Lecture10
Pharmaceutical Biotechnology

Hepatic protein metabolism

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Hepatic Protein Metabolism

- Aside from renal and gastrointestinal metabolism, the liver may also play a major role in the metabolism of protein therapeutics.
- Exogenous as well as endogenous proteins undergo proteolytic degradation to dipeptides and amino acids that are reused for endogenous protein synthesis.

- Proteolysis usually starts with endopeptidases that attack in the middle part of the protein, and the resulting oligopeptides are then further degraded by exopeptidases,
- The rate of hepatic metabolism is largely dependent on the specific amino acid sequence of the protein.

- A prerequisite for protein metabolism is the uptake of proteins into the hepatocytes.

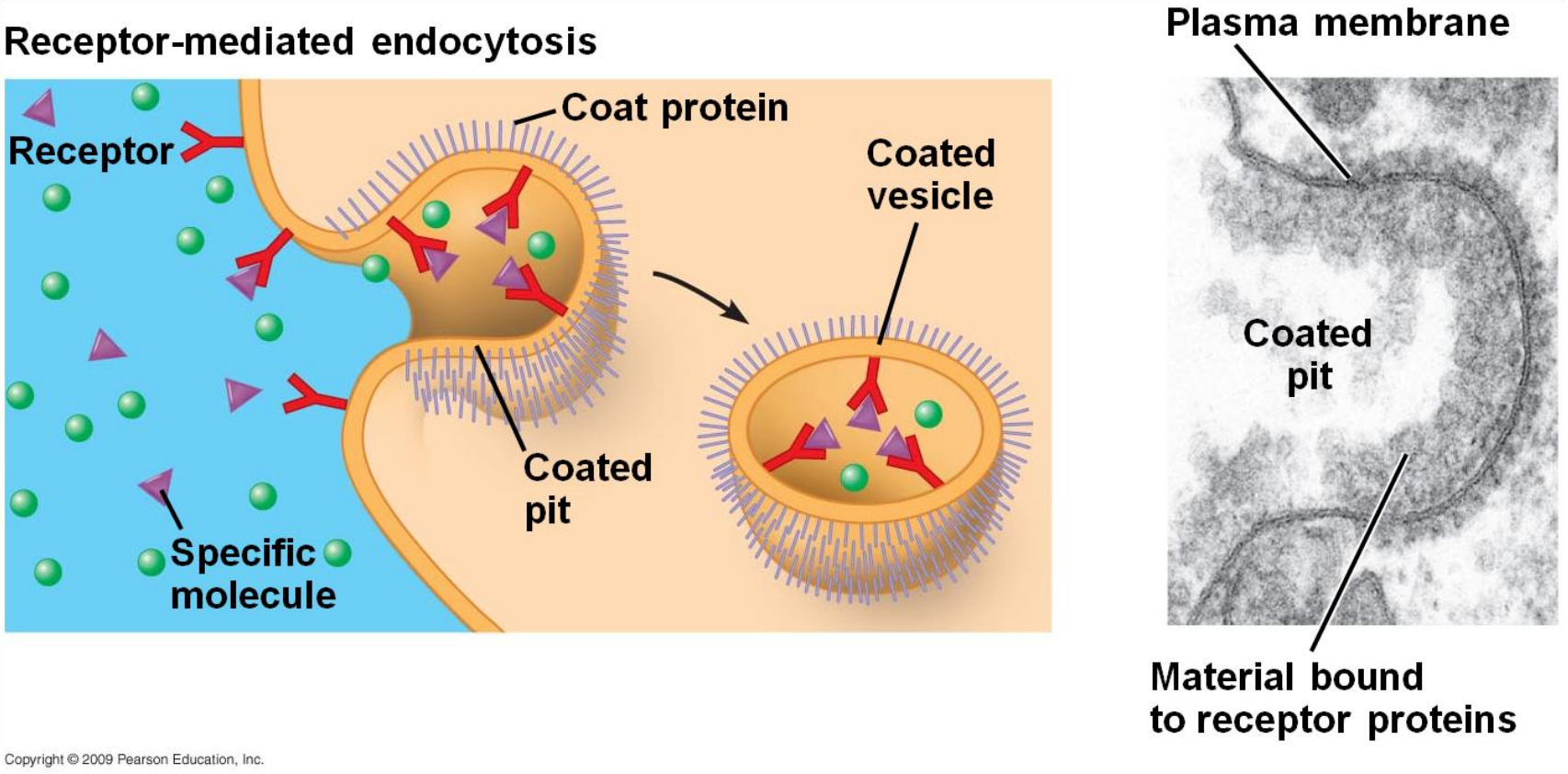
1. Small peptides may cross the hepatocyte membrane via simple passive diffusion if they have sufficient hydrophobicity. Peptides of this nature include the cyclosporine (cyclic peptides).
2. Other cyclic and linear peptides of small size (<1.4 kDa) and hydrophobic nature (containing aromatic amino acids), such as cholecystokinin-8 (CCK-8; 8 aminoacids) are taken up by the hepatocytes by a carrier-mediated transport, in this case of CCK-8 by the organic anion transporting polypeptide OATP-8 (SLCOIB3)

