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REVIEW

Sonja-V. Albers · Jack L.C.M. Van de Vossenberg · Arnold J.M. Driessen · Wil N. Konings

Bioenergetics and solute uptake under extreme conditions

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Abstract The ion and particularly the proton and sodium ion permeabilities of cytoplasmic membranes play crucial roles in the bioenergetics of microorganisms. The proton and sodium permeabilities of membranes increase with 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 constant (*homeoproton permeability*). Thermophilic bacteria are an exception. They rely on the less permeable sodium ions to generate a sodium motive force, which is subsequently used to drive energy-requiring membrane-bound processes. Transport of solutes across bacterial and archaeal membranes 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 uptake systems. Several high-affinity ATP-binding cassette (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.

Key words ABC transporter · Secondary transporter · Solute-binding protein · Bioenergetics · Proton motive force · Sodium motive force

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S.-V. Albers · J.L.C.M. Van de Vossenberg[1] · A.J.M. Driessen · W.N. Konings (✉)
Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands
Tel. +31-50-3632150; Fax +31-50-3632154
e-mail: w.n.konings@biol.rug.nl

Present address:
[1]Rumen Microbiology, Agresearch Grasslands, Palmerston North, New Zealand

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Introduction

The number of microorganisms that have been found in extreme environments increases steadily. Members of this group, termed extremophiles, thrive in environments in which the physical parameters such as temperature, salinity, pH, or pressure were previously thought to be hostile to any form of life. A great part of these extremophiles belong to the kingdom of the archaea (Woese et al. 1990), but bacteria and eukarya can also tolerate some of these extreme conditions.

Biological cells are surrounded by cytoplasmic membranes. These membranes function as barriers between the cytoplasm and the extracellular environment. Such an impermeable barrier is essential for maintaining optimal internal conditions for metabolism and energy transduction in cells. For the passage of solutes, which are needed for metabolism to proceed, specific transport proteins are present in these membranes.

Membranes are very complex structures and consist of a bilayer or monolayer of lipid molecules, which form a matrix for various membrane proteins. The basic properties of these membranes are described by the fluid-mosaic model (Singer and Nicholson 1972). The lipid composition of membranes is the main determinant of its fluidity and permeability properties. Organisms are able to adapt the properties of their cytoplasmic membranes in response to changes in the environment by changing the lipid composition. The different strategies that extremophiles use to adapt their membrane and membrane proteins to the various extreme conditions in which they grow are described.

Composition of the membrane

The lipid composition of cell membranes is very complex and differs among organisms. It is tightly regulated and dependent on environmental conditions. Bacterial and eukaryal lipids are mainly composed of two acyl chains bound via an ester linkage to glycerol (Fig. 1 A). These lipids

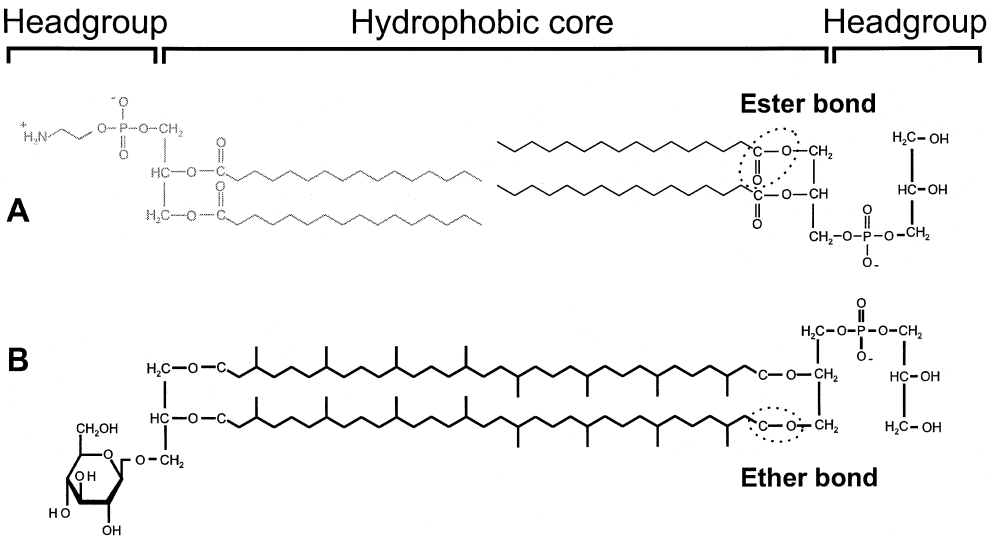

Fig. 1. Lipids from archaea and bacteria. **A** Bilayer-forming lipids in bacteria: Phosphatidylaethanolamine (PE). The acyl chain is straight (not in all cases: some bacterial lipids have a methyl branch, or a cyclohexyl group, at the end of the acyl chain; other lipids have one or more unsaturated bonds). The connection of the acyl chain to the glycerol is via an *ester linkage*. **B** Monolayer-forming lipids in thermoacidophilic archaea: Main glycophospholipid (MPL) of *Thermoplasma acidophilum*. The phytanyl chain contains isoprenoid-like branches. The connection of the phytanyl chain with the *headgroup* is via an *ether linkage*. Archaeal membranes also contain bilayer-forming diether lipids. Some acidophilic tetraethers contain cyclopentane rings

are organized in a bilayer in which the polar headgroups stick into the water phases, while the carbon chains are directed toward the inner side of the membrane.

