CH1131 Biomolecular Engineering

Syllabus

Intracellular Organelles

INTRACELLULAR ORGANELLES

Mitochondrial Structure & Function

I. Mitochondria are large enough to be visible in light microscope

A. Found to be osmotically active early on – they swell in hypotonic media, shrink in hypertonic media; this suggested they were surrounded by a semipermeable membrane like that around cell

II. Morphological characteristics & physiological properties of typical mitochondria – they possess recognizable morphological characteristics, but also exhibit considerable variability in appearance

A. In EM, usually sausage-shaped;

B. Dynamic organelles- probably change shape & size & move from place to place within cytoplasm, depending upon the particular species, tissue & physiological conditions

III. Their size, number & intracellular location vary from cell to cell

IV. Mitochondria possess two membranes: an inner (complex) membrane and an outer membrane

A. Outer membrane completely encloses mitochondrion; serves as its outer boundary

B. Part of inner membrane is just inside the outer membrane but much of it is thrown into deep folds or invaginations (**cristae**); they greatly increase the surface area for aerobic respiration machinery

1. In some cells (mammalian liver, brown adipose tissue), cristae are wide sheets that cut across the entire width of the mitochondrion

C. Mitochondrial membranes divide the mitochondrion into 2 aqueous compartments

1. Matrix - within mitochondrion interior (inside inner mitochondrial membrane); gel-like consistency due to high [protein] (≤500 mg/ml)

2. Intermembrane space between inner & outer membranes – seems limited but swells during active respiration

V. Mitochondrial membranes - traits of 2 mitochondrial membranes; they have very different properties

A. Outer membrane - ~50% lipid by weight; contains many enzymes involved in diverse activities: epinephrine oxidation, tryptophan degradation, fatty acid elongation, etc. .

B. Inner membrane – very high protein/lipid ratio (3:1 by weight; ~1 protein/every 15 phospholipids)

1. Highly impermeable with special transporters for virtually all molecules & ions that enter matrix

2. Its composition & organization are keys to mitochondria bioenergetic activities; its architecture & apparent bilayer fluidity facilitate interactions of membrane components needed to make ATP
3. Also contain a variety of transport systems in addition to most of the ATP synthesis machinery

VI. Mitochondrial matrix - many enzymes, ribosomes (smaller than in cell cytoplasm) & several usually circular double-stranded DNAs (encode inner membrane proteins; nuclear DNA codes for some, too)

CYTOPLASMIC MEMBRANE SYSTEMS: STRUCTURE, FUNCTION, AND MEMBRANE TRAFFICKING

LECTURE OUTLINE

An Overview of the Endomembrane System and Its Dynamic Nature

I. Early investigation pointed to an extensive membrane network in cytoplasm

A. Membrane-bound vesicles of varying diameter; containing material of different electron density

B. Long channels bounded by membranes that radiate through cytoplasm; form an interconnected network of canals

C. These studies & subsequent biochemical studies showed that eukaryotic cell cytoplasm was subdivided into a variety of distinct membrane-bound compartments

II. These organelles are part of dynamic, integrated network; materials are shuttled between parts of cell

A. Transport vesicles shuttle things between organelles; form by budding from donor compartment

B. Transport vesicles move in directed manner, often pulled by motor proteins operating on tracks formed by microtubules & microfilaments of the cytoskeleton

C. When they reach their destination, they fuse with acceptor compartment, which receives vesicles' soluble cargo & membrane wrapper

D. Exhibit repeated cycles of budding & fusion that move a diverse array of materials along numerous pathways traversing the cell

III. Several distinct pathways through cytoplasm have been identified; they fall into two groups: a biosynthetic (secretory) pathway & an endocytic pathway

IV. Biosynthetic (secretory) pathway – synthesis in ER (protein) or Golgi (lipid, carbohydrate); altered as pass through Golgi, sent from there to various locations (membrane, lysosome)

A. Many materials made in ER (proteins) & Golgi (complex polysaccharides) fated for secretion from cell

B. Two types of secretory activity - constitutive & regulated

1. Constitutive - synthesis & secretion into extracellular space occurs in continual, unregulated manner; most cells do it to form extracellular matrix & plasma membrane itself
2. Regulated - secretory materials are often stored in large, densely packed, membrane-bound secretory granules in cell periphery; secreted after correct stimulus
   a. Endocrine cells release hormones
   b. Pancreatic acinar cells release digestive enzymes
   c. Nerve cells release neurotransmitters

