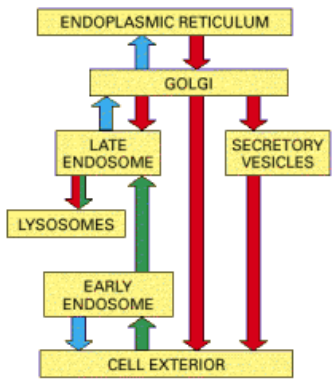
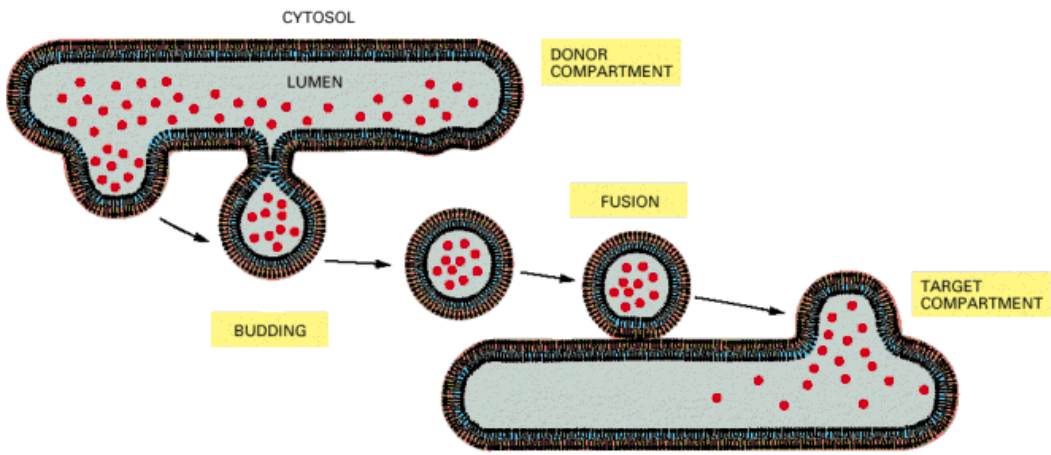
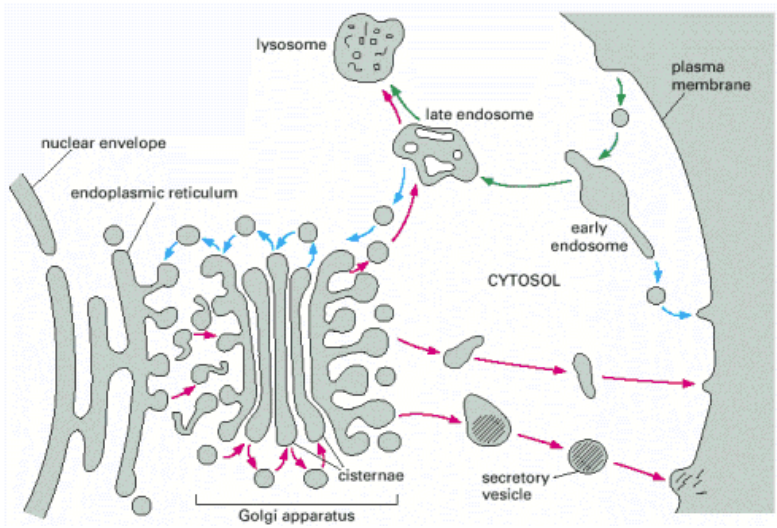
Μεταφορά με κυστίδια, οδοί έκκρισης και ενδοκυττάρωσης



