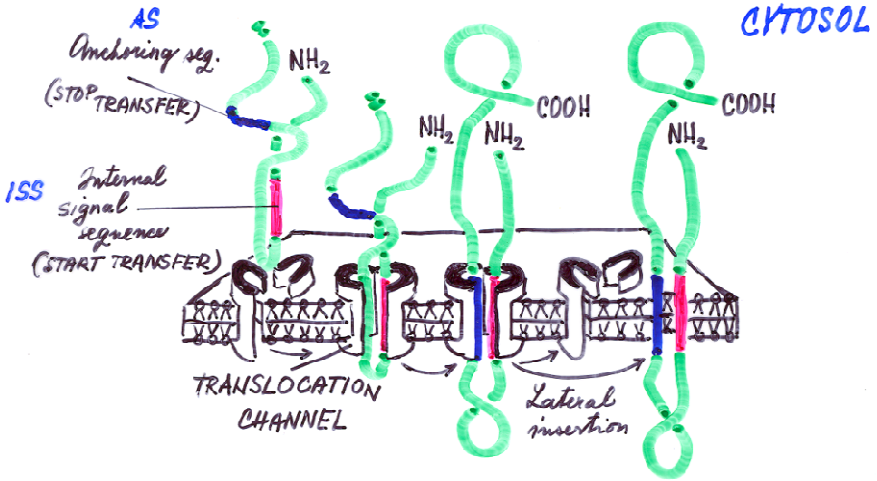


FIGURE 12-68 Schematic diagram of the mitochondrial protein import machinery. See the text for a description. [After Voos, W., Martin, H., Krimmer, T., and Pfanner, N., *Biochim. Biophys. Acta* **1422,** 237 (1999).]

INSERTION OF PROTEIN WITH TWO TRANSMEMBRANE SEGMENTS INTO MEMBRANE OF ER



Several ISS and AS allow various segments of protein to be oriented on each side of membrane = proteins with more transmembrane regions

AS : hydrophobic
always a signal for docking in the membrane

ER and GOLGI FUNCTIONS

ENDOPLASMIC RETICULUM

1) • correct folding of proteins in the presence of chaperones
   - only correctly folded proteins are transported/processed/used further; **oxidative environment = formation of S–S bridges**

   unfolded protein response (UPR) = endoplasmic reticulum stress response : arrest of PS + misfolded proteins are retrotranslocated (after reduction of S–S bridges (ERdj5 reductase)) to cytosol for degradation by the Ubiquitin–Proteasome system (ERAD)

   XBP1 (X-box binding protein 1 = TF) controls expression of genes required for UPR : binds to promotor elements of genes that encode chaperone proteins that assist with protein folding in ER (ERAD = ER-associated protein degradation)

   [KO of Xbp1 gene in mice : embryonic lethal
   in liver in adult : reduction of biosynthesis of fatty acids + cholesterol by 85–90% !!]

2) • glycosylation – in blocks of 14 monosaccharides to NH_2– of Asn

3) • formation of antibodies – assembly from 4 polypeptide chains
   – glycosylation : determination of Ab activity ?

GOLGI APPARATUS

1) • completion of glycosylation – glycid chain trimming + further modification
   oligosaccharides serve as markers for the sorting process

   mannose 6-P labelling of hydrolases (enzymes) to become recognized by receptors of vesicles traveling/delivering cargo to LYSOSOMES

   ABO blood group antigens : = glycoproteins
   also secreted in saliva, urine, milk, semin. fluid, gastric juice

	antigen	Terminal position of the antigen
A group	A	: GalNAc
B group	B	: Gal
0 group	H	: Ø
AB group	A+B	: GalNAc + Gal

   (A,B group carriers : resistance to cholera)

2) • secretion pathways :
   secretory vesicles formation
   membranous vesicles formation

