NA+ AS COUPLING ION IN ENERGY TRANSDUCTION IN EXTREMOPHILIC BACTERIA AND ARCHAEA

Speelmans, G.; Poolman, Berend; Konings, W.N

Published in:
World Journal of Microbiology & Biotechnology

DOI:
10.1007/bf00339136

Publication date:
1995

World Journal of Microbiology & Biotechnology 11, 58–70

Na^+ as coupling ion in energy transduction in extremophilic Bacteria and Archaea

G. Speelmans, B. Poolman and W.N. Konings*

For microorganisms to live under extreme physical conditions requires important adaptations of the cells. In many organisms the use of Na^+ instead of protons as coupling ion in energy transduction is associated with such adaptation. This review focuses on the enzymes that are responsible for the generation and utilization of Na^+ gradients in extremophilic microorganisms. Aspects that are dealt with include: bioenergetics and ion homeostasis in extremophilic Bacteria and Archaea; the molecular mechanism of Na^+ translocation; and (dis)advantages of Na^+ as coupling ion in energy transduction.

Key words: Bioenergetics, energy transduction, extremophiles, Na^+, transport.

Our knowledge about the functioning of the prokaryotic cell is, for the greater part based on observations made on cells functioning under 'moderate' or 'normal' physical conditions, i.e. at a pressure of 1 atm, near neutral pH, moderate osmolarities and a temperature of 30–37°C. Many microorganisms belonging to the Archaea and Bacteria have been discovered that live in the most diverse and bizarre environments, and these organisms have developed special properties to survive and prosper in these habitats. Adaptations are found in the cellular lipids, proteins and other macromolecular compounds, and in the mechanisms for energy transduction and the regulation of the intracellular milieu. The following groups of extremophiles have been discriminated: (1) alkali- and acido-philes, growing optimally at high and low pH, respectively; (2) halophiles, growing optimally at high ionic strength; (3) barophiles, living at high pressure; and (4) psychro- and thermo-philes, growing optimally at low and high temperatures, respectively. Furthermore, some microorganisms can even grow optimally in milieus in which two or more of these extreme physical conditions exist simultaneously.

All living cells have a cytoplasmic membrane that functions as the major barrier between the cytoplasm and the environment. The barrier properties of this membrane are mainly determined by the lipid molecules, which expose their polar headgroups to the water phases and their apolar fatty acid tails to each other. The cytoplasmic membrane is responsible for the selective passage of compounds from the cytoplasm to the environment and vice versa. It forms a matrix in which the energy-transducing enzymes and carrier proteins are embedded and by which specific solute concentration gradients can be generated and maintained (Poolman *et al.* 1993). The cytoplasmic membrane therefore plays a crucial role in maintaining the energy status of the cell (Hellingwerf & Konings 1985), and regulating the intracellular milieu (Booth 1985; Abee & Konings 1990), the turgor pressure (Higgins & Booth 1987) and other energy-transducing/consuming processes.

Usually, the ion concentrations of the environment differ considerably from those of the intracellular milieu. Various ion pumps in the cytoplasmic membrane are used to generate ion concentration gradients in order to maintain an optimal intracellular ion composition (Booth 1985; Skulachev 1987; Walderhaugh *et al.* 1987; Abee & Konings 1990). Only under certain conditions, when parameters of the external milieu are similar to the internal ones, e.g. pH 7.5, low Na^+ concentrations and high concentrations of K^+ and other nutrients and with ATP being formed by substrate-level phosphorylation, are bacteria able to grow without ion homeostasis and without a proton motive force (Harold & Van Brunt 1977; Kinosita *et al.* 1984).

In this review we describe the energy-transducing mechanisms in extremophilic microorganisms. Special attention is

G. Speelmans was and B. Poolman and W.N. Konings are with the Department of Microbiology, Biology Centre, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands; fax: 31 50 632154. G. Speelmans is now with the Department of Biochemistry of Membranes, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands. * Corresponding author.

paid to the nature of the coupling ion in energy transduction and the possible advantages of having a H^+ cycle, Na^+ cycle or both are discussed. The important features of enzyme systems that use Na^+ as coupling ion are described.