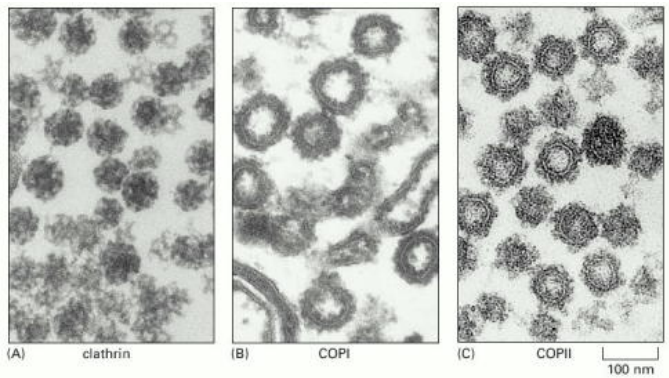
Μεταφορά με κυστίδια



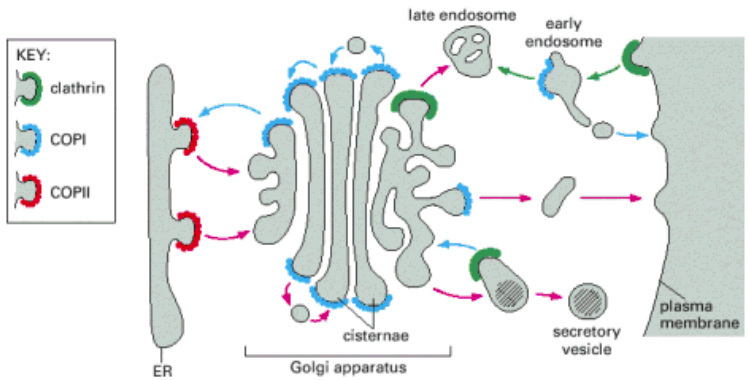
Κυτταρικά διαμερίσματα που επικοινωνούν μεταξύ τους μέσω κυστιδίων μεταφοράς