In contrast, archaeal lipids consist of two phytanyl chains that are linked via an ether bond to glycerol or other alcohols such as nonitol. Such C_{20} diether lipids also form bilayer membranes just as their bacterial and eukaryal counterparts do. In some extremophilic archaea, ether lipids can be found in which the phytanyl chains are fused together to a C_{40} core. These lipids are called tetraether lipids. They form a monolayer in which the tetraether lipids span the whole membrane (Relini et al. 1996) (Fig. 1B). Freeze-fracturing revealed that cleavage between the leaflets in a lipid bilayer can occur, but not in tetraether lipid membranes. In tetraether lipid membranes, therefore, the water-facing sides of the membrane are connected and cannot be separated (Beveridge et al. 1993; Choquet et al. 1992; Elferink et al. 1992). Ether lipids are much more stable at high temperature than ester lipids, and they are more resistant to oxidation. Moreover, ether lipids are not susceptible to degradation at alkaline pH or to enzymatic degradation by phospholipases (Choquet et al. 1994) and are more tolerant to high salt concentrations. Liposomes composed of archaeal lipids are more stable than those composed of bacterial lipids and have a lower proton permeability at a given temperature (Elferink et al. 1994; Van de Vossenberg et al. 1995, 1998a).

Bioenergetics

The cytoplasmic membrane plays an essential role in the generation of metabolic energy. Metabolic energy can be generated during catabolism by substrate-level phosphorylation processes or by energy transduction in the cytoplasmic membrane. This latter process is catalyzed by proteins located in the cytoplasmic membrane (Mitchell 1961). Specific pumps translocate protons or sodium ions from the cytoplasm to the external medium across the membrane, thus generating electrochemical gradients of proton or sodium ions (Lolkema et al. 1994; Speelmans et al. 1993). When protons are extruded, the generated electrochemical proton gradient exerts a force on the proton: the proton motive force (PMF). This PMF consists of two components: the chemical proton or pH gradient, the ΔpH; and the membrane potential generated by the transport of electrical charge, the $\Delta\psi$. The PMF (mV) is expressed by the following equation:

$$PMF = \Delta\psi - 2.3(RT/F)\Delta pH(mV)$$

Here R is the gas constant, T the absolute temperature (K), and F the Faraday constant. The effect of 1 unit pH difference is 2.3 (RT/F), which equals 59 mV at 25°C and 70 mV at 80°C. Under physiological conditions the PMF is negative and the driving force on the protons is directed into the cell. In neutrophiles (around pH 7), both components of the PMF are negative. In a similar way, a sodium motive force (SMF) can be generated by sodium ion pumps ($SMF = \Delta\psi + 2.3\,Z\log[Na^+_{in}]/[Na^+_{out}]$).

The PMF or SMF can be used to drive the conversion of adenosine diphosphate (ADP) and phosphate to ATP via the membrane-bound ATPase, the transport of substrates across the membrane via specific transport proteins, the rotation of flagella, and the maintenance of the intracellular pH. Efficient generation and maintenance of a PMF or SMF is possible only if the cytoplasmic membrane has a low permeability for protons and sodium ions.

Bioenergetic problems of extremophiles

Extremophiles face different problems in maintaining viable proton or sodium-ion gradients across their membranes, depending on the environment that they inhabit. This topic has been reviewed recently for archaea (Schäfer et al. 1999).