Energy Transduction can Occur with H^+ and Na^+ as Coupling Ion

Light or chemical energy can be converted by energy-transducing enzymes into an electrochemical transmembrane H^+ gradient (inside alkaline and negative). This gradient exerts a force on the protons, the proton motive force (Δp); which in turn can be used to drive energy-consuming processes. This mechanism of energy coupling as a means to drive endergonic reactions at the cytoplasmic membrane has been termed the chemiosmotic hypothesis (Mitchell 1961). The proton motive force generated upon H^+ extrusion is composed of an electrical potential ($\Delta\psi$) and a chemical gradient of H^+ (the pH gradient, ΔpH):

$$\Delta p = \Delta\psi - Z\Delta pH \text{ (mV)}$$

where $Z = 2.3(RT/F)$, R is the gas constant, T the absolute temperature, and F the Faraday constant.

Until 1980, H^+ was considered to be the primary coupling ion in chemiosmotic processes in bacteria (Dimroth 1987). However, it is now well established that energy transduction in bacteria is not uniquely dependent on H^+ cycling. Electrochemical gradients of Na^+, which are generated/consumed by similar types of transport mechanism as those that form and consume a Δp, are frequently encountered in microorganisms. These organisms use a sodium motive force (Δs) exclusively or in addition to a proton motive force for their vectorial energy-transducing machinery (Skulachev 1985, 1987, 1989; Dimroth 1992a, b). This 'sodium motive force' is also composed of an electrical and chemical component, given by the equation:

$$\Delta s = \Delta\psi - Z\Delta pNa \text{ (mV)}$$

Interconversion of the different ion motive forces is achieved by secondary transport mechanisms, such as Na^+/H^+ antiporters (see below).

H^+ and Na^+ Cycles in Prokaryotes

Energy transduction in most bacteria is performed by combining a primary H^+ pump with a secondary transport system for the generation of a Na^+ gradient. The contribution of the Na^+ gradient to energy transduction can vary from marginal to essential. Organisms such as *Lactococcus lactis* rely completely on H^+ as coupling ion while others, like the enterobacterium *Escherichia coli*, take an intermediate position (Poolman 1993; Poolman & Konings 1993). In *E. coli* the Δp is generated by a primary pump (respiration-linked or ATP-driven H^+ translocation) but a Na^+/H^+ antiporter is needed when the Na^+ concentration is high and the medium is relatively alkaline. Some solute transport systems in *E. Coli* are Na^+-coupled, but usually more than one mechanism exists (or can be expressed) for the same solute, so that the organism is not strictly dependent on a Na^+ gradient (Dibrov 1991).

The presence of a Na^+ cycle is essential in extremely alkaliphilic bacteria, and an electrogenic Na^+/H^+ antiporter is used to keep the intracellular pH below the extracellular one (Ivey *et al.* 1992). In the alkaliphiles, all secondary symport systems studied to date are Na^+-coupled (see below). In aerobic thermophiles, rumen bacteria and extreme halophiles, the Δs is also required for solute transport processes (see below). *Clostridium fervidus* is perhaps the most extreme in this regard, since its energy transduction at the membrane is exclusively dependent on Na^+ (Speelmans *et al.* 1993a)

Primary and Secondary Na^+-transport Systems in Bacteria and Archaea

In Bacteria and Archaea the proton and sodium gradients can be formed by:

(1) Redox-linked primary ion pumps (cyclic and linear electron transfer chains);
(2) The light-driven proton pump (bacteriorhodopsin in halophilic bacteria);
(3) Membrane-bound ATPases;
(4) Membrane-bound pyrophosphatases;
(5) Membrane-bound decarboxylases;
(6) Methyltransferases in methanogens (and acetogens);
(7) Secondary transport processes.

Upon re-uptake of H^+ and/or Na^+ the proton and/or sodium cycle is completed. This uptake of Na^+ has occasionally been shown to be rate-determining in the Na^+ cycle (Krulwich *et al.* 1985). The downhill flux of H^+ and/or Na^+ can be used to drive endergonic reactions that take place at the cytoplasmic membrane. In most cases, a particular enzyme uses either H^+ or Na^+ as coupling ion. Occasionally, however, enzymes (transporters) can choose between H^+ and Na^+ (Li^+) (see below). The following Δp- and Δs-consuming processes have been described.