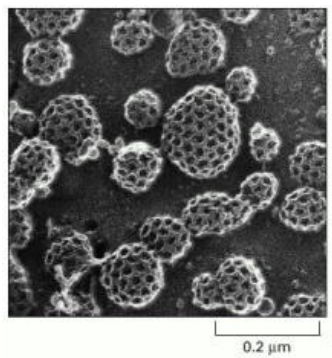
Καλυμμένα κυστίδια

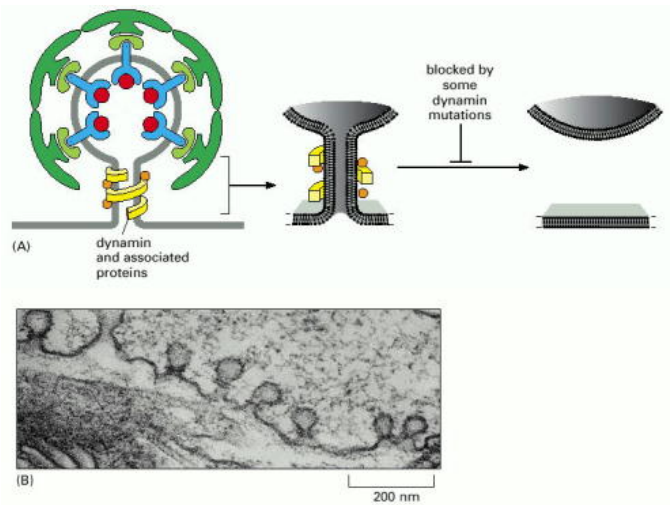


Πρωτεΐνες κάλυψης και διαδρομές

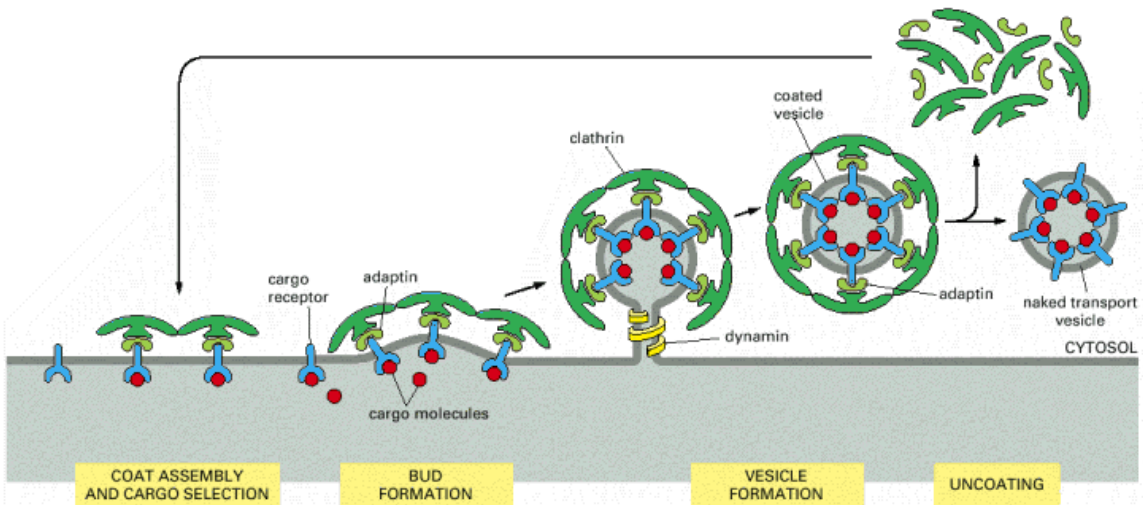


Κυστίδια καλυμμένα με κλαθρίνη

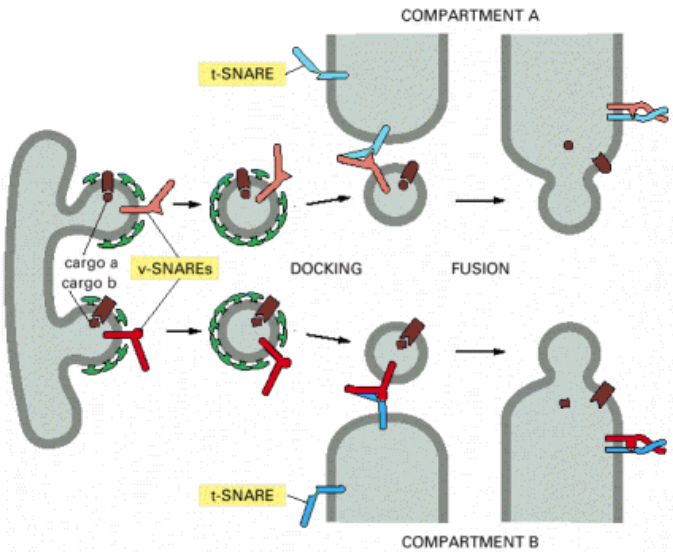




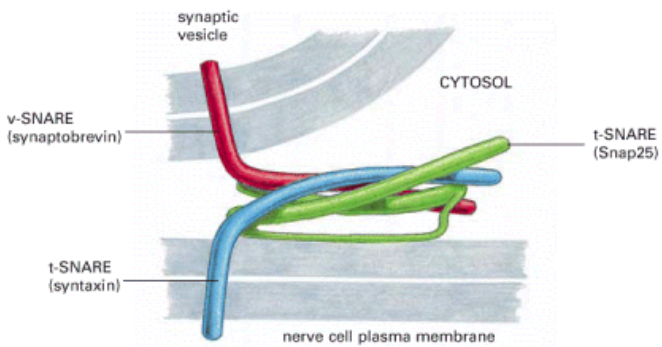
Επιλεκτική μεταφορά μέσω κυστιδίων καλλυμένων με κλαθρίνη

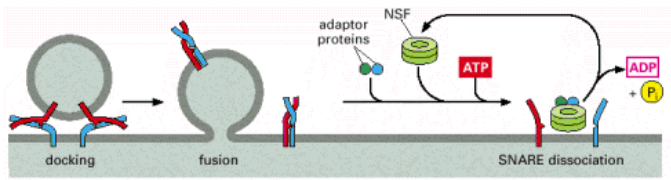


Ένα μοντέλο προσάραξης των κυστιδίων μεταφοράς

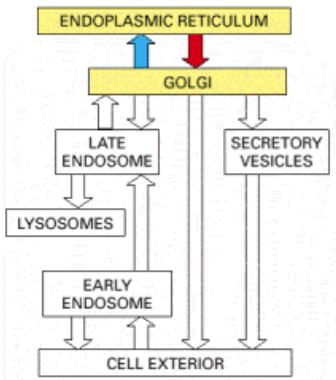


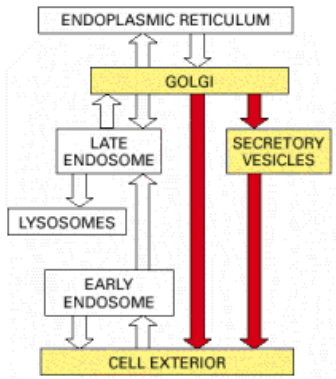
Οι πρωτεΐνες SNARE παίζουν κύριο ρόλο στη σύντηξη των μεμβρανών