Temperature

Growth at high temperatures requires not only high-temperature stability of enzymes and other macromolecules (Adams 1993) but also limited permeability of the membranes for ions. The ion permeability of all membranes increases with temperature, and it becomes more difficult or even impossible for the organism to establish a sufficient PMF/SMF. However, psychrophilic and mesophilic bacteria and all archaea adjust the lipid composition of their membranes in such a way that the proton permeability at the temperature of growth is maintained within a narrow permeability window (H^+ permeability coefficient near 10^{-9} cm s^{-1}) (Fig. 2) (Van de Vossenberg et al. 1995) Consequently, these organisms can use protons as coupling ions in energy transduction at all temperatures at which they grow. This homeostasis of proton permeability has been termed the "homeo-proton permeability adaptation." Confirmation of such homeo-proton permeability adaptation mechanisms was obtained in studies of *Bacillus subtilis* grown at and within the boundaries of its growth temperature range: 13°C to 50°C (Van de Vossenberg et al. 1999a). Over this whole temperature range, the proton permeabilities of the *B. subtilis* membranes at the respective growth temperature were constant. Interestingly, the fluidity of the membrane did not remain constant but increased significantly with temperature. Prokaryotes such as the thermophilic bacteria *Bacillus stearothermophilus* and *Thermotoga maritima* and the hyperthermophilic archaeon *Pyrococcus furiosus* have been found to be unable to restrict sufficiently the proton permeability of their membranes (Fig. 2) (Van de Vossenberg 1999). These organisms must rely on other mechanisms. Some organisms can counteract the high rate of proton influx by drastically increasing the rate of respiration and consequently the rate of excretion of protons (De Vrij et al. 1988). Most thermophilic bacteria use the much less permeable sodium ions instead of protons as coupling ions for energy transduction. In such organisms, for example, the anaerobe *Caloramator fervidus* (growth temperature 70°C), sodium ions are the only coupling ions used in energy transduction (Fig. 3B). Sodium ions can be excreted in thermophilic bacteria by specific sodium pumps such as ATPases, electron transfer systems, decarboxylases, or methyltranferases. The high SMF generated can be used by energy-requiring membrane processes. At their growth temperature, the studied thermophilic bacteria have a high SMF but a very low PMF. In *C. fervidus,* both $\Delta\psi$ and ΔpH are low. The internal pH follows the external pH, and *C. fervidus* can therefore live only in environments with near neutral pH. In other organisms such as the thermoalkaliphile *Anaerobranca gottschalkii*, the PMF is a result of a reversed ΔpH (acidic inside) and a normal $\Delta\psi$ (negative inside) (Prowe et al. 1996). This reversed ΔpH is most likely generated by the passive influx of protons in response to the $\Delta\psi$ generated by Na^+ pumping.

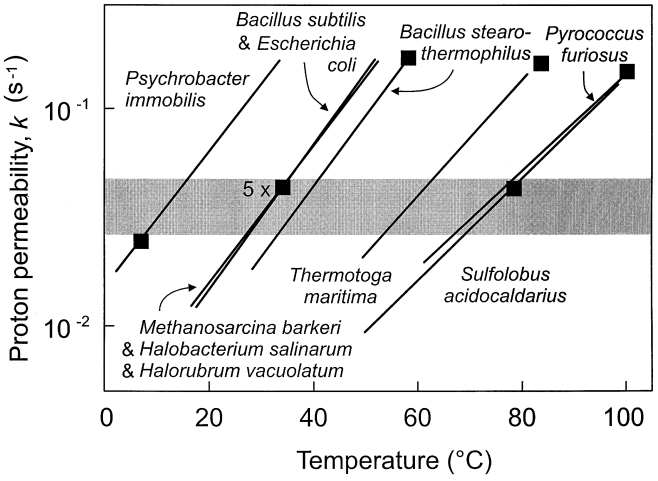

Fig. 2. Schematic presentation of the proton permeabilities of membranes from archaea and bacteria that live at different temperatures. Data were obtained by measuring the proton permeabilities of liposomes made of the lipids of the respective organism at different temperatures (Van de Vossenberg et al. 1995, 1998a,b). At the respective growth temperatures, the proton permeabilities fall within a narrow window (*gray bar*). The bacteria *Thermotoga maritima* and *Bacillus stearothermophilus* and the archaeon *Pyrococcus furiosus* have a permeability that is higher than in the other organisms. The diagram is reprinted from Albers *et al.* (2000)

Salt

Halophiles, such as *Halobacterium salinarium*, generate an electrochemical proton gradient across the membrane by respiration and/or the light-driven pump bacteriorhodopsin (Michel and Oesterhelt 1980). This organism generates an SMF by an H^+/Na^+ antiporter, which keeps the cytoplasmic concentration of sodium ions low (Murakami and Konishi 1988). The proton and sodium permeabilities of halobacterial membranes do not differ from those of nonhalophilic organisms that live at the same temperature. Membranes of halophiles and haloalkaliphiles are mainly adjusted to high salt concentration and to a lesser extent to pH (Van de Vossenberg et al. 1999b).

pH

Extreme acidophiles experience a very high ΔpH across the membrane. This ΔpH can be up to 4 pH units in *Picrophilus oshimae* (Van de Vossenberg et al. 1998a; Schleper et al. 1995). The PMF consisting of the ΔpH is very high, and acidophiles compensate for this by inverting the $\Delta\psi$ from negative inside to positive inside (Fig. 3D). The inversion of the $\Delta\psi$ is formed by active influx systems for positively charged ions such as K^+. The large ΔpH can be maintained only when the proton permeability of the membrane is very low. The proton permeability of the membranes of thermoacido-