(1) Secondary solute transport; uptake of substrates, such as amino acids, sugars, nucleotides and ions, and excretion of metabolic end-products, toxic compounds etc, can be coupled to Δp and/or Δs. A special case is the Na^+/H^+ antiport system, which interlinks the H^+ and Na^+ cycles.
(2) ATP synthesis; ATP formed from ADP and P_i at the expense of Δp or Δs.
(3) Motility; the flagellar motor has been shown to be driven either by the Δp or Δs.

(4) Other membrane-linked endergonic reactions, such as protein translocation, DNA uptake during transformation, conjugation and phage infection, cell wall synthesis, N_2-fixation, regulation of cell volume and cell turgor, heat production and transhydrogenases, have only been described as Δp-dependent processes.

Redox-linked Na^+ Pumps

Respiration-driven Na^+ pumps can be found in marine and halotolerant/alkalitolerant bacteria. In *Vibrio alginolyticus*, two NADH:quinone reductases are present: NQR1 and NQR2. NQR1 is specifically stimulated by Na^+; while NQR2 is Na^+-independent [for a recent review see Unemoto & Hayashi (1993)]. Na^+-dependent, NADH:quinone reductases have also been described for other bacteria. Most of the organisms are Gram-negative, marine and moderately halophilic or moderately alkaliphilic bacteria. With the exception of the alkalitolerant *Bacillus* FTU (Semeykina & Skulachev 1992), NADH-quinone reductases in Gram-positive, moderately halophilic bacteria translocate H^+ across the membrane (Unemoto *et al.* 1992).

Other redox-linked primary Na^+ pumps comprise the terminal cytochrome *o* oxidase from *Vitreoscilla* sp. This enzyme complex has been purified and reconstituted into liposomes and has clearly been shown to be a primary Na^+ pump (Efiok & Webster 1990). *Bacillus* FTU and *E. coli* grown under conditions of low Δp have also been reported to contain terminal oxidases that extrude Na^+ (Avetisyan *et al.* 1991). In *E. coli* a cytochrome *d* oxidase and in *Bacillus* FTU an *o*-type oxidase have been claimed to be Na^+ pumps (Aveytisian *et al.* 1992) (Muntyan & Skripnikova 1993), but the data have not yet been obtained with purified enzymes or well-defined membrane systems.

In methanogenic microorganisms (belonging to the Archaea), some steps in the reduction of CO_2 to methane are dependent on Na^+, i.e. an endergonic reaction in the pathway is driven by Δs whereas an exergonic reaction generates a Δs (Becher *et al.* 1992; Müller & Gottschalk, 1992; Schönheit 1992). In *Acetobacterium woodii* the conversion/reduction of methylene-tetrahydrofolate to methyl-Co(III)-corrinoid is probably involved in the generation of a Δs (Heise *et al.* 1989, 1993).

Na^+-pumping ATPases and Δs-driven ATPsynthases

ATP-driven Na^+ and H^+ pumps can be categorized into: (1) F-type; (2) V-type (vacuolar ATPase); and (3) P-type (or E_1E_2-ATPase). F-type ATPases comprise the F_0F_1-ATPase of bacteria, chloroplasts and mitochondria. In respiratory and phototrophic bacteria, the F_0F_1-ATPase normally functions in the synthesis mode (i.e. the enzyme utilizes the Δp and/or Δs to synthesize ATP). In fermentative bacteria, on the other hand, the enzyme usually works in the opposite direction (a Δp and/or Δs is generated upon hydrolysis of ATP).