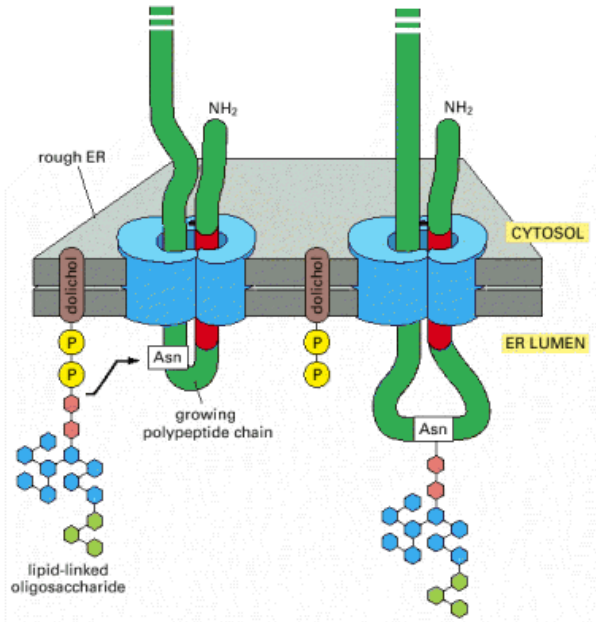


Μεταφορά από το ΕΔ στην κυτταρική επιφάνεια, διαμέσου της συσκευής Golgi

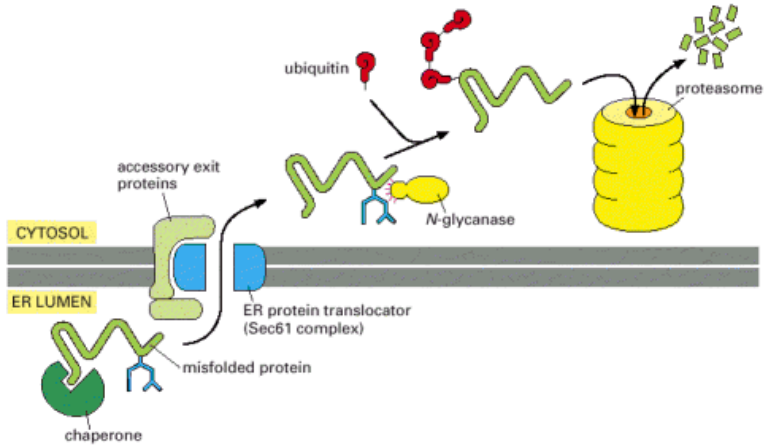




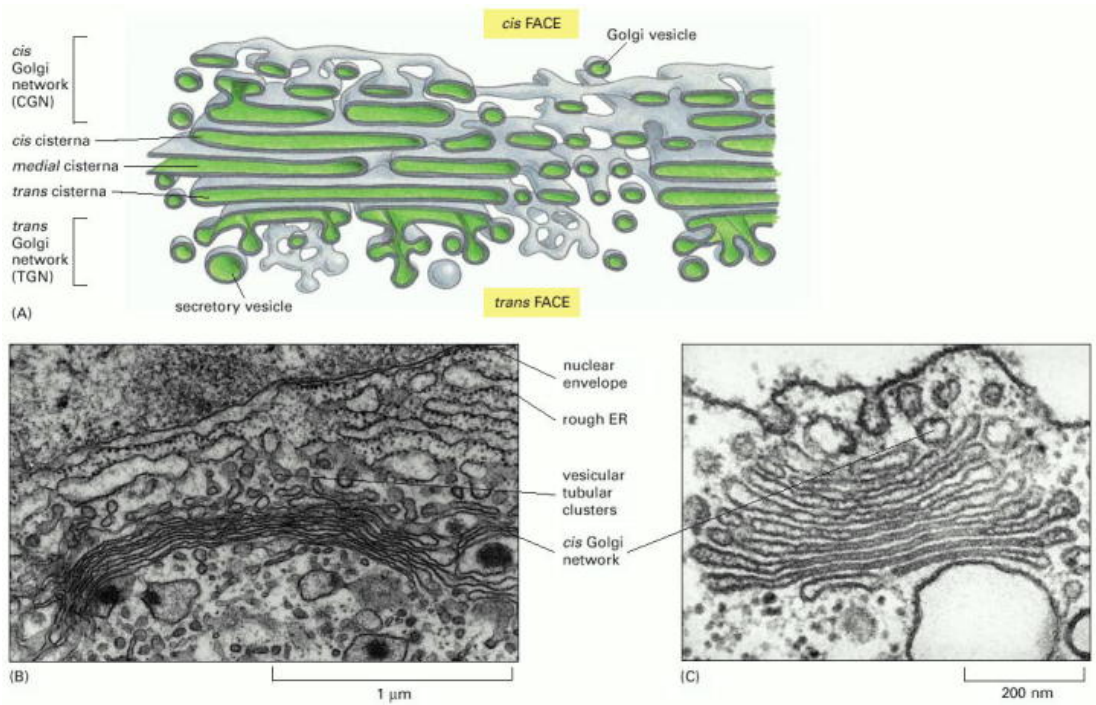
Η γλυκοζυλίωση των πρωτεϊνών στο ΕΔ