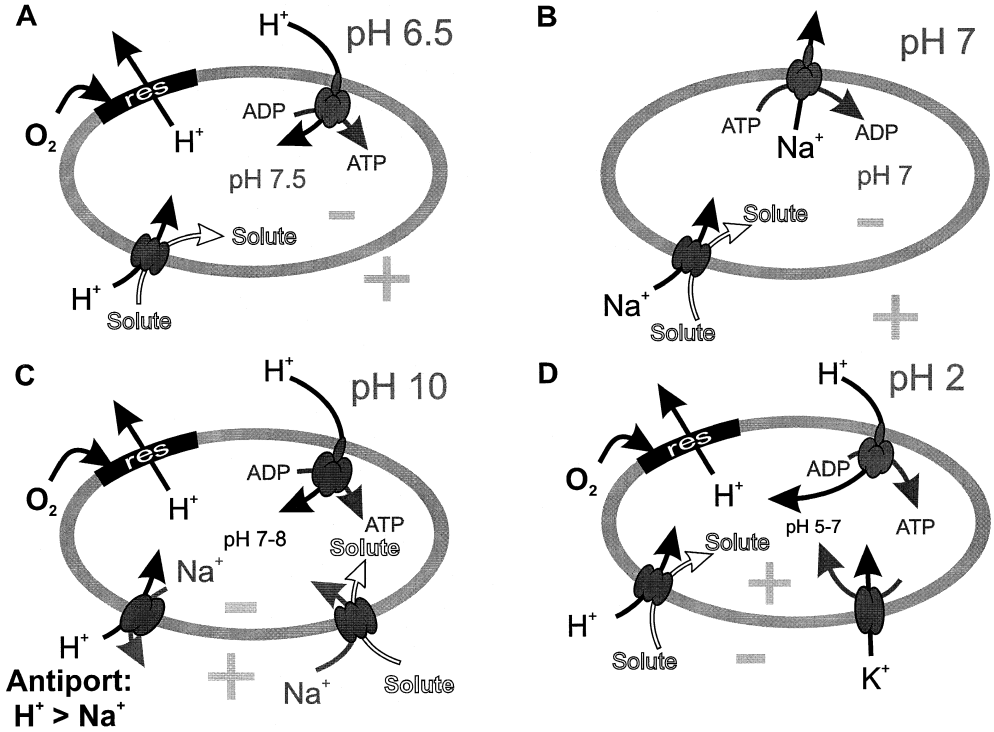

Fig. 3A–D. Energy transduction in membranes of bacteria and archaea. **A** H^+ cycling in aerobic mesophiles such as *E. coli* and *B. subtilis*. The respiratory chain excretes protons, thereby generating a proton motive force (PMF). This PMF drives the influx of protons, which is coupled to ATP hydrolysis or to the uptake of solutes from the environment. **B** Na^+ cycling in anaerobic thermophilic bacteria such as *Caloramator fervidus* (Speelmans et al. 1993). At the growth temperature, the membranes of this organism are leaky for protons. The organism relies on a sodium-extruding ATPase to build up a sodium motive force (SMF), and this SMF drives Na^+-coupled uptake of solutes. **C** H^+ and Na^+ cycling in organisms such as *Bacillus alcalophilus* that live in alkaline environments (Krulwich 1995). Protons are extruded by the respiratory chain. This results in a high PMF. H^+ ions are electroneutrally exchanged with Na^+, resulting in a reversed ΔpH (acid inside) and a high SMF. The SMF is then mainly used to drive transport of solutes into the cell. **D** H^+ cycling in organisms such as *Picrophilus oshimae* that live in an acid environment (Van de Vossenberg et al. 1998a). The respiratory chain excretes protons against a large pH gradient. The PMF is retained within physiological values by an inversion of the $\Delta\psi$ by cation influx (usually K^+). *Res*, respiratory chain

philic archaea is as low at the elevated temperatures at which the organisms grow as that of the membranes of mesophilic bacteria grown at mesophilic growth temperatures (Fig. 2).

Alkaliphiles have to maintain an intracellular pH that is much lower than the external pH. Since the ΔpH is reversed, a high $\Delta\psi$ (negative inside) is needed to maintain a sufficient PMF (Fig. 3C). Aerobic mesophilic alkaliphiles use a Na^+/H^+ antiporter in combination with H^+-coupled respiration to regulate their intracellular pH (Krulwich 1995; Speelmans et al. 1995).

It can be concluded that temperature has the most dramatic effect on the proton permeability of membranes. The changes in the lipid composition of cytoplasmic membranes in archaea and psychrophilic and mesophilic bacteria serve to keep the ion permeability of the membrane at a viable level during growth.

