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The cell membrane plays a crucial role in survival of bacteria and archaea in extreme environments

Wil N. Konings[1,*], Sonja-Verena Albers[1], Sonja Koning[1] & Arnold J.M. Driessen[1]
[1]*Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands (*Author for correspondence; E-mail: w.n.konings@biol.rug.nl)*

Key words: energy transduction, extreme environments, ion-permeability, membrane, solute transport

Abstract

The cytoplasmic membrane of bacteria and archaea determine to a large extent the composition of the cytoplasm. Since the ion and in particular the proton and/or the sodium ion electrochemical gradients across the membranes are crucial for the bioenergetic conditions of these microorganisms, strategies are needed to restrict the permeation of these ions across their cytoplasmic membrane. The proton and sodium permeabilities of all biological membranes increase with the temperature. Psychrophilic and mesophilic bacteria, and mesophilic, (hyper)thermophilic and halophilic archaea are capable of adjusting the lipid composition of their membranes in such a way that the proton permeability at the respective growth temperature remains low and constant (homeo-proton permeability). *Thermophilic* bacteria, however, have more difficulties to restrict the proton permeation across their membrane at high temperatures and these organisms have to rely on the less permeable sodium ions for maintaining a high sodium-motive force for driving their energy requiring membrane-bound processes. Transport of solutes across the bacterial and archaeal membrane is mainly catalyzed by primary ATP driven transport systems or by proton or sodium motive force driven secondary transport systems. Unlike most bacteria, hyperthermophilic bacteria and archaea prefer primary ATP-driven uptake systems for their carbon and energy sources. Several high-affinity ABC transporters for sugars from hyperthermophiles have been identified and characterized. The activities of these ABC transporters allow these organisms to thrive in their nutrient-poor environments.

Abbreviations: PMF – proton motive force; SMF – sodium motive force

Introduction

An increasing number of microorganisms are found to flourish in environments in which the physical parameters such as temperature, salinity, pH or pressure, are extreme with respect to the conditions in which eukaryotic organisms live preferentially. Most of these extreme environments were previously thought to be too hostile for any form of life. The organisms that live in these extreme environments are termed *extremophiles*. Most extremophiles belong to the kingdom of the archaea, but also certain bacteria and even some eukarya can tolerate some of these extreme conditions.

Biological cells are surrounded by a cytoplasmic membrane, which functions as a barrier between the cytoplasm and the extracellular environment. Such membranes are usually impermeable for most ions and solutes, a property, which is essential for controlling the composition of the cytoplasm. The cytoplasmic membrane therefore has a crucial function in maintaining optimal internal conditions for metabolism and energy transduction. Solutes and ions have to pass the membrane for metabolism to proceed and specific transport proteins catalyze the transfer of these compounds across these membranes.

Membranes are very complex structures. They consist of a bilayer or monolayer of lipid molecules, which form a matrix in which various membrane proteins float. The fluid-mosaic model describes the basic properties of these membranes. The fluidity and permeability properties of the membranes are mainly determined by their lipid composition. Organisms are

able to adjust the lipid composition of their cytoplasmic membrane in response to changes in the environment. The different strategies, which extremophiles use to adapt their membrane and membrane proteins to the various extreme conditions in which they grow, are described.

The cytoplasmic membrane and bioenergetics

Biological cells generate metabolic energy by two basically distinct mechanisms. One mechanism is substrate level phosphorylation, in which chemical energy released in catabolic processes is stored in ADP to form ATP. The second mechanism takes place at the level of the cytoplasmic membrane. This membrane contains energy transducing systems which convert chemical energy, or in phototrophs light energy, into electrochemical energy of ions or *vice versa*. Both metabolic energy-generating processes are closely linked and together they determine the energy status of the cell.

Energy transduction in the cytoplasmic membrane is catalyzed by integral membrane proteins that translocate ions across the membrane into the external medium at the expense of other forms of energy. Electron transfer systems and the membrane bound ATPases are examples of such systems. The activity of these pumps results in the generation of electrochemical gradients of the translocated ions (Figure 1) (Speelmans et al. 1993a; Lolkema et al. 1994). These ions are termed energy-coupling ions and so far only protons and sodium ions have been found to carry out this function. Proton pumping from the cytoplasm to the external medium results in the generation of an electrochemical gradient of protons which is composed of a pH-gradient, inside alkaline versus acid outside, and an electrical potential across the membrane, inside negative versus outside. These two gradients, the pH-gradient or Δ pH, and the electrical potential across the membrane (also termed membrane potential), the $\Delta\Psi$, exert an inward directed force on the protons, the proton motive force (or PMF). The formula of the PMF is:

$$\mathrm{PMF} = \Delta\Psi - 2.3(RT/\mathrm{F})\Delta\mathrm{pH}$$

The PMF is expressed in mV, in which R is the gas constant, T the absolute temperature (K), and F the Faraday constant. The effect of 1 unit pH difference between cytoplasm and external medium is 59 mV at 25 °C, and 70 mV at 80 °C. Both components of the electrochemical proton gradient contribute equally to the force on the protons. In most organisms the resulting PMF has a negative value and the driving force on the protons is directed into the cell. In organisms that live around pH 7 (neutrophiles), both the membrane potential and the pH-gradient have a negative value.