C. Proteins, lipids & complex polysaccharides are transported through cell along biosynthetic or secretory pathway; discussion will center on several distinct classes of proteins

1. Soluble proteins discharged from cell
2. Integral proteins of various membranes
3. Soluble proteins that reside within various compartments enclosed by endomembranes (like lysosomal enzymes)

V. Endocytic pathway - moves materials or membrane surface into cell from outside to cytoplasmic compartments (endosomes, lysosomes); movement direction is opposite to that of secretory pathway

The Endoplasmic Reticulum (ER): Background Information and General Functions

I. Endoplasmic reticulum (ER) is divided into 2 broad categories - rough & smooth; both enclose space so cytoplasm divided into cytosolic & luminal (cisternal) space; contents of the 2 spaces are quite different

A. Smooth ER (SER) - typically tubular; interconnecting pipeline system; curves through cytoplasm; lacks associated ribosomes; when cells are homogenized, it fragments into smooth-surfaced vesicles

B. Rough ER (RER) – extensive organelle with ribosomes attached to RER on cytosolic surface; made mostly of cisternae (interconnected network of flattened sacs); space inside appears continuous

1. RER is continuous with nuclear envelope outer membrane (it has ribosomes on cytosolic surface)
2. When cell is homogenized, RER fragments into rough-surfaced vesicles
3. Because they have different densities, rough & smooth vesicles can be readily separated by density gradient centrifugation & then studied

II. Smooth ER functions - extensively developed in many cells (skeletal muscle, kidney tubules, steroid-producing endocrine cells); its specific proteins vary cell-to-cell depending on functions of cell's SER

A. Synthesis of steroid hormones in gonad & adrenal cortex endocrine cells

B. Detoxification in liver of many organic compounds (barbiturates & ethanol), whose chronic use can lead to SER proliferation in liver cells; detoxification carried out by oxygen-transferring enzymes

C. Release of glucose from glucose-6-phosphate in liver cells by the enzyme glucose-6-phosphatase

1. Large glycogen reserves are stored as granules on outside of liver SER membranes; if chemical energy is needed, phosphorylase enzymatically converts glycogen to glucose 6-phosphate

2. SER membrane glucose 6-phosphatase removes phosphate group, generating glucose molecules

3. Glucose leaves cell (membrane is permeable to it); gets into bloodstream & then to body tissues

D. Sequestering Ca^{2+} ions within cisternal space; their release triggers specific cell activities

III. Rough ER functions - predominantly export or membrane protein synthesis (pancreatic acinar cells, mucus-secreting cells of digestive tract lining; early studies done on these cells)

A. Organelles of protein-secreting

1. Nucleus & extensive RER cisternae found near cell basal surface near blood supply; RER is site of synthesis proteins, carbohydrate chains & phospholipids that move through cytomembrane system
2. Golgi complex is located in central region of cell
3. Apical surface faces duct lumen that will carry secretory product out of organ
4. Cell apical end contains membrane-bound secretory vesicles whose contents are released upon arrival of appropriate signal

The Endoplasmic Reticulum (ER): Synthesis of Proteins on Membrane-Bound vs. Free Ribosomes

I. Polypeptides are synthesized at 2 distinct locales within cell

A. Some proteins are made on ribosomes attached to cytosolic surface of RER membranes

1. Proteins secreted from cells
2. Integral membrane proteins
3. Soluble proteins that reside within compartments of endomembrane system (ER, Golgi complex, lysosomes, endosomes, vesicles)

B. Other polypeptides made on "free" ribosomes (not attached to ER) & then released into cytosol

1. Proteins destined to remain in cytosol (enzymes of glycolysis, cytoskeleton proteins)

2. Peripheral proteins of inner cell membrane surface (spectrins, ankyrins; weakly bonded to membrane's cytoplasmic surface)
3. Proteins that are transported to nucleus
4. Proteins to be incorporated into peroxisomes, mitochondria

II. Steps in synthesis of secretory/lysosomal protein on membrane-bound ribosomes

A. mRNA for secretory/lysosomal protein binds to free ribosome (same as those used for domestic proteins) from pool; these ribosomes are not attached to a cytoplasmic membrane

