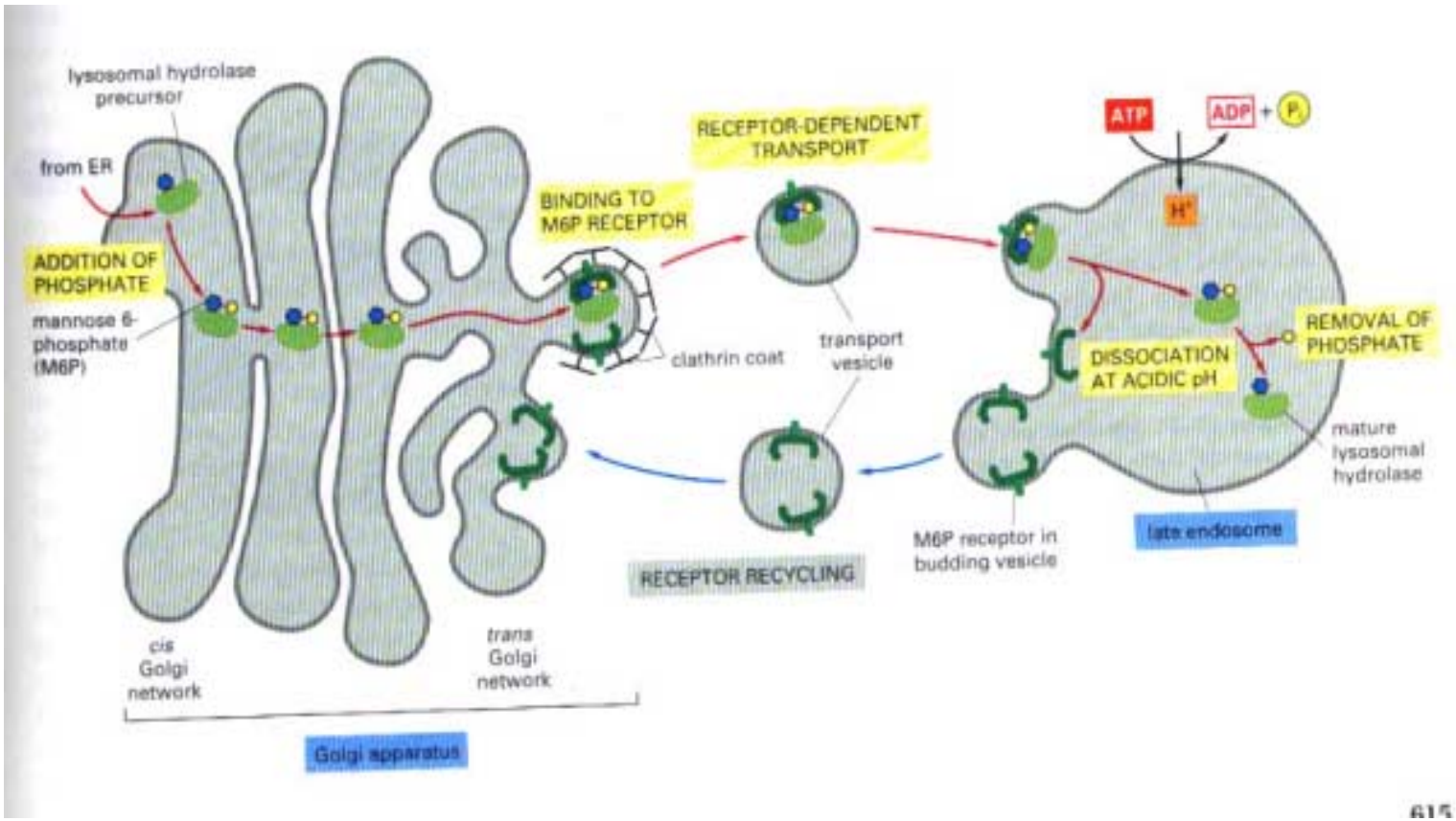
Lüsosoomide ensüümid.

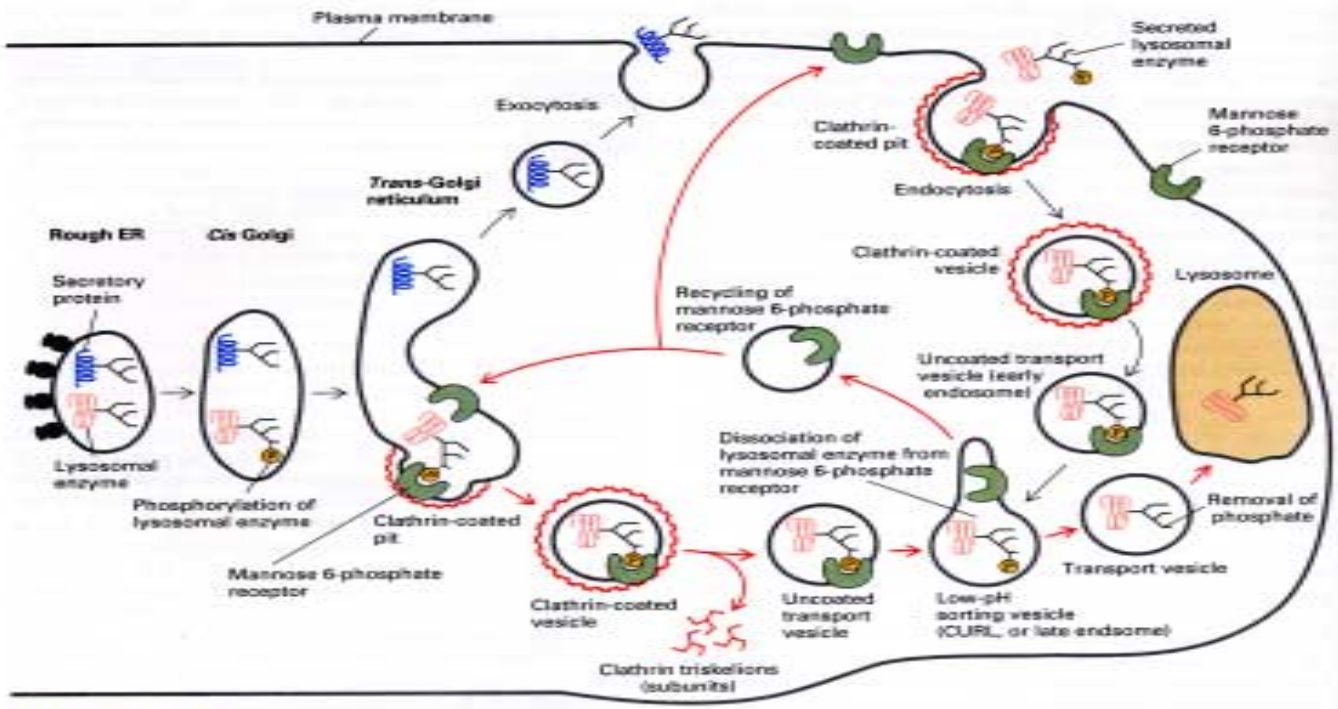
- Kõik lüsosoomide ensüümid on **happelised hüdrolaasid** ja kannavad unikaalset markerit **mannoos-6-fosfaati**.
- proteaasid
- nukleaasid
- glükosidaasid
- lipaasid
- fosfataasid
- sulfataasid
- fosfolipaasid
    - nende pH optimum on 4,8-5,0
- Lüsosoomide membraan sisaldab **transportvalke** ja **H^+ pumpa**, mis kasutab ATP energiat et hoida lüsosoomi sees madalamat pH kui tsütosoolis.

Värkelt sünteesitud lüsosomaalse hüdrolaasi transport lüsosoomi.



N-atsetüülglükoosamiini fosfotransferaas katalüüsib **mannoos-6-fosfaat grupi** teket

Lüsosomaalsete ensüümide suundumine lüsosoomidesse.



▲ FIGURE 16-37 The targeting of lysosomal enzymes to lysosomes. During biosynthesis, lysosomal enzymes migrate from the rough ER (left) to the *cis* Golgi, in which one or more mannose residues are phosphorylated. In the trans-Golgi reticulum, the enzymes bind to the membrane-attached, highly specific mannose 6-phosphate receptor, which directs the enzymes into vesicles coated with the fibrous protein clathrin. The clathrin surrounding these vesicles is rapidly depolymerized to its clathrin triskelion subunits, and the uncoated transport vesicle fuses with a CURL vesicle (also called a late endosome). The low pH of the CURL vesicle (~5.5) causes the phosphorylated enzyme to dissociate from its receptor. The receptor recycles back to the Golgi, and the phosphorylated enzyme is incorporated into a different transport vesicle that buds from the CURL. In this vesicle the protein loses its phosphate group, and the vesicle soon fuses with a lysosome. The sorting of lysosomal enzymes from secretory proteins (left) thus occurs in the trans-Golgi reticulum, and these two classes of proteins are incorporated into different vesicles, which take different routes after they bud from the Golgi.

The mannose 6-phosphate receptor is also found on the plasma membrane (upper right), where it binds extracellular phosphorylated lysosomal enzymes that are occasionally secreted. These receptor-ligand complexes are internalized from the cell surface in clathrin-coated vesicles which also lose their clathrin coats and fuse with the CURL organelle. [See G. Griffiths et al. 1988. *Cell* **52**:329–341; S. Kornfeld. 1992 Ann. Rev. Biochem. **61**:307–330; and G. Griffiths and J. Gruenberg. 1991. *Trends Cell Biol.* **1**:5–9.]

Lagundatava matrjali jõudmine lüsosoomi:

- 1. Makromolekulid võetakse väliskeskkonnast **endotsütoosi** teel *varajastesse endosoomidesse*. Osa neist läheb tagasi plasmamembraani, osa *hilistesse endosoomidesse*.