Transport of solutes in extremophiles

Up to 60% of prokaryotic membranes consists of membrane proteins. Whereas much is known about the features of lipids from extremophiles, only limited data from this group of organisms are available on proteins that catalyze the transport of solutes across the membranes. Membrane proteins involved in energy transducing processes, such as bacteriorhodopsin (Michel and Oesterhelt 1980), cytochrome oxidases, and ATPases, have been described and reviewed (Schäfer et al. 1999).

Transport systems are classified into five groups in bacteria and archaea, depending on the molecular architectures of the transport proteins and the driving force of transport: (i) channels (Fig. 4A); (ii) secondary transporters that use electrochemical gradients of protons or sodium ions to drive transport of substrates across the membrane (Fig. 4B); (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 (Fig. 4C); (iv) primary transporters, which use the chemical energy of ATP or other compounds to drive substrate translocation. Well-studied examples are ion-transducing respiratory chains, bacteriorhodopsin, and ATP-binding cassette (ABC) transporters. The latter transporters consist of two transmembrane proteins, which form the translocator pathway, and two ATP-binding proteins (Higgins 1992). ABC transporters involved

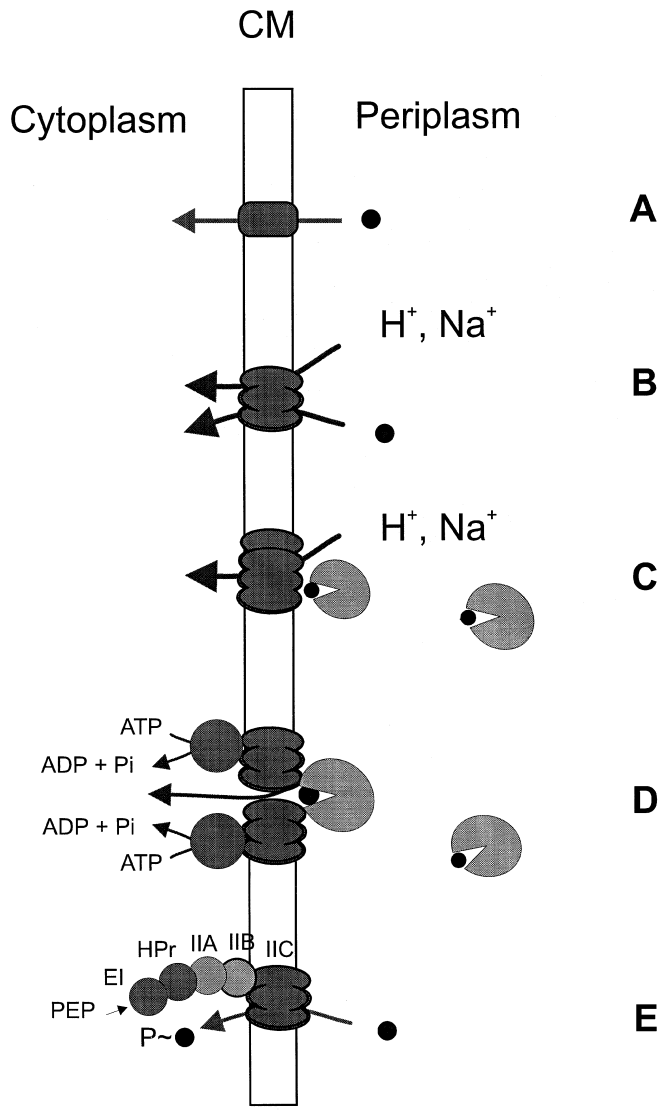

Fig. 4. Different classes of solute transporters. *A*, channel; *B*, secondary transporter; *C*, binding-protein-dependent secondary transporter (also described as tripartite ATP-independent transporter, TRAP-T); *D*, binding-protein-dependent ABC transporter; *E*, phosphoenolpyruvate-dependent phosphotransferase system (PTS). *CM*, cytoplasmic membrane

in the uptake of solutes also contain a high-affinity periplasmic binding protein (Fig. 4D); (v) phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS), which couples the transport of a sugar to phosphorylation (Fig. 4E).

In general, all classes of transporters are present in extremophiles, with the exception of PTS systems. In none of the archaeal genomes have PTS systems been identified, and the same is true for the hyperthermophilic bacteria *Thermotoga maritima* (Nelson et al. 1999) and *Aquifex aeolicus* (Deckert et al. 1998). PTS systems seem to be restricted to mesophilic bacteria, which may be an indication that these systems evolved relatively late.

The transport systems that have been identified in extremophiles are listed in Tables 1, 2. The study of transport systems in archaea is greatly hampered by the lack of a suitable membrane vesicle system. The isolation of membrane vesicles is complicated by the presence of the S-layer. Only from Halobacteria have functional membrane vesicles been isolated and used for amino acid uptake studies (Greene and MacDonald 1984). Another approach that can be used to study archaeal transport proteins is to express these proteins in mesophiles and study their properties in the membrane vesicles of these organisms. Thus far, it has not been demonstrated that genes for archaeal primary or secondary transport proteins expressed heterologously are active. Bacteriorhodopsin seems to be an exception. Also, the complementation of a Mg^{2+}-uptake null mutant of *Salmonella typhimurium* by a CorA homologue of *Methanococcus jannaschii* (Smith et al. 1998) has been demonstrated.

