Oxford Tutorial

PART A:

1. When a person eats, food is absorbed and broken down into its components. When the food reaches the small intestine, it is already an amino acid. An amino acid is a simple organic compound which contains a carboxyl, an amino group, a hydrogen, and a side chain specific to each amino acid. They are the building blocks of proteins.
2. The small intestine is the primary site of amino acid and glucose absorption into the blood. It is critical for this to happen since the supply of amino acids must be reached to all tissues.
3. Most of the internal and external body surfaces such as the intestines are covered with epithelial cells called epithelium. Many epithelial cells transport ions or small molecules from one side of the epithelium to the other. The epithelium lining the small intestine transport products of digestion such as glucose and amino acids into the blood. These epithelial sheets are connected by specialized regions of the plasma membrane called cell junctions. These provide strength and rigidity to the tissue and prevent the water-soluble material on one side of the sheet from moving across to the other side. **Amino acid is POLAR and TOO BIG, that's why it cannot go directly through the membrane.** The epithelial cell is polarized because one side differs in function and structure from the other.
4. The plasma membrane has 2 regions, each with different sets of transporter proteins. In epithelial cells that line the intestine, portion of the plasma membrane facing the intestine, the *apical* surface, is specialized for absorption; the rest of the plasma membrane, the lateral and basal surfaces, often referred to as the *basolateral surface*, mediates transport of nutrients from the cell to the surrounding fluids which lead to the blood.
5. The apical surface of the intestinal epithelial cells have finger-like projections called microvilli. The microvilli increase surface area for absorption. Increase in apical surface causes an increase in transport proteins it can contain. Apical surface makes direct contact with lumen.
6. To enter the epithelial cell, the amino acid in the gut lumen has to pass through the membrane transport proteins.
7. The amino acid passes through an import: Na+/amino acid symport on the apical surface (in microvillar membrane). It functions by active transport, therefore energy is needed (imported against its concentration gradient). This symporter couples the energetically unfavorable inward movement of one amino acid to the energetically favorable inward transport of Na^+ ions. The Na+/amino acid symport is coupled with Na+/K+ ATPase antiport. This is found on the basolateral surface of plasma membrane. Pumps 2K+ in and 3Na+ out. It maintains internal concentration of Na+ used to drive uptake of amino acids. It is coupled to ATP hydrolysis and Pi changes shape.

8. Once in the apical surface of the cell, the amino acid must be loaded onto a tRNA molecule. The amino acid gets delivered to a ribosome for incorporation into polypeptide chain in direct interaction with mRNA codon. The tRNA molecule is an adaptor molecule that is specific to an amino acid. The tRNA holds the amino acid in place while interacting directly and specifically with a codon in mRNA. The enzyme used is aminoacyl-tRNA synthetase.
9. Loading of tRNA: Enzyme's active site binds ATP and amino acid forming aminoacyl-AMP and 2 phosphates. The amino acid bound at AMP is now "activated." The activation uses ATP hydrolysis to make addition of amino acid to tRNA energetically favourable. Activated amino acid is transferred to tRNA. The finished aminoacyl-tRNA comes off, AMP come off amino acid, and the amino-acyl tRNA is ready for translation.

PART B:

1. The tRNA delivers the amino acid to the ribosome. The amino acid will get incorporated into a polypeptide chain that will be incorporated into the membrane as a transport molecule. The genes coding for transport protein has already been transcribed in the nucleus in the cell from DNA to mRNA. The mRNA transcript will be used to synthesize the membrane protein. The mRNA codes specifically for membrane protein and incorporation into epithelial cells.
2. Translation initiation: Initiator tRNA loaded with met(methionine) binds to small ribosomal subunit (loaded with proteins known as translation initiator factors); binds tightly to P site of small ribosomal subunit (only initiator tRNA can do this). Loaded ribosomal subunit binds 5' end of an mRNA molecule (signalled by 7m-G cap(guanine nucleotide with methyl group)). Small subunit slides along mRNA looking for the first AUG. When the AUG is reached, several initiation factors diffuse off, allowing the large ribosomal subunit to bind. Next charged tRNA can move into A site, and strand synthesis can begin.
3. Translation: The strand is ALWAYS read 5' to 3'. Aminoacyl tRNA carrying the correct anticodon for the mRNA codon diffuses into A site; its anticodon binds to codon on mRNA (pretty much simultaneous with 'spent' tRNA exiting the E site). Peptide bond forms between amino acid on aminoacyl tRNA in A site and growing polypeptide on tRNA in P site. It is catalyzed by enzymatic site containing ribozymes. The rRNA molecules with specific tertiary structure allows it to catalyze. Ribosome (first large subunit, then small subunit) moves along three bases and all three tRNAs move down one position
    - tRNA in the E site exits
    - tRNA in P site moves to E site
    - tRNA in A site moves to P site

New aminoacyl tRNA can move into A site. The process can begin all over again. At some point the mRNA codon will code for the original amino acid, which is how it will get incorporated into the necessary protein.