- 2. **Autofaagia**, mille käigus lagundatakse raku enda vananenud komponente *autofagosoomides* ja hiljem *autofagolüsosoomides*.

- 3. **Fagotsütoos** esineb rakkudes, mis on spetsialiseerunud suuremate osakeste ja mikroorganismide fagotsüteerimisele. See toimub *fagosoomis*, mis hiljem ühineb endosoomiga moodustades *fagolüsosoomi*.

Lagundamise rajad lüsosoomis.

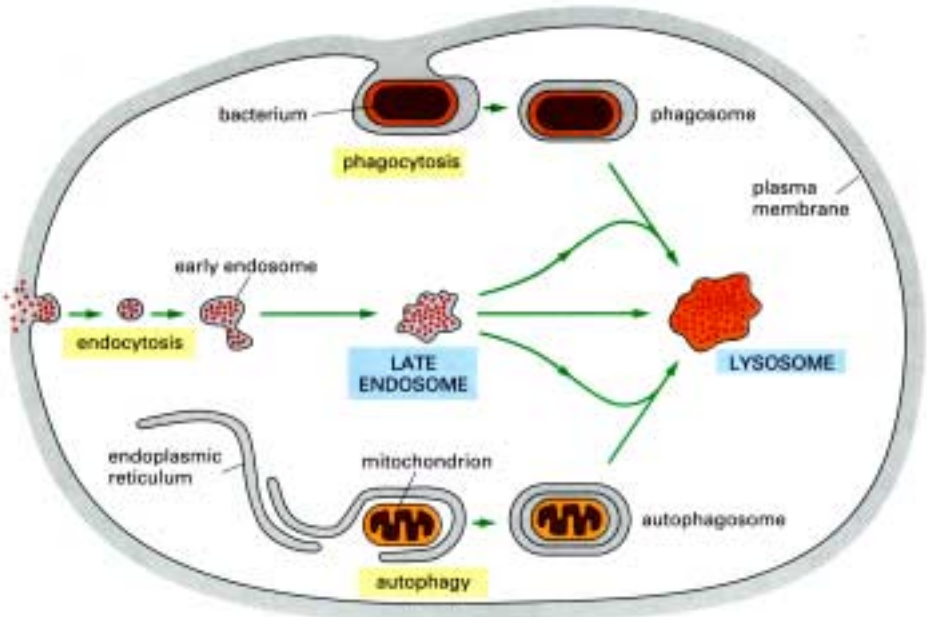


Figure 13–22 Three pathways to degradation in lysosomes. Each pathway leads to the intracellular digestion of materials derived from different source. The compartment resulting from the three pathways c sometimes be distinguished morphologically—hence the terms "autophagolysosome," "phago-lysosome," and so on. Such lysosomes, however, may differ onl because of the different materials they are digesting.

Lüsosomaalsete ensüümide defektidest tingitud haigused.

- Lüsosomaalsed **ladestushaigused** on põhjustatud geneetilistest defektidest, mille tõttu üks või mitmed lüsosomaalsed hüdrolaasid puuduvad.
- 1. **I-rakkude haigus** (ingl.k. *I-cell disease* e. *inclusion-cell disease*), mille korral fibroblastides ja makrofaagides puuduvad kõik hüdrolüütilised ensüümid. Puudub N-atsetüülglükoosamiini fosfotransferaas, mis katalüüsib lüsosomaalsete ensüümide fosforüleerimist, s. o. mannoosi fosforüleerimine puudub (ei teki mannoos-6-fosfaati).
- 2. **Hunter'**i **ja Hurler'**i **sündroom** on tingitud ensüümide defektist, mis on vajalikud sulfaaditud mukopolüsahhariidide lagundamiseks.
- 3. **Tay-Sachs'**i **sündroom** on põhjustatud **β-N-heksoosaminidaasi** defitsiidist, mille tõttu on häiritud gangliosiid GM2 lagundamine.

Rakusisese proteolüütilise süsteemi ülesandeks on:

- a) ära tunda ja elimineerida kokkupakkimata valgud
- b) kõrvaldada kahjustatud või valesti pakitud valgud
- c) lagundada lühikese poolestusajaga valgud (näit. tsükliinid)

- Tsütosoolis lagundatavad valgud suunatakse suurtesse valgu kompleksidesse, mida nimetatakse **proteasoomideks**. Nendes lagundatakse valke, mis on ära märgitud neile kovalentselt seostunud **ubikvitiini** poolt.

Ubikvitiinist sõltuv valkude lagundamine.

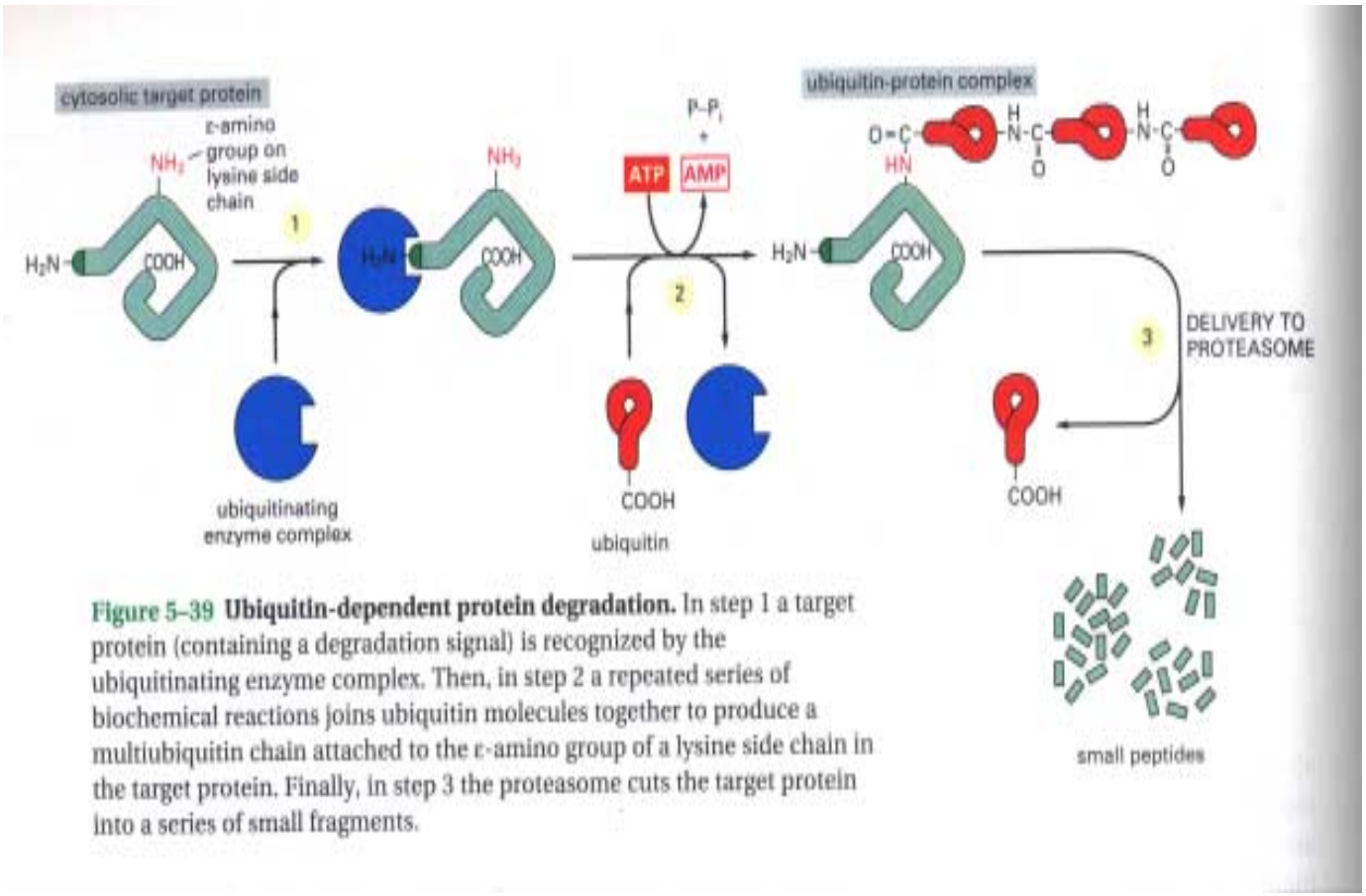
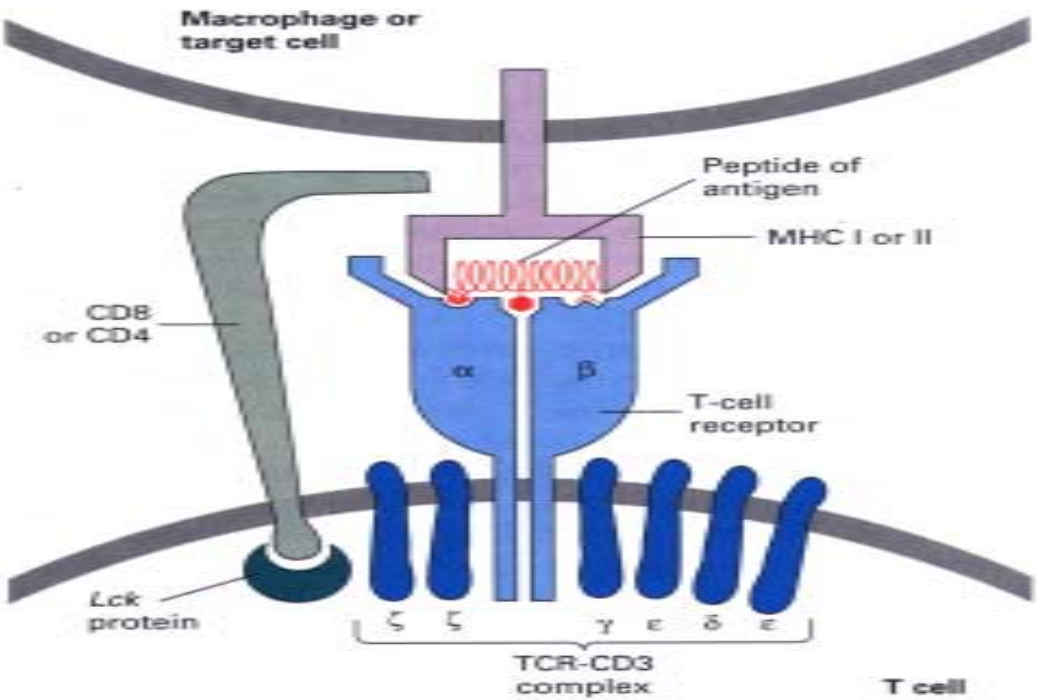