- After internalization into the cytosol, these peptides are usually metabolized by microsomal enzymes (cytochrom P-450 3A for cyclosporine A) or cytosolic peptidases (CCK-8).
- Substances that enter the liver via carrier-mediated transport are typically excreted into the bile by active export transporters.
- These hepatic clearance pathways are identical to those known for most small hydrophobic drug molecules.

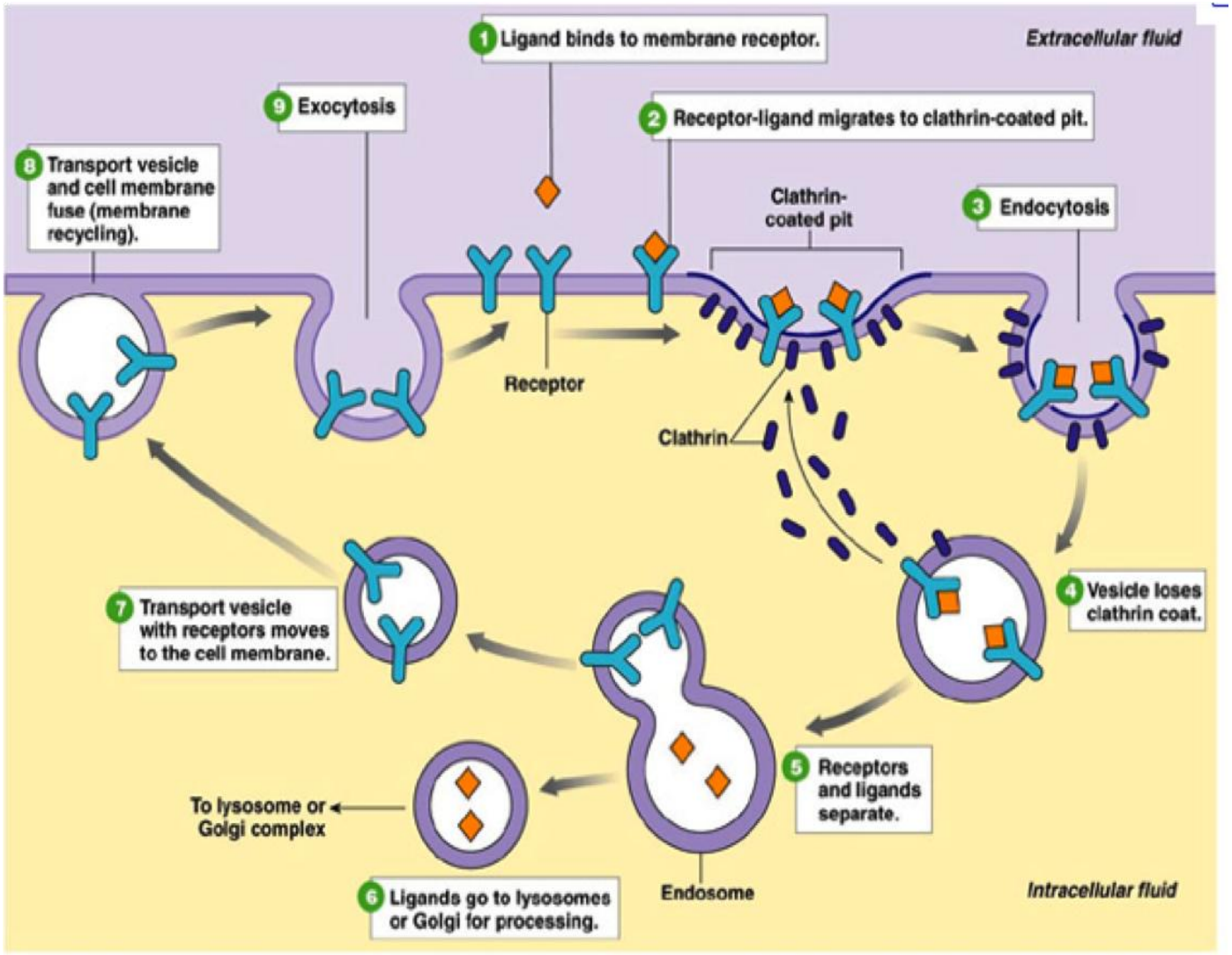
3. Uptake of larger peptides and proteins is facilitated via various carrier-mediated, energy-dependent transport processes.

