1 Transport Proteins

Transport proteins work together to transfer glucose from the intestine to the blood stream.

The internal side of the lumen is covered by vili, increasing the surface area, within which epithelial cells are located. On each vili there are microvili, increasing the surface area even more.

The basal and lateral sites of a vilus are very similar. Outside of vili blood or extracellular fluids are flowing.

Glucose is thus goes from low concetration inside the lumen to the high concentration in the cytosol of the epithelial cell and then tho the low concentration in the extracellular fluid on the basolateral side of the epithelial cell, where GLUT uniporters and Na^+ and K^+ pumps are located.

Transcellular transport of clucose requires the asymmetric distribution of membrane proteins. There are specific areas called tight junctions which restrict the location of transport proteins, keeping Na^+/glucose symporter on the apical membrane and GLUT2 uniporter and Na^+/K^+ pum on the basolateral plasma membrane.

Tight junctions also stop intercellular molecular movement.

2 ATP-Driven Pumps

There are three kinds of transport ATPases: P-type pumps, F-type(and V-type) proton pumps and ABC transporters.

2.1 F-type and V-type ATPase

F-type and V-type ATPases are structurally related, but have opposite modes of action.

F-type ATPases (present in mitochondria, chloroplasts and bacteria) use the H^+ gradient to drive the synthesis of ATP. On the other hand, V-type ATPases (present in lysosomes and plant vacuoles) use ATP and pump H^+

For example, ATP synthases use the H^+ electrochemical gradient to produce ATP (F-type), while H^+ pumps use ATP to pump H^+ against the electrochemical gradient (V-type) and acidify the lumen.

3 Membrane Potential

Membrane potential is the difference in electrical charge on two sides of the membrane, which is used by symporters and antiporters to carry out secondary active transport (in animals and plants). Moreover, membrane potential provides action potential in nerve cells.

3.1 Generation of Membrane Potential

K^+ leak channels play a makor role in membrane potential by securing an outward flow of K^+.

Na^+/K^+ pump, on the other hand, has the following characteristics:

- Na^+ gradient with low cytosolic [Na^+]
- K^+ gradient with high cytosolic [K^+]
- Elecrogenic
  - Net 1+ ion pumped out
  - Responsible for 10% of the membrane potential

In animal cells, ions in solution are present in pairs, with more (+) charge on the outside because of Na^+ and K^+ and more (-) charge on the inside due to Cl^- and fixed anions. At equilibrium, the resting membrane potential can be measured, and is equal to -70 mV on average.

4 Intracellular Compartments

Cytosol usually assumes half the cell volume. Cytosol, where protein synthesis and degradation occur, is also the location of intermediary metabolism.

Definition 4.1. An organelle is a subcellular compartment or large macromolecular complex, often membrane-enclosed, that has a distinct structure, composition, and function.

e.g. nucleus, endoplasmic reticulum, Golgi apparatus, nucleolus

Rough endoplasmic reticulum is the location of synthesis of transmembrane, organellar and secreted proteins, while smooth endoplasmic reticulum is where fats and steroid hormones are metabolised.

There are a lot more membranes in the cell than around the cell.

We can compare liver hepatocytes and pancreatic exocrine cells. A standard liver cell, liver hepatocyte, is involved with detoxification. Pancreatic exocrine cells secrete digestive enzymes. ERs constitute approximately 50% of both cells. In liver hepatocyte there are significantly more SER, however.

Percentage composition of cells varies from one cell to another.

4.1 Dynamics of Intracellular Compartments

Intracellular compartments exchange lipids and proteins. Together organelles form the endomembrane system.

The endomembrane system is involved in biosynthetic/secretory pathways (biosynthesis (proteins and lipids are produced and shared with other organelles) and secretion (proteins, exocytosis)) and endocytic pathway (*eg.* endocytosis).

During exocytosis vesicle contents are delivered to extracellular space, while vesicle membrane becomes a part of the plasma membrane. During endocytosis, the plasma membrane forms the vesicle membrane, with vesical luminal contents coming from the extracellular space.

5 Vesicular Transport

Definition 5.1. A **vesicle** is a small, membrane-enclosed organelle in the cytoplasm of a eukaryotic cell. Vesicles shuttle components back and fioth in the endomembrane system.

Transmembrane proteins can select specific proteins to go inside a vesicle from the donor compartment to the recipient compartment.

Specific proteins are targeted to different organells in the following steps. First, mRNA arrives in the cytoplasm and translation starts on ribosomes in the cytosol. A cytosolic protein is then translated in the cytosol and has no sorting signal.

Mitochondria and chloroplasts have their own genome and ribosomes, but most proteins are nuclear-encloded.

Nuclear-encoded proteins are translated in the cytosol and then targeted by a signal sequence, which imports them into the organelle post-translationally.

Proteins remain unfolded in the cytosol by association with hsp70 chaperones.

The path of a secreted protein from translation to secretion can be described as follows.

First, mRNA arrives in the cytoplasm and translation starts on ribosomes in the cytosol. While translation is still occuring, insertion of the protein into the endoplasmic reticulum starts, as directed by the signal sequence. In this way, **co-translational translocation** occurs.

SRP stops the translation on detection of the signal sequence, and taking the ribosome to the translocator by binding with the SRP receptor, which then associates with the plug in a translocus. In this way, the protein gets made while it moves into the ER lumen, and then a signal peptidase cuts off the signal after it has been used.

Pancreatic cells make lots of secreted proteins. These cells can be used to follow the path of newly synthesised proteins. First, the cells are provided with a short pulse of radioactive amino acids. The path of these aminoacids can then be followed as they are incorporated into proteins. This technique is called a **pulse-chase experiment**.

Proteins move from the rough endoplasmic reticulum, proceed to the Golgi apparatus and then get to the secretory vesicles.

The secretory pathway can be of two types:

- constitutive (continual production of secreted proteins)

  e.g. collagen

- regulated (proteins are stored in secretory granules ready for export in response to a stimulus)

  e.g. neurotransmitters

6 Protein Sorting Mechanisms

- Gated (proteins move between the cytosol and nucleus through nuclear pore complexes)
- Transmembrane (a translocon protein is needed to transport specific proteins across a membrane)

- Vesicular (membrane-enclosed transport vesicles ferry proteins from one compartment to another)

6.1 Signal Sequences

A signal sequence is a stretch of the amino acid sequence of a protein that directs the protein to the correct destination. Each signal sequence specifies a specific destination in the cell (to a nucleus, mitochondria, ER, peroxisomes, etc). Signal sequences are recognized by sorting receptors that take proteins to their destination.

Signal sequences are often found at the N-terminus of the protein. However, some signal sequences are internal stretches of AA which remain part of the protein. For proteins with N-terminal signal sequences there are signal peptidases removing the signal sequence from the protein.

Secreted proteins have N-terminal signal sequences.

7 Review

Sorting of a secreted protein proceeds as follows:

- translation starts on cytosolic ribosomes
- signal sequence of the amino-terminal end directs the protein to the ER (the signal sequence is hydrofobic)
- protein inserted throuch the membrane by the translocon lumen