Figure 5–39 Ubiquitin-dependent protein degradation. In step 1 a target protein (containing a degradation signal) is recognized by the ubiquitinating enzyme complex. Then, in step 2 a repeated series of biochemical reactions joins ubiquitin molecules together to produce a multiubiquitin chain attached to the ε-amino group of a lysine side chain in the target protein. Finally, in step 3 the proteasome cuts the target protein into a series of small fragments.

Ubikvitiin-sõltuva süsteemi abil lagundatakse denatureeritud, valesti pakitud või oksüdeerunud amiinohappeid sisaldavaid valke.

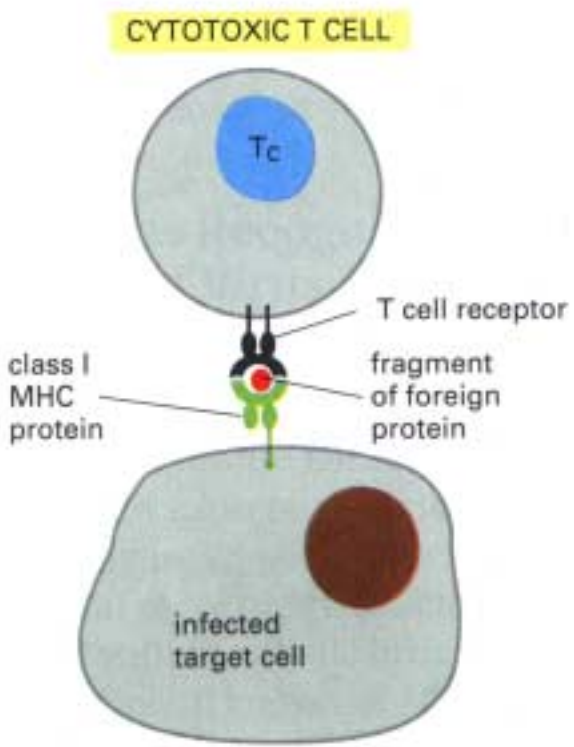
Põhilise koesobivuskompleksi (MHC) ja T-raku retseptori seostumine.



MHC I klassi molekulid on kõigil tuumaga rakkudel,
MHC II klassi molekulid on ainult antigeeni esitlevatel rakkudel (B-lümfotsüüdid, makrofaagid, dendriitrakud)

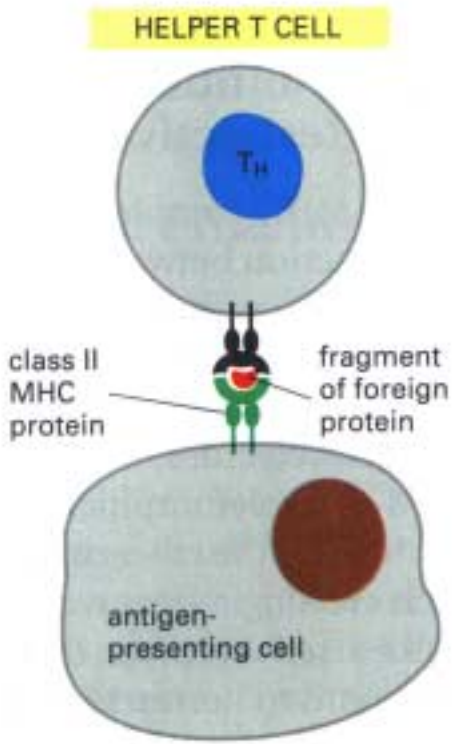
Tsütotoksiline ja abistaja T rakk tunnevad ära erinevaid MHC komplekse.

Tsütotoksiline T-lümfotsüüt



Nakatunud märklaudrakk

Abistaja T-lümfotsüüt



Antigeeni esitlev rakk

Ekstratsellulaarse valgulise antigeeni töötlemine esitlemaks T abistajarakkudele.

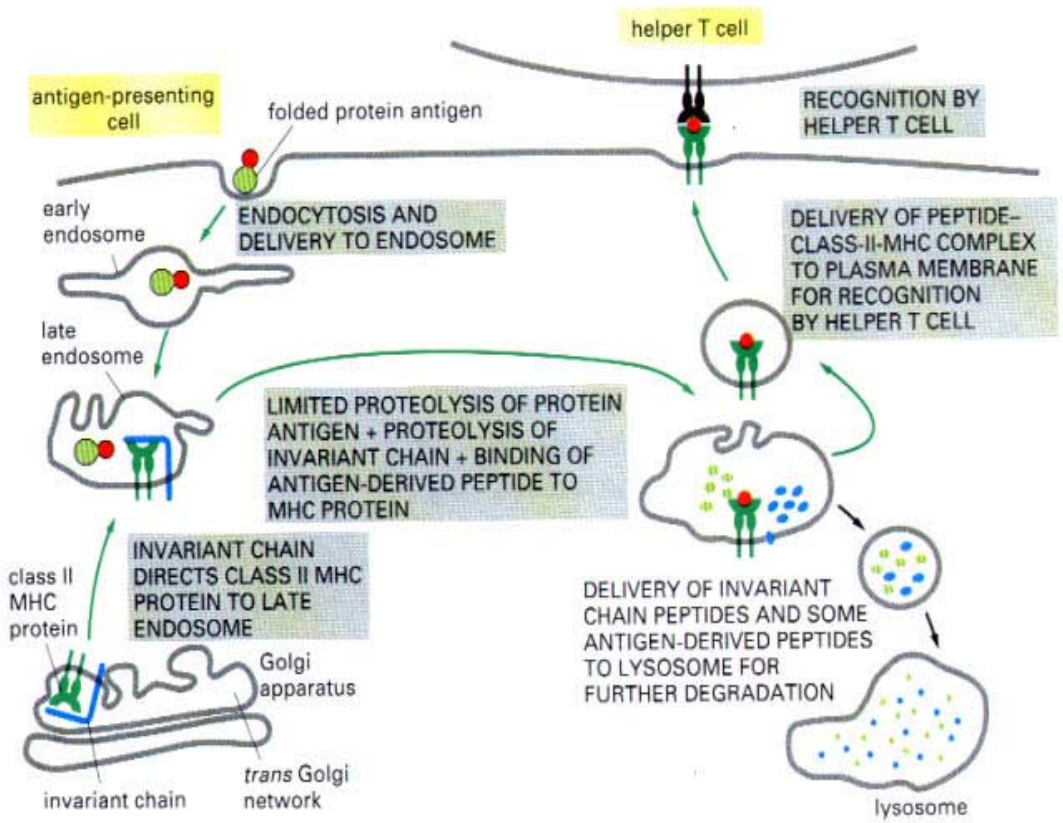
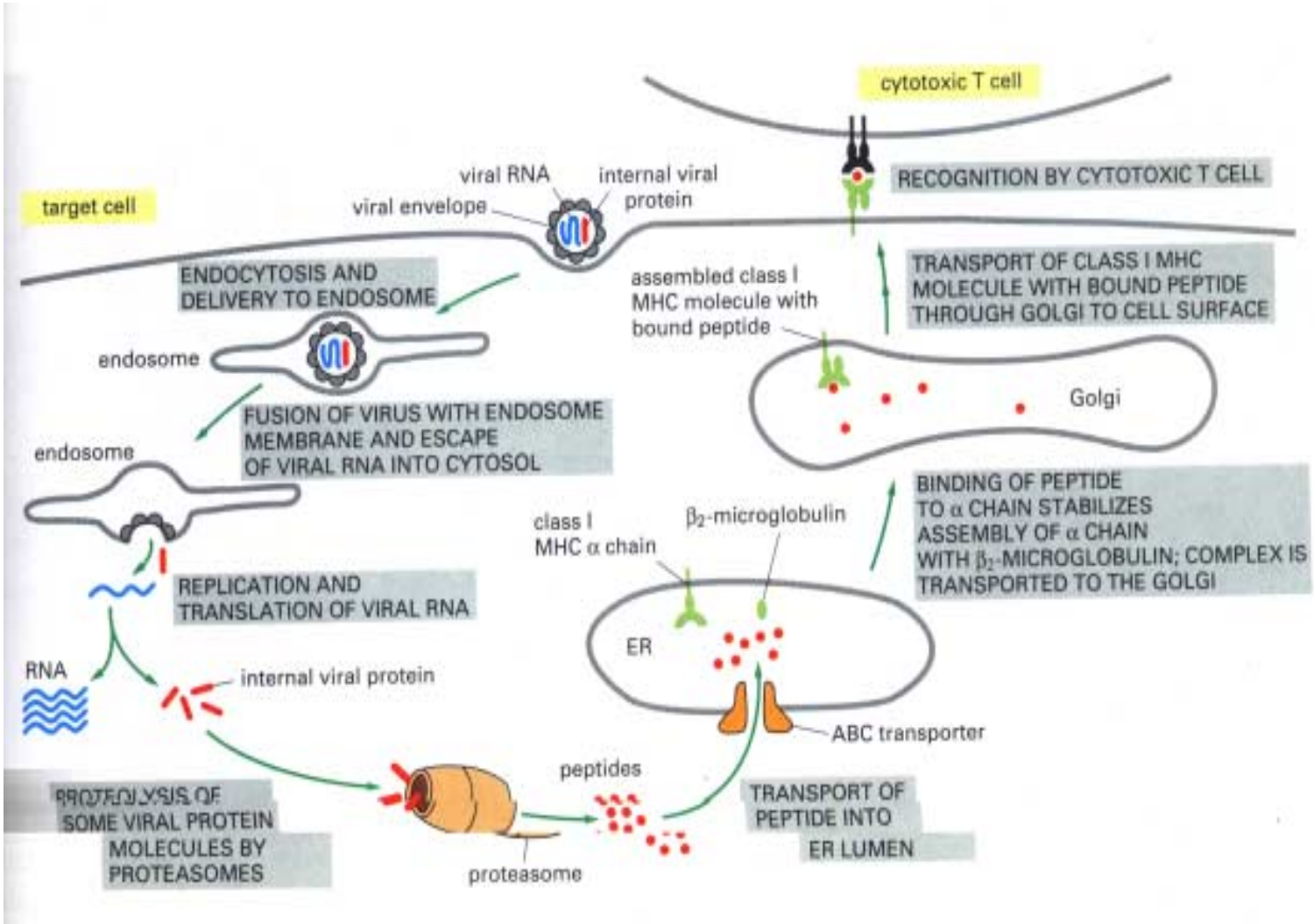