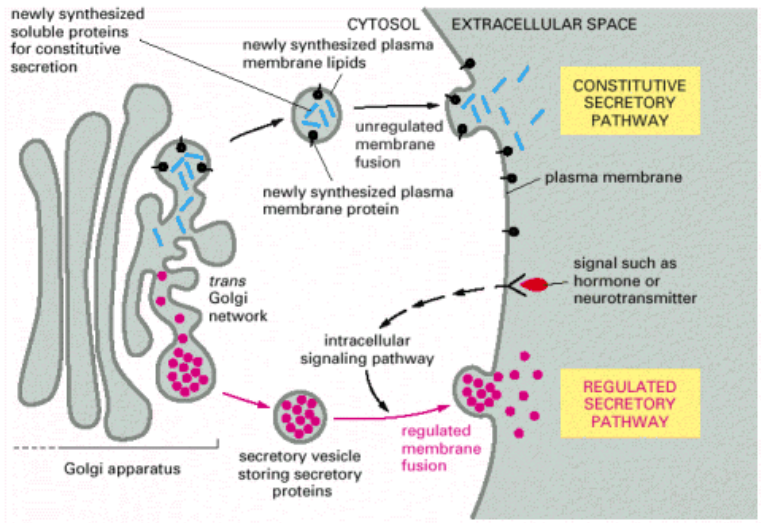
Ποιοτικός έλεγχος στο ΕΔ



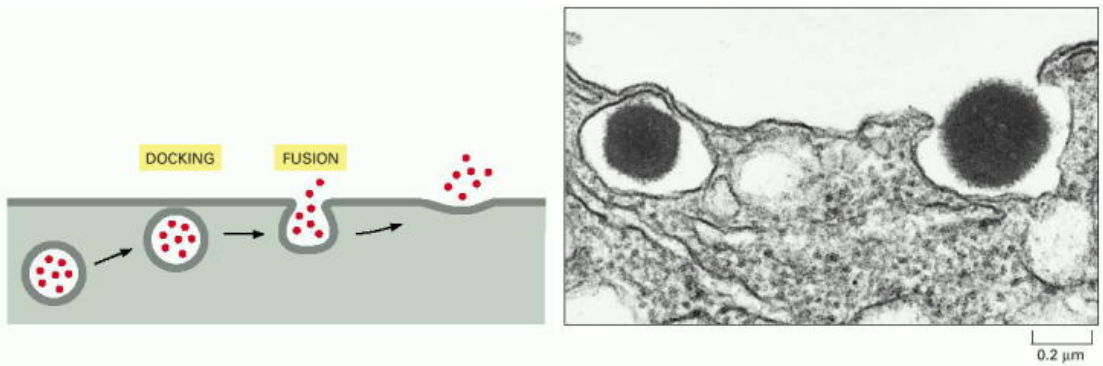
Η συσκευή Golgi



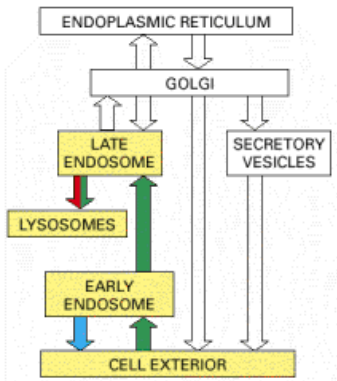
Η ρυθμιζόμενη και η ιδιοσύστατη οδός της εξωκυττάρωσης