B. N-terminal aminos emerge from ribosome with signal sequence (6-15 hydrophobic amino residues); targets nascent polypeptide & ribosome for ER

C. Signal sequence is recognized by signal recognition particle as it exits ribosome; SRP in mammalian cells consists of 6 distinct polypeptides & a small RNA molecule (the 7S RNA)

D. SRP binds to signal sequence on nascent polypeptide & ribosome; arrests further synthesis; cessation of synthesis lasts until the complex collides with & attaches specifically to an ER membrane

E. SRP tag allows entire complex (SRP-ribosome-nascent polypeptide) to bind specifically to SRP receptor on ER cytosolic surface; this binding occurs through at least 2 distinct interactions

1. First distinct interaction is between SRP & SRP receptor
2. The other interaction is between ribosome & protein-lined membrane channel (**translocon**)

F. After ribosome is bound tightly to the ER membrane, SRP is released from its ER receptor & the signal sequence on the nascent polypeptide is inserted into narrow aqueous channel of translocon

1. Signal sequence binds to translocon internal site & is thought to trigger a conformational change that greatly widens the channel to ER lumen
2. Translation resumes & polypeptide is translocated through channel into ER lumen
3. Upon translation termination & movement of completed polypeptide through translocon, the membrane-bound ribosome is released; ribosome subunits separate & can be reused
4. Channel reverts to original, narrow conformation

III. After protein enters RER, many events occur; it is acted on by a variety of membrane or luminal enzymes

A. Carbohydrates are added to protein by enzyme oligosaccharyltransferase (integral membrane protein)

B. RER contains molecular chaperones: BiP (**Bi**nding **P**rotein)

The Endoplasmic Reticulum (ER): Synthesis of Integral Membrane-Bound Ribosomes

I. Integral membrane proteins (except for those of mitochondria, chloroplasts & peroxisomes) are also synthesized on membrane-bound ribosomes of ER

A. They are translocated into ER membrane as they are synthesized (cotranslationally) using same machinery used for the synthesis of secretory & lysosomal enzymes
   1. But integral proteins contain one or more hydrophobic transmembrane segments of amino acids (**stop-transfer sequences**) that block further movement of protein into ER lumen
   2. Stop-transfer sequences typically include ≥15 continuous hydrophobic or uncharged amino acids
   3. Stop-transfer sequences allow stable integration into ER lipid bilayer (membrane), once the chain is released from the translocation channel
   4. It is thought that translocation channel somehow opens along its side & expels the transmembrane segment into lipid bilayer
   5. It is unclear whether polypeptide transmembrane segments leave the channel one or two at a time as polypeptide is synthesized or only after the entire polypeptide is synthesized & folded

B. Many integral membrane proteins have a single segment in nascent chain that serves as both a signal sequence for binding SRP & stop-transfer sequence for insertion into lipid bilayer
   1. Segments that have this combined function are called **signal-anchor sequences**

The Endoplasmic Reticulum (ER): Membrane Biosynthesis in the ER

I. Membranes thought to arise only from pre-existing membranes (not *de novo* [new entities from pools of proteins & lipids])
   A. Membranes grow as newly made proteins & lipids are inserted into existing membranes in ER; each compartment has unique membranes

II. Synthesis of membrane lipids
   A. Most membrane lipids are produced entirely in ER membrane
   B. Lipids are carried from ER to Golgi complex & plasma membrane as part of bilayers making up transport vesicle walls

The Endoplasmic Reticulum (ER): Glycosylation in the Rough Endoplasmic Reticulum

I. Most proteins made on RER are glycosylated & thus become glycoproteins, whether integral proteins of membrane, soluble lysosomal or vacuolar enzymes or parts of ECM
   A. Carbohydrate groups – have key roles in function of many glycoproteins (e. g., binding sites in their interactions with other macromolecules); also aid in proper folding of protein to which they are attached
   B. Oligosaccharide sugar sequence assembled by group of membrane-bound enzymes (**glycosyltransferases**)
      1. Glycosyltransferases transfer specific monosaccharide from an appropriate sugar donor to an appropriate sugar acceptor