Figure 23–53 The processing of an extracellular protein antigen for presentation to a helper T cell. A current view of how peptide–class-II-MHC complexes are formed in endosomes and delivered to the surface of an antigen-presenting cell.

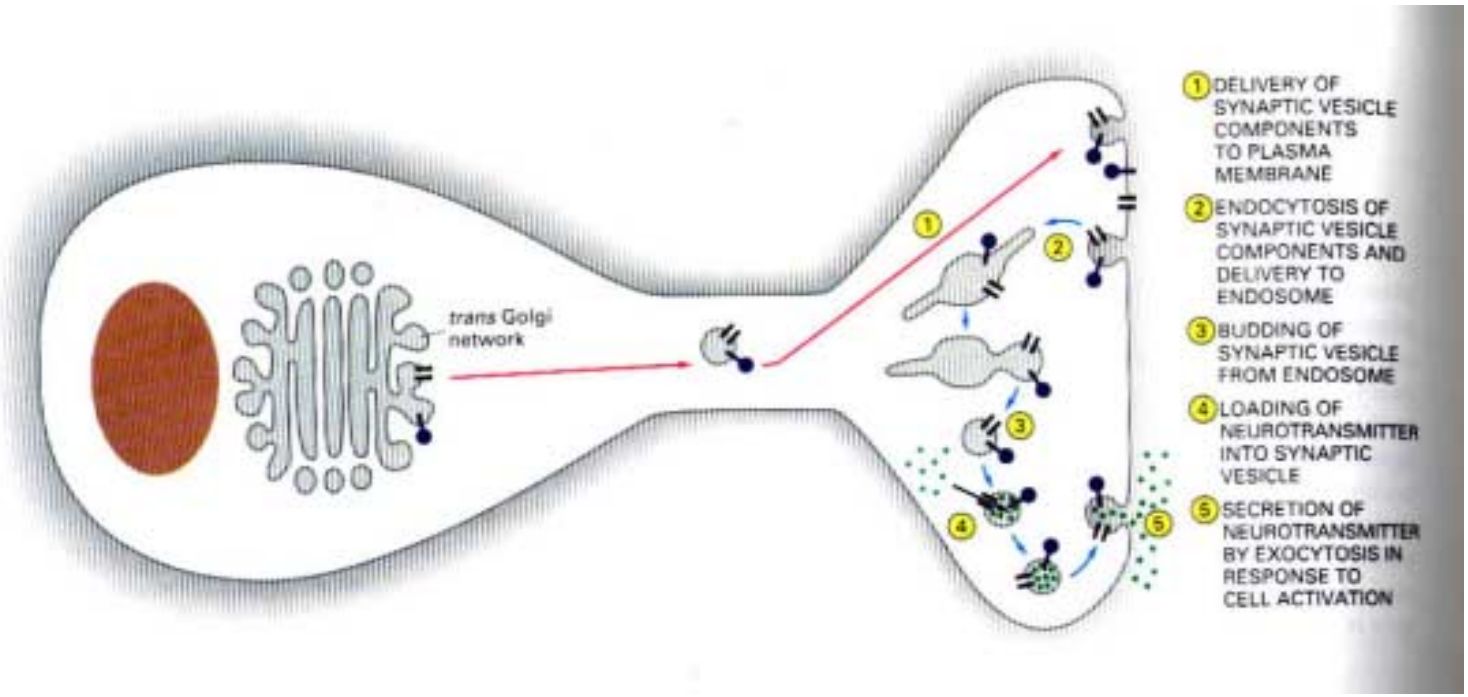
Viirusvalgu töötlemine selleks, et teda esitleda tsütotoksilisele T-lümfotsüüdile.



Eksotsütoosi teed.

- Ekso- ja endotsütoos on membraaniga ümbritsetud **transpordivesiikulite teke** ja nende ühinemine kas raku välismembraaniga (eksotsütoos) või endosoomi membraaniga (endotsütoos).
- 1. **Pidev e. konstitutiivne tee.**
- **Transpordivesiikulid** kannavad uusi membraani komponente Golgi kompleksist välismembraani. Organismile vajalike valkude eksotsüteerimine rakust väliskeskkonda.
- 2. **Reguleeritud tee.**
- Sekreteeritavad ained kogutakse **sekretoorsetesse vesiikulitesse** ning need ühinevad välismembraaniga pärast keskkonnast tulevat signaali.

Sünaptiliste vesiikulite formeerumine.



Endotsütoosi tüübid.

- 1. **Pinotsütoos**-lahustunud makromolekulide sissevõtmine väikeste vesiikulite abil.
  - a) retseptor-vahendatud endotsütoos
  - b) vedela faasi endotsütoos
- 2. **Fagotsütoos**-suurte partiklite (mikroorganismid, surnud rakkude osad jne.) sissevõtmine.

Klatriinse katte teke ja lagunemine.

membrane vesicle, the clathrin coats on vesicles are constructed in a similar way from 12 pentagons plus a larger number of hexagons. (A, from E. Ungewickell and D. Branton, *Nature* 289:420–422, 1981, © 1981 Macmillan Journals Ltd.; B, from I.S. Nathke et al., *Cell* 68:899–910, 1992. © Cell Press; C, from G.P.A. Vigers, R.A. Crowther, and B.M.F. Pearse, *EMBO J.* 5:2079–2085, 1986.)

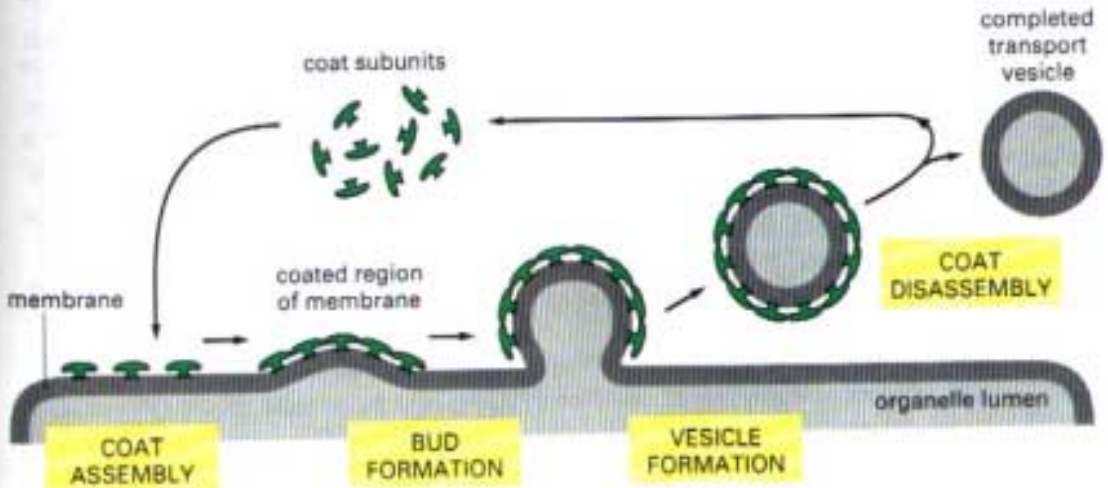


Figure 13–51 The assembly and disassembly of a clathrin coat. The assembly of the coat is thought to introduce curvature into the membrane, which leads in turn to the formation of uniformly sized coated buds. The pinching off of the bud to form a vesicle involves the more complex process of membrane fusion, which we discuss later. Although coats consist of multiple protein components, only clathrin is shown in this simplified schematic drawing. Whereas the coat of clathrin-coated vesicles is rapidly removed shortly after the vesicle forms, we shall see later that coatomer coats are removed after the vesicle docks on its target membrane.

