Membrane Protein Function

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Synonyms

7-Transmembrane Domain Receptors

Definition

The varied functions performed by specific membrane proteins and peptides including, for example, enzymatic reactions, membrane transport, cell communication, light harvesting, membrane disruption, and cell adhesion.

Introduction

Membrane proteins account for approximately 30% of the proteins encoded by a typical genome (Wallin and von Heijne *1998*). This abundance gives some indication of the diversity of function of this class of protein. Typically, they are involved in regulating the cell's response to, and interaction with, the environment. Each of these processes is critical to correct cellular function, and the examples below serve to highlight the importance of this class of proteins (Smith *2010*).

Receptors

Receptors on the cell surface transduce signals from the external environment across the cellular membrane, leading to downstream responses. They detect a wide variety of stimuli and result in an equally diverse array of cellular responses. Due to the difficulty in obtaining structural data on membrane-spanning proteins, receptors are often classified by their predicted topology – the simplest consists of a single transmembrane helix while G protein-coupled receptors (GPCRs) have seven transmembrane helices. Receptors can be broadly divided into three domains. The extracellular domain detects (normally be direct binding) the stimulating molecule. The transmembrane domain transduces the signal across the membrane, possibly involving a

conformational change. Finally, the intracellular domain interacts with downstream signaling components within the cell (e.g., GPCRs) or possesses an intrinsic enzymatic activity which induces the signaling cascade (e.g., insulin receptor which functions via tyrosine kinase activity).
G protein-coupled receptors (GPCRs) are the largest family of cell surface receptors and are characterized by seven transmembrane alpha-helices. They detect a wide variety of ligands and signal via heterotrimeric guanine nucleotide-binding proteins (G proteins). In the unstimulated state, the Gα, Gβ, and Gγ subunits form a heterotrimeric G protein in which the Gα subunit is bound to GDP. When the receptor is stimulated, it undergoes a conformational change, leading to association with the G protein and a concomitant change in the Gα structure leading to the release of GDP. This GDP is replaced by GTP, and the Gα subunit dissociates from the Gβγ dimer and the receptor. The Gα and Gβγ subunits activate a wide variety of effectors, depending on their exact identity. Signaling is terminated by hydrolysis of the GTP to GDP and reformation of the heterotrimeric G protein.
GPCRs can be divided into six classes by sequence identity and ligand:

. Class A – Rhodopsin like
. Class B – Secretin
. Class C – Metabotropic glutamate
. Class D – Fungal pheromones
. Class E – cAMP
. Class F – Frizzled/Smoothened

Rhodopsin is found in the retina and is involved in transduction of light signals and is one of the best characterized GPCR (Smith *2010*). In contrast to many GPCRs, it does not detect its ligand directly. Instead, it contains a chromophore – retinal – which lies horizontally in the protein and is photobleachable by light. Photons cause the isomerization of 11- *cis*-retinal into all-trans-retinal and an associated conformational change in rhodopsin structure. This leads to activation of transducin (Figure 1). Rhodopsin absorbs most strongly in the green-blue region of the spectrum and is therefore purple in color.

Bacteriorhodopsin is found in a variety of archaea including *Halobacteria*. Although this protein consists of seven transmembrane domains and contains the retinal chromophore which detects light, it is not a GPCR. Instead of coupling to a G protein, stimulation by light results in isomerization of the retinal and a conformational change which allows protons to move across the membrane through bacteriorhodopsin. The Nobel Prize in Chemistry in 2012 was awarded for work on GPCRs.
Tyrosine kinase receptors (TKRs) are transmembrane receptors for growth factors, cytokines and hormones. In neoplastic tissues, they play a focal role in cancer biology during angiogenesis, tumor development, proliferation and metastasis (Boonstra et al al., 2016).

