Membrane Proteins: Structure and Organization

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Synonyms

Integral Membrane Protein Structure

Definition

Membrane proteins are those which are integral to, or associated with, the various lipid membranes within and around cells.

Introduction

Membrane proteins are classified by their method of attachment into four main groups: integral, peripheral, partially penetrating, and lipid-anchored. Additionally, integral proteins can be further divided by the type of fold they possess – either alpha-helical or beta-barrel. These proteins have been challenging to study due to their hydrophobic nature and low natural abundance.

Integral Membrane Proteins

Integral membrane proteins constitute a significant proportion of the total protein of most cells and are characterized by insertion through the plasma membrane, resulting in permanent attachment. As such their removal can require relatively harsh methods including detergent solubilization or the use of nonpolar solvents. The advent of some detergent-free methods has enabled the extraction of membrane proteins whilst retaining the surrounding lipids. Such lipids have been implicated in the physiological activity of certain integral membrane proteins. As expected, the regions of the protein in contact with the lipids tend to contain hydrophobic residues. Integral membrane proteins can be divided into two classes, based upon the precise nature of their interaction with the membrane. Monotopic membrane proteins are those which are attached to the membrane from one side only. These proteins/domains carry out their function on the side of the membrane in which they are

located. Examples of these kinds of proteins include carnitine palmitoyltransferase-2 from rat, which is located to the inner leaflet of the mitochondrial inner membrane; prostaglandin H2 syntheses 1 and 2; microsomal prostaglandin E synthase; and the monotopic domains of fatty acid amide hydrolase and monoamine oxidase B. The latter two examples also contain transmembrane (TM) domains; however, the majority of the proteins including the active site are located monotopically, on one side of the membrane. In contrast, polytopic or transmembrane proteins span the membrane completely, and are able to carry out functions on both sides of the membrane. They comprise over one-quarter of the total protein within an organism and have many important cellular roles: acting as receptors, forming ion channels and translocons, as well as energy-generating pumps and reaction centers. These proteins can have varying numbers of transmembrane domains ranging from one, such as the receptor tyrosine kinase family, to several, such as the seven TM G protein-coupled receptors and are classified as either single- or multi-pass. Integral membrane proteins can adopt two types of fold: alpha-helical or beta-sheet (Figure 1). Alpha-helices are by far the most represented structure for a protein in a bilayer due to energetically favorable hydrogen-bond formation within the protein backbone. Beta-barrels are also low energy structures present in bacteria, mitochondria, and chloroplasts, although they have only been studied in detail in the outer membrane of Gram-negative bacteria.

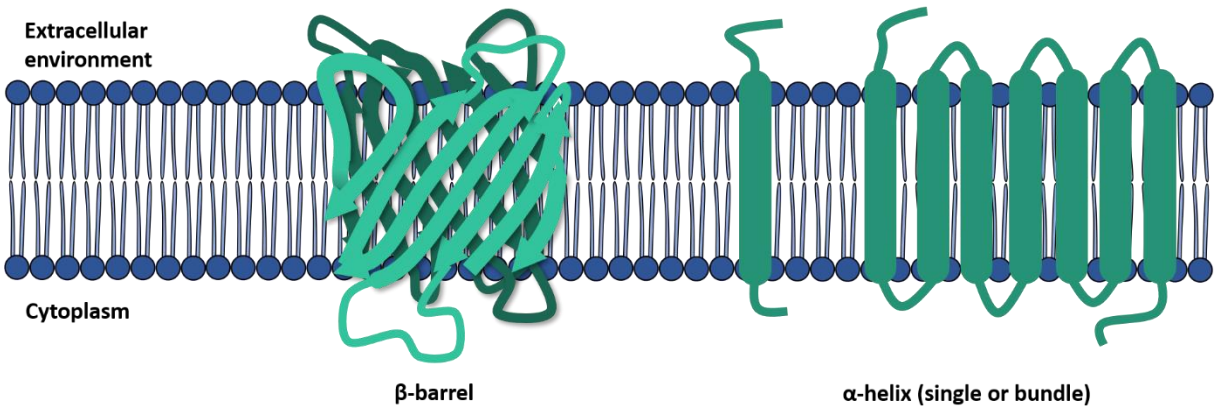

Figure 1: Schematic of integral membrane protein structures.

Peripheral Membrane Proteins

Peripheral membrane proteins are associated with the polar head group region of the lipid bilayer. This association can be mediated by non-covalent interactions, covalent binding such as lipid anchors, as well as ionic interactions. Unlike integral membrane proteins they do not contact the hydrophobic interior of the bilayer. Typically, peripheral proteins are maintained in close proximity to the bilayer through interaction with other membrane proteins. The loose membrane association means that this group of proteins can be isolated from the bilayer using relatively mild conditions without the need for detergents. Cytochrome *c* is one such protein which interacts with components of the electron transport chain but does not penetrate the bilayer.

Partially Penetrating Membrane Proteins

In addition to those proteins which are peripheral or integral to the membrane are a class of proteins which associate with both the polar heads of the bilayer and also with the hydrocarbon tails of the lipids. These are described as partially penetrating membrane proteins. These proteins may use a surface loop, exposed via a conformational change, to interact with the membrane. Additionally, membrane-targeting domains and peripheral membrane proteins may also be partially penetrating during the interaction process. Examples of this type of protein include the antimicrobials magainin and dermaseptin.