V-type ATPases have been defined as ion (proton) motive ATPases associated with membranes of eukaryotic organelles other than mitochondria and chloroplasts. On the basis of sequence similarities, archaeal H^+-ATPases of *Sulfolobus acidocaldarius* (Lübben & Schäfer 1987), *Methanosarcina barkeri* (Inatomi 1986) and *Halobacterium halobium* (Mukohata & Isoyama 1987) have been categorized in this family. V-type ATPases have also been recognized in bacteria such as *Enterococcus hirae* (Kakinuma & Igarashi 1990; Takase *et al.* 1993), and possibly *Thermus thermophilus* (Yokoyama *et al.* 1990) and *Clostridium fervidus* (Speelmans *et al.* 1993a). With the exception of the V-type ATPase of *E. hirae* and the putative V-type ATPase of *C. fervidus*, which have been shown to transport Na^+, the other examples of V-type ATPases translocate H^+ rather than Na^+ (see below). V-type ATPases are specifically inhibited by NO_3^-, in contrast to F_0F_1-ATPases for which N_1N'-dicyclohexylcarbodiimide (DCCD) is often used as diagnostic inhibitor.

P-type ATPases form two covalent acyl phosphate intermediates as part of their reaction cycle (E_1 and E_2), which differ in conformation. The P-type ATPases are quite specifically inhibited by micromolar concentrations of ortho-vanadate. P-type ATPases have been described for the translocation of H^+ or Na^+ but members of the same family of enzymes have also been shown to transport various other compounds (Siebers & Altendorf 1992).

Na^+-ATPsynthases have frequently been detected in organisms that use primary Na^+ translocation for Δs generation (Dimroth 1992b). Solid biochemical evidence for the ion specificity of translocation catalysis by the enzymes is limited and some controversies exist. For instance, it has been suggested that ATP synthesis in marine bacteria, such as *Vibrio alginolyticus*, is driven by Δs (Skulachev 1987). However, the purified and reconstituted ATPase of *V. alginolyticus* exhibits H^+- rather than Na^+- pumping activity (Krumholz *et al.* 1990; Dmietriev *et al.* 1991). The most thoroughly characterized Na^+-linked ATPsynthase is from the strictly anaerobic, marine bacterium *Propionigenium modestum*, which is a typical member of the F_0F_1 family (Laubinger & Dimroth 1987). The F_0F_1-ATPase complex is activated by sodium ions (Laubinger & Dimroth 1988a), but the enzyme not only pumps Na^+ but also H^+, i.e. when the Na^+ concentration is low (Laubinger & Dimroth 1988b). The specific activation of the ATPase activity by Na^+ is lost upon dissociation of the F_1 moiety from the membrane-bound F_0 subunits, and the activation by sodium can be restored by reconstitution of the enzyme complex in liposomes (Laubinger & Dimroth 1988b). Interestingly, a functional hybrid ATPase composed of F_1 from *E. coli* and F_0 from *P. modestum* generates a Na^+ gradient (Laubinger *et al.* 1990), or can synthesize ATP when a Δs is applied (Dmietriev *et al.* 1993). These experiments strongly indicate that the ion specificity is conferred by the F_0 moiety.

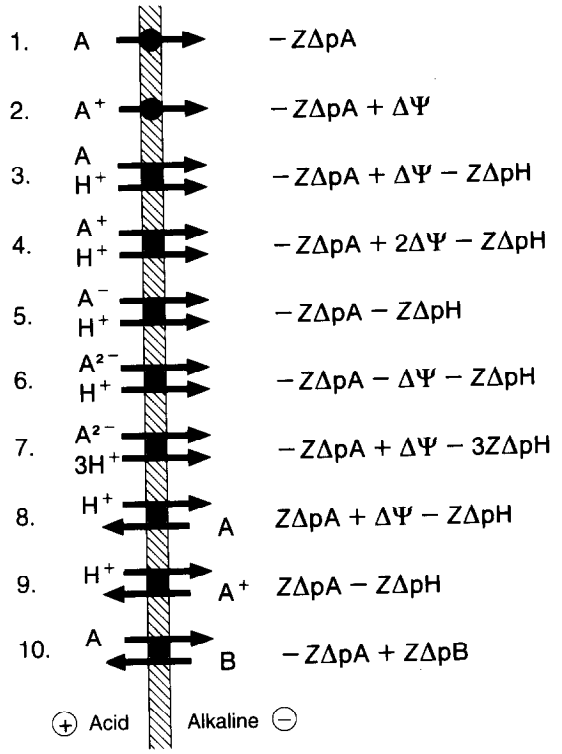

Figure 1. Different types of secondary transport in bacteria. The driving force on solute A is indicated for each transport mechanism. For transport systems that use Na^+, the chemical Na^+ gradient (ΔpNa) provides the driving force instead of the H^+ gradient (ΔpH).