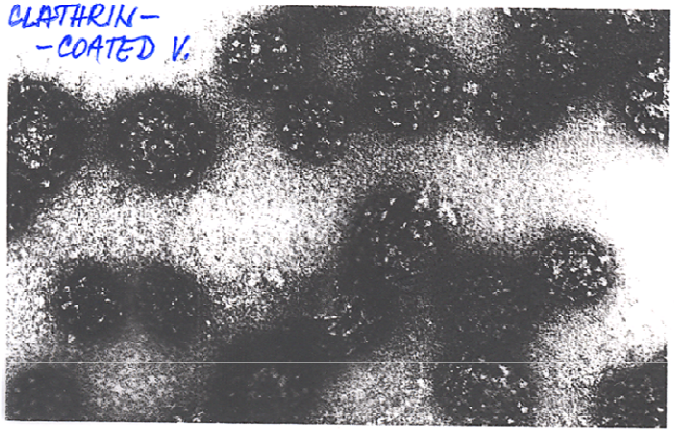
23-3B). The
s, each com
anous tubu
pposite the
er the Golgi
), through wl
inations. Th
different ty
, each of which contains differ-
ocessing enzymes.
e end of the Golgi stack to the

3 types of coated vesicles

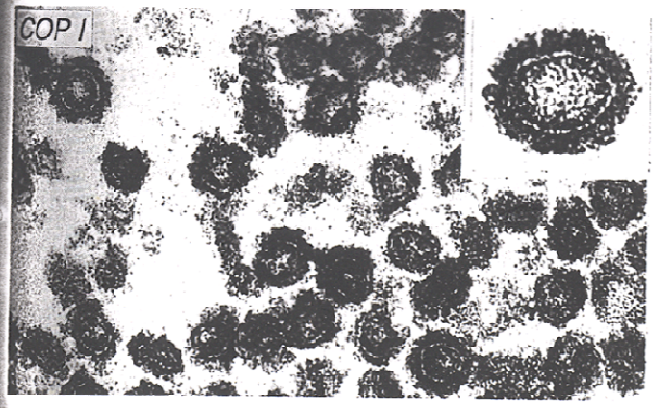
(60 – 150 nm diameter sacs)

FIGURE 12-52 Electron micrographs of coated vesicles. (*a*) Clathrin-coated vesicles. Note their polyhedral character. [Courtesy of Barbara Pearse, Medical Research Council, Cambridge, U.K.] (*b*) COPI-coated vesicles. (*c*) COPII-coated vesicles. The insets in Parts *b* and *c* show the respective vesicles at higher magnification. [Courtesy of Lelio Orci, University of Geneva, Switzerland.]

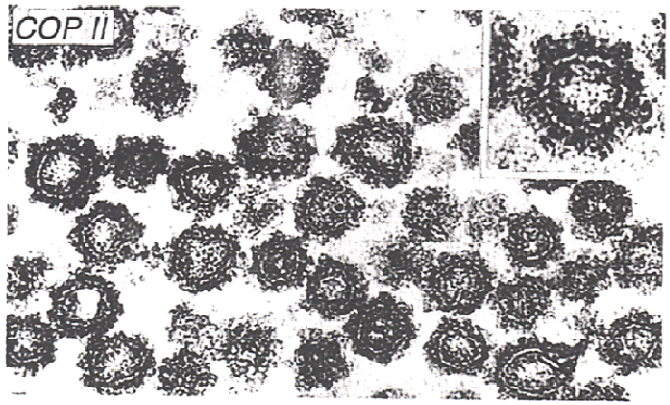
(*a*)



(*b*)