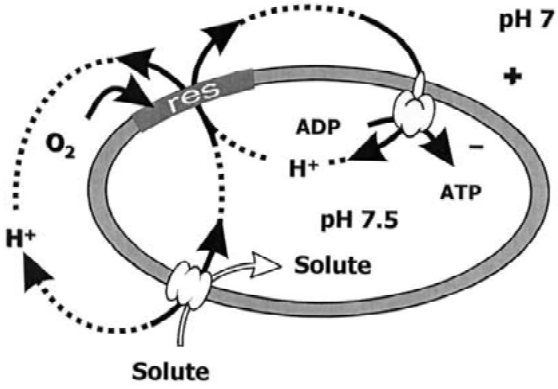

Figure 1. Energy transduction in the cell membrane of aerobic mesophilic bacteria. The extrusion of protons by the respiratory chain results in the generation of a $\Delta\Psi$, inside negative, and a ΔpH, inside alkaline. The resulting PMF drives the synthesis of ATP by the membrane bound ATPase and the transport of solutes by secondary transporters and other metabolic-energy requiring membrane processes.

A number of organisms use sodium ions as coupling ions in energy transduction. In these organisms sodium ions are pumped from the cytoplasm into the external medium. In analogy with the PMF these sodium ion pumps can generate a sodium motive force (SMF), which is composed of a membrane potential, $\Delta\Psi$, and the chemical gradient of the sodium ions. In formula:

$$\mathrm{SMF} = \Delta\Psi + 2.3RT/F\log([\mathrm{Na}^+_{\mathrm{in}}]/[\mathrm{Na}^+_{\mathrm{out}}])$$

The PMF and the SMF can be used to drive metabolic energy requiring processes such as ATP synthesis from ADP and phosphate, the transport of specific solutes across the membrane, flagellar rotation, and maintenance of the intracellular pH (Booth 1985) and turgor (Figure 1). Obviously, this type of energy transduction can only operate if the electrochemical gradients of H^+ or Na^+ can be maintained and this will only be possible if the biological membranes have a limited permeability for these ions and free energy is available to maintain these gradients.

It has been stated above that the cytoplasmic membrane functions as a barrier between the cytoplasm and the environment and controls the movement of solutes (ions and nutrients) into or out of the cell. Biological

membranes are usually composed of a bilayer of lipids in which proteins are embedded. The lipids have polar head groups that stick into the water phase and hydrophobic hydrocarbon chains that are oriented to the interior of the membrane. At the growth temperature of a given organism, the membranes are in a liquid crystalline state (Melchior 1982). Non-covalent bonds such as Van der Waals bonds and electric interactions mainly hold the structure of biological membrane together. The barrier function of the cell membrane is critical for controlling the concentrations of molecules and ions inside the cell. Most solutes cannot or only very slowly cross the lipid bilayer of biological membranes by diffusion. Although lipophilic solutes dissolve readily in the membranes these compounds also cross the membranes very slowly. Specific transport proteins are needed to catalyze the transfer of these solutes across the membrane at rates required for growth.

The lipid layer forms a suitable matrix for transport proteins that can generate and maintain specific solute concentration gradients across the membrane. Under physiological conditions the lipids in the membrane are ordered and in a liquid crystalline state that allows optimal functioning of the membrane proteins. By adjusting the lipid composition bacteria and archaea can control the permeability of their cell membrane for solutes and ions (see below). A low permeability limits the energy needed for maintaining ion and solute gradients.

Lipid composition of bacterial and archaeal membranes

The lipid composition of cell membranes is very complex and differs strongly between organisms. It is tightly regulated and dependent on environmental conditions. Bacterial and eukaryal lipids are composed of two fatty acyl chains that are ester-linked to glycerol (Figure 2). The third hydroxyl group of the glycerol is linked to hydrophilic phospho- or glyco-containing headgroups. These lipids are organized in a bilayer so that the polar head-groups stick into the water phases while the alkane chains are directed towards the inner side of the membrane.

In contrast to bacterial and eukaryal lipids, archaeal lipids consist of two phytanyl chains which are linked via an ether bond to glycerol or other alcohols like nonitol (Figure 2). The acyl chains of these archaeal lipids are usually fully saturated isoprenoids (De Rosa et al. 1991). Most archaea growing under moderate conditions contain bilayer forming lipids with a C_{20}*di*-ether lipid core (Kates et al. 1993; Upasani et al. 1994; Kates 1996). Such C_{20} *di-ether* lipids also form bilayer membranes just as their bacterial and eukaryal counterparts (Koga et al. 1993; Yamauchi et al. 1995; Kates 1996). In extreme thermophilic and acidophilic archaea (De Rosa et al. 1991) membrane spanning (bolaform amphiphilic) ether lipids are found in which the phytanyl chains of two diether lipids are fused to a C_{40} core. These so-called *tetraether* lipids form a monolayer in which the lipids span the whole membrane (Figure 2). Freeze-fracturing of these membranes reveals that cleavage between two leaflets of the membrane does not occur, indicating that the water facing sides of the membrane are connected and cannot be separated (Choquet et al. 1992; Elferink et al. 1992; Beveridge et al. 1993). The observation that tetraether lipids from *Thermoplasma acidophilum* and *Sulfolobus solfataricus* form black lipid membranes with a thickness of 2.5-3.0 nm (Stern et al. 1992) is in accordance with the length of the tetraether lipids (Gliozzi et al. 1983).

Membranes composed of ether lipids have a higher stability than those of ester-lipids (Elferink et al. 1992; Thompson et al. 1992), most likely as a result of the reduced segmentary motion of tertiary carbon atoms (i.e., rotation of carbon atoms that are bound to three other C-atoms, resulting in kinks in the acyl chain). This restriction in hydrocarbon chain mobility may also result in a reduced permeability of the archaeal membranes.

Bioenergetic problems of extremophiles

Extremophiles living in various harsh environments face different problems in maintaining a viable proton motive force or sodium motive force across their membranes.