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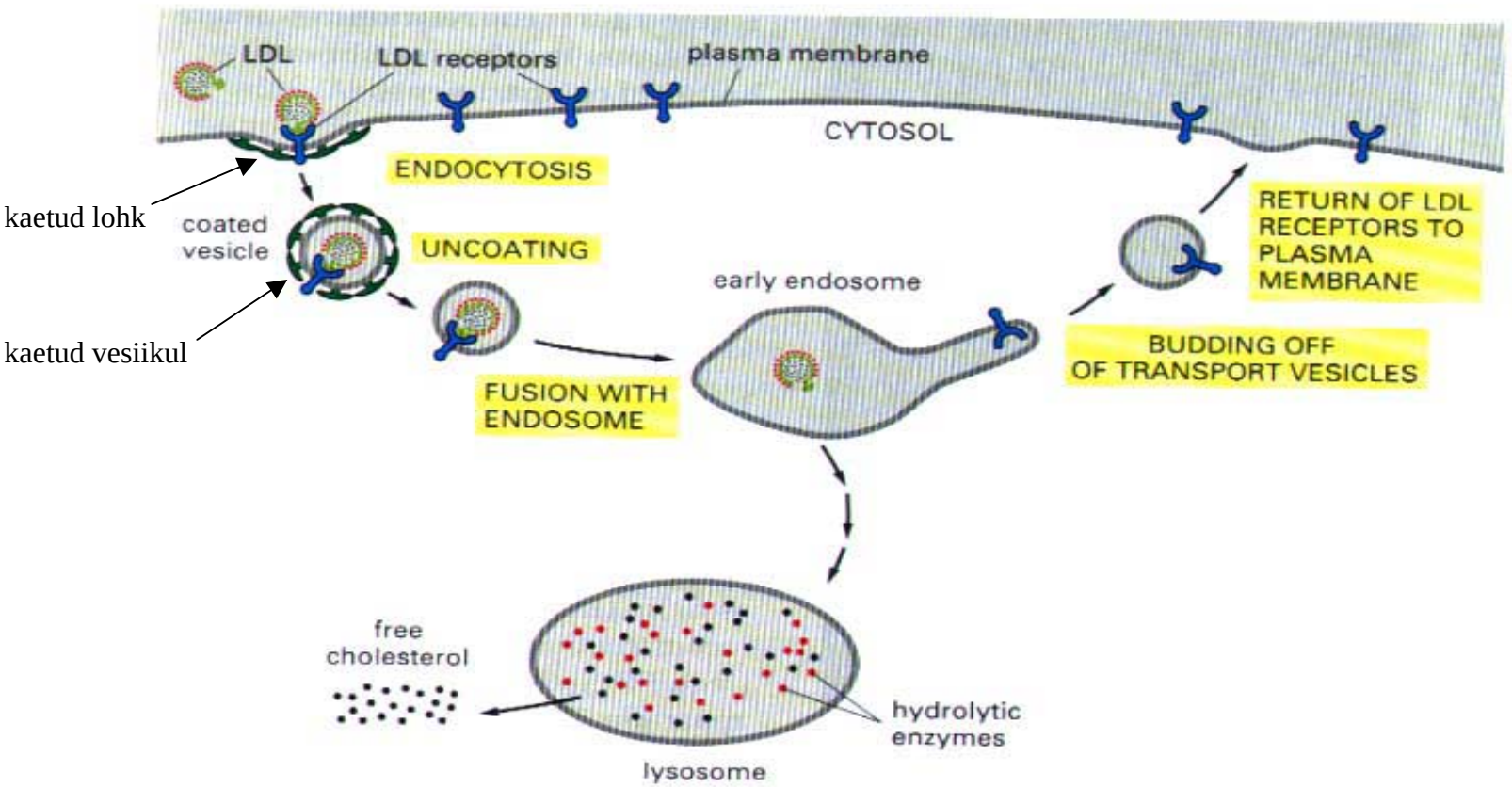
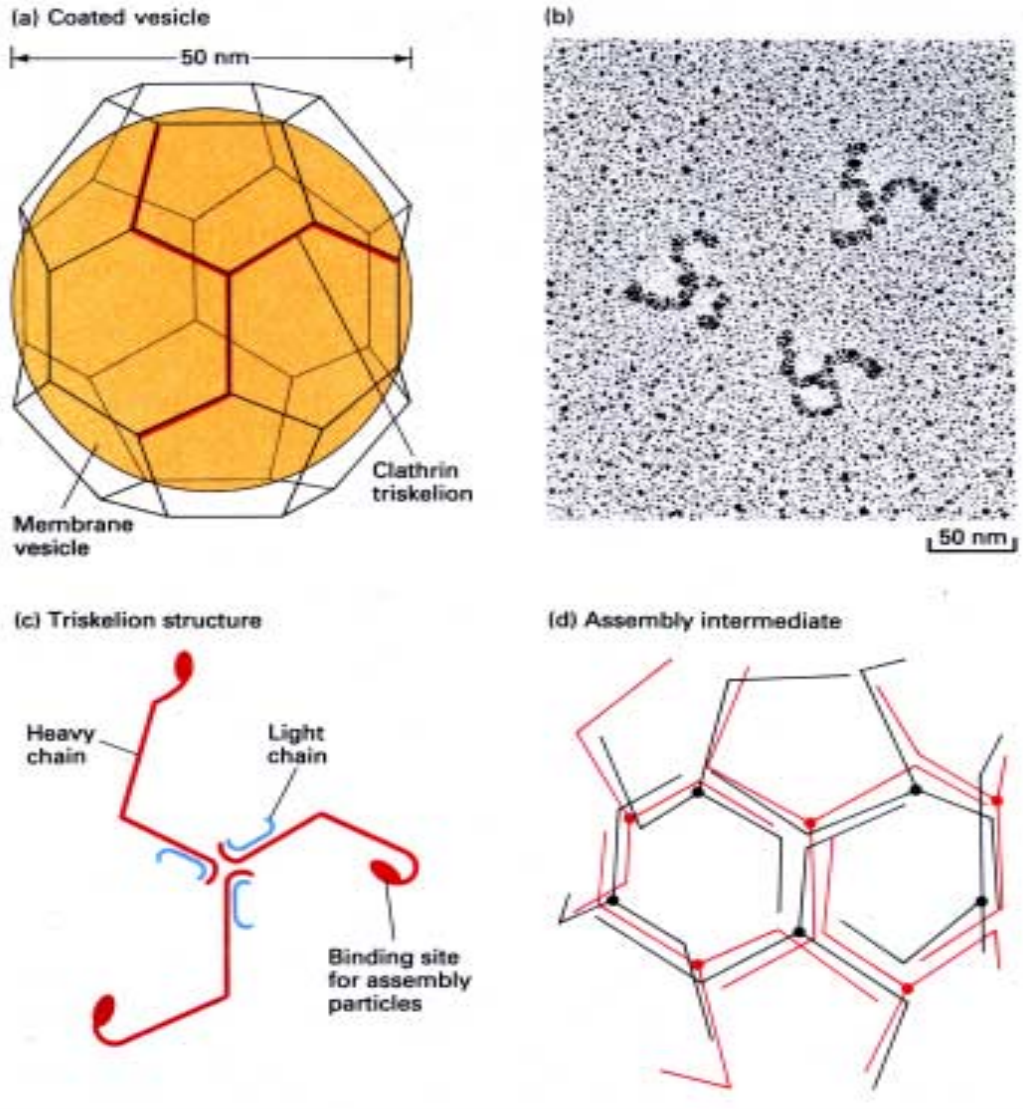
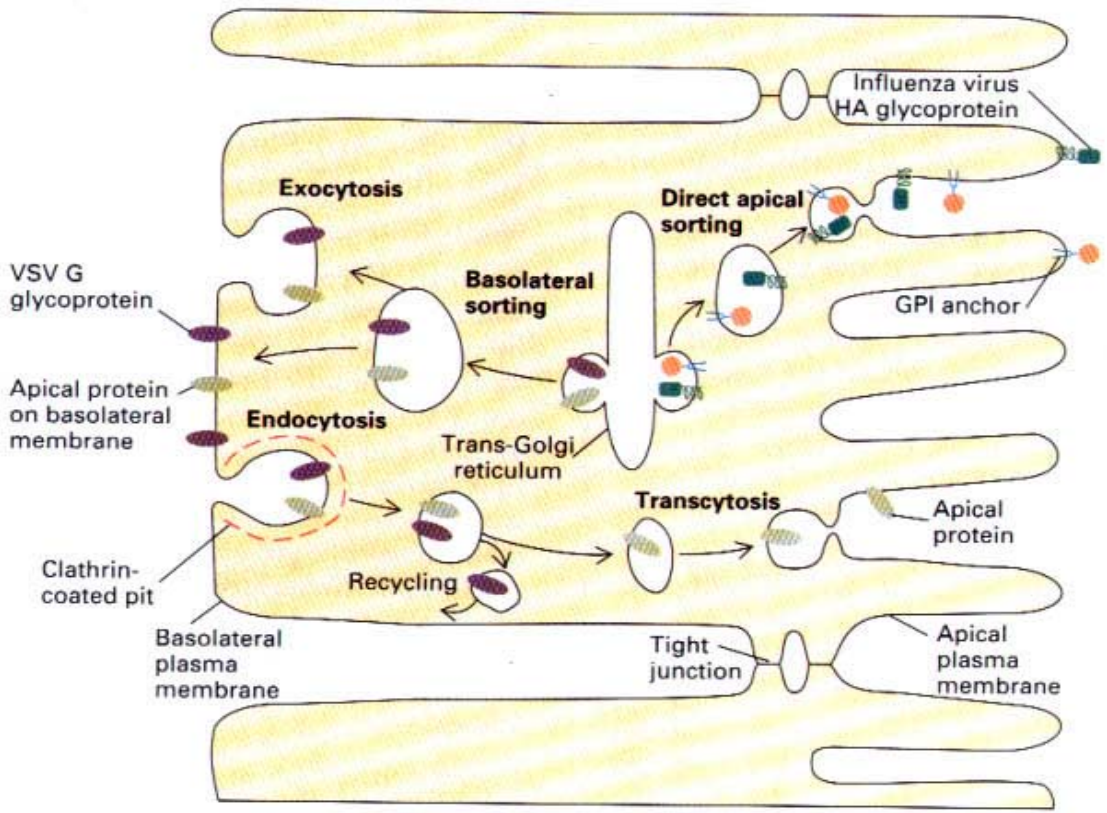
Madala tihedusega lipoproteiini (LDL) retseptor-vahendatud kolesterooli endotsütoos.