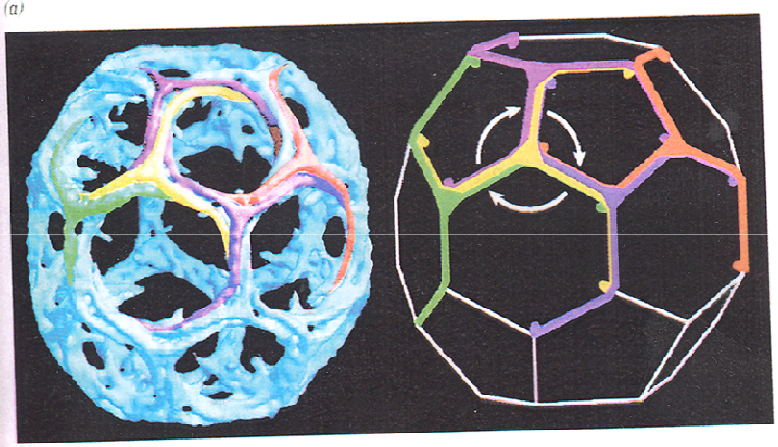


(*c*)

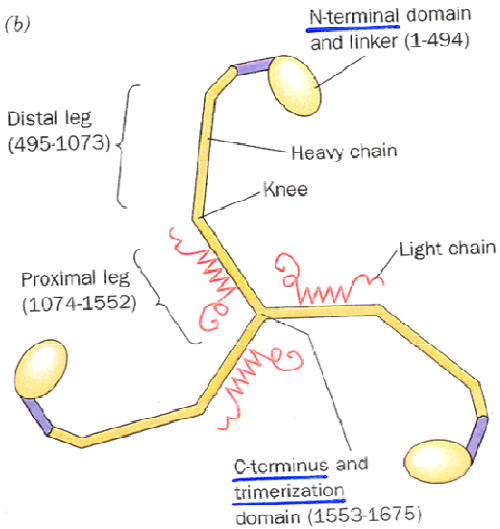


CLATHRIN - FORMED CAGE

(a)



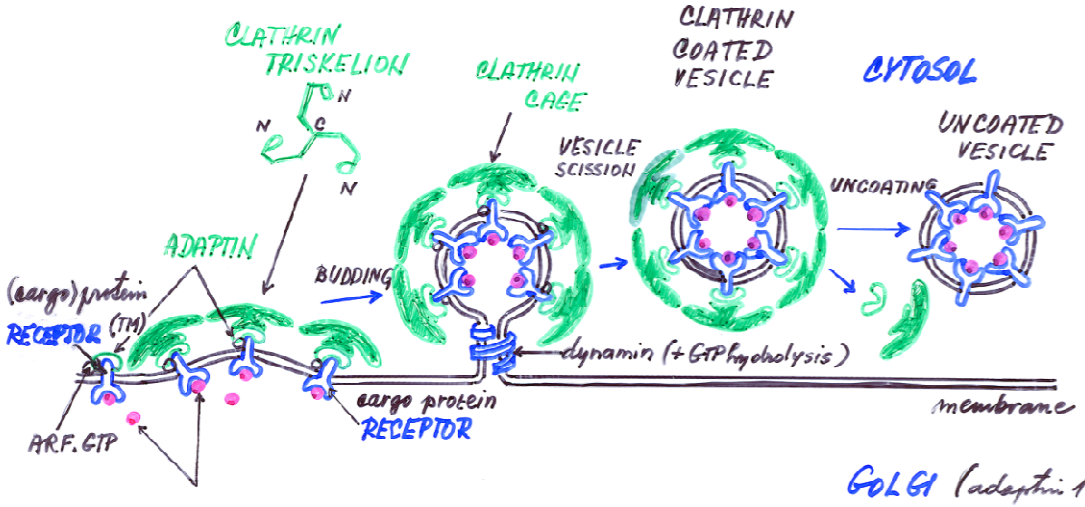
(b)