Secondary transporters

Secondary transporters are driven by electrochemical gradients (usually of protons or sodium ions). Three modes of secondary transport can be distinguished: uniport (only one solute is translocated, and this solute equilibrates along its electrochemical gradient); symport (solute is cotransported with the ion); and antiport (solute is exchanged for an ion or another substrate). Most of the secondary transporters in Table 2 couple transport of the solute to sodium ions. Na^+ ions are common coupling ions for bacteria and archaea that inhabit environments rich in Na^+ (Krulwich and Guffanti 1989; Prowe et al. 1996). Thermophilic bacteria studied so far have been shown to depend on an SMF and require Na^+ for growth. In *Caloramator fervidus* and the anaerobic thermoalkaliphilic strain *Anaerobranca gottschalkii* (*Thermoalkalibacter bogoriae*) LBS3 (Prowe et al. 1996), energy transduction and amino acid uptake are strictly dependent on sodium ions (Speelmans et al. 1989).

ABC transporters

Most of the studied transport systems of extremophiles belong to the family of ABC transporters (Table 1). These ABC transporters require periplasmic solute-binding proteins with high affinities for their specific substrates. Most ABC transporters are involved in sugar uptake. Three ABC transporters have been described for *Haloferax volcanii* and have been shown to be essential for nitrate respiration (Wanner and Soppa 1999). A whole range of sugar ABC transporters have been found recently in *Sulfolobus solfataricus* and *Pyrococcus furiosus* (Albers et al. 1999b; Elferink et al. 2001; Koning et al. 2001). These transporters fall into two groups: (i) the glucose, arabinose, trehalose-systems of *S. solfataricus* and the maltose/trehalose and maltotriose system of *P. furiosus*, which show homology 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 homology with bacterial di/oligo-peptide transport-

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. 1999b
	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 and Soppa 1999
	Molybdate	–	–	
	Inorganic anions	–	–	
M. thermoautotrophicum	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

[a] Solute binding to binding protein
[b] not determined

Table 2. Described secondary solute transporters in extremophiles

Secondary transporter	Substrate	K_m for uptake (μM)	Coupling ion	Reference
Archaea				
H. volcanii	Glucose	–[a]	Na^+	Tawara and Kamo 1991
H. halobium	Glutamate	–	Na^+	Kamo et al. 1988
	All amino acids except cysteine and aspartate	–	Na^+	Greene and 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] not determined

ers. This latter observation is surprising since this kind of transporter is so far restricted to di/oligo-peptide transport only. In all sequenced genomes of archaea and in the thermophilic bacteria *Thermotoga maritima* and *Aquifex aeolicus*, a large number of ABC transporters can be identified that belong to the class of di/oligo-peptide transporters. *T. maritima*, for example, contains 11 members of the di/oligo-peptide transporter family, of which nine are located in an operon with genes in sugar metabolism. Nelson et al. (1999) postulated that peptide and sugar degradation are coordinately regulated. However, it is more likely that most of these ABC transporters are transporters for sugar-oligomers instead of peptides. In the recently completed genome of *Thermoplasma acidophilum*, an ABC transporter (Ta1325–Ta1329) that has been designated oligopeptide transporter OPP1 (Ruepp et al. 2000) is highly similar to the maltose transporter of *S. solfataricus* (Elferink et al. 2001).

Solute-binding proteins

The majority of the binding proteins in the ABC transporters listed in Table 1 bind sugars. Although these proteins bind very similar substrates, their features differ according to their phylogenetic origins. All characterized archaeal binding proteins exhibit a very high binding affinity for their substrates, which can be in the nanomolar range (see Table 1). This is partly achieved by more pronounced hydrogen bonding between the substrate and the protein while apolar contacts are reduced (Diez et al. 2001; Evdokimov et al. 2001). The maltose-binding proteins of *T. maritima* and *Thermoanaerobacter ethanolicus* also accept maltotriose and trehalose as a substrate (Jones et al. 2000), whereas the maltose-binding protein of *Escherichia coli* binds only maltose and maltotriose. Like other bacterial binding proteins