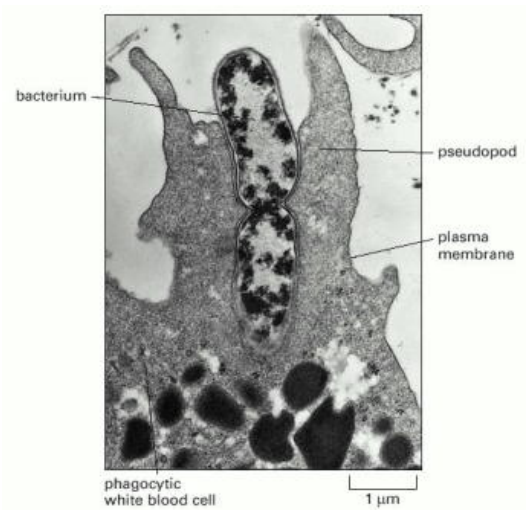
Εξωκυττάρωση εκκριτικών κυστιδίων

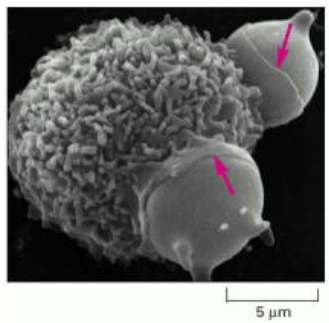


Ενδοκυττάρωση

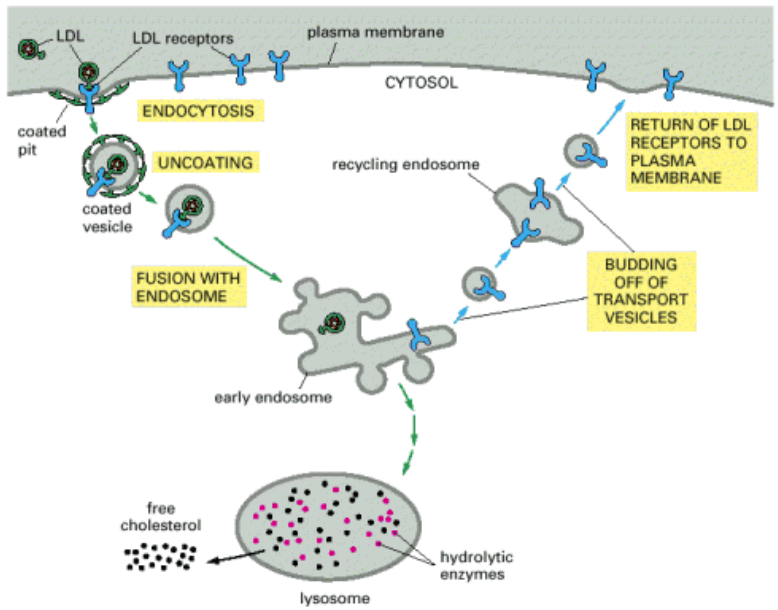


Φαγοκυττάρωση

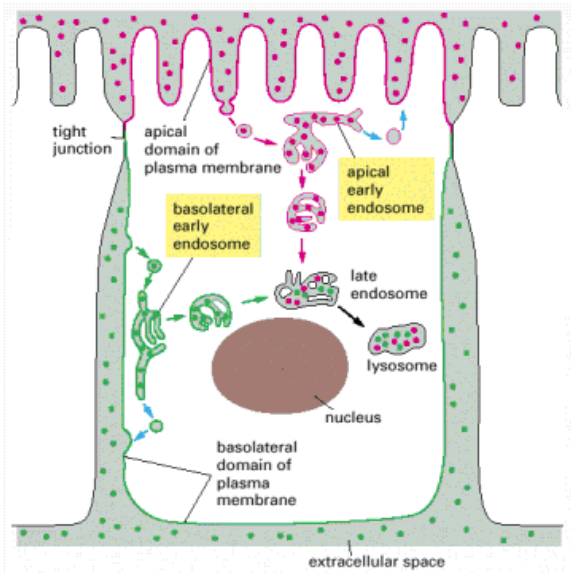




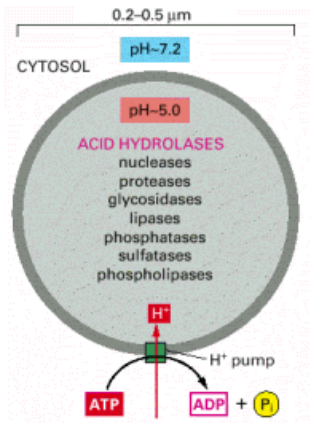
Ενδοκυττάρωση της LDL μέσω υποδοχέων



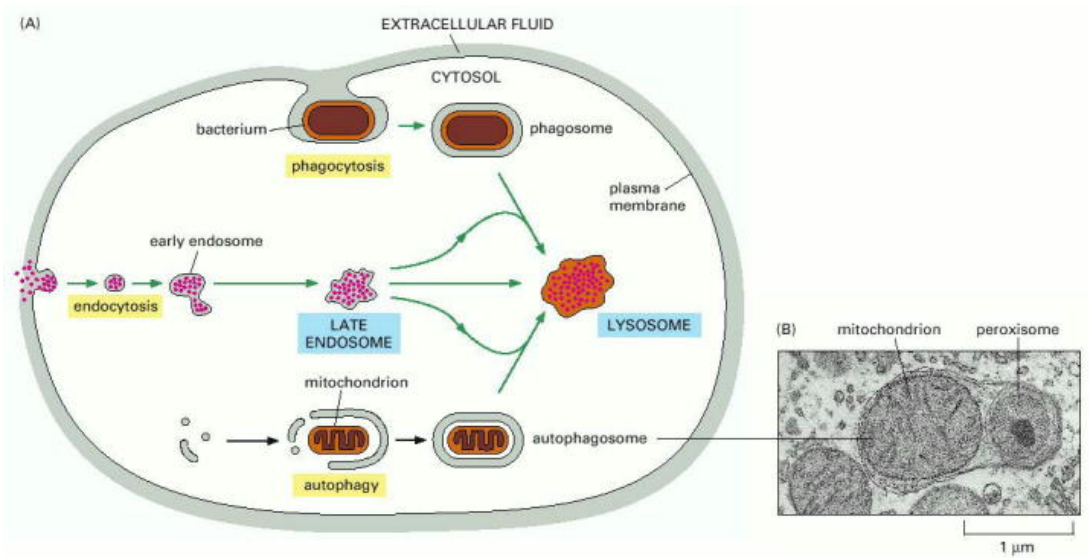
Πιθανή τύχη των υποδοχέων που συμμετέχουν στην ενδοκυττάρωση



Λυσοσωμάτιο



Τρεις διαδρομές προς αποδόμηση στα λυσοσωμάτια

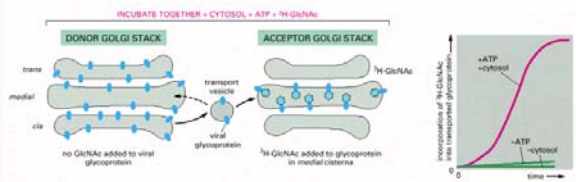


Στρατηγικές που χρησιμοποιήθηκαν για τη μελέτη των μοριακών μηχανισμών που εμπλέκονται στη μεταφορά μέσω κυστιδίων

CELL-FREE SYSTEMS FOR STUDYING THE COMPONENTS AND MECHANISM OF VESICULAR TRANSPORT

Vesicular transport can be reconstituted in cell-free systems. This was first achieved for the Golgi stack. When Golgi stacks are isolated from cells and incubated with cytosol and with ATP as a source of energy, transport vesicles bud from their rims and appear to transport proteins between cisternae. By following the progressive processing of the oligosaccharides on a glycoprotein as it moves from one Golgi compartment to the next, it is possible to follow the process of vesicular transport.

To follow the transport, two distinct populations of Golgi stacks are incubated together. The "donor" population is isolated from mutant cells that lack the enzyme *N*-acetylglucosamine (GlcNAc) transferase I and that have been infected with a virus; because of the mutation, the major viral glycoprotein fails to be modified with GlcNAc in the Golgi apparatus of the mutant cells. The "acceptor" Golgi stacks are isolated from uninfected wild-type cells and thus contain a good copy of GlcNAc transferase I, but lack the viral glycoprotein. In the mixture of Golgi stacks the viral glycoprotein acquires GlcNAc, indicating that it must have been transported between the Golgi stacks—presumably by vesicles that bud from the *cis* compartment of the donor Golgi and fuse with the *medial* compartment of the acceptor Golgi. This transport-dependent glycosylation is monitored by measuring the transfer of ^{3}H-GlcNAc from UDP-^{3}H-GlcNAc to the viral glycoprotein. Transport occurs only when ATP and cytosol are added. By fractionating the cytosol, a number of specific cytosolic proteins have been identified that are required for the budding and fusion of transport vesicles.