- One of the possibilities is receptor-mediated endocytosis, such as for insulin and epidermal growth factor.
- In receptor-mediated endocytosis, circulating proteins are recognized by specific hepatic receptor proteins.

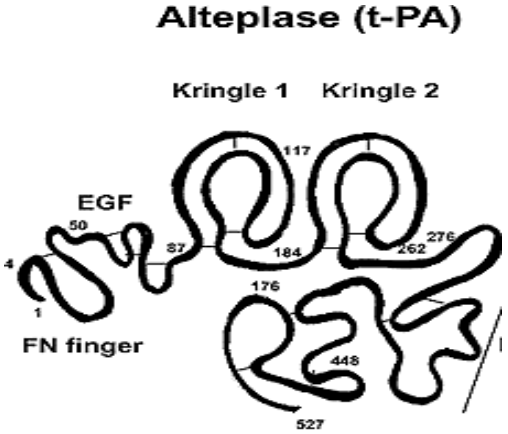
Receptor-mediated endocytosis



- The receptors are usually integral membrane glycoproteins with an exposed binding domain on the extracellular side of cell membrane.
- After the binding of the circulating protein to the receptor, the complex is already present or moves in coated pit regions, and the membrane invaginates and pinches off to form an endocytotic coated vesicle that contains the receptor and ligand (internalization).
- The vesicle coat consists of proteins (clathrin, adaptin, and others), which are then removed by an uncoating denosine triphosphatase (ATPASE).
- The vesicle parts, the receptor, and the ligand dissociate and are targeted to various intracellular locations.
- Some receptors, such as the low-density lipoprotein (LDL), asialoglycoprotein and transferrin receptors, are known to undergo recycling.
- Other receptors, such as the interferon receptor, undergo degradation. This degradation leads to a decrease in the concentration of receptors on the cell surface (receptor down-regulation).
- Others, such as insulin receptors, for example, undergo both recycling and degradation.