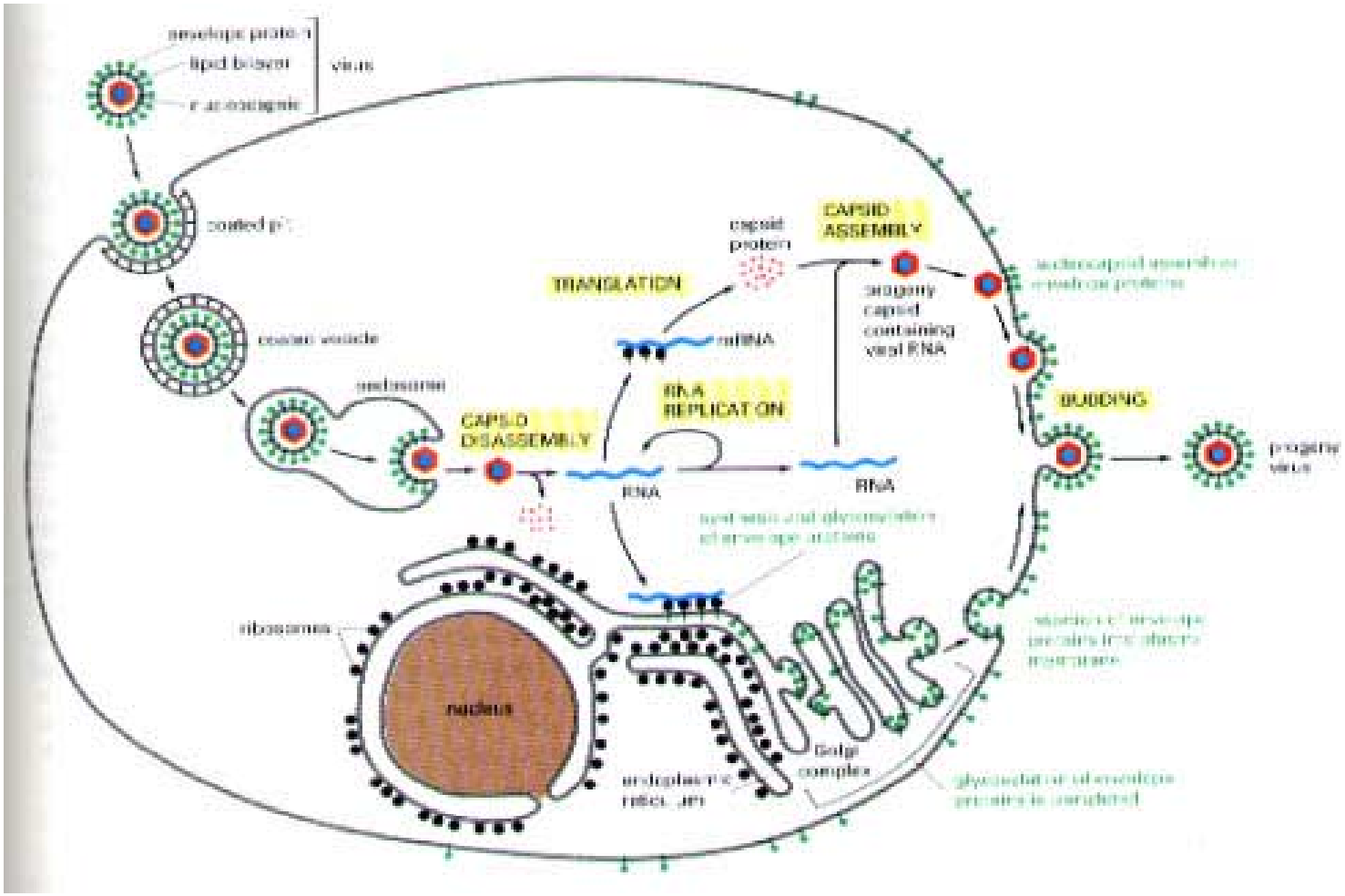
Klatriiniga kaetud vesiikuli struktuur.



Eksotsütoos, endotsütoos ja transtsütoos.



Semiliki metsaviiruse elutsükkel.



Antikeha poolt esilekutsutud fagotsütoos.

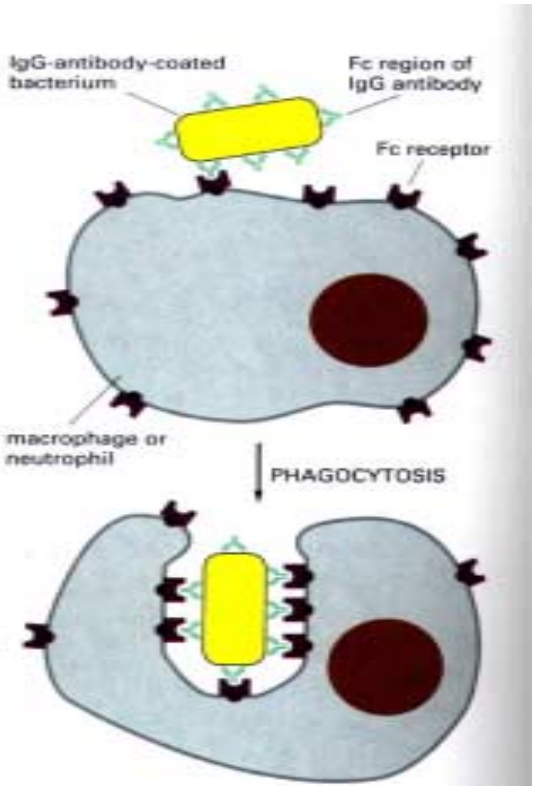


Figure 23–20 Antibody-activated phagocytosis. An IgG-antibody-coated bacterium is efficiently phagocytosed by a macrophage or neutrophil, which has cell-surface receptors able to bind the Fc region of IgG molecules. The binding of the antibody-covered bacterium to these Fc receptors activates the phagocytic process.

Valkude liikumine viisid rakus.

- 1. **Väravtransport** esineb tuuma ja tsütoplasma vahel *tuuma pooride kompleksi* kaudu.
- 2. **Transmembraanne transport** toimub membraaniseoseliste *translokaator-molekulide* abil.
- 3. **Vesikulaartransport** membraaniga ümbritsetud *transpordi-vesiikulite* abil.
  - a) **konstitutiivne sekretoorne suund** läheb endoplasmaatilisest retiikulumist Golgi kompleksi ja sealt raku välispinnale
  - b) **lüsosomaalne suund** kus *mannoos-6-fosfaadi* poolt märgistatud valgud suunatakse läbi endosoomi lüsosoomi.
  - c) **reguleeritud sekretoorne suund** esineb spetsialiseeritud rakkudes, kus on nn. *kontrollitud eksotsütoos*. Valkudel peavad olema signaaljärjestused, mis määravad nende jõudmise trans-Golgi sekretoorsetesse vesiikulitesse.

Vesikulaartransport rakkudes.

- See ühendab omavahel:
- ER-i
- Golgi kompleksi (cis-, kesk-, ja trans-Golgit)
- Endosoome
- Lüsosoome
- Sekretoorseid vesiikuleid
- Raku välispinda

- **Klassikaline sekretoorne rada:**

- ER → Golgi → Sekretoorsed vesiikulid (kontrollitud eksotsütoos)
  Golgi → eksotsütoos rakust (plasmamembraan)
  Golgi → Mannoos-6-fosfaat lüsosoomi

Vesikulaartransport rakkudes.

Vesiikulite teke

Katalüüsivad nn. katte valgud (ingl. k. *coat proteins*)

Tuntakse kahte tüüpi vesiikuleid:

a) klatriiniga kaetud vesiikulid

b) koatomeeriga kaetud vesiikulid (COP valgud)

COP I (GK → ER)

COP II (ER → GK)

Koatomeerse katte teke ja lagundamine on sõltub GTP-seoselisest valgust **ARF** (võib osaleda ka klatriinse katte tekkes).

Vesikulaartransport rakkudes.

Õigete molekulide pakkimine vesiikulitesse.

Seostumine retseptoriga (mannoos-6-retseptor, retseptorvahendatud endotsütoos)

Õige sihtmembraani äratundmine rakus.

Organelle markeerivad SNARE valgud (vSNARE ja tSNARE)

Vesiikuli ühinemine sihtmembraaniga. Membraanide ühinemist katalüüsivad nn. ***fusogeensed valgud***

SNARE= SNAP retseptor

SNAP=soluble NSF-attachment protein

NSF=N-ethylmaleimide sensitive factor

Organellidesse suunavad signaalid valkude koostises.

Organell	**Paiknemine valgus**	**Signaaljärjestuse äralõikamine**	**Koostis**
ER	N-terminus	+	6-12 hüdrofoobsest aminohappest koosnev südamik, millele eelneb üks või mitu aluselist aminohapet
Mitokonder	N-terminus	+	3-5 mittejärjestikust Arg või Lys, tihti koos Ser ja Thr
Kloroplast	N-terminus	+	Pole ühiseid motive, esineb palju Ser ja Thr ning ei esine Glu ja Asp
Peroksüsoom	C-terminus		Tavaliselt Ser-Lys-Leu
Tuum	internaalne		Üks klaster, mis koosneb viiest aluselisest aminohappest (Lys, Arg) või kahest väiksemast klastrist, mis on eraldatud teineteisest 10-11 aminohappega

Vesikulaartransport. Sekretoorsed ja endotsütootilised rajad.