4. Termination: Presence of one of several STOP codons in the mRNA (UAA, UAG, UGA). Are not recognized by a tRNA; do not specify an AA. There is no corresponding tRNA. any STOP codon that reaches the A site will bind a release factor that alters catalytic activity, causing addition of a water rather than forming a peptide bond
5. Folding begins during translation, long before termination and disassembly of ribosomes
   Although it occurs 'spontaneously' (no energy required), often assisted by proteins called **molecular chaperones**
   Some chaperone proteins bind to ribosome near 'tunnel' where growing peptide exits
6. Levels of folding: Primary Structure- Amino acid sequence, complete upon termination of translation
   Secondary Structure-different folding structures, generated by various sequences, single polypeptides, hydrogen bonds between amino and carboxyl groups in polypeptide backbone
   Alpha Helix: spiral staircase, placing similar subunits next to each other in the same repetitive relationship relative to the one before, Hydrogen bond between every fourth amino acid (H and O), coiled-coiled: multiple alpha helixes
   Beta Sheets: Hydrogen bonds between segments of polypeptide lying side by side, parallel" runs back and forth on itself, anti-parallel: segments run opposite to neighbors
   Tertiary Structure- combination of non-covalent interactions. Vander waals (dipole-dipole), hydrophobic factors (water forces together by repulsion), electrostatic attractions (between positive and negative charges on polar molecules) and disulfide bonds (S-S bon between cyesine)
   Quaternary Structure- Interactions between different polypeptide chains; dimer (2 proteins, same affinity), helix (of multiple), and ring (cyclic structure).
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PART C:

1. Post Transitional Modifications: Chemical modification of protein structure (modification of the amino acids from the 20 basic structures we talked about)

Generally involve addition of functional groups/small molecules to protein
Eg. Addition of carbohydrates, lipids, phosphates, methyl group, acetyl group, cleavage of peptide bonds
It is important for protein diversity.

2. Proteins get embedded in apical plasma membrane of epithelial cell. Unattached, folded protein will only partially cross and become embedded in the membrane. Protein gets guided to necessary location by signal sequence.

3. -proteins must *unfold* to be imported, then refold and signal sequence removed

-Proteins all enter ER from cytosol
-Synthesis of all proteins starts on free ribosomes
-As mRNA is translated, ribosomes bind to it forming a polyribosome
-Membrane bound ribosomes are attached to ER making it "rough" ER- proteins translocated into ER
-Water soluble proteins completely cross ER membrane released into lumen
-Transmembrane proteins partially cross the ER- embedded in membrane

4. Protein directed to ER by signal sequence (small hydrophobic amino acid). Guided by signal recognition particle (SRP) which binds to signal sequence. This creates the SRP ribsosomal complex, which binds to SRP receptor in the ER membrane. SRP released (and SRP receptor) acting as matchmakers, they pass the complex to protein translocation channel in ER membrane. Signal sequence opens translocation channel. Signal sequence remains bound to channel while rest of protein chain is threaded through membrane in a loop. The protein is released into ER lumen. The signal sequence is cleaved off by signal peptidase. Signal sequence released into membrane and is rapidly degraded.

5. There are 3 cases of **TRANSMEMBRANE PROTEINS**

i) Simplest case: Single membrane spanning segment

- N-terminal sequence initiates translocation (N-terminal is on signal sequence)

- Additional sequence of hydrophobic amino acid(stop transfer sequence) halts transfer process in peptide chain

- stop transfer sequence is released laterally from translocation channel to drift into lipid bilayer

- forms membrane spanning segment that anchors protein in membrane

ii) Internal Signal Sequence

- Used to start protein transfer
- Continues until stop transfer sequence is reached
- Two hydrophobic sequences released into bilayer and remain anchored

iii) Complex multi-pass protein
- Additional start (signal sequence) and stop sequence come into play

- Reinitiate translocation further down polypeptide chain
- Happens multiple times forming a “stitching” pattern into lipid bilayer as being synthesized