Temperature

Bacteria and archaea can respond to changes in ambient temperature through adaptations of the lipid composition of their cytoplasmic membranes (Gaughran 1947). These changes are needed to keep the membrane in a liquid crystalline state (Russell et al. 1990) and to limit the proton permeation rates. At higher

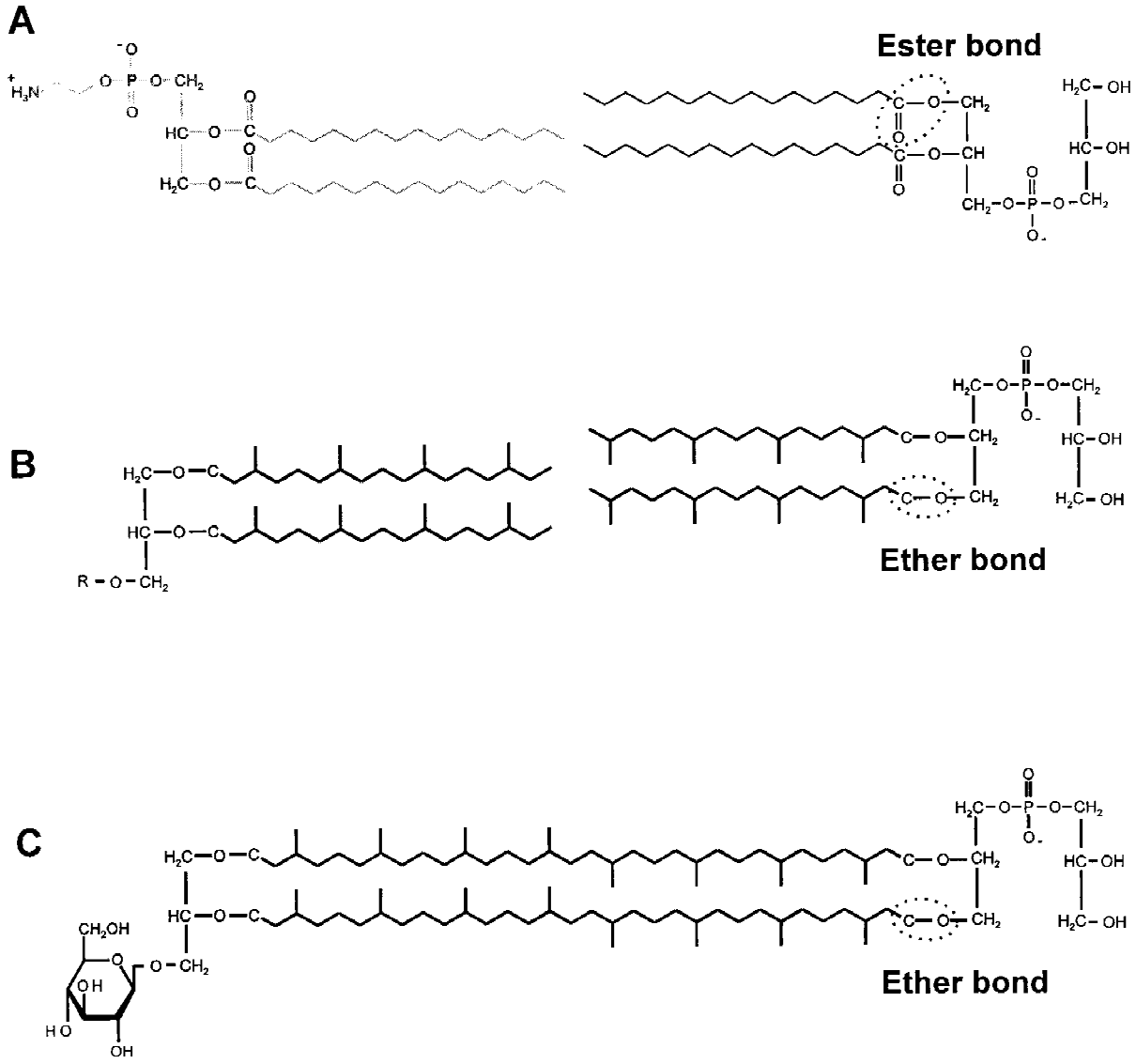

Figure 2. Lipids from archaea and bacteria. (A) Bilayer forming lipids in bacteria: Phosphatidylglycerol (PG) from Eschericia coli. The acyl chain can be branched, contain a cyclohexyl group at the end of the acyl chain, or contain one or more unsaturated bonds. The connection of the acyl chain with the headgroup is an ester. (B) Bilayerforming diether lipids from archaea. The connection of the phytanyl chain with the headgroup is an ether linkage. The phytanyl chain contains isoprenoid-like branches. Some acidophilic tetraethers contain cyclopentane rings. (C) Monolayer forming tetraether lipids in thermoacidophilic archaea.

temperatures, this can be done in bacteria by increasing the chain length of the lipid acyl chains, the ratio of *iso*/*anteiso* branching and/or the degree of saturation of the acyl chain (Reizer et al. 1985; Prado et al. 1988; Svobodová and Svobodová, 1988). The archaeal *Sulfolobales* species, *Sulfolobus solfataricus* and *Thermoplasma acidophilum*, contain a high percentage of tetraether lipids in their membranes (above 90%). The degree of cyclization of the C_{40} isoprenoid in the tetraether lipids increases with the growth temperatures (De Rosa and Gambacorta 1988). In *Thermoplasma* cells grown at 40 °C the ratio of acyclic/monocyclic/bicyclic chain is 62/37/1 and 25/50/24 for cells grown at 60 °C (Langworthy 1982). By increasing the cyclization of the C_{40} isoprenoid chains the lipids can be packed more tightly, which results in a more restricted motion of the lipids and prevents that the membrane becomes too fluid. In the euryarchae-

ote *Methanococcus janaschii* an increase of growth temperature results in a decrease of the diether lipid content and an increase of the content of the more thermostable tetraether lipids (Sprott et al. 1991). Also, in this organism cyclization of the isoprenoid chains tends to decrease the motion of the lipids and therefore contributes to acceptable membrane fluidity at elevated growth temperature.