Every cell must communicate with its environment. In a procaryotic cell all of this communication takes place across the plasma membrane: digestive enzymes, for example, are secreted to the cell exterior, and the small metabolites generated by digestion are then taken up by transport proteins in the plasma membrane. Eucaryotic cells, by contrast, have evolved an elaborate internal membrane system that allows them to take up macromolecules by a process called *endocytosis* and deliver them to digestive enzymes that are stored intracellularly in lysosomes; as a consequence, metabolites generated by digestion are delivered from the lysosomes directly to the cytosol as they are produced. Besides providing for regulated digestion of macromolecules by the *endocytic pathway*, the internal membrane system provides a means whereby eucaryotic cells can regulate the delivery of newly synthesized proteins and carbohydrates to the exterior. Because each molecule that travels along this *biosynthetic-secretory pathway* passes through multiple compartments, the cell can modify the molecule in a series of controlled steps, store it until needed, and then deliver it to a specific cell-surface domain by a process called *exocytosis*. The endocytic and biosynthetic-secretory pathways are shown in color in Figure 13–1.

Compartmental Diversity

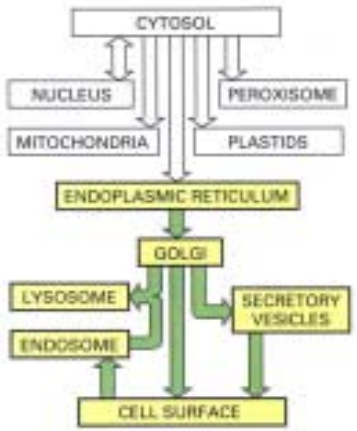


Figure 13–1 The secretory and endocytic pathways. In this "road map" of biosynthetic protein traffic, which was introduced in Chapter 12, both the secretory and endocytic pathways are colored.

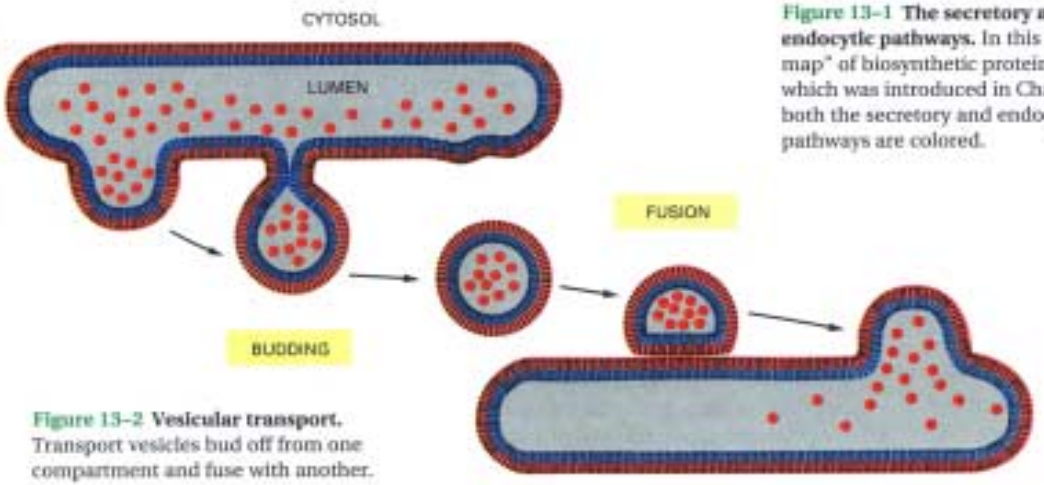
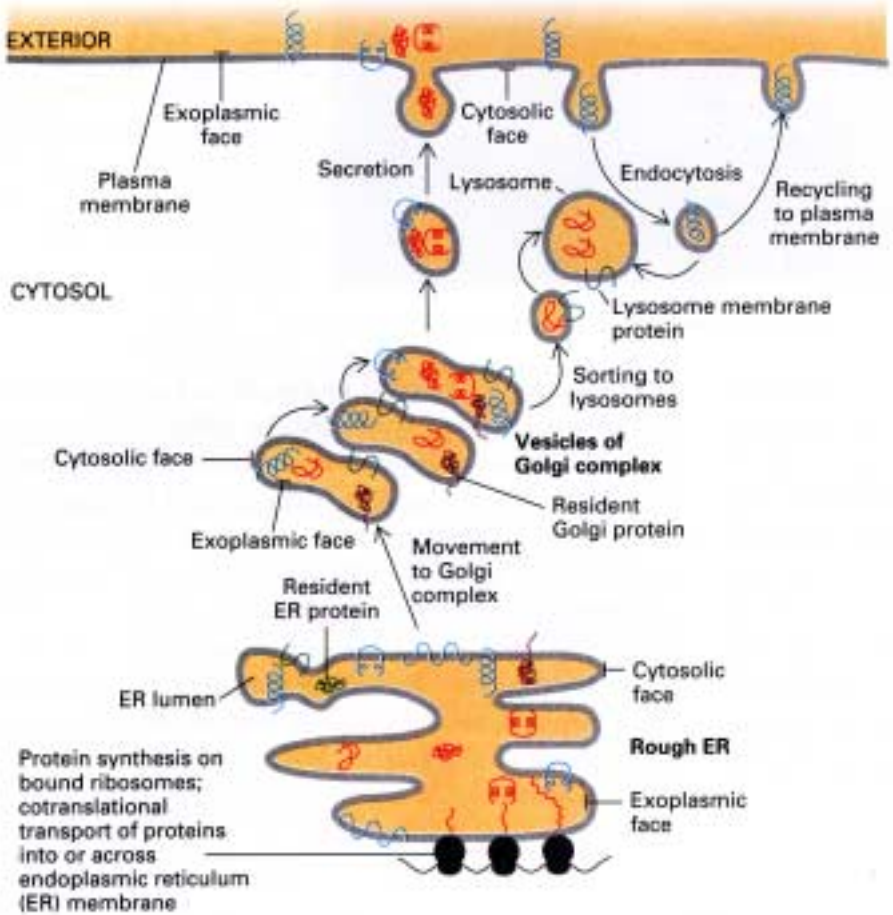


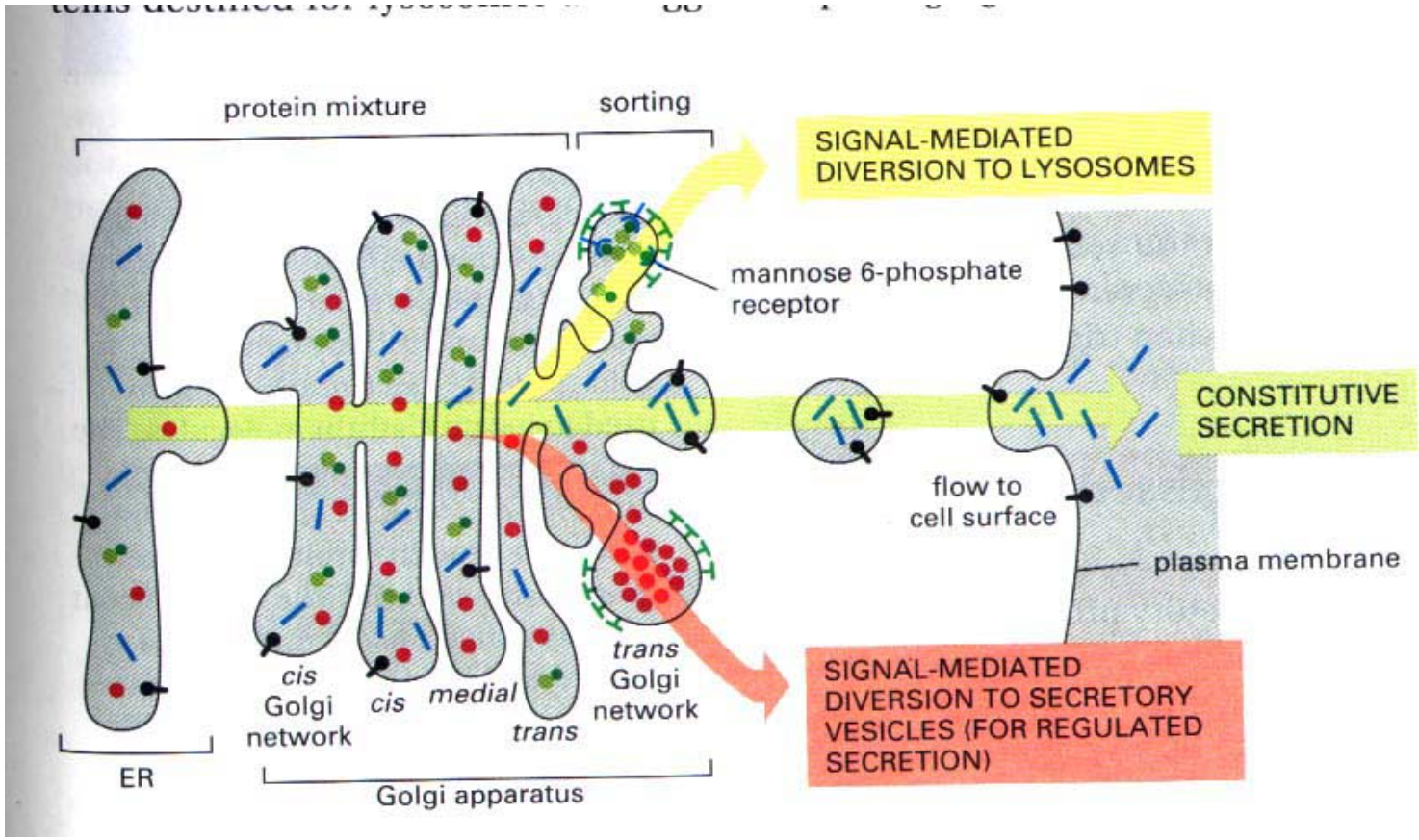
Figure 13–2 Vesicular transport. Transport vesicles bud off from one compartment and fuse with another.

