MEMBRANE ADAPTATION AND SOLUTE UPTAKE SYSTEMS

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Summary

This article considers the structure of membranes and how their transport proteins are arranged to carry out the functions of moving ions, water, and other molecules into or out of the cell, with special focus on the influence of membrane lipid composition on the different modes of transport in microorganisms that live at low temperatures. The modulation of transport systems by changes in lipid composition in response to decreases in temperature is put in the context of how the cold-adaptation of nutrient uptake systems may play a role in the ecology of cold-adapted microorganisms.

1. Introduction

Cold-adapted microorganisms (that is, bacteria, yeasts, fungi, and microalgae) have successfully colonized the naturally cold habitats of water, snow, ice, soils (including those that are permanently frozen), as well as artificial cold habitats, particularly those associated with chilled and frozen foods. To do this they have necessarily adapted their cellular compositions to function at low temperatures. Enzyme proteins perform these functions, and the molecular basis of their cold adaptations is discussed in *Catalysis and Low Temperature: Molecular Adaptation*. In addition, there is another class of cellular proteins whose activity is crucial to the survival of all cells, namely those membrane

proteins that are responsible for the uptake of essential nutrients and the expulsion of unwanted compounds. These proteins are known as "transport proteins" or "carriers," and sometimes by their historical name of "permeases."

In cold-adapted microorganisms, it follows that these proteins must have specific adaptations in order to enable them to function at low temperatures. However, we know nothing of the molecular adaptations of membrane proteins: all the available information to date has come from soluble cytoplasmic enzymes, and we can only guess at how membrane proteins might have evolved to be cold active. Those guesses might well be inaccurate, because the interactions between membrane proteins and the hydrophobic moieties of lipids are very different to those that soluble proteins make with solvent (water). Moreover, the structural arrangement of membrane proteins, particularly those that span membranes, is quite different to that of soluble proteins. Our knowledge is deficient because it is difficult to purify transport proteins for two reasons: they are very hydrophobic and they have relatively low abundance in membranes. The technology of gene cloning has enabled the amino acid sequences of transport proteins to be derived from their gene (DNA) base sequences, but three-dimensional structures can only be inferred from such information. In contrast, we know a lot about the cold-adaptations of membrane lipids that form the basis of the maintenance of a fluid lipid bilayer at the core of membrane structure. The interplay between how these changes regulate membrane fluidity and the rate of uptake of nutrients is considered in this article.

All cells must regulate their internal environment, and microorganisms are no exception. A major contribution to this function is made by the cytoplasmic membrane, which is not only responsible for the entry and exit of small molecules and some large molecules too, but also controls the intracellular ionic environment by virtue of the activity of various ion pumps that move particularly Na^+, K^+, and H^+ across the membrane. The movement of H^+ is especially important in the creation of the so-called "proton-motive force" (PMF), which is a combination of chemical and electrical potential across the cell membrane that has the capacity to drive energetic processes, including the uptake of molecules by transport proteins. The PMF (Δp) is defined as:

$$\Delta p = \Delta \psi - Z\Delta pH$$

in which

$\Delta \psi$ = transmembrane electrical potential difference,

ΔpH = transmembrane pH difference, and

Z = a factor that converts pH units into millivolts units.

Protons (H^+) can be moved either by ATPases or through the action of the respiratory chain enzymes and electron carriers that are found in the cytoplasmic membrane of prokaryotic microorganisms (bacteria) and the inner mitochondrial membrane of eukaryotic microorganisms (yeasts, fungi, and microalgae). Some bacteria also use Na^+ gradients across the cytoplasmic membrane to energize transport systems. Under

anaerobic conditions, a microorganism growing on carbohydrate may expend 20–30% of its total energy balance on taking up nutrients by active transport, which serves to emphasize just how critical transport processes are to the existence of the cell. Besides the activity of specialized transport proteins that control the passage of molecules across the membrane in a facilitated fashion, there is passive movement of molecules through the lipid bilayer. This passive process is about four orders of magnitude (10^4) times slower than facilitated transport, but can be equally important in habitats where there is a high concentration of a particular nutrient or essential ion. Because passive transport occurs through the lipid phase of the membrane, it is particularly influenced by cold-adaptations in lipid composition, and there is a theory of thermal adaptation that emphasizes the importance of passive proton permeability of membranes (see Section 5.1).