The increased motion of the lipid molecules in the membranes causes an increased proton permeability of the membranes at high temperature. Due to this increased motion more water molecules are trapped in the lipid core of the membranes. Protons can hop from one water molecule to the other and the higher water content of the membrane thus leads to a higher permeation of protons. Unlike protons, other ions cross the membrane by diffusion. This is a temperature dependent process and results in an increased ion-permeability of the membrane at higher temperatures. When the membrane permeation of the coupling ions, protons or sodium ions, becomes too high, the organism will encounter difficulties in establishing a PMF or SMF of sufficiently high magnitude. The permeability of the cytoplasmic membrane therefore is a major factor in determining the maximum temperature of growth. To determine the proton and sodium ion permeability's of the cell membrane of bacteria and archaea, the lipids have been extracted from their isolated membranes and used for the formation of liposomes (van de Vossenberg et al. 1995). The proton permeability of these liposomal membranes can be determined by following the fluorescence change of the externally present pH probe pyranine upon proton influx, while the sodium ion permeability can be assessed by following the leakage of radioactive sodium ions from preloaded liposomes (van de Vossenberg et al. 1999b). In all liposomes studied, the proton permeability increases with temperature (Figure 3). However, a most important finding of these studies was that the proton permeability of membranes from psychrophilic and mesophilic bacteria and of all archaea studied so far is maintained within a narrow window (H^+-permeability coefficient near 10^{-9} cm s^{-1}) at their growth temperature. Above this growth temperature the membranes become highly permeable for protons. These organisms can apparently use protons at their growth temperature as coupling ions for energy transduction. However, at higher temperatures the proton permeation becomes too high and a PMF of viable magnitude can not be maintened.

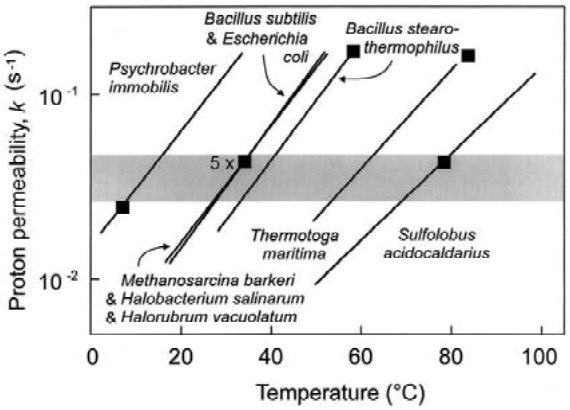

Figure 3. Temperature dependency of the proton permeability of liposomal membranes derived from various archaea and bacteria that live at different temperatures. The membrane of psychrophilic and mesophilic bacteria and of most archaea has at their growth temperatures a proton permeability that falls within a narrow window (grey bar). The membrane of the thermophilic bacteria *T. maritima* and *B. stearothermophilus*, however, has at their growth temperature, a much higher proton permeability than the other organisms. Squares indicate the proton permeability at the growth temperature.

The proton permeability of the membrane of psychrophilic and mesophilic bacteria and archaea is controlled by the lipid composition. This homeostasis of proton permeability has been termed 'homeo- proton permeability adaptation'. Strong support for the homeo-proton permeability theory was supplied by studies of *Bacillus subtilis*, grown at and within the boundaries of its growth temperature range: 13 to 50 °C (van de Vossenberg et al. 1999a). The proton permeability of the membranes of *B. subtilis* grown at different temperatures remained constant over the whole growth temperature range (Figure 4). Interestingly, in contrast to the proton permeability the fluidity of the membranes is not maintained constant but increases significantly with temperature. These observations strongly indicate that the growth temperature-dependent alterations in fatty acyl chain composition are mainly aimed at maintaining the proton permeability of the cytoplasmic membrane at a rather constant level. The observations also clearly demonstrate that the upper temperature of growth is determined by the proton permeability of their membranes.

The situation in *thermophilic bacteria* is significantly different from those in psychrophilic and mesophilic bacteria and archaea. In thermophilic bacteria, the membrane proton permeability at their respective growth temperatures has found to be extremely high. As a result, these organisms have extreme difficulties in generating a PMF of viable magnitude (Figure 3)

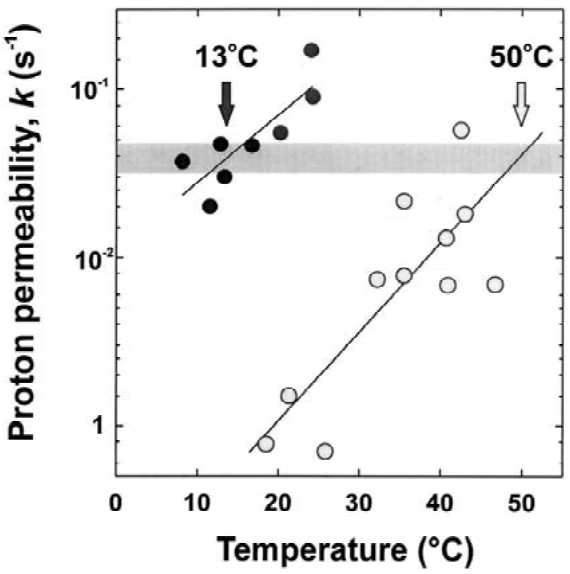

Figure 4. The temperature dependency of the proton permeability of membranes of *Bacillus subtilis* grown at different temperatures in the range from 13 to 50 °C. Only the data for 13 and 50°C are shown. The proton permeabilities of all membranes fall within a narrow window.