Na^+ Extrusion by Membrane-bound Decarboxylases

The membrane-bound, oxaloacetate decarboxylase of *Klebsiella pneumoniae* was the first primary Na^+ pump to be discovered in bacteria (Dimroth 1980). Later, methylmalonyl-CoA and glutaconyl-CoA decarboxylases were also found to be Na^+-translocating enzymes (Dimroth 1987, 1991). The species in which these enzymes are found are not closely related phylogenetically. Decarboxylases are membrane-associated enzymes that are composed of three or four different polypeptides (Dimroth 1992a): (1) a hydrophilic α-subunit of 60 to 65 kDa, which performs the decarboxylation reaction and in which a H^+ is consumed and CO_2 is formed independent of Na^+; (2) a membrane-bound β-subunit of 33 to 34 kDa, which possesses the Na^+-binding/translocation site; (3) a membrane-bound γ-subunit of either 9 kDa (in the case of oxaloacetate decarboxylases) or 19 to 24 kDa (in the case of methylmalonyl-CoA and glutaconyl-CoA decarboxylase). The biotin moiety is either bound to the α-subunit (in the case of oxaloacetate decarboxylases) or to the γ-subunit. In the latter case a δ-subunit of 14 to 16 kDa, with unknown function, is also present.

Secondary Na^+-coupled Transport Systems

Three categories of secondary transport can be distinguished in bacteria (Mitchell 1968; Poolman & Konings 1993): (1) Uniport systems, which function independent of any coupling ion; (2) Symport systems, in which solutes move in the same direction [the (electro)chemical gradient of one solute is used to drive the uphill transport of the other]; and (3) Antiport systems, in which solutes move in opposite directions. The solutes transported by secondary systems can be neutral or negatively or positively charged and different numbers of solutes may be co- or counter-transported. The driving forces of these processes therefore vary considerably [see Figure 1; for a review of Poolman & Konings (1993; Poolman *et al.* 1994)]. In the examples 3 to 9 in Figure 1, protons are shown as coupling ion but, in most cases, similar systems have been described which use Na^+ as co- or counter-transported species. In general, Na^+-coupled secondary transport is found more frequently in extremophilic Bacteria and Archaea growing at high temperatures, alkaline pH or high salinity, whereas H^+-coupled transport is more dominant in acidophiles and non-extremophilic Bacteria. However, several exceptions to this rule have been reported (see below), and in many cases Na^+- and H^+-coupled transport systems can be expressed in one and the same organism. For an overview of Na^+-dependent cotransport the reader is referred to the review of Maloy (1990), updated by Speelmans (1993).

Na^+/H^+ Antiport

Padan & Schuldiner (1992) stated that all bacteria have Na^+/H^+ antiporter(s). The anaerobic thermophile *C. fervidus* appears to be an exception to this rule, since a functional Na^+/H^+ antiporter could not be demonstrated in intact cells of or membrane vesicles derived from this organism (Speelmans *et al.* 1993a, b). In addition to a role in the Na^+ cycle, necessary for Δs-consuming processes or in creating an energy buffer for Δp (Skulachev 1987), the Na^+/H^+ antiporters also serve important roles in control mechanisms and homeostasis of the cell, e.g. by lowering of the intracellular Na^+ concentration to prevent Na^+ toxicity and regulating the internal pH (Booth 1985; Ivey *et al.* 1992; Schuldiner & Padan 1992). In methanogens the Na^+/H^+ antiporter has been suggested, in some circumstances, to work in the opposite direction and to generate Δp at the expense of Δs (Kaesler & Schönheit 1989).