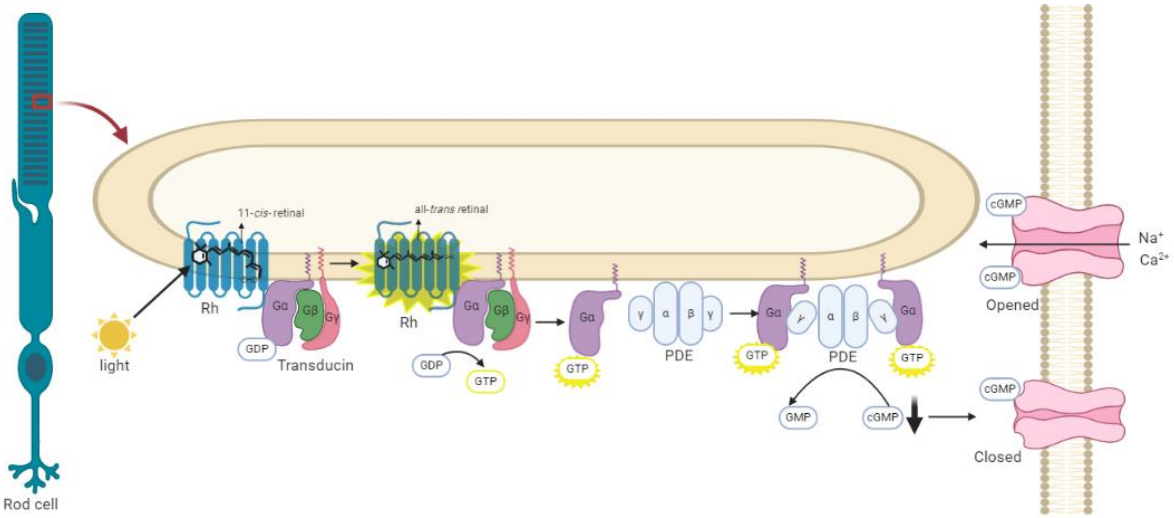

Figure 1: Schematic representation of the phototransduction cascade. Depicted is a rod and the outer membrane disk in a rod. Upon light absorption by rhodopsin (Rh), 11-cis-retinal is isomerized into all-trans-retinal. Transducin is activated and the α subunit dissociates from subunits β and γ, catalyzing its activation by the release of bound GDP in exchange for cytoplasmic GTP. The activated α subunit binds the γ subunits of the phosphodiesterase (PDE) activating its α and β subunits. The activated PDE hydrolyzes cGMP to GMP. Reduced cGMP caused cyclic nucleotide gate channels to close preventing a further influx of Na^+ and Ca^{2+}. Created with Biorender.com.

Ion Channels

Ion channels are complexes of integral membrane proteins, present in all cell types, which facilitate the flow of ions such as sodium or potassium down an electrochemical gradient. Ion channels form aqueous pores across the bilayer which are highly conductive to the passage of various ion species both into and out of the cell. These proteins have a central role in cell processes, including transmission of nerve impulses, muscle contraction, and insulin release, and are therefore clinically important as their malfunction leads to a number of disease states, the most well known being cystic fibrosis which affects a chloride channel. Furthermore, ion channels pose as important drug targets for the treatment of auto-immune diseases such as multiple sclerosis. The mechanism by which a channel is opened and closed is normally referred to as gating, and gating mechanisms are used in a classification system. Voltage-gated channels are controlled by the membrane potential and include those for sodium, potassium and calcium. Ligand-gated channels respond to small molecules which bind to specific sites in the extracellular part of the channel and include the nicotinic acetylcholine receptor and γ-aminobutyric acid-gated $GABA_A$ receptor. Other gating mechanisms include mechanical stress, temperature, and second messenger binding on the cyctoplasmic side of the channel. Channel structure varies with ion type and gating mechanism but typically consists of several transmembrane helices surrounding a central channel; a loop region is inserted into the channel in a number of cases which confers specificity. Most research with ion channels uses the technique of patch clamping which measures the flow of ions through a single channel; the development of which was awarded the Nobel Prize for medicine in 1991 (Neher et al. *1978*). Determination of the structure of the voltage-gated potassium channel from *Streptomyces lividans* won the Nobel Prize for chemistry in 2003 (MacKinnon et al. *1998*).