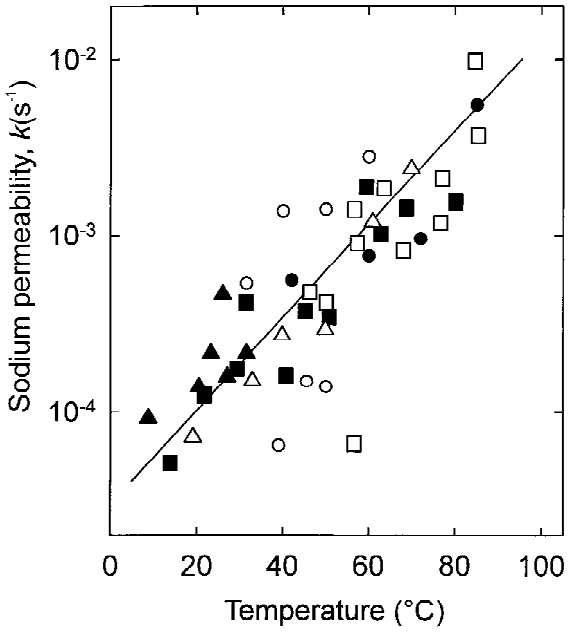

Figure 5. Temperature dependency of the sodium permeability of liposomes derived from various bacteria and archaea. *P. immobilis* sp (▲), *M. barkeri* (△), *E. coli* (○), *B. stearothermophilus* (■), *T. maritima* (●), and *S. acidocaldarius* (□).

(van de Vossenberg et al. 1995). Evidently, thermophilic bacteria, such as *B. stearothermophilus, Thermotoga maritima* and *Calaromator fervidus,* are not able to restrict the proton permeability of their membrane at the high growth temperatures by adjusting the lipid composition. The sodium ion permeability of the membrane of all bacteria and archaea studied also increases exponentially with temperature (Figure 5). However, the increase of the sodium-ion permeability is the same for all organisms. These results indicate that the lipid composition of the membrane affects the membrane permeability of sodium ions only to a minor extent and that the temperature is the main determining factor of the rate of sodium ion permeation. However, since the sodium permeability is several orders of magnitude lower than the proton permeability, the generation of a high SMF is possible in all bacteria and archaea even at high temperatures.

Aerobic bacteria, such as *Bacillus stearothermophilus,* which live at the lower range of thermophilic temperatures (around 60 °C), can compensate for the high proton leakage of their membranes by drastically increasing the rate of respiration and consequently the rate of proton excretion (de Vrij et al. 1988). Since the permeability of the membranes for sodium ions is several fold lower than for protons, metabolic energy can be saved by rapidly transducing the PMF into an SMF with the help of proton/sodium exchange systems. In this way a high SMF can be generated that can subsequently be used to drive energy requiring membrane processes such as secondary solute transport (Figure 6).

Most thermophilic bacteria, however, have to rely completely on the less permeable sodium ion as coupling ion for energy transduction (Figure 7). This strategy is used by *Caloramator fervidus* (previously *Clostridium fervidus*) (Speelmans et al. 1993a,b), an organism that can grow at a higher temperature than *B. stearothermophilus*, i.e., 70 °C (Esser & Souza 1974; Patel et al. 1987). *C. fervidus* has a Na^+-translocating ATPase that excretes sodium ions at the expense of ATP. These V-type ATPases have interesting properties which will not be further discussed in this review (Speelmans et al. 1993a; Boekema et al. 1999). As a result, a SMF is generated that is the driving force for energy requiring processes such as solute transport.

The high proton leakage of the membranes of *C. fervidus* has also other consequenties for growth. Due to the high proton permeability of its membrane, *C. fervidus* is not able to control its intracellular pH. Consequently, growth of *C. fervidus* is confined to a narrow niche, i.e., an environment with a pH near neutrality.

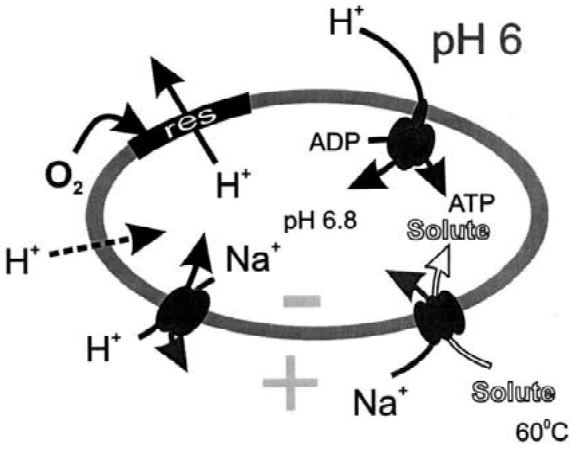

Figure 6. Energy transduction in the membranes of *Bacillus stearothermophilus.* The respiratory chain pumps at a high rate protons from the cytoplasm to the external medium thus generating a PMF. This PMF drives the synthesis of ATP via the membrane bound ATPase. The high passive influx of protons results in rapid dissipation of the PMF. A Na^+/H^+ antiporter converts the PMF into a SMF and this SMF is subsequently used for other energy requiring processes such as secondary transport.