1 Ligand binds to membrane receptor.
Extracellular fluid
9 Exocytosis
2 Receptor-ligand migrates to clathrin-coated pit.
8 Transport vesicle and cell membrane fuse (membrane recycling).
Clathrin-coated pit
3 Endocytosis
Receptor
Clathrin
7 Transport vesicle with receptors moves to the cell membrane.
4 Vesicle loses clathrin coat.
5 Receptors and ligands separate.
To lysosome or Golgi complex
Endosome
6 Ligands go to lysosomes or Golgi for processing.
Intracellular fluid

- For glycoproteins, if a critical number of exposed sugar groups (mannose, galactose, fucose, N-acetylglucosamine, N-acetylgalactosamine, or glucose) is exceeded, receptor-mediated endocytosis through sugar-recognizing receptors, is an efficient hepatic uptake mechanism.
- Important carbohydrate receptors in the liver are the asialoglycoprotein receptor in hepatocytes and the mannose receptor in Kupffer and liver endothelial cells.
- The high-mannose glycans in the first kringle domain of t-PA, for example, have been implicated in its hapatic clearance.



- Low density lipoprotein receptor-related protein (LRP) is a member of the low-density lipoprotein (LDL) receptor family responsible for endocytosis of several important lipoproteins, protease, and protease-inhibitor complexes in the liver and other tissues.

- Uptake of proteins by liver cells is followed by transport to an intracellular compartment for metabolism.
- Protein internalized into vesicles via an endocytotic mechanism undergo intracellular transport towards the lysosomal compartment near the center of the cell. There, the endocytotic vehicles fuse with or mature into lysosome, which are specialized acidic vesicles that contain a wide variety of hydrolases capable of degrading all biological macromolecules.
- Proteolysis is started by endopeptidases (mainly cathepsin D) that act on the middle part of the proteins. Oligopeptides-as the result of the first step-are further degraded by exopeptidases.
- The resulting amino acids and dipeptidases reenter the metabolic pool of the cell.

- The hepatic metabolism of glycoproteins may occur more slowly than the naked protein because protecting oligosaccharide chains need to be removed first.
- Metabolised protein and peptides in lysosomes from hepatocytes, hepatic sinusoidal cells and kupffer cells may be released into blood.
- Degraded proteins in hepatocyte lysosomes can also be delivered to the bile canaliculus and excreted by exocytosis.

- The receptor-mediated uptake of protein drugs by hepatocytes, followed by intracellular metabolism, **some-times causes dose-dependent plasma disposition curves** due to the saturation of the active uptake mechanism at higher doses.

Direct shuttle or transcytotic pathway

- A second intracellular pathway for proteins is the direct shuttle or transcytotic pathway.
- The endocytotic vesicle formed at the cell surface traverses the cell to the peribiliary space, where it fuses with the bile canalicular membrane, releasing its content by exocytosis into bile.
- This pathway, described for polymeric immunoglobulin A, bypasses the lysosomal compartment completely.

Receptor-Mediated Protein Metabolism

- Receptor-mediated metabolism is often a substantial elimination pathway for those protein therapeutics that bind with high affinity to membrane-associated receptors on the cell surface.
- The interaction of the protein therapeutic with the membrane receptor is frequently part of pharmacologic effect of the drug, i.e., the receptor is the target structure the protein therapeutic is directed at.
- This binding can lead to receptor-mediated uptake by endocytosis and subsequent intracellular lysosomal metabolism.

- Receptor-mediated uptake and metabolism via interaction with these generally high-affinity, low-capacity binding sites is not limited to a specific organ or tissue type.
- Thus, any tissue, including the therapeutic target cells that express receptors for the drug can contribute to the elimination of the protein therapeutic (Meibohm and Derendorf, 2003).
- Since the number of protein drug receptors is limited, receptor-mediated protein metabolism can usually be saturated within therapeutic concentrations, or more specifically at relatively low molar ratios between the protein drug and the receptor.
- As a consequence, the elimination clearance of these protein drugs is not constant but dose dependent, and decreases with increasing dose.
- Thus, receptor mediated elimination constitutes a major source for nonlinear pharmacokinetic behavior of numerous peptide and protein drugs, i.e., systemic exposure to the drug increases more than proportional with increasing dose (Tang et al., 2004).