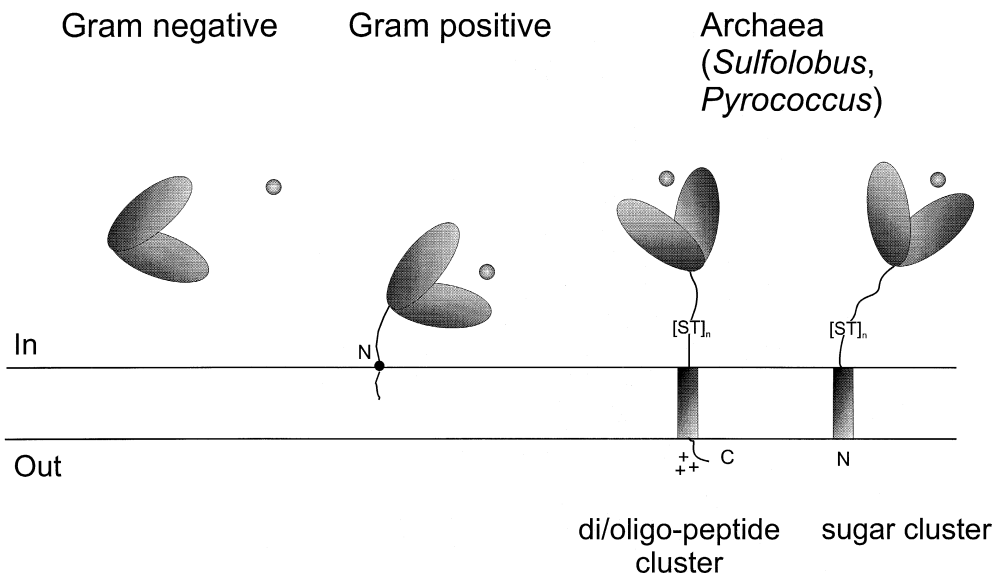

Fig. 5. Anchoring modes of solute-binding proteins. In gram-negative bacteria, the binding proteins (BP) are floating freely in the periplasm. BPs of gram-positive bacteria are anchored to the membrane via a fatty acid attached to the N-terminus. Archaeal BPs are most probably inserted into the membrane via a C-terminal transmembrane segment, in the case of a di/oligo-peptide cluster, or an N-terminal transmembrane segment in the case of a sugar cluster. These BPs also contain a C-terminal hydrophobic domain, which could also be inserted into the membrane (not shown). *[ST]$_n$*, flexible linker region with high percentage of hydroxylated amino acids (mainly serine and threonine)

(Tam and Saier 1993), the maltose-binding protein of the gram-negative *T. maritima* is present in the periplasm, while that of the gram-positive *T. ethanolicus* is attached to the membrane via a lipid anchor (Fig. 5) (Jones et al. 2000). This is also the case for the maltose/maltodextrin-binding protein of *Alicyclobacillus acidocaldarius* (Hülsmann et al. 2000). Archaeal binding proteins are bound to the membrane, but via a hydrophobic transmembrane segment (Albers et al. 1999a; Koning et al. 2001). This segment is held to the membrane by the very positively charged amino acids at the cytoplasmic surface (positive-inside rule) (von Heijne 1990). It is interesting that this positive-inside rule also applies to extreme acidophiles, which have an opposite $\Delta\psi$, inside positive (Van de Vossenberg et al. 1998c). Binding proteins of *S. solfataricus* and cellobiose binding protein (CbtA) from *P. furiosus* are all glycosylated. They contain very clear hydrophobic domains, which most likely anchor the proteins to membranes as described above (Fig. 6) (Albers et al. 1999b; Koning et al. 2001). In the trehalose/maltose binding protein (TMBP) of *Thermococcus litoralis*, the situation is less clear: the only hydrophobic domain present is part of the predicted N-terminal signal sequence, and this sequence is followed by a typical bacterial fatty acid attachment site (Gilson et al. 1988; Horlacher et al. 1998). It has not been possible to determine the N-terminal sequence of the mature TMBP, and it is not yet clear whether this binding protein is lipidated (Horlacher et al. 1998). It also is yet to be elucidated whether the *T. litoralis* TMBP belongs structurally to the sugar cluster of the binding proteins of *Sulfolobus* (Fig. 6). The hydrophobic part of the binding proteins of *S. solfataricus* and *P. furiosus* is followed or preceded (Figs. 5, 6) by a stretch of up to 30–60 hydroxylated amino acids. This region is a flexible linker region. In