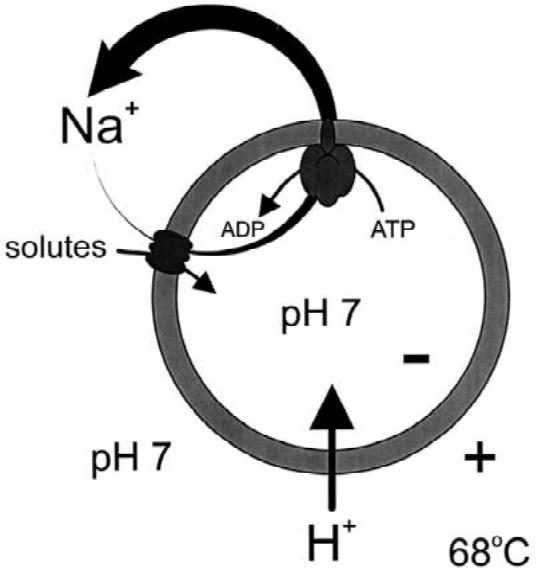

Figure 7. Energy transduction in the membrane of *Calaromator fervidus*. ATP is generated by substrate level phosphorylation. ATP hydrolysis by the membrane-bound V-type ATPase results in the extrusion of sodium ions and the generation of a SMF. This SMF is used to drive energy-requiring membrane processes such as secondary solute transport. The internal pH equilibrates with the external pH by the passive influx of protons.

At their growth temperature, the thermophilic bacteria have a high SMF, but a very low PMF. In *C. fervidus* both the $\Delta\Psi$ and the ΔpH are low. In other organisms such as the thermoalkaliphile *Anaerobranca*

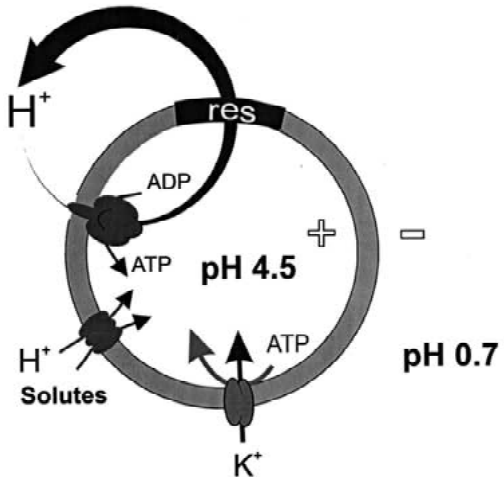

Figure 8. Energy transduction in the membrane from the thermophilic archaea such as *Solfolobus solfataricus* and *Picrophilus oshimae.* The proton pumping respiratory chain extrudes protons and generates a PMF and increases the internal pH to 4.5. In order to lower the PMF to viable values cation/H^+ symport systems translocate positive charges from outside to inside and generate a reversed $\Delta\varphi$, inside positive. The PMF drives energy requiring processes such as secondary transport.

gottschalkii (*Thermoalkalibacter bogoriae*) the low PMF is the result of a reversed ΔpH (inside acid) and a normal $\Delta\Psi$ (inside negative). This reversed ΔpH is most likely generated by the passive influx of protons in response to the $\Delta\Psi$ generated by Na^+ pumping.

Salt

Halophiles, such as *Halobacterium salinarum*, generate an electrochemical proton gradient across the membrane by respiration and/or the light driven pump bacteriorhodopsin. This organism keeps the cytoplasmic concentration of sodium ions low by using the PMF to drive sodium ions out of the cells via a H^+/Na^+ antiporter. In doing so also a high SMF is generated.

The proton and sodium permeabilities of membranes from the halophile *Halobacterium salinarium* and the haloalkaliphile *Halorubrum vacuolatum* have found to be very similar to those of non-halophilic organisms living at the same temperature. In contrast to membranes of *E. coli* those of halophiles and haloalkaliphiles remain stable up to 4 M NaCl or KCl and also at higher pH values. Obviously these membranes are well adapted to the halophilic conditions (van de Vossenberg et al. 1999b).

pH

Extreme acidophiles maintain an internal pH close to neutrality. As a consequence, these organisms experience a very high ΔpH (inside alkaline) across their cell membrane. This ΔpH can be up to 4 pH units in organisms such as *Picrophilus oshimae* that grow optimally at medium pH-values of 0.5–1 (Schleper et al. 1995). Such a large ΔpH can only be maintained with a membrane that has very low proton permeability. The very high ΔpH in these organisms needs to be compensated by an inverted ΔΨ (negative outside vs positive inside) in order to keep the PMF within viable values (Figure 8). This inversion of the ΔΨ is mainly realized by potassium ion uptake systems.

Some thermophilic archaea can grow at very low pH-values. An organism such as *Sulfolobus solfataricus* can grow up to 90 °C at pH values of 2.5–3.5. It has been described above that the proton permeability of the membranes of these thermo-acidophilic archaea, is at the high growth temperature as low as that of membranes of mesophilic bacteria grown at the mesophilic growth temperatures.

Alkaliphiles also have to maintain an intracellular pH close to neutrality in their very alkaline environment. The internal pH is therefore significantly lower than the external pH. Since the ΔpH is reversed (inside acid vs. outside), a very high ΔΨ (inside negative) is needed to create a sufficiently high PMF (Figure 9). To keep the internal pH close to neutrality, aerobic mesophilic alkaliphilic bacteria use Na^+/H^+-antiporters in combination with H^+-coupled respiration. Many alkaliphiles live in sodium-rich environments. As a result of the high chemical gradient of sodium ions and the high ΔΨ in these organisms a high SMF is generated.