Motility

Cell mobility is achieved by the action of flagella. In most organisms studied to date, rotation of the flagellum is driven by the proton motive force (Khan 1990). In marine bacteria (Tokuda 1992) and alkaliphilic bacteria (Hirota & Imae 1983; Sugiyama *et al.* 1985; Bogachev & Skulachev 1993) flagellar motility is Na^+-dependent at alkaline pH. In the marine bacterium, *V. alginolyticus*, flagellar rotation is Δp-driven at neutral pH. *Vibrio parahaemolyticus* possesses two types of flagella. The single polar flagellum, suited for

swimming in liquid medium, is driven by the Δs, whereas the lateral flagella, suited for swarming over viscous surfaces, are Δp-driven (Atsumi *et al.* 1992).

Molecular Mechanism of Na^+ Translocation

An intriguing phenomenon is the interchangeability of Na^+ and H^+ as coupling ion in some Na^+-, H^+-translocating enzymes. The F_oF_1-ATPase of *Propionigenium modestum* (Laubinger & Dimroth 1988b), the alanine carrier of PS3 (Hirata *et al.* 1984), and the melibiose carrier (MelB) of *E. coli* (Tsuchiya & Wilson 1978) can use either Na^+ (Li^+) or H^+ as coupling ions. The observation that a number of enzymes involved in energy transduction use either Na^+ or H^+ as coupling ion could be reflected in the actual catalysis of cation transport. Boyer (1988) suggested that transport systems that choose between Na^+ and H^+ as coupling ion may actually use H_3O^+ (the steric analogue of Na^+) instead of H^+ as transported species. Such a mechanism would account for competition between protons and sodium ions for a single cation-binding site. Also, an evolution from proton to sodium-ion coupling and *vice versa* can be easily envisaged when protons and sodium ions use a similar translocation mechanism.

H^+ translocation in the electron transfer chain of respiring and phototrophic organisms can occur by the proton motive Q cycle, which involves flavosemiquinone and/or ubisemiquinone radicals as intermediates (Mitchell 1976; Sone 1990; Trumpower 1990). It is unlikely that the quinols can function as Na^+ carriers. Therefore, in redox-linked Na^+ translocation one has to invoke a coupling mechanism in which Na^+ transport is enzyme mediated, e.g. as proposed for the Na^+ motive NADH:quinone reductase in *Vibrio alginolyticus* (Tokuda 1992). It has also been suggested that terminal oxidases, such as the cytochrome *o* oxidase of *Vitreoscilla* (Efiok & Webster 1990) and *Bacillus* FTU (Muntyan & Skripnikova 1993) and the cytochrome *d* oxidase of *E. coli* (Avetisyan *et al.* 1992), pump Na^+.

Some details of the coupling mechanism of the Na^+ pump methylmalonyl-CoA decarboxylase have been elucidated by following the flux of Na^+ ions using the isotope $^{22}Na^+$. It has been shown that the enzyme pumps two Na^+ out per decarboxylation reaction. The enzyme also catalyses exchange of external and internal Na^+ (Hilpert & Dimroth 1991), but there is no evidence that the enzyme can translocate H^+ (H_3O^+).

A strong phylogenetic relationship between the $Na^+(H^+)$-translocating ATPase of *P. modestum* and the H^+-translocating ATPases of other organisms has been found (Dimroth 1992b). This has important implications for the mechanism of ATP synthesis and hydrolysis via F_oF_1-type enzymes. All models in which a specific role for H^+ has been proposed that cannot be performed by another cation are likely to be incorrect. Studies on the Na^+ (H^+)-ATPase of *P. modestum* indicate that Na^+ (or H^+) binding at the F_o moiety triggers a conformational change which brings about ATP synthesis in the F_1 moiety (Dimroth 1992b). The observation that purified F_o of the ATPase of *P. modestum* catalyses Na^+/Na^+ counterflow (Kluge & Dimroth 1992) indicates that distinct cation-ion binding site(s) are present on F_o. This cation conduction through F_o is unlikely to proceed via a network of hydrogen-bonded chains, at least not when Na^+ is translocated.