This article focuses on the ways in which lipid adaptations to low temperatures influence membrane transport processes, and how in turn this has a role to play in the ecology of cold-adapted microorganisms. Even a single bacterial, yeast, fungal, or algal cell may take up many different types of compounds from the external surroundings. But overall as a group, microorganisms are capable of transporting an extraordinary diversity of different molecules, including water, ions (for instance, H^+, Na^+, K^+, Ca^{2+}), small nutrient molecules (for example, sugars and oligosaccharides, sugar alcohols, amino acids, peptides, fatty acids, organic acids, nitrogenous compounds), vitamins and cofactors, antibiotics, iron (Fe), and macromolecules (for example, proteins and DNA). Given this plethora of transport substrates and different types of organism, it is not possible in this overview to discuss specific transport systems in detail. Instead, the focus is on generic types of transport system and how the lipid environment of the membrane influences their activity. Therefore, first it is necessary to consider membrane structure and lipid organization.

2. Membrane Structure and Lipid Organization

The majority of cellular membranes of microorganisms are made up of roughly equal amounts of proteins and lipids (there are some specialized exceptions, such as gas vesicle membranes that are comprised only of proteins), together with a smaller amount of carbohydrate in the form of glycoproteins, glycolipids, or other specialized molecules. These components are organized in much the same way as in the membranes of animals and plants, as embodied in the fluid-mosaic model of membrane structure. The basic framework of the membrane is a bilayer of lipid, consisting of two opposed monolayers of lipids, with their hydrophobic fatty acyl chains facing inwards and their hydrophilic headgroups outwards. It is within this two-dimensional bilayer structure that proteins float, able to diffuse in the lateral plane of the membrane but having restricted vertical movement. Microbial membrane lipids consist mainly of phospholipids together with glyco(phospho)lipids (the latter are found particularly in Gram-positive bacteria); in eukaryotic microorganisms there are also sterols, whilst some bacteria contain hopanoids that are saturated equivalents of sterols. The fluid-mosaic model also presupposes that the lipid bilayer is in the liquid-crystalline phase, in which the lipid molecules are highly mobile and undergo rapid rotational and vibrational motions. As temperature falls, lipids will lose fluidity and eventually pass through a transition to form a gel phase, in which the molecules are packed much more tightly with greatly

reduced motions. The temperature of this transition (T_m) depends largely on the fatty acyl composition of the lipid, and can be measured most readily in model systems containing a single type of lipid (Table 1).

Phospholipid	Transition temperature (°C) (*Liquid-crystalline to gel phase transition temperature*)
Di-16:0-phosphatidylcholine (*Fatty acid nomenclature is given as "number of carbon atoms: number of double bonds"*)	62
Di-16:0-phosphatidylcholine	41
Di-16:0-phosphatidylglycerol	41
Di-*cis*18:1-phosphatidylethanolamine	-16
Di-*cis*18:1-phosphatidylcholine	-17
Di-*cis*18:1-phosphatidylglycerol	-18
Di-*iso*16:0-phosphatidylcholine	22
Di-*anteiso*16:0-phosphatidylcholine	-3
Di-*cis*16:1-phosphatidylcholine (*Double bond positional isomer is palmitoleic acid*)	-36
Di-17:0-phosphatidylcholine	49
sn-1-18:0, *sn*-2-18:0-phosphatidylcholine	56
sn-1-18:0, *sn*-2-18:1-phosphatidylcholine (*Double bond positional isomer is oleic acid*)	6
sn-1-18:0, *sn*-2-18:2-phosphatidylcholine (*Double bond positional isomer is linoleic acid*)	-16
sn-1-18:0, *sn*-2-18:3-phosphatidylcholine (*Double bond positional isomer is linolenic acid*)	-13

Table 1. The effect of fatty acyl composition on the liquid-crystalline to gel phase transition temperatures of some phospholipids

However, in natural membranes there is usually a mixture of lipid types with different combinations of fatty acyl chains, so no abrupt transition to a gel phase is generally seen. Instead as the temperature is lowered, domains of gel-phase lipid form, that increase in size as the temperature continues to fall. Experiments with mutants of *E. coli* and with the wall-less bacterium *Acholeplasma laidlawii*, in both of which the fatty acyl composition of lipids can be controlled and varied widely, have shown that cells remain viable and their membranes function normally with at least a quarter of the lipid in the gel phase. Proteins may be excluded from gel-phase domains of lipid, which results in the membranes becoming leaky, so there is a limit to the amount of gel phase that can be tolerated. This is relevant to life in the cold because if the temperature falls suddenly, gel-phase domains may form, and membrane functions and cellular viability will be impaired. In eukaryotic microorganisms the liquid-crystalline to gel-phase transition temperature of the membrane lipids depends on the phospholipid/sterol ratio, as well as

the fatty acyl composition. Therefore, thermal adaptation to growth at low temperatures involves changes in both of these parameters in yeast, fungi, and microalgae (see Section 4).