Lipid-Anchored Membrane Proteins

Many proteins associate with the membrane via covalent lipid modifications. Fatty acids can be directly attached to the peptide backbone and can also be easily incorporated into lipid bilayers. The two most common examples are palmitoylation and myristoylation. Modification with fatty acids increases the overall hydrophobicity of the protein and mediates membrane attachment. S-palmitoylation involves that attachment of fatty acids, most commonly palmitic acid, to cysteine residues via an ester bond. Due to the nature of this bond, the attachment is reversible and the precise palmitoylation state of the protein may be key in modulating activity. The reverse reaction is catalyzed by palmitoyl protein thioesterases. Myristoylation is the irreversible co-translational attachment of the myristate-derived myristoyl group via an amide bond to the alpha-amino group of the N-terminal amino acid of the peptide. The most common site for such attachment is glycine, although attachment at other amino acids occurs. The process is catalyzed by N-myristoyltransferase (NMT). Prenylation involves the attachment of farnesyl or geranyl-geranyl to the C-terminus of a protein via a conserved CaaX motif, where “C” is the cysteine residue that is prenylated and “a” is an aliphatic amino acid. The identity of residue “X” determines the nature of prenylation by determining which enzyme acts upon the protein substrate. Prenylation is catalyzed by farnesyl and geranyl-geranyl transferases, CaaX protease, and methyl transferase.
Proteins can also be attached to membranes via a GPI anchor (glycophosphatidylinositol). This is attached posttranslationally to the C-terminus of the protein. Firstly, GPI-anchored proteins contain a signal sequence which directs the protein to the endoplasmic reticulum (ER). The C-terminus is hydrolyzed and amino acids remain inserted in the ER membrane. The hydrolyzed end is cleaved and replaced by the GPI anchor. The protein is then targeted to vesicles, then golgi, and finally to the extracellular membrane, where it remains attached. The GPI anchor can be cleaved by phospholipases for controlled release.

Alpha-Helical Membrane Proteins

The vast majority of transmembrane proteins are contained in this group. Alpha-helical proteins can be further classified according to orientation and number of membrane spans. Type I proteins are single-pass with an extracellular or luminal N-terminal domain. Type II proteins are single-pass but with the N-terminus located on the intracellular or cytoplasmic side. Type III proteins or multi-pass proteins contain more than one transmembrane domain and can be in either orientation. Type IV membrane proteins consist of complexes of multiple domains from separate polypeptide chains. The membrane-spanning helix forms due to energetically favorable hydrogen-bond formation within the protein backbone and can be either right or left handed. A typical helix contains 3.6 residues per turn, although helices with 3.10 residues are also found, and is around 30 Å in length. The majority

of residues within a transmembrane helix are hydrophobic, and membrane-spanning regions can be identified using computational algorithms/hydrophobicity plots to identify regions of hydrophobic sequence. Examples of alpha-helical transmembrane proteins include G protein-coupled receptor, ion channels, translocons, and light-harvesting complexes.

Beta-Barrel Membrane Proteins

A transmembrane protein which spans the bilayer as a beta-barrel structure comprised of antiparallel beta-strands forming a rigid and stable barrel shape which usually forms a channel for substrates. Known structures of beta-barrels contain between 8 and 22 strands, allowing flexibility in the size of the barrel channel. In general, beta-barrels can be identified by their alternating pattern of lipid-exposed hydrophobic residues and interior hydrophilic residues with both the N- and C-termini located on the intracellular side. The tilt or inclination of the barrel in the membrane can be defined by the number and stagger of the strands. Both oligomeric and monomeric beta-barrels are found in biological membranes. Beta-barrel membrane proteins commonly form pores known as porins in the outer membrane of Gram-negative bacteria, chloroplasts, and mitochondria where the variation in barrel structure enables selectivity of a wide range of substrates. They are also found in microbial toxins.

Membrane Protein Insertion and Folding

The process by which membrane proteins insert into the bilayer and assemble to form stable structures has been studied in greater depth for alpha-helices than beta-barrels. In both cases membrane insertion can occur spontaneously; however, alpha-helices usually require the assistance of a translocon and an input of energy. Following insertion, proteins may preferentially localise in discrete domains within the membrane depending on their precise characteristics.
In order to conceptualize the membrane protein folding process, a number of models have been proposed which consider isolated alpha-helical segments. The two-stage model of membrane protein folding was proposed by Popot and Engelman in 1990 (Popot and Engelman *1990*) and describes the establishment of stable individual helical domains across the bilayer as the first step followed subsequently by lateral association of the helices. This model was later refined to include a third step describing the insertion of cofactors and formation of quaternary complexes (Engelman et al. *2003*). In 1999 a further model was proposed by Wimley and White which added additional stages to describe the formation of helical domains which involves partitioning of the helical segments into the bilayer interfacial region proceeded by helix formation and then bilayer insertion (White and Wimley *1999*). This four-stage process of partitioning, folding, insertion, and association was described using thermodynamic principles. The development of hydrophobicity scales, to describe the energetic cost of inserting each amino acid into the bilayer, has greatly improved our understanding of this aspect of membrane protein folding. The lateral association of helices within the bilayer has also been extensively explored leading to the discovery of specific residues and motifs that promote helix association such as GxxxG motif, where two small glycine residues are separated by three amino acids, thus placing the two glycines on the same face of the helix where their small size facilitates an interaction. This motif was first characterized in glycophorin A which is the model for association of transmembrane domain (Engelman and Russ *2000*). Other

determinants of interaction include motifs of small polar residues such as serine and threonine, as well as leucine zippers similar to those found in soluble proteins.

Cross-References

Ion Channels
Lipids
Membrane Protein Function
Protein Folding
Rhodopsin – Stability and Characterization of Unfolded Structures
Rhodopsin: Stability and Structural Organization in Membranes

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