Similar cell-free systems have been used to study transport from the *medial* to the *trans* Golgi network, from the *trans* Golgi network to the plasma membrane, from endosomes to lysosomes, and from the *trans* Golgi network to late endosomes.

GENETIC APPROACHES FOR STUDYING VESICULAR TRANSPORT

Genetic studies of mutant yeast cells defective for secretion at high temperature have identified more than 25 genes that are involved in the secretory pathway. Many of the mutant genes encode *temperature-sensitive* proteins. These function normally at 25°C, but when the mutant cells are shifted to 35°C, some of them fail to transport proteins from the ER to the Golgi apparatus, others from one Golgi cisterna to another, and still others from the Golgi apparatus to the vacuole (the yeast lysosome) or to the plasma membrane.

Once a protein required for secretion has been identified in this way, one can identify genes that encode proteins that interact with it by making use of a phenomenon called multicopy suppression. A temperature-sensitive mutant protein at high temperature often has too low an affinity for the proteins it normally interacts with to bind to them. If the interacting proteins are produced at much higher concentration than normal, however, sufficient binding occurs to cure the defect. For this reason yeast cells with a temperature-sensitive mutation in a gene involved in vesicular transport are often transfected with a yeast plasmid vector into which random yeast genomic DNA fragments have been cloned. Because this plasmid is maintained in cells at high copy number, those that carry intact genes will overproduce the normal gene product, allowing rare cells to survive at the high temperature. The relevant DNA fragments, which presumably encode proteins that interact with the original mutant protein, can then be isolated from the surviving cell clones.

The genetic and biochemical approaches complement each other, and many of the proteins involved in vesicular transport have been identified independently by biochemical studies of mammalian cell-free systems and by genetic studies in yeast.

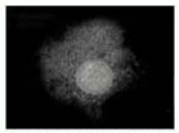
GFP-FUSION PROTEINS HAVE REVOLUTIONIZED THE STUDY OF INTRACELLULAR TRANSPORT

One way to follow the whereabouts of a protein in living cells is to construct fusion proteins, in which green fluorescent protein (GFP) is attached by genetic engineering techniques to the protein of interest. When a cDNA encoding such a fusion protein is expressed in a cell, the protein is readily visible in a fluorescent microscope, so that it can be followed in living cells in real time. Fortunately, for most proteins studied the addition of GFP to a protein does not perturb the protein's function.

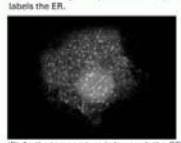
GFP fusion proteins are widely used to study the location and movement of proteins in cells. GFP fused to proteins that shuttle in and out of the nucleus, for example, is used to study nuclear transport events and their regulation. GFP fused to mitochondrial or Golgi proteins is used to study the behavior of these organelles. GFP fused to plasma membrane proteins is used to measure the kinetics of their movement from the ER through the secretory pathway. Dramatic examples of such experiments can be seen as movies on the CD that accompanies this book.

The study of GFP fusion proteins is often combined with FRAP and FLIP techniques (discussed in Chapter 10), in which the GFP in selected regions of the cell is bleached by strong laser light. The rate of diffusion of unbleached GFP fusion proteins into that area can then be determined to provide measurement of the protein's diffusion or transport in the cell. In this way, for example, it was determined that many Golgi enzymes recycle between Golgi apparatus and the ER.

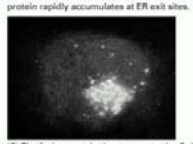
(A–D *right*, courtesy of Jennifer Lippincott-Schwartz Lab.)



(A) In this experiment, GFP fused to the vesicular stomatitis virus coat protein was expressed in cultured cells. The viral protein is an integral membrane protein that normally moves through the secretory pathway from the ER to the cell surface, where the virus would be assembled if cells also expressed the other viral components. The viral protein contains a mutation that allows export from the ER only at a low temperature. Thus, at the high temperature shown, the fusion protein labels the ER.



(B) As the temperature is lowered, the GFP fusion protein rapidly accumulates at ER exit sites.



(C) The fusion protein then moves to the Golgi apparatus.

(D) Finally, the fusion protein is delivered to the plasma membrane. From such studies the kinetics of each step in the pathway can be determined.