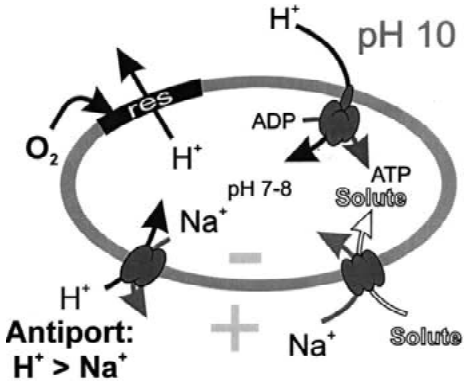

Figure 9. Energy transduction in the cell membrane of aerobic alkaliphiles. The respiratory chain pumps protons from the cytoplasm to the external medium. This results in a rapid generation of a ΔΨ, inside negative. This ΔΨ drives ATP-synthesis and electrogenic H+/Na+ exchange, in a ratio >1, resulting in a decrease of the internal pH and in the generation of a SMF. This SMF drives secondary transport processes.

Solute transport proteins

Membrane proteins form a large part of the mass of prokaryotic membranes (up to 60% w/w). Many of these membrane proteins are involved in energy transducing processes, such as electron transfer proteins (cytochromes, etc.), ATPases and solute transport systems. Membrane proteins have been studied extensively in bacteria and to a much lesser extent in archaea and hardly in thermophilic and hyperthermophilic archaea. Membrane proteins involved in energy transducing processes, such as cytochrome oxidases and ATPases, have been described and characterized, e.g. from *S. acidocaldarius* (reviewed in Schäfer et al. 1999). The genome sequences of bacteria and archaea, which are now available, indicate that up to 30% of the genes encode for membrane proteins and that a large fraction of these proteins (up to 20%) are transport proteins. Very few studies have been carried out on transport systems of extremophiles.

The transport systems of solutes across biological membranes which have been described so far can be classified according to their molecular architectures and their driving force of transport. Five classes of transport systems have been discriminated: (i) channels (Figure 10); (ii) secondary transporters which use of electrochemical gradients of protons or sodium ions to drive transport of substrates across the membrane (Figure 10); (iii) binding-protein-dependent secondary transporters (TRAP transporters), which consist of a periplasmic binding protein and a membrane translocator. These systems use the PMF or the SMF to drive uptake of solutes (Figure 10); (iv) primary transporters, which use light energy or the chemical energy of ATP or of other compounds to drive substrate translocation. Well-studied examples are ion-translocating respiratory chains and bacteriorhodopsin and the ABC- (ATP-binding cassettes) transporters. The latter transporters consist of two transmembrane proteins, that form the translocation pathway, and two cytosolic ATP-binding proteins. ABC transporters which catalyse the uptake of solutes contain in addition a high-affinity periplasmic binding protein (Figure 10); (v) the group translocation systems, i.e., the phosphoenolpyruvate (PEP) dependent phospho-

Table 1. Described solute transporters in extremophiles

ABC-transporter	Substrate	K_m for uptake (nM)	K_d for solute binding[a] (nM)	Reference
Archaea				
T. litoralis	maltose/trehalose	22/17	160	Xavier et al. 1996; Horlacher et al. 1998
S. solfataricus	glucose	2000	480	Albers et al. 1999
	cellobiose + cellooligomers	–[b]	–	Elferink et al. 2001
	trehalose	–	–	"
	maltose/maltotriose	–	–	"
	arabinose	–	130	"
P. furiosus	cellobiose + cellooligomers	175	45	Koning et al. 2001
	maltodextrin	–	270	Evdokimov et al. 2001
H. volcanii	glucose (anaerobic)	–	–	Wanner et al. 1999
	molybdate	–	–	"
	Inorganic anions	–	–	"
A. fulgidus	glycine betaine, proline betaine	–	–	Holtmann et al. 2000
M. thermoautrophicum	phosphate	25	–	Krueger et al. 1986
Bacteria				
T. maritima	maltose/maltotriose/trehalose		300	Wassenberg et al. 2000
T. ethanolicus 39E	maltose/maltotriose/trehalose	40	270	Jones et al. 2000
A. acidocaldarius	Maltose/maltodextrin	1500	1500	Hülsmann et al. 2000
Secondary transporter	Substrate	K_m for uptake (μM)	Coupling ion	Reference
Archaea				
H. volcanii	glucose	–[b]	Na^+	Tawara & Kamo 1991
H. halobium	glutamate	–	Na^+	Kamo et al. 1988
	all amino acids except cysteine and aspartate	–	Na^+	Greene & MacDonald 1984
Bacteria				
T. thermophilus	nitrate/nitrite	–	–	Ramirez et al. 2000
C. fervidus	amino acids	–	Na^+	Speelmans et al. 1989
Bacillus TA2.A1	glutamate	290	Na^+	Peddie et al. 1999
	sucrose	33	Na^+	Peddie et al. 2000
B. acidocaldarius	methylthio-β-galactoside	–	H^+	Krulwich et al. 1978
A. gottschalkii LBS3	leucine	–	Na^+	Prowe et al. 1996

[a]Solute binding to binding protein.
[b]Not determined.

transferase systems (PTS), which couple the transport of sugars to phosphorylation (Figure 10).

Members of each of these classes of transporters have been identified in extremophiles, except for PTS systems. In none of the completed genomes from archaea genes encoding membrane components PTS-systems have been found, and also in the hyperthermophilic bacteria *T. maritima* and *Aquifex aeolicus* this kind of transporter is absent. PTS-systems seem to be restricted to mesophilic bacteria, which may be an indication that these systems evolved relatively late.