In secondary transport systems, the residues that participate in Na^+ or H^+ transport across the membrane are often not known but, with the aid of mutagenesis techniques and kinetic analysis of the mutant proteins, some amino acids essential for ion translocation have been identified (Poolman & Konings 1993). Similar to the situation with the ATPase, the observation that some homologous proteins can either translocate H^+ and/or Na^+ argues against the operation of a H^+ relay network in these carrier proteins as has been proposed for the lactose carrier of *E. coli* (Kaback 1990). For the melibiose carrier protein (MelB) of *E. coli*, a number of aspartic acids present in transmembrane segments I, II, and IV have been implicated in Na^+-coupling of transport (Pourcher *et al.* 1993; Zani *et al.* 1993). Although these studies showed that the *N*-terminal portion of the MelB protein is important for cation recognition, no universal or easily recognizable Na^+-binding site can be identified. Further support for a role of the amino-terminal transmembrane segments in cation recognition has come from studies in which various portions of the MelB protein of *E. coli* were exchanged with the corresponding domains of the H^+-(Li^+)-dependent MelB protein of *Klebsiella pneumoniae* (Hama & Wilson 1993). However, the analysis of chimeric MelB proteins has also indicated that, for different sugar substrates, different parts of the carrier participate in cation recognition/transport, which makes it virtually impossible to define the Na^+-binding site without further structural information on the protein.

Ion Homeostasis

The ion composition of the cytoplasm is kept within certain limits for optimal metabolic activity (Booth 1985; Skulachev 1987; Walderhaugh *et al.* 1987; Abee & Konings 1990). Since the ion composition usually differs from that of the environment, active pumps are needed in the membrane to maintain this concentration difference. Usually, the internal pH is kept near neutrality (Booth 1985; Abee & Konings 1990) and the K^+ concentration inside is maintained at a higher concentration (Walderhaugh *et al.* 1987) and the Na^+ concentration at a lower concentration (Skulachev 1987) than in the surrounding medium.

pH Homeostasis

Since most biomolecules (including proteins and nucleic

acids) have groups that undergo protonation/deprotonation by which conformations and thus biological (physiological) functions will be affected, regulation of cytoplasmic pH serves an important physiological function. The intracellular pH of neutrophilic bacteria is usually set between 7.6 and 7.8 (Schuldiner & Padan 1992) whereas for acidophiles it is around pH 6.5 (Matin 1990b) and for alkaliphiles around 8.2 (Ivey *et al.* 1992). In order to maintain a constant pH under conditions that a primary proton pump is active, the rate of proton extrusion has to be regulated and/or compensated by an appropriate rate of H^+ uptake. Na^+/H^+ and K^+/H^+ antiporters as well as the F_0F_1-ATPase have been implicated in pH homeostasis of the bacterial cell (Kroll & Booth 1981; Kobayashi *et al.* 1984; Nakamura *et al.* 1984; Schuldiner & Padan 1992). An important factor of this regulation is the modulation of the activities and/or expression levels of the antiporters and the F_0F_1-ATPase as a function of the intracellular pH.

Na^+ Homeostasis

In marked contrast to the homeostasis of the cytoplasmic pH, the intracellular Na^+ concentration is not constant in *E. coli* (Castle *et al.* 1986a, b). The Δs is constant at a given pH, but the intracellular Na^+ concentration varies with pH and the external Na^+ concentration. These observations indicate that the extracellular pH affects the intracellular Na^+ concentration. At neutral pH and at 400 to 500 mM extracellular Na^+, the intracellular Na^+ concentration of *E. coli* has been estimated to be 40 to 50 mM (Padan & Schuldiner 1992; Schuldiner & Padan 1992). These intracellular Na^+ concentrations can apparently be tolerated without bacteriostatic effects (Schuldiner & Padan 1992). *Escherichia coli* cells react to a high Na^+ load, i.e. when the intracellular concentration approaches the threshold concentration of 40 to 50 mM, by swimming away from the toxic ion and at the same time by increasing the expression of NhaA, one of the Na^+/H^+ antiporters of the cell (Padan & Schuldiner 1992; Schuldiner & Padan 1992).