CLATHRIN HAEVY CHAIN TRIMER
=
TRISKELION

FIGURE 12-55 Anatomy of a clathrin-coated vesicle. (*a*) A cryoelectron microscopy–based image of a clathrin cage at 21 Å resolution with its triskelions differently colored. Its adaptor protein–containing core has been removed for clarity. As the accompanying diagram (*right*) indicates, a triskelion is centered on each of this polyhedral cage's 36 vertices, the cage edges are formed by the antiparallel legs of adjacent triskelions, and the linker and N-terminal domains project inward. Clathrin forms polyhedral cages with a large range of different sizes (number of hexagons); That shown here is only ~600 Å in diameter, whereas clathrin-coated membranous vesicles are typically ~1200 Å in diameter or larger. [Electron micrograph by Barbara Pearse and courtesy of H.T. McMahon, MRC Laboratory for Molecular Biology, Cambridge, U.K.] (*b*) Schematic diagram of a triskelion indicating its structural subdivisions.

FORMATION OF CLATHRIN-COATED VESICLES
= A COMPLEX PROCESS



CARGO PROTEINS carry specific TRANSPORT SIGNALS, which are recognized by CARGO RECEPTORS → mannose 6-P labeling of hydrolases for lysosomes

Function of CLATHRIN CAGE: to determine the shape of the vesicle ?

MOVEMENT OF VESICLES

1) by diffusion - short distances (ER → GOLGI app.)

2) by motor proteins (myosin, dynein, kinesin) alongside of cytoskeletal microtubules
+ ATP driven "crawling"

Mechanisms of vesicle movement in the cell

1. Vesicles that travel only short distances (< 1 µm) do so via diffusion, a process that typically takes from 1 to several minutes

2. Vesicles that have longer distances to commute (e.g. from trans-GOLGI to the plasma membrane) are actively transported along cytoskeletal microtubules by the motor proteins dynein and kinesin, which unidirectionally crawl along microtubule "tracks in an ATP-driven process.

1. SECRETORY VESICLE FUSION

On arriving at its target membrane, a vesicle fuses with it, thereby releasing its contents on the opposite side of the target membrane:

e.g. TRANSMISSION OF NERVE IMPULSES via SYNAPSIS (junctions between neurons, and neurons and muscles)

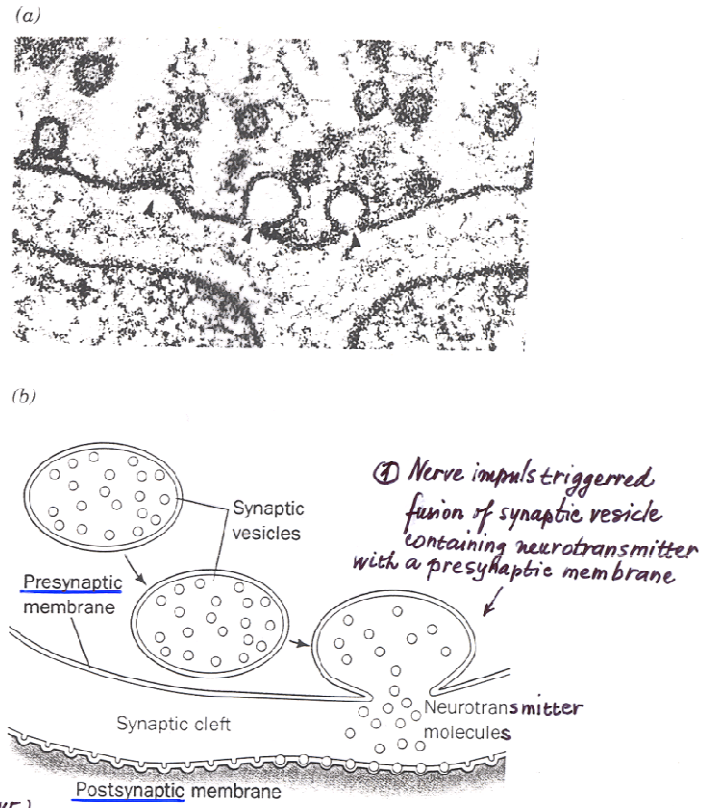


FIGURE 12-61 Transmission of nerve impulses across a synaptic cleft. (*a*) Electron micrograph of a frog neuromuscular junction in which the synaptic vesicles are undergoing exocytosis (*arrows*) with the presynaptic membrane (top) [Courtesy of John Heuser, Washington University School of Medicine, St. Louis, Missouri.] (*b*) ② The neurotransmitter, which is thereby discharged into the synaptic cleft, rapidly (in < 0.1 ms) diffuses to the postsynaptic membrane, where it binds to transmembrane receptors, triggering a new nerve impulse = continuation of the nerve impulse in the postsynaptic cell.