2. Donor is always a nucleotide sugar - CMP-sialic acid, GDP-mannose, GDP-fucose, UDP-galactose, UDP-*N*-acetylglucosamine; acceptor of transferred sugar is growing end of carbohydrate chain
3. Sequence of sugar transfer during oligosaccharide assembly depends on sequence of glycosyltransferases participating in process
4. Glycosyltransferase sequence depends on location of specific enzymes within the various secretory pathway membranes
5. Thus sugar arrangement in oligosaccharide chains of a glycoprotein depends on spatial localization of certain enzymes in pathway membranes

II. Carbohydrate chains are attached to protein by N-linkages (asparagine N atom) or O-linkages (to serine or threonine O or collagen hydroxylysine residue) of both soluble & integral membrane proteins

From the ER to the Golgi Complex: The First Step in Vesicular Transport

I. RER cisternae are typically interconnected facilitating membrane & luminal protein movement from synthesis site to sites facing central cell regions

The Golgi Complex

I. Discovered by Camillo Golgi

II. Characteristic morphology - flattened, disk-like membranous cisternae with dilated rims & associated vesicles & tubules (smooth membranes so found with smooth microsomes)

III. Golgi cisternae polarized - *cis* face (entry face closest to ER); *trans* face (exit face at opposite end of stack; closer to plasma membrane)

A. Golgi complex is divided into several functionally distinct compartments arranged along a *cis*-*trans* axis; new materials enter *cis* face & pass to *trans* face where they exit Golgi complex
1. *cis*-most face composed of interconnected network of tubules (*cis* Golgi network; CGN); CGN & seems to be mostly a sorting station (ships some proteins on further into Golgi, some back to ER)
2. Bulk of Golgi complex consists of a series of large, flattened cisternae divided into 3 regions: the *cis* cisternae, *medial* cisternae, *trans* cisternae
3. *Trans*-most face has distinct network of tubules & vesicles (*trans* Golgi network; TGN); also sorting station; proteins placed into different vesicle types (either to membrane or elsewhere in the cell)

B. Membranous elements of Golgi complex may be supported mechanically by a peripheral membrane skeleton or scaffold composed of a variety of proteins, including:

C. Golgi complex composition is not uniform from *cis*- to *trans*-end; polarized; differences in composition of membrane compartments (polarization) reflects primary processing plant role

1. Newly synthesized membrane proteins (also secretory & lysosomal proteins) leave the ER & enter the Golgi complex at its *cis*-face & then pass across the stack to the *trans* face

D. As they move along the stack, proteins originally synthesized in RER are sequentially modified in specific ways; for example:

IV. Glycosylation in Golgi complex - synthesis site of most of cell's complex polysaccharides

A. In ER, glucose residues had just been removed (see above) from the ends of core oligosaccharide of *N*-linked CHO chains

B. In Golgi, as in RER, sequences in which sugars are inserted into oligosaccharides is determined by spatial arrangement of specific glycosyltransferases that contact new proteins as they pass through

C. Unlike N-linked oligosaccharides, whose synthesis starts in ER, those attached to proteins by *O*-linkages are assembled wholly within Golgi complex

The Types of Vesicle Transport and Their Function: Background Information

I. Materials carried between membrane compartments by membrane-bound vesicles (typically 50 - 75 nm dia), which bud from donor membranes & fuse with acceptor membranes

A. Most budding vesicles covered on cytosolic surface by fuzzy, electron-dense layer

1. The dark-staining layer consists of a protein coat formed from soluble proteins that assemble on the donor membrane cytosolic surface at sites where budding takes place

2. Each coated bud pinches off to form a coated vesicle; vesicles of similar size & structure have been formed in cell-free systems

B. Protein coats have at least two distinct functions:

1. They act as a mechanical device that causes the membrane to curve & form a budding vesicle

2. They provide a mechanism for selecting components (& thus soluble cargo) to be carried by vesicle

C. Components selected for transport can include:

1. Cargo to be transported (secretory, lysosomal, & membrane proteins)

2. Machinery required to target & dock the vesicle to an acceptor membrane

II. Three distinct classes of coated vesicles have been identified and have distinct role in cell trafficking

A. COPII-coated vesicles - move materials forward from ER to ERGIC (intermediate compartment between ER & Golgi) & Golgi complex; COP is acronym for ***co***at ***p***roteins