The transport systems, which have been identified so far in extremophiles, are listed in Table 1. Most secondary transporters listed couple the transport of a solute to sodium ions. This property of the thermophilic bacteria, studied so far, is consistent with

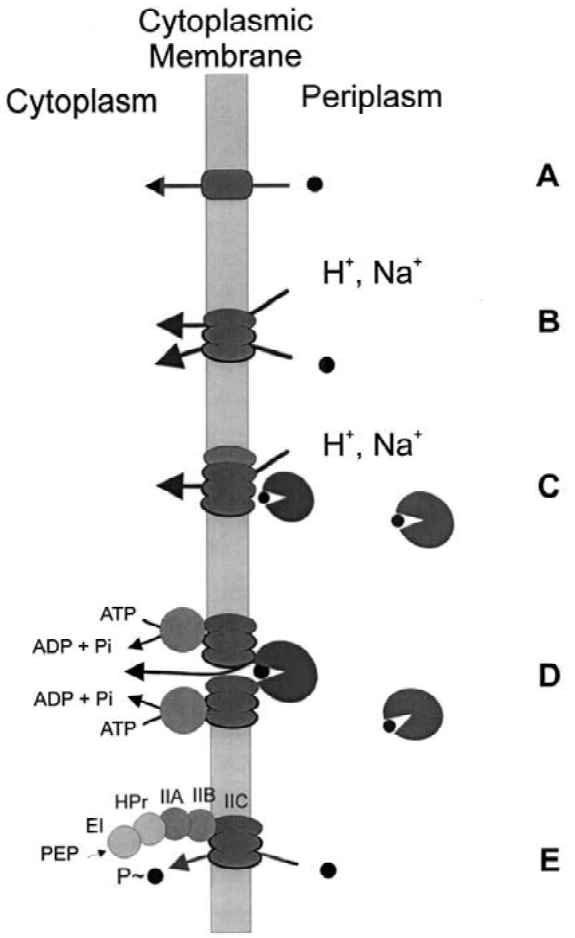

Figure 10. Solute transport systems found in bacteria and archaea. (A) Channels. (B) Secondary transport systems. (C) Binding protein-dependent secondary transport systems. (D) ATP-binding cassette (ABC) transporters. (E) Phosphoenolpyruvate dependent phosphotransferase systems (PTS).

the above described complete dependency of these organisms on a SMF. In *C. fervidus*, and the anaerobic thermoalkaliphilic strain *A. gottschalkii* LBS3, energy transduction and amino acid uptake are strictly dependent on sodium ions.

In all three domains of life, ABC transporters have been found to catalyze transport of a wide variety of different substrates. ABC transporters can catalyze the uptake of solutes or the excretion of products of metabolism or of cytotoxic compounds. ABC transporters from archaea and thermophilic bacteria, described so far, are uptake systems and require a periplasmic solute-binding protein to bind the substrate with high affinity.

Most of the known transport systems of extremophiles are sugar uptake systems which belong to the family of ABC transporters (Table 1). A whole range of sugar ABC transporters have been found recently in *Sulfolobus solfataricus* and *Pyrococcus furiosus* (Albers et al. 1999; Elferink et al. 2001a; Koning et al. 2001). These transporters fall into two groups: (i) the glucose, arabinose, trehalose-systems of *S. solfataricus* and the maltose/trehalose and maltodextrin system of *P. furiosus*, which show similarity to the sugar ABC transporters of bacteria, and (ii) the cellobiose transporters of both organisms and the maltose/maltotriose transporter of *S. solfataricus,* which exhibit the highest similarity with bacterial di/oligopeptide transporters. This latter observation is surprising since this group of transporters has been found to transport only di/oligopeptide.

In the sequenced genomes of archaea and the thermophilic bacteria *T. maritima* and *Aquifex aeolicus,* a large number of ABC transporters have been identified which belong to the group of di/oligopeptide transporters. *T. maritima*, for example, contains eleven members of the di/oligopeptide transporter family, of which nine are located in an operon with genes in sugar metabolism. It has been postulated that peptide and sugar degradation are coordinately regulated. However, the information presented above suggests strongly that most of these ABC transporters catalyze the transport of sugar-oligomers instead of peptides (Nelson et al. 1999).

All sequenced genomes of archaea and of the thermophilic bacteria *T. maritima* and *A. aeolicus* contain a large number of genes, which encode transport proteins (Table 1). In all these organisms a large number of ABC transporters have been found and transport studies and sequence comparisons indicate that these transporters are mainly involved in the uptake of organic solutes. *T. maritima* possesses 25 putative secondary transporters of which only 10 are putative transporters for organic solutes. Most of the predicted secondary transporters are putative inorganic ion transporters. On the other hand, this organism has 55 ABC-type transporters. Most of these transporters are most likely involved in the uptake of organic solutes although at this moment only one has been identified as a transporter of maltose, maltotriose and trehalose. In contrast, in *E. coli* secondary transporters are the more predominant transporters for organic solutes.

The preference of (hyper)thermophiles (the majority of sequenced genomes from extremophiles are

from hyperthermophilic organisms) for ABC-type transporters seems to be important for their survival strategy in their natural habitat. In nutrient-poor environments, such as hydrothermal vents or sulfuric hot springs, in which these organisms thrive, ABC transporters have the advantage that they can scavenge solutes at very low concentrations due to the high binding affinities ($K_d < 1$ μM) of their binding proteins. Furthermore these transporters can catalyze transport at a high rate and high internal concentrations of solutes can be achieved. In contrast, secondary transport systems exhibit binding affinities in the micro or millimolar ranges, which make these systems less suitable for growth in oligotrophic extreme environments.

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