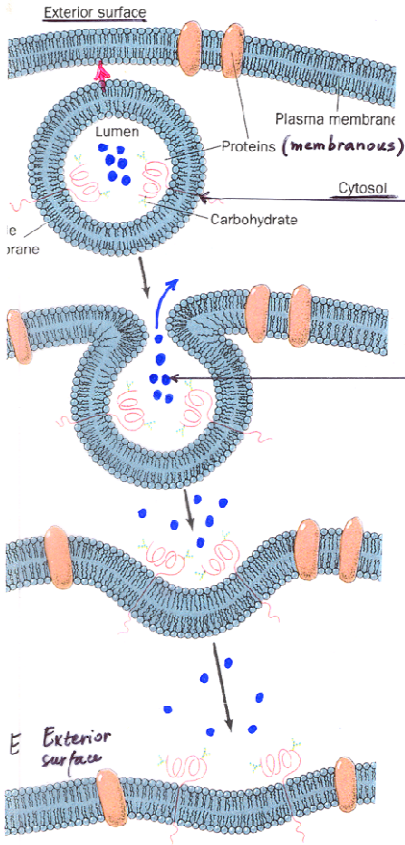
PROTEINS REQUIRED FOR VESICLE FUSION (ACTIVE) PROCESS:

- Rab (GTPases)
- SNARE
  - R-SNARE: assoc. with vesicle membr.
  - Q-t-SNARE: assoc with target membr.

  by association they anchor vesicles to their target membranes = DOCKING
- SM proteins

indicating the presence of an **NEM-sensitive fus protein.** NSF is a cytosolic ATPase that does n membranes unless a **soluble NSF attachme (SNAP)** is also present. SNAPs bind to membr

2. THE FUSION OF A VESICLE WITH THE PLASMA MEMBRANE



↑ fusion direction

I. a) transport of a segment of membrane

b) transport of proteins for secretion = EXOCYTOSIS

Proteins destined for SECRETION (ad b)

i) constitutive

ii) induced (insulin)

12-53 The fusion of a vesicle with the plasma membrane preserves the orientation of the integral proteins embedded in the vesicle bilayer. The inside of the vesicle and the exterior of the cell are topologically equivalent because the same side of the protein is always immersed in the cytosol. Any soluble proteins contained within the vesicle will be secreted. In fact, proteins destined for secretion are packaged in membranous secretory vesicles that subsequently fuse with the plasma membrane as shown.

VESICLE FUSION requires the coordinated action of many proteins. It is an active process.

Being negatively charged, biological membranes do not spontaneously fuse! They strongly repel one another at short distances.

~ Four classes of (membrane) proteins appear to participate in the fusion:

1) Rab (GTPases): recognition of the target membrane

2) SNARE

R-SNARE: in the cargo vesicle

Q-SNARE: in the target membr.

by their association (via complementarity) they anchor vesicles to their target membranes = DOCKING = TRANS-SNARE COMLEX FORMATION