Although a great variety of lipids are found in different microbial classes, the one property they have in common is that they are amphiphilic, that is, they have both polar and nonpolar parts to their structure. This enables them to be integrated into a bilayer structure, and this even applies to the outer membrane of Gram-negative bacteria, in which the outer half of the bilayer leaflet is made up of lipopolysaccharide (LPS) instead of phospholipids. When tested as single types, some lipids such as phosphatidylglycerol or phosphatidylcholine will generally form a bilayer, whereas others tend to form non-bilayer phases (for example, phosphatidylethanolamines containing unsaturated fatty acyl chains form hexagonal phases). However, microbial membranes always contain a mixture of lipids so that the overall structure is a bilayer. The presence of lipids that have a tendency to form non-bilayer phases gives a certain tension to the membrane, and may be important in helping to drive processes such as membrane fusion and cell division. Non-bilayer-forming lipids may also have special architectural roles in helping to pack irregularly shaped proteins within the bilayer. The balance of bilayer and non-bilayer lipids is important in relation to transport for two reasons. First, it is the spatial organization of membrane lipids that is crucial to the passive permeability properties; and second, the transport proteins interact with, and their activity is influenced by, membrane lipids (see Section 5.1).

3. Structure of Transport Proteins

There are broadly two ways in which membrane proteins (and therefore transport proteins) interact with the lipid bilayer. Extrinsic (or peripheral) proteins interact electrostatically through polar non-covalent bonds such as hydrogen bonds and salt bridges with the polar head-groups of lipids or the exposed surfaces of other membrane proteins. They are found almost exclusively on the inner surfaces (the cytoplasmic face) of membranes. Intrinsic (or integral) proteins penetrate into the hydrophobic core of the membrane, and are arranged with part of their structure in the membrane bilayer and part exposed on the surface; the exposed polar domain is usually on the inner face of the membrane, although a few such proteins have the opposite orientation (for example, the substrate-binding proteins of ABC transporters in Gram-positive bacteria—see Section 5.1). Some intrinsic proteins span the membrane, so have polar domains exposed on the inner and outer surfaces of the membrane. However, all of them make both polar and nonpolar interactions with the surface and interior respectively of the membrane. The interactions within the nonpolar core of the membrane are via hydrophobic bonding with fatty acyl chains of lipids, and in protein complexes with intramembranous domains of other intrinsic proteins. These proteins penetrate and cross membranes by means of several α-helices, which are coiled regions of the protein polypeptide containing at least 19 amino acid residues that act like hydrophobic "plugs." By definition, since transport proteins carry solutes from the outside to the inside (or *vice versa*) of the cell, they must be membrane-spanning intrinsic proteins and contain α-helices within their structures.

The α-helix is a secondary structure in which the protein polypeptide backbone is coiled like a spring in such a way that it is stabilized by intramolecular hydrogen bonds; the side chains of the amino acid residues are on the outside of the coiled helix and in transmembrane helices are mainly hydrophobic. Thus they interact not only with each other through hydrophobic bonding to give the helix stability, but also with the hydrophobic core of the lipid bilayer that is comprised of the fatty acyl (or alkyl) chains of the lipids. This gives particular stability to the membrane and the transport proteins. Prokaryotic and eukaryotic microorganisms have biosynthetic systems for ensuring that integral membrane proteins are inserted correctly into the membrane during their synthesis. These systems consist of protein "machines" that protect the helices and align them so that they enter the membrane hydrophobic core correctly. This process and the correct folding of some proteins may also involve lipids.

The archaeal protein bacteriorhodopsin (that acts as a light-driven proton pump) from *Halobacterium halobium* is the only transport protein to have been crystallized in pure form and its three-dimensional structure determined. Consequently, it is used as a paradigm for the folding and insertion of transport proteins. All structures of other transporters have been inferred from polypeptide sequences that are derived from DNA base sequences of the cloned genes. From such information it is possible, for instance, to identify the transmembrane α-helices. Structures can also be compared with those of some other types of membrane proteins from *E. coli* that have been studied extensively. However, in relation to transport proteins in general, bacteriorhodopsin is unusual in that it has 7 transmembrane α-helices, so caution must be exercised in extrapolating too widely. In fact, transmembrane proteins generally span the membrane through a series of 6–14 α-helices, most commonly 10 or 12, and the same is true of transport proteins. Among families of different types of transporters there may be great conservation of secondary and tertiary (three-dimensional) structure (despite there being large differences in primary amino acid sequence), not only between the Bacterial and Archaeal kingdoms, but also extending to Eukaryotes. For example, 24 different transporters, members of the so-called APC superfamily, all of which have 12 membrane-spanning domains, mediate amino acid transport across the cytoplasmic membrane in yeast. Homologues of the yeast APC superfamily are found in bacteria, plants, and animals, the transporters having 12 or 14 membrane-spanning domains.