Translocons

Translocons are membrane protein complexes which regulate and facilitate both the transport of proteins across, and the integration of membrane proteins into, the cell membrane. In bacteria,

protein transport across the cytoplasmic membrane occurs by two major systems, the most common being the general secretory pathway or Sec system and the twin-arginine translocase or Tat system for the transport of folded proteins. In eukaryotes, the Sec61 channel translocates proteins into the endoplasmic reticulum from the cytosol. Translocation is coupled to translation in eukaryotes with the nascent chain emerging from the ribosome and being translocated immediately through the channel. Proteins which are to be transported usually contain a specific sequence at their N-terminus which may be directly recognized by the translocon or by specific chaperons which guide the protein to the translocon (examples include the SecA component of the bacterial Sec system and the signal recognition particle of the eukaryotic system). In general, the translocation of proteins requires an input of energy in the form of ATP hydrolysis. The bacterial and eukaryotic Sec systems are involved in the insertion of membrane proteins into the bilayer; hydrophobic segments are recognized by the translocon, and translocation is arrested to allow the lateral movement of the segment out of the channel and into the bilayer. Mutations of the Sec61subunits can result in common variable immunodeficiencies (CVID). Other protein translocons include the Toc and Tic complexes of the chloroplast membranes and the Tom and Tim complexes of the mitochondria, both of which couple translocation across two bilayers.

Photosynthesis

The light-dependent reactions of photosynthesis, whether in bacteria or chloroplasts, take place at the plasma membrane. In chloroplasts, the thylakoid membrane separates the lumen from the stroma (the site of the dark reactions). This membrane contains light-harvesting complexes, photosystems I and II (PSI and PSII), cytochrome b_6f, and ATP synthase. Light-harvesting complexes (antenna complexes) comprise the light-harvesting system which surrounds the reaction center of photosystems and transfer electrons to a single chlorophyll a molecule within it. Light-harvesting complexes contain a number of molecules such as chlorophyll b, carotenes, and xanthophylls which enable the plant to absorb a wide wavelength of light. These complexes effectively funnel energy to the reaction center of the photosystems. Photosystem II splits water into electrons, protons, and molecular oxygen. The electrons are transferred to plastoquinone (a mobile electron carrier in the membrane). Electrons then pass to cytochrome b_6f, a transmembrane proton pump, and then on to plastocyanin (a water-soluble electron carrier). The proton gradient established by cytochrome b_6f is then used to generate ATP via the transmembrane ATP synthase which exports protons and generates ATP. The electrons from plastocyanin pass to photosystem I where they are transferred to NADPH or back to cytochrome b_6f. ATP and NADPH are then used to synthesize organic molecules from CO_2. The light-dependent reactions of photosynthesis involve a variety of different membrane proteins to move electrons and protons and to perform chemical reactions.

Transport Proteins

Transport across biological membranes may be passive (no energy required) or active (energy required). Primary active transport utilizes chemical energy, e.g., ATP, whereas secondary active transport involves the use of electrochemical gradients. Transport proteins can function via a variety of mechanisms. Uniporters transport a single substrate in one direction. Examples of such transporters are GLUT1 (involved in the movement of glucose across cell membranes in mammalian cells) and valinomycin (a nonpeptide potassium transporter derived from *Streptomyces*). Symporters

transport two or more different molecules across membranes. A prime example of this is lactose permease (LacY) which transports lactose, along with protons, into bacterial cells. In humans, the glucose/Na^+ transporter moves glucose across the luminal membrane of endothelial cells to allow it to be taken up into the bloodstream. Antiporters move one substrate across the membrane in each direction – the electrochemical gradient of one substrate is used to drive the transport of the other. Examples of this type of transport mechanism include the ADP/ATP exchanger and the Na^+/H^+ exchanger.