B. COPI-coated vesicles - move materials in retrograde direction from ERGIC & Golgi stack backward toward ER

C. Clathrin-coated vesicles - move materials from TGN to endosomes, lysosomes & plant vacuoles

1. Also move materials from plasma membrane to cytoplasmic compartments along endocytic pathway

2. Also implicated in trafficking from endosomes & lysosomes

COPII-Coated Vesicles: Transporting Cargo from the ER to the Golgi Complex

I. COPII-coated vesicles mediate the first leg of journey through the biosynthetic pathway from ER to ERGIC & CGN

II. COPII-coated vesicles are thought to be able to select & concentrate certain components that they transport

A. ER integral membrane proteins are selectively captured because they interact specifically with COPII proteins of vesicle coat; several types of membrane proteins are included in this group:

1. Enzymes that act at later stages of biosynthetic pathway, like glycosyltransferases of Golgi complex

2. Membrane proteins involved in docking & fusion of the vesicle with the target compartment

3. Membrane proteins that bind soluble cargo (secretory proteins)

COPI-Coated Vesicles: Transporting Escaped Proteins Back to the ER

I. COPI-coated vesicles mediate the retrograde transport of proteins; they have been implicated in the movement of:

A. Golgi-resident enzymes in a *trans*-to-*cis* direction (like mannosidase II)

B. ER-resident enzymes from the ERGIC & the Golgi complex back to the ER

II. Retaining & retrieving resident ER proteins – if vesicles continually bud from membrane compartments, how does each compartment retain its unique composition?

Clathrin-Coated Vesicles: Sorting Lysosomal Proteins at the TGN

I. How does particular protein synthesized in ER get targeted toward particular cellular destination?

A. Cell must be able to distinguish among the various proteins it manufactures

B. Protein sorting occurs in the last of the Golgi compartments, the *trans* Golgi network (TGN), which functions as a major branch point in the movement of materials along the secretory pathway

1. The TGN is the site of assembly of clathrin-coated vesicles

2. Clathrin coats mediate cargo sorting at TGN & clathrin-coated vesicles carry hydrolytic enzymes & membrane proteins from there to endosomes, lysosomes & plant vacuoles

II. Lysosomal protein sorting & transport - made on membrane-bound RER ribosomes, carried to *cis* Golgi cisternae with other protein types; this is the best understood post-Golgi pathway (for lysosomal enzymes)

A. Once in Golgi cisternae, soluble lysosomal enzymes recognized by enzymes catalyzing 2-step addition of phosphate group to certain *N*-linked CHO chain mannose sugars

1. Unlike other glycoproteins sorted at the TGN, lysosomal enzymes possess phosphorylated mannose residues, which act as recognition signals

B. Lysosomal enzymes with mannose 6-phosphate signal are recognized & captured by mannose-6-phosphate receptors

C. Lysosomal enzymes are transported from TGN in clathrin-coated vesicles; coats of the vesicles contain:

D. Lysosomal enzymes are escorted from the TGN by a recently discovered family of adaptor proteins called GGAs

1. Each GGA molecule has several domains, each capable of grasping a different protein involved in vesicle formation
2. The outer ends of GGA adaptors bind to clathrin molecules, holding the clathrin scaffolding onto the surface of the vesicle
3. On their inner surface, GGA adaptors bind to sorting signals in the cytosolic tails of the mannose 6-phosphate receptors
4. The MPRs, in turn, bind to soluble lysosomal enzymes within the vesicle lumen
5. As a result of these interactions with GGA adaptors, MPRs in TGN membrane & lysosomal enzymes within TGN lumen become concentrated into clathrin-coated vesicles

E. Once the vesicle has budded from the TGN, the clathrin coat is lost & the uncoated vesicle proceeds to its destination, which may be an endosome, lysosome or plant vacuole

F. Once the vesicle reaches its destination organelle, the MPRs dissociate from the lysosomal enzymes & return to the TGN for another round of lysosomal enzyme transport

Lysosomes

I. Lysosome morphology & contents – typically contain ~50 different hydrolytic enzymes made in RER & targeted for lysosomes; lysosomes are an animal cell's digestive organelles

A. Lysosomal enzymes taken together can hydrolyze virtually every type of biological macromolecule, resulting in low MW products that can be transported across the lysosomal membrane into cytosol

B. All of the enzymes have pH optimum at acid pH (acid hydrolases) suited to the low pH of the lysosomal compartment; lysosome interior pH is ~ 4.6