3) Pore forming protein VO

4) SM proteins

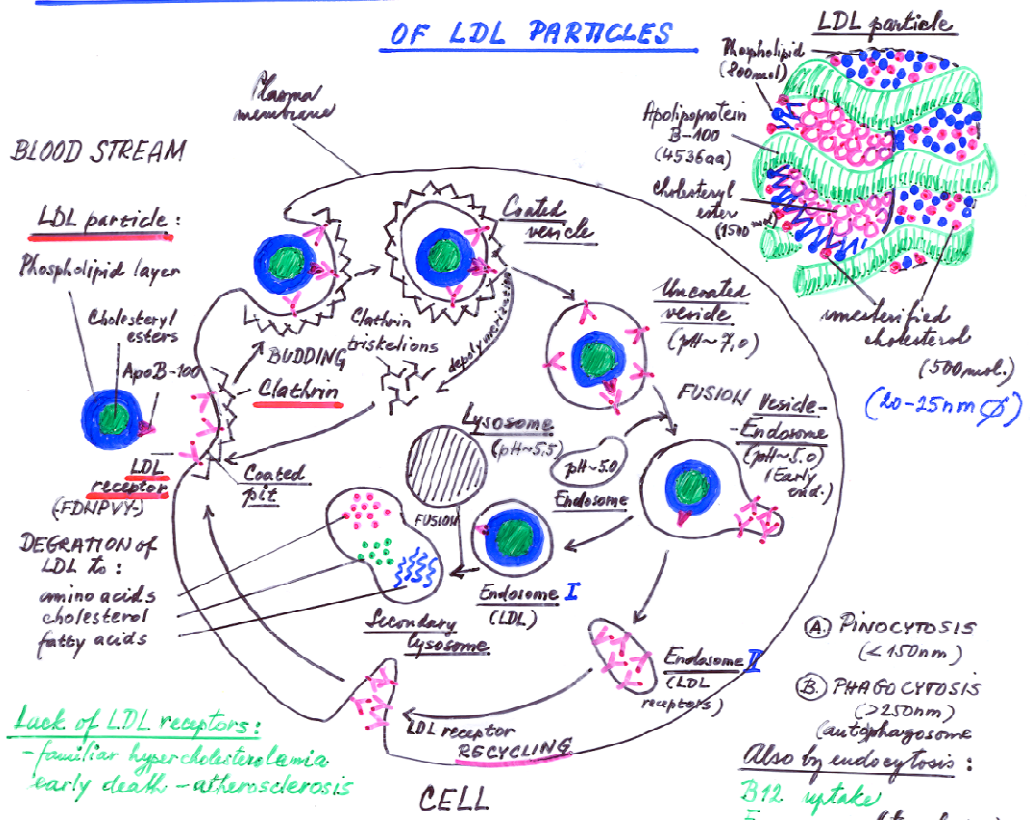
Tet Tx binds to inhibitory neurons ⇒ = spastic paralysis

Bot Tx (1-7) binds to motor neurons ⇒ = flaccid paralysis

TETANUS and BOTULINUS TOXINS specifically cleave (proteases) SNAREs = inhibition of release of neurotransmitters into synapses

ially encased on their outer (cytosolic) faces by oteins that act as flexible scaffolding in prom formation. A vesicle buds off from its memt in and later fuses to its target membrane. *preserves the orientation of the transmembrane (p. 12-53), so that the lumens of the ER and the*

SEQUENCE OF EVENTS IN THE RECEPTOR-MEDIATED ENDOCYTOSIS OF LDL PARTICLES



Lack of LDL receptors:
- familiar hypercholesterolemia
early death - atherosclerosis

Ⓐ PINOCYTOSIS (< 150nm)

Ⓑ PHAGOCYTOSIS (> 250nm) (autophagosome)

Also by endocytosis:
B12. uptake
Fe —"— (transferrin)
virus internalization (influenza, HIV, etc.)

+ active movement:
a) actin-mediated
b) microtubular motor: dynein induced (molecules)

VESICLE – coated
– uncoated

ENDOSOME: dissociation of LDL from its receptor by pH~5.0

- endosome I: vesicular portion of the endosome = accumulates LDL

- endosome II: membrane portion with attached tubular structure = concentrates LDL receptors

LYSOSOME: degradation of LDL – cholesterol → membranes