ABC Transporters

ABC (ATP-binding cassette) transporters use ATP to enable active transport of a range of substrates across membranes. They are found in both prokaryotes (where they may import or export substrates) and eukaryotes (where they only export). ABC transporters consist of two domains – the transmembrane domain (TMD) and the nucleotide binding domain (NBD). The minimal unit of the ABC transporter is two TMDs and two NBDs. The TMD consists of six alpha-helices such that the dimer has 12 in total. This domain can be classified as type I, II, or III depending upon the fold. The NBD is cytoplasmic and consists of two domains. The first is the catalytic core which is involved in ATP hydrolysis and consists of beta sheets. The second domain is a unique alpha-helical subdomain. The genes encoding ABC transporters often produce a fusion polypeptide TMD-NBD-TMD-NBD which ensures correct orientation and stoichiometry of the subunits.

Secondary active transporters

Secondary active transporters utilize an electrochemical gradient for the symport of a substrate and a solute. The transport is generally performed using an alternating-access mechanism which means that substrate and solute can bind to the transporter from both sides of the membrane. During transport, the protein switches from an inward facing to an outward facing state or the other way around. Rocker-switch, rocking-bundle or elevator proteins using either a moving or fixed barrier facilitate the transport. However, the transport is only initialized if both substrate and solute are bound to the protein. This energy-requiring step and the fact that they can transport substrates against a concentration gradient makes them active transporters. The MFS (major facilitator superfamily), LeuT and NhaA fold are the three main folds found for secondary active transporters. They consist out of either 10 or 12 transmembrane helices and share little to no sequence similarity. Their main structural similarities are a twofold pseudosymmetry axis and discontinuous helices which are important for substrate and solute binding and the transport itself.

Membrane Pumps

Membrane proteins involved in the active transport of solutes across cell membranes in an ATP-dependent manner are known as pumps. ATP-dependent pumps are classified by type (type-P I-V, type-F, and type-V) based on sequence homology and structure. Type-P pumps function by phosphorylation of the alpha subunit, leading to a conformational change. Examples of type-P pumps are the sodium potassium ATPase which functions as an antiport ion pump in the plasma membrane, actively transporting sodium ions out of the cell in exchange for the entry of potassium, and the calcium ATPase which serves to remove calcium from the cell cytoplasm to enable its

continued use as a signaling molecule. Type-V pumps are not phosphorylated; they are found in the endosomal, lysosomal, and vacuolar membranes where they are responsible for maintaining the proton gradient. Type-F pumps again only transport protons and are not modified by phosphate; they are present in the membranes of bacteria, mitochondria, and chloroplasts. This type of pump is can also be known as an ATP synthase as it utilizes the energy from the electron transport chain to generate ATP.

Porins

Porins are transmembrane proteins arranged in a beta-barrel structure forming a cylindrical aqueous channel to aid the diffusion of small molecule solutes (typically less than 1,500 Da) into and out of the cell, typically sugars, ions, and amino acids. Porins are highly represented in bacterial outer membrane, with the best studied example being OmpF (Nikaido *2003*), and are also present in chloroplast and mitochondrial outer membranes. They influence antibacterial drug resistance and play a role in the host-pathogen interaction. The structure of the channel is formed by a varying number of beta strands which defines the size of solutes which can pass through the pore, and an alternating pattern of hydrophobic and hydrophilic amino acids which, interact with the lipid membrane and form the lining of the pore, respectively. An external loop between the strands is believed to form an "eyelet" which additionally restricts the size of the channel regulating solute passage. Porin monomers are typically arranged as trimers in the membrane but with each monomer functioning as an individual pore. Both general and substrate-specific porins are found, and expression of different porins is controlled by the cell in response to environmental stress such as osmotic pressure and temperature (OmpC/OmpF) as well as chemicals such as phosphate (PhoE).