Sekreteeritavate valkude sünteesi ja sortimise rajad.

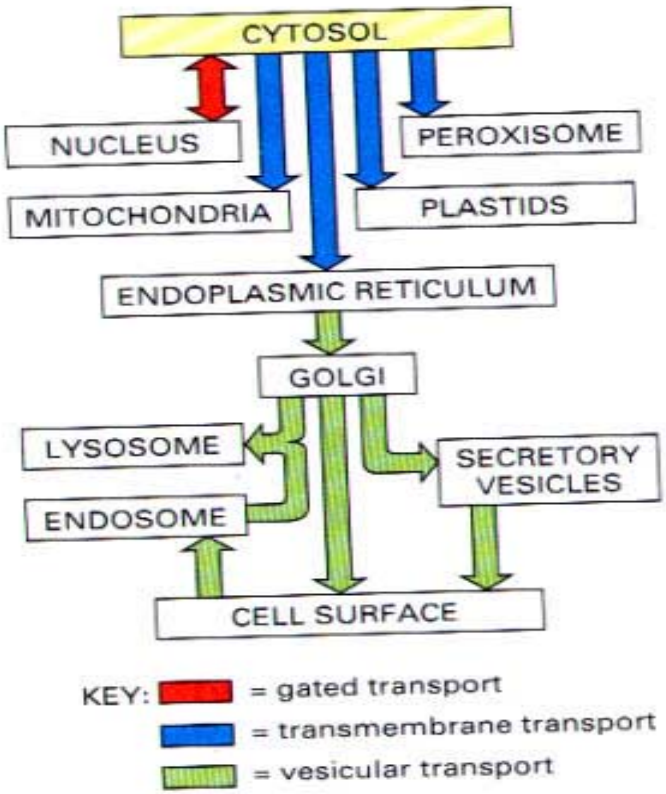


◀ FIGURE 16-1 The secretory pathway of protein synthesis and sorting. Proteins synthesized on ribosomes bound to the rough endoplasmic reticulum (ER) enter the secretory pathway. This class includes both integral membrane proteins that become inserted in the ER membrane and proteins that become soluble in the lumen of the ER. The proteins are subsequently sorted to their different destinations. Some (rough ER enzymes or structural proteins) remain resident in the ER. The remainder move via transport vesicles to the three classes of Golgi vesicles, where certain of them remain. The rest of the proteins move, via secretory vesicles, either to the plasma membrane—where soluble proteins are secreted—or to lysosomes. Plasma membrane proteins also move *via* transport vesicles from the rough ER to the Golgi to the plasma membrane; frequently they are internalized by endocytosis and then either sorted to lysosomes for degradation or recycled back to the plasma membrane. Each integral membrane protein (blue) retains its membrane orientation during all the sorting steps: some segments always face the cytosol (gray); others always face the exoplasmic space (yellow; i.e., the lumen of the ER, the Golgi vesicles, and other transport vesicles and the outside of the cell).

Valkude sorteerimide teed trans-Golgi võrgustikus.



Valkude liikumise teed rakus.



SNARE-de osa vesikulaarse transpordi juhtimises.

Fusogeensed valgud

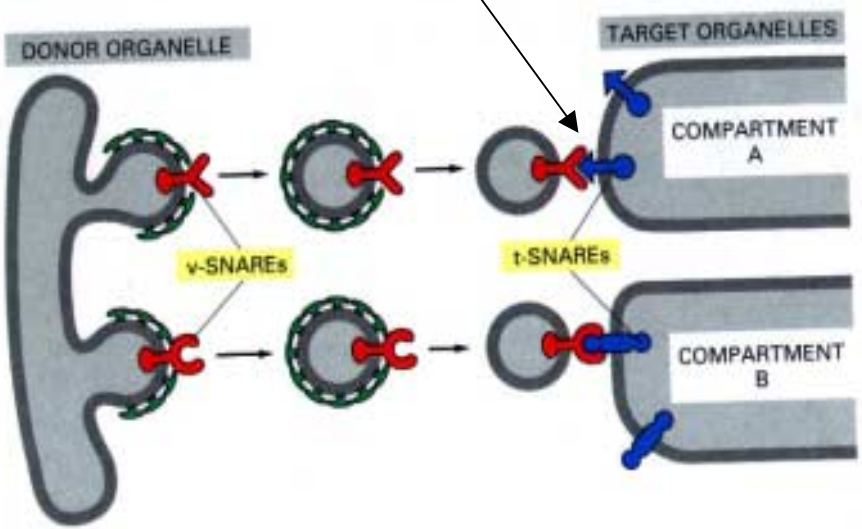
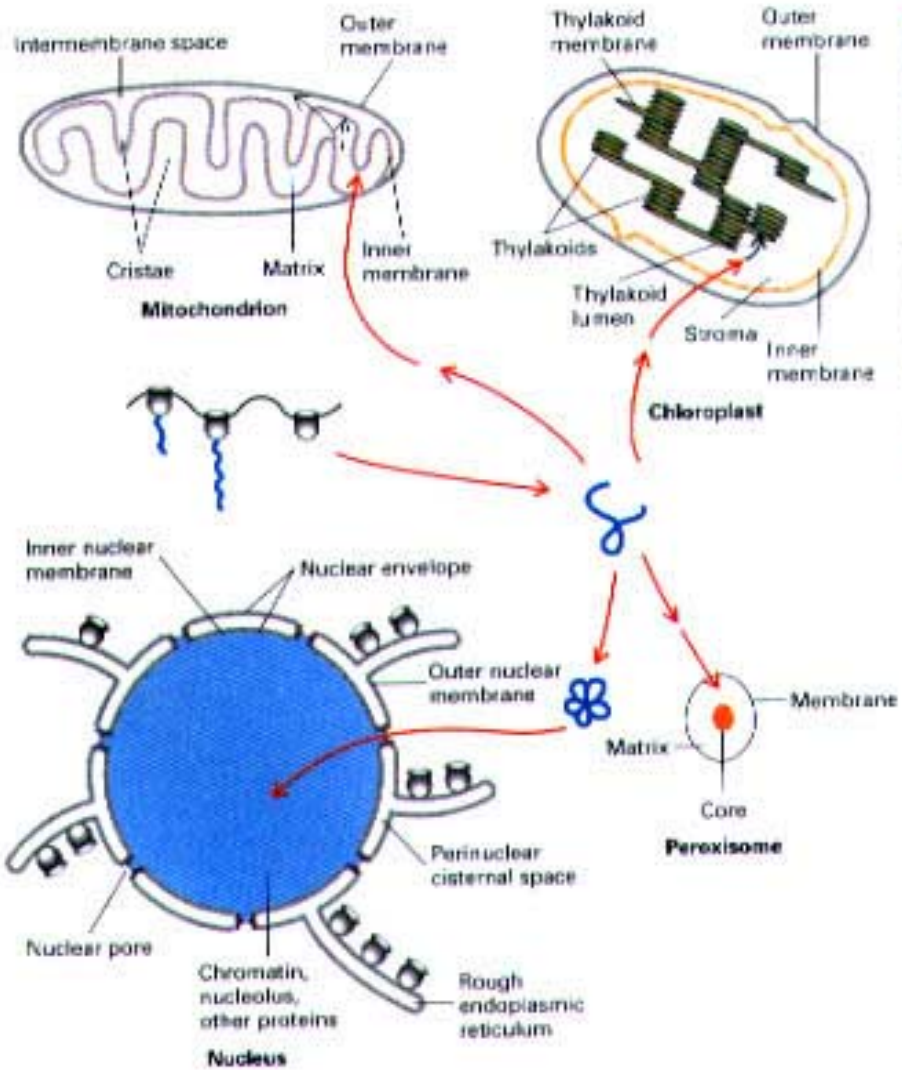


Figure 13–56 The postulated role of SNAREs in guiding vesicular transport. Complementary sets of vesicle-SNAREs (v-SNAREs) and target-membrane SNAREs (t-SNAREs) determine the selectivity of transport-vesicle docking. v-SNAREs, which are co-packaged with the coat proteins during the budding of transport vesicles from the donor membrane, bind to complementary t-SNAREs in the target membrane.

Valkude import tsütosoolist mitokondrisse, kloroplasti, peroksüsoomi ja tuuma.



◀ FIGURE 19-1 Import of proteins from the cytosol into the mitochondrion, chloroplast, peroxisome, and nucleus. Immediately after their synthesis on free cytosolic ribosomes (those not bound to membranes), these proteins are released into the cytosol. Mitochondrial proteins insert into the outer membrane, or cross the outer and inner mitochondrial membranes to enter the matrix space. Some remain there, others (black arrows) cross into the intermembrane space or become incorporated into the inner membrane. Similarly, chloroplast proteins cross the outer and inner chloroplast membranes to enter the stroma. Some remain there, others (black arrow) enter the thylakoids. Peroxisomal proteins cross the peroxisome membrane, and nuclear proteins enter through the nuclear pores. Ribosomes and mRNAs exit the nucleus through the pores.