**MEMBRANES AND RECEPTORS - SESSION 1**

**LIPIDS, PROTEINS AND MEMBRANE STRUCTURE**

**LECTURE 1.2 - MEMBRANE PROTEINS, MEMBRANE ASYMMETRY AND THE CYTOSKELETON**

**AIMS**

To consider

- the distribution and role of proteins in membrane structure

- the importance of an asymmetric distribution of membrane proteins

- mechanisms for the correct insertion of membrane proteins into the lipid bilayer and - the structure of the erythrocyte cytoskeleton.

**Lipid mosaic theory of membrane structure (Singer - Nicholson Model)**

Biological membranes are composed of a lipid bilayer with associated membrane proteins which may be deeply embedded in the bilayer (integral) or associated with the surface (peripheral).

Peripheral membrane proteins

- bound to the surface of membranes by electrostatic and hydrogen bond interactions.

These proteins can be removed by changes in pH or ionic strength. 18

Integral membrane proteins

- interact extensively with the hydrophobic regions of the lipid bilayer. These proteins can not be removed by manipulation of pH or ionic strength but require agents (detergents, organic solvents) that compete for the non-polar interactions in the bilayer.

**Asymmetrical orientation of membrane proteins**

Asymmetrical orientation of proteins in biological membranes is important for function e.g. a receptor for a hydrophilic extracellular messenger molecule, such as insulin, must have its recognition site directed towards the extracellular space to be able to function.

**The erythrocyte membrane** - **a model plasma membrane**

Erythrocyte ghosts can be prepared by osmotic haemolysis to release cytoplasmic

components. Analysis of ghost membranes by gel electrophoresis reveals over 10 major proteins. The major ones have been numbered 1, 2, 3, 4.1, 4.2, 5, 6 and 7 etc.. Most of these proteins are released when ghost membranes are treated with high ionic strength medium or by changing the pH and are, thus, peripheral proteins. These peripheral proteins must be located on the cytoplasmic face since they are susceptible to proteolysis only when the cytoplasmic face of the membrane is accessible.

Protein bands 3 and 7 can only be dissociated from the red cell membrane by detergents and are, thus, integral proteins. Both proteins contain covalently attached carbohydrate units and are, thus, *glycoproteins*. The highly hydrophilic nature of the extracellular carbohydrate groups acts to lock the orientation of the protein in the membrane by preventing flip-flop rotation. A great variety of carbohydrate structures

is possible on different proteins. Specific carbohydrate groups on membrane proteins 19

may be important for cellular recognition to allow tissues to form and in immune

recognition.

**Cytoskeleton (Membrane skeleton)**

The peripheral membrane proteins removed from erythrocyte membrane preparations by low ionic strength washes compose a membrane skeleton on the cytoplasmic face of the membrane. The erythrocyte cytoskeleton is a network of spectrin and actin molecules. Spectrin is a long, floppy rod-like molecule. and subunits wind together to form an antiparallel heterodimer and two heterodimers then form a head-to-head association to form a heterotetramer of 22 . These rods are crosslinked into networks by short actin protofilaments (~14 actin monomers), and band 4.1 and adducin molecules which form interactions towards the ends of the spectrin rods. The spectrin-actin network is attached to the membrane through adapter proteins. Ankyrin (band 4.9) and band 4.1 link spectrin and band 3 protein and glycophorin A, respectively. Attachment of integral membrane proteins to the cytoskeleton restricts the lateral mobility of the membrane protein. 20

**Haemolytic anaemias**

The erythrocyte cytoskeleton is a very important structure in maintaining the deformability necessary for erythrocytes to make their passage through capillary beds without lysis. In the common dominant form of **Hereditary Spherocytosis** spectrin levels may be depleted by 40 - 50%. The cells round up and become much less resistant to lysis during passage through the capillaries and are cleared by the spleen. The shortened *in vivo* survival of red blood cells and the inability of the bone marrow to compensate for their reduced life span lead to **haemolytic anaemia**. Other forms of hereditary spherocytosis also exist where mutated cytoskeletal elements with dysfunctional binding sites for other components are expressed. Similarly, in **Hereditary Elliptocytosis,** a common defect is a spectrin molecule that is unable to form heterotetramers resulting in fragile elliptoid cells. Even simple treatment with cytochalasin drugs, which cap the growing end of polymerizing actin filaments, can alter the deformability of the erythrocyte.

**Membrane protein synthesis directs protein orientation**

Like cytosolic proteins, membrane proteins and those to be secreted or targeted to lysosomes are synthesised against the messenger RNA template by ribosomes. However, before synthesis progresses very far the translation of these proteins is halted until the ribosome has been transferred to the rough ER (Figure). A characteristic hydrophobic amino acid sequence of

18 - 30 amino acids flanked by basic residues at the N-terminus of the nascent polypeptide, termed the ***signal*** or ***leader*** sequence, is recognised by a large protein/RNA complex called the ***signal recognition particle*** *(****SRP****).* Binding of the SRP to the growing polypeptide chain and the ribosome locks the ribosome complex and prevents further protein synthesis while the ribosome is in the cytoplasm.

On the ER the SRP is recognised by a ***SRP receptor*** or ***docking protein***. In making the interaction with the docking protein, the SRP is released from the signal sequence of the nascent polypeptide removing the inhibitory constraint on further translation. The 21

signal sequence then interacts with a ***signal sequence receptor*** *(****SSR****)* within a ***protein***

***translocator complex (Sec61)*** in the ER membrane, which directs further synthesis through the ER membrane. The ribosome becomes anchored to this pore complex, through which the growing polypeptide chain is extruded. In the case of a secreted or lysosomal protein, synthesis is completed and the nascent protein is translocated into the lumen of the ER. For membrane proteins the passage of the protein through the membrane must be arrested. The ***stop transfer signal*** for this is a region of highly hydrophobic primary sequence in the growing polypeptide of between 18 and 22 amino acids long followed directly by charged amino acids which, in -helical form, is long enough to span the hydrophobic core of the bilayer. This sequence forms the transmembranous region of the protein. A lateral gating mechanism releases the membrane protein from the protein translocator into the lipid bilayer. The ribosome then presumably detaches from the ER and protein biosynthesis continues in the cytoplasm. The result is a transmembrane protein with it‟s N-terminal directed in to the lumen and it‟s C-terminal to the cytoplasm. For both secretory proteins and membrane incorporated proteins, the signal sequence is cleaved from the new protein by ***signal peptidases*** even before protein synthesis is completed.