Aquaporins

Aquaporins (AQPs) are integral membrane proteins regulating the passage of water across the membrane. They are highly selective for water and are controlled by the osmotic gradient. Studies have shown that AQPs also transport small, uncharged molecules such as glycerol and urea, CO_2, ammonia, H_2O_2, boric acid, silicic acid, antimonite, arsenite. They have a six-transmembrane-domain architecture, with the N- and C-terminal halves of the protein being sequence related. Each half consists of three transmembrane domains with the loops between helices two and three, in one half, and five and six in the other forming hemipores with each traversing one leaflet of the bilayer. The pores close together during water transport to form the full pore defined as an hourglass structure. This arrangement was confirmed by cryo-electron microscopy and atomic force microscopy. The narrowest diameter of the channel was found to be 2.8 Å – just large enough for a water molecule to pass. Aquaporins form a tetrameric assembly in the membrane, but each monomer alone is capable of acting as a channel.

Antimicrobial Peptides

Antimicrobial peptides act as effective broad-spectrum antibiotics. They generally consist of a short polypeptide (12–50 amino acids) with a high hydrophobic content. Members of this family can adopt four different conformations: alpha-helical, e.g., magainin; extended, e.g., indolicidin; beta sheet

stabilized by disulphide bonds, e.g., defensin; and mixed, e.g., protegrin-1. Many of these peptides are actually disordered in solution and only order upon partitioning into membranes. These proteins tend to be amphipathic to enable partitioning into the membrane. They act via two mechanisms, either the formation of a pore or penetration into the cell and subsequent interaction with essential cellular components. These peptides are relatively selective for prokaryotic cells due to the differences in membrane composition when compared to eukaryotes. Prokaryotes typically have many zwitterionic phospholipids in their membranes, creating an overall charge and allowing electrostatic interaction with the peptide. However, eukaryotic cells generally have an uncharged outer membrane, prohibiting hydrophobic interactions with the cationic peptides.

Fusogenic Peptides

As their name suggests, fusogenic peptides promote fusion of lipid membranes. A prime example is hemagglutinin from influenza virus. Hemagglutinin is a spike-shaped protein found on the viral surface. As expected, the exposed portion is largely hydrophilic and recognizes sialic acid on the surface of host cells. Binding of hemagglutinin to sialic acid causes the viral particle to be endocytosed into the host cell. As part of the host defense, the endosome is then acidified. This causes partial unfolding of hemagglutinin and exposes a hydrophobic region of the protein. This region then inserts into the endosomal membrane. As the rest of the protein adopts its new conformation at lower pH, the viral and endosomal membranes are drawn closer together, resulting in fusion.

Cell Adhesion Molecules and Scaffold Proteins

Cell adhesion molecules (CAMs) are displayed on the cell surface and interact with other cells or components of the extracellular matrix. They are tethered to the cytoskeleton on the intracellular side and are classified according to calcium dependency. The immunoglobulin superfamily and integrins are both calcium independent, while the cadherins such as snares and selectins are calcium dependent.

Some proteins act as a scaffold on to which other proteins can be tethered and localized to the plasma membrane. These proteins usually form part of signaling cascades and include receptors such as the epidermal growth factor receptor and the platelet-derived growth factor receptor. Upon activation by extracellular ligand binding, the membrane scaffold protein becomes phosphorylated on the cytoplasmic side and is then able to recruit downstream signaling components. In this way, the scaffold protein orchestrates the signaling cascade through the formation of membrane-localized complexes. Scaffold proteins play a role in regulating and coordinating signal transduction by reducing or enhancing the downstream signal, through the recruitment of specific proteins, and are therefore central to feedback loops.

Cross-References

Ion Channels
Membrane Protein Structure
Membrane Proteins: Structure and Organization

Membrane Transport, Energetics and Overview
Potassium Channels: Their Physiological and Molecular Diversity
Thermodynamics of Lipid Interactions

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