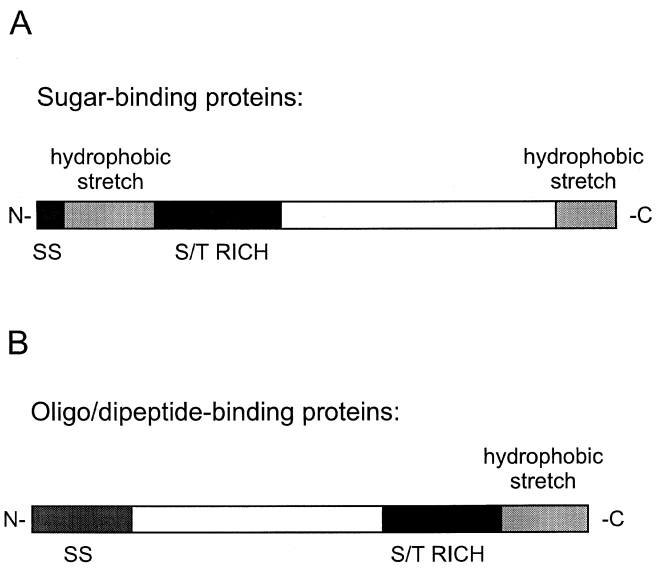

Fig. 6A,B. Domain organization of the two groups of archaeal sugar-binding proteins. **A** sugar-cluster. **B** di/oligo-peptide cluster. *SS*, signal peptide; *S/T rich*, linker region with high percentage of hydroxylated amino acids (mainly serine and threonine)

archaeal proteins, such as the S-layer, cytochrome $b_{558/566}$, and pullulanase (Erra-Pujada et al. 1999; Hettmann et al. 1998; Sumper et al. 1990), this site has been shown to be a glycosylation site.

A remarkable difference between the sugar and di/oligo-peptide cluster of binding proteins is the occurrence of a very short and unusual signal sequence. This signal sequence was first observed in the glucose-binding protein

of *S. solfataricus* and is very similar to the signal sequences of archaeal flagellins (Albers et al. 1999a). These signal sequences are homologous to the signal peptides from type IV pilins (Faguy et al. 1994).

ATP-binding domain

In all three kingdoms of life, ABC transporters have been found to transport a wide variety of different substrates (Boos and Shuman 1998; Higgins 1992). An important question is how ATP-hydrolysis can drive the transport process. Very recently, the 3-D structure of MalK, the ATP-binding protein of the maltose transporter of *Thermococcus litoralis*, was solved (Diederichs et al. 2000). This protein consists of two domains: (i) an ATP-binding domain very similar to HisP, the ATP-binding protein of the *Salmonella typhimurium* histidine transporter (Hung et al. 1998); and (ii) a unique domain at the C-terminus showing predominantly β-sheets organized in a β-barrel-like structure. This domain is a C-terminal extension that is found more often in bacterial and archaeal ATP-binding proteins. In *E. coli* MalK, this domain interacts with MalT, the activator of the *mal* operon (Kuhnau et al. 1991). Further investigation will show whether these domains fulfill similar functions in the regulation of expression of transporters and metabolic routes in archaea.

Distribution of transporters

All sequenced genomes of archaea and the thermophilic bacteria *T. maritima* and *Aquifex aeolicus* contain a large number of genes that encode transport proteins (Table 3) (Smith et al. 1997). In all these organisms, a large number of ABC transporters are found, and transport studies and sequence comparisons indicate that these transporters are mainly involved in the uptake of organic solutes. *T. maritima*, for example, possesses 25 putative secondary transporters, of which only ten are putative transporters for organic solutes. Most of the predicted secondary transporters are putative inorganic ion transporters (Paulsen et al. 2000). 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 to transport maltose, maltotriose, and trehalose (Wassenberg et al. 2000) (Tables 1, 2, 3). In contrast, in *E. coli*, secondary transporters are the 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 could be important for their survival strategy in their natural habitat. In the 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 their 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.

Table 3. Distribution of primary and secondary transporters in extremophiles as deduced from the genome sequences

Organism	Number of predicted	
	Secondary transporters	ABC-type transporters
T. maritima	25 (10)[a]	55
A. aeolicus	26 (3)	14
M. jannaschii	24 (2)	14
M. thermoautothrophicum	19 (3)	15
P. horikoshii	34 (12)	23
E. coli	194 (180)	74

[a] In parentheses is the number of putative secondary organic transporters out of the total number of predicted secondary transporters

Conclusions

Of all extreme conditions that extremophiles have to face, temperature has the most pronounced effect on the membrane. The membranes of (hyper)thermophiles are well adapted to this environmental stress factor. The lipids are not sensitive to oxidation and are very thermostable. Moreover, by altering the lipid composition, hyperthermophilic organisms are able to restrict the ion permeability of their membranes to a level comparable to the permeabilities of membranes of mesophiles. Also, hyperthermophilic bacteria and archaea show a preference for binding-protein-dependent ABC transporters for the uptake of organic solutes, whereas thermophilic and alkaliphilic bacteria mainly depend on Na^+-coupled secondary transporters. The features of the components of hyperthermophilic membranes make them attractive for biotechnical applications. Due to their long-term stability, archaeal lipids could, for example, be used in liposomes as a tool for drug delivery. Lipids and membrane proteins may form matrices for the construction of biosensors. Expression of some archaeal membrane proteins was achieved in *E. coli* (unpublished data), which makes these proteins well suited for structural and functional analyses (e.g., 3-D crystallization).

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