Bioenergetics and Ion Homeostasis in Extremophiles

Important insights into the bioenergetics of microorganisms have originated from studies on Bacteria and Archaea that were isolated from extreme environments. For instance, the high stability of some integral membrane-protein complexes in thermophiles has facilitated the purification and subsequent biochemical analysis of the enzymes (Yoshida *et al.* 1977). In this section, the bioenergetics and ion homeostasis of extremophiles will be reviewed and compared with that of non-extremophiles for which some general properties have already been outlined above. In Table 1, an overview of various types of extremophiles, their definition, and their distribution among the domains of Bacteria and Archaea are given.

Acidophiles

Obligate acidophiles have an optimum pH of growth which is extremely low (pH 1 to 4). To shield the intracellular enzymes and other components from a low medium pH, the organisms maintain a large pH gradient across the membrane. Special lipids are present in the acidophiles which may minimize leakage of H^+ down the pH gradient and stabilize protein complexes at low pH values (Russell & Fukunaga 1990). For instance, ω-cyclohexyl groups in lipids of the thermophilic bacterium *Bacillus acidocaldarius*, membrane-spanning, tetraether lipids containing cyclopentane rings in the thermophilic Archaea such as *Sulfolobus acidocaldarius*, and other unusual fatty acids have been implicated as special adaptations to growth at extremely low pH values (Russell & Fukunaga 1990).

Acidophiles maintain the cytoplasmic pH at around 6.5, which is at least one to two pH units lower than the intracellular pH of neutrophiles and alkaliphiles (Krulwich & Ivey 1990; Matin 1990b). The large ΔpH (3 to 4 pH units) in (aerobic) acidophiles is partly compensated by a $\Delta\psi$, positive inside; this polarity of the $\Delta\psi$ is opposite to that in neutrophiles (Bakker 1990; Matin 1990a). The reversed $\Delta\psi$ is formed by electrogenic K^+ uptake which allows the cells to extrude more H^+, and thus to maintain the internal pH at a higher level (Skulachev 1987; Walderhaugh *et al.* 1987; Bakker 1990). The large ΔpH formed in acidophiles has only been observed in intact cells. In membrane vesicles at low external pH, a smaller ΔpH and $\Delta\psi$ of 'normal' magnitude and polarity (negative inside) is formed upon addition of respiratory substrates (Guffanti *et al.* 1984).

In *S. acidocaldarius* H^+ is pumped out by the respiratory chain (Schäfer & Moll 1988) and the Δp is used to drive ATP synthesis (Moll & Schäfer 1988). Also, amino acid uptake in *B. acidocaldarius* (W. De Vrij, unpublished work) and ATP synthesis in *Thiobacillus ferrooxidans* (Apel *et al.* 1980) are H^+-coupled processes. A scheme for energy transduction in aerobic acidophiles is presented in Figure 2A. As with the other schemes presented below, generalizations of energy transduction have been made on the basis of the best-studied examples of a particular class of organisms.

Alkaliphiles

Obligate alkaliphiles have an optimum pH of growth which is high, and these organisms are not able to grow at or near neutral pH. The best studied obligate alkaliphiles belong to the genus *Bacillus*. These organisms contain highly branched lipids and lipids that are more unsaturated than those of facultative alkaliphiles (Clejan & Krulwich 1988; Dunkley *et al.* 1991). The internal pH of obligate alkaliphiles is kept at 8.2, which is at least two units more acidic than the external pH (Krulwich & Ivey 1990; Ivey *et al.* 1992) and somewhat higher than the cytoplasmic pH of neutrophiles. In alkaliphilic bacilli H^+ is pumped out by the

Table 1. Definition and classification of extremophiles.

Extremophile	Growth definition*	Aerobe/ anaerobe†	Archaea/ Bacteria†	Other possible extremophilic properties
Acidophile	pH 1 to 4	Aerobe	Archaea/Bacteria	Thermophilic, barophilic, marine environment
Alkaliphile	pH > 10.5	Aerobe	Archaea/Bacteria	Halophilic
Halophile	> 2.5 M [NaCl]	Aerobe/anaerobe	Archaea	Alkaliphilic
Marine	0.5 M [NaCl]	Aerobe/anaerobe	Archaea