Those domains of carrier proteins that are exposed on the outer and inner surfaces of the membrane provide recognition sites for the small molecules or ions that are transported, and in the same fashion as occurs during enzyme catalysis, they undergo a series of conformational changes resulting in transfer of the substrate molecule from one side of the membrane to the other. By analogy with enzymes, which reduce the activation energy for converting substrate(s) to product(s), transport proteins overcome the energy barrier of moving a hydrophilic substrate through a hydrophobic milieu. Consequently, transport can be analyzed kinetically in much the same way as is used for enzymes, for example, using Michaelis–Menten kinetics (see Section 5.1).

An exception to the α-helical organization for membrane-spanning transport proteins is seen in the structure of porins, which are responsible for the facilitated diffusion of a wide range of molecules through the outer membrane of Gram-negative bacteria. Unusually for intrinsic membrane proteins, the porins do not have any long stretches of

hydrophobic amino acids, and the polypeptide is arranged in a series of 8 or 16 antiparallel β-strands that criss-cross the membrane bilayer, tilted at an angle of 30–60° and forming the "staves" of a membrane-spanning barrel that encloses a water-filled channel. Some types of porin are organized as trimers in the outer membrane, each trimer having three channels for the passage of molecules; the center of the trimer is very hydrophobic, giving it stability. Other porins remain as monomers in the outer membrane. The loops connecting the multiple β-strands are very short on the inner face of the membrane, but much longer, and containing acidic amino acid residues, on the outer face, where they interact with the negatively charged carbohydrate residues of LPS through divalent cation bridges. In contrast, the channels are more hydrophilic, which explains why lipophilic solutes are excluded from the porins that ferry hydrophilic substrates such as sugars, sugar alcohols, ions, nucleosides, and iron chelates across the outer membrane. The selectivity for compounds with particular sizes is explained by the quite rigid structure of the channel, which has wide entrance and exit dimensions plus a central constriction that excludes substrates above a certain size. Selectivity is also achieved by having specific amino acid residues placed near the entrance of the channel: for instance, a positively charged residue at the entrance gives selectivity towards anions and against cations.

Another Gram-negative outer membrane protein with a β-barrel structure is the ferric hydroxamate uptake receptor (FhuA) of *E. coli*, for which the X-ray structure is also known. Like the porins, FhuA interacts with LPS, making extensive van der Waals interactions with its lipid A component, the fatty acyl chains of which are ordered on the surface of the transport protein approximately parallel to its β-barrel staves.

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Biographical Sketch

Nick Russell was a Prize Scholar and gained a First Class Honors degree in Biochemistry at the University of Cambridge in 1969, followed by a Ph.D. in 1973 at the same university, for his studies of the cold adaptation of amino acid transport in a psychrotolerant bacterium. After postdoctoral studies in the University of Cambridge, he was appointed successively as Lecturer, Senior Lecturer, and Reader in the Department of Biochemistry at the University of Cardiff, where his research focused on the mechanisms of microbial adaptation to extreme environments, particularly low temperature or high salinity. Low-temperature research has included topics such as adaptive changes in cell membrane lipids, the cold activity of enzymes, the biochemistry of cold-adapted food-poisoning bacteria, and climate-change effects on cold-adapted microbial ecosystems. Some of this research has been conducted in the field during several international expeditions to Antarctica working in a diverse range of cold habitats. In 1995 he was appointed as Professor of Food and Environmental Microbiology, and then Head of Biological Sciences at Wye College, University of London. Following the merger of Wye with Imperial College London, he was appointed Director of Research. Professor Russell has coordinated several EU research programs, including EUROCOLD and COLDZYME, which integrated European research on cold-adapted microorganisms with a particular focus on how their enzymes function and can be exploited in novel biotechnological applications. He is the author of more than 200 original publications, has served on the Editorial Boards of *Microbiology (UK)* and *Extremophiles* journals, and on the Council of the UK Society for Microbiology, and is a member of the Sub-Committee for Evolutionary Biology of the Scientific Committee for Antarctic Research.