1. The high internal proton concentration is maintained by a proton pump (transporter; an H^+-ATPase) present in the lysosome's boundary membrane

II. Lysosomal functions

A. Materials brought into cell (protozoa, macrophages, neutrophils) from extracellular environment are enzymatically broken down; resulting nutrients cross membrane into cytosol; best-studied function

1. Many single-celled organisms ingest food particles, which are disassembled in lysosome
2. In mammals, phagocytic cells (macrophages, neutrophils) act as scavengers, ingesting debris & potentially dangerous microorganisms; highly phagocytic cells may have up to 1000 lysosomes
3. Ingested bacteria are usually inactivated by low pH & then digested enzymatically; some are not
4. Peptides made by the above process are posted on cell surface; they alert immune system to presence of foreign agent

B. Organelle turnover (**autophagy**) – regulated destruction of cell's own organelles & their replacement

1. During process, organelle (e. g., mitochondrion) is surrounded by a double membrane derived from an ER cisterna; ER membrane then fuses with lysosome to produce **autophagolysosome**
2. Often see this in EM (mitochondrion or other organelle); it is calculated that 1 mitochondrion undergoes autophagy about every 10 min in mammalian liver cell
3. If nutrient supply drops, autophagy rate increases to provide missing nutrients & thus energy; cell cannibalizes its own organelles to acquire energy to maintain life
4. Once digestive process in autophagolysosome is completed, organelle is called **residual body**
5. Depending on cell type, residual body contents may be eliminated from cell by exocytosis or retained within cytoplasm indefinitely as **lipofuchsin granule**
6. Lipofuchsin granules rise in number with age of individual; accumulation is particularly evident in long-lived cells (neurons) where granules are considered a major characteristic of aging process

Cellular Uptake of Particles and Macromolecules: Background Information and Overview

I. Cells take in materials too large to pass through membrane by uptake of extracellular materials into vesicles derived from folds or invaginations of cell membrane

A. Two separate categories of uptake of extracellular materials into cytoplasmic vesicles, which occur by different mechanisms - **phagocytosis** & **endocytosis**

1. Phagocytosis – the uptake of particulate matter
2. Endocytosis – the uptake of fluid, dissolved solutes & suspended macromolecules

B. Phagocytic vesicles usually ~10X larger than endocytic ones (1 - 2 μm vs. 0.1 - 0.2 μm in dia)

II. Phagocytosis (cell eating) - uptake of relatively large particulate matter (>0.5 μm in dia); extensive in a few cell types specialized for uptake of particulate matter from environment & delivery to lysosomes

A. Single-celled heterotrophs (amoebae, ciliates) make their livelihood this way; trap food particles & smaller organisms & enclose them within folds of plasma membrane, engulfing food particles

1. Folds fuse to form vacuole (**phagosome**) that pinches off inwardly from plasma membrane

2. Phagosome then fuses with lysosome forming **phagolysosome**, within which material is digested

3. Process is somewhat similar to digestion of cytoplasmic organelle by autophagy

B. In most animals, phagocytosis by certain cells is protective mechanism rather than mode of feeding

1. Mammals possess a variety of professional phagocytes (macrophages, neutrophils) – wander through blood & tissues phagocytizing invaders, damaged & dying cells, aging RBCs, debris

2. These materials are recognized & bound by highly selective surface receptors on surface of phagocyte prior to uptake; started by contact of cell with right target

3. Once inside the phagocyte, microorganisms killed by lysosomal enzymes or oxygen free radicals generated in phagosome lumen

III. Endocytosis - uptake of fluid, dissolved solutes, suspended macromolecules; divided into 2 broad categories: bulk phase & receptor-mediated endocytosis

A. Bulk phase endocytosis – nonspecific uptake of extracellular fluids without recognition by membrane

1. Any molecules (large or small) that happen to be present in enclosed fluid are taken into cell as well

2. Visualized by adding substance to culture medium (dye lucifer yellow; enzyme horseradish peroxidase); taken up non-specifically

3. Occurs continually in certain cell types where it may function primarily to convert plasma membrane into cytoplasmic membrane; keeps cell from accumulating too much plasma membrane

4. This conversion is required in cells that have been engaged in secretion & have had large numbers of secretory vesicles fuse with the plasma membrane

B. Receptor-mediated endocytosis (RME) – brings about uptake of specific extracellular macromolecules (ligands) that bind to receptors on external plasma membrane surface

C. Rate of both processes can be remarkably rapid - up to half membrane surface can be internalized in as little as 30 min

1. Despite rapid inward movement of plasma membrane, there is no shrinkage of cell surface

2. Nor is there any immediate need for synthesis of new membrane components

3. Membrane is simply cycled between surface & cell interior so that membrane is added to surface as fast as it is removed; exocytosis rate equals that of endocytosis (membrane is recycled)

Cellular Uptake of Particles and Macromolecules: Receptor-Mediated Endocytosis (RME) and the Role of Coated Pits

I. RME provides means for selective & efficient uptake of macromolecules that may be present at relatively low concentrations in extracellular fluid

A. Cells have receptors for the uptake of many different types of ligands (hormones, growth factors, enzymes, plasma proteins)

1. Substances that enter cell by RME bind receptors that collect in specialized areas of plasma membrane (**coated pits**)
2. Receptors are concentrated in coated pits to 10 - 20X that in rest of membrane

B. Coated pits – membrane surface sites that are indented & covered on cytoplasmic face by bristly, electron dense protein layer containing clathrin & adaptors

1. Clathrin is the same protein in clathrin-coated vesicles formed at TGN
2. Coated pits invaginate into cytoplasm & then pinch free of plasma membrane & form coated vesicles

III. Like clathrin-coated vesicles budding from TGN, coated vesicles formed during endocytosis also contain a layer of adaptor complexes situated between clathrin lattice & vesicle surface facing cytosol

Cellular Uptake of Particles and Macromolecules: The Endocytic Pathway

I. Molecules taken into a cell by endocytosis are routed through a well-defined endocytic pathway

II. 2 different types of receptors are subjected to endocytosis

A. Housekeeping receptors – responsible for uptake of materials that will be used by cell; best-studied examples are transferrin & LDL receptors; mediate delivery to cells of iron & cholesterol, respectively

1. Endocytosis of these receptors leads typically to the delivery of the bound materials (like iron & cholesterol) to the cell & return of the receptor to the cell surface for additional rounds of uptake

B. Signaling receptors – responsible for binding extracellular ligands that carry messages that change cell activities; these ligands (hormones like insulin; growth factors like EGF) do not actually enter cell

1. Instead, they bind to the surface receptor & signal a physiological response inside the cell
2. Their endocytosis leads typically to destruction of receptor **(receptor down-regulation**), which has the effect of reducing the cell's sensitivity to further stimulation by the hormone or growth factor
3. Receptor down-regulation is a mechanism by which cells regulate their ability to respond to extracellular messengers
4. Usually marked for endocytosis & subsequent destruction

III. Endocytic pathway begins with a dynamic network of tubules & vesicles known collectively as endosomes

A. Endosome lumen fluid is acidified due to activity of endosome membrane H^+-ATPase (H^+ pump)

B. Endosomes are divided into 2 distinct classes - distinguished from one another on basis of buoyant density (allows them to be isolated in different fractions on density gradient), pH, protein composition

1. Early endosomes - typically located near peripheral region of cell
2. Late endosomes - typically located in more interior part of cell, closer to nucleus

IV. Receptors taken up by endocytosis are transported in endocytic vesicles to an early endosome, which serves as a sorting station that directs different types of receptors & ligands along different pathways

A. Housekeeping receptors dissociate from their bound ligands in the acidified endosomal environment

B. In contrast, bound ligands (e.g., LDL) of housekeeping receptors, once released from receptors, are concentrated into a more spherical sorting compartment before being sent to late endosomes

C. Signaling receptors previously marked are also sorted, sent off to late endosomes & ultimately destroyed

V. Steps along endocytic pathway from an early endosome to a lysosome

A. Molecules that travel along endocytic pathway in a late endosome are ultimately directed to a lysosome, the terminal compartment of the endocytic pathway; this movement occurs by 2 major routes

1. Maturation of late endosomes into lysosomes – in addition to getting material from early endosomes, late endosomes get newly made lysosomal enzymes from TGN (carried by receptors)
2. Fusion of late endosomes with preexisting lysosomes

B. Once in a lysosome, membrane receptors & other macromolecules are destroyed, but transported materials like cholesterol are typically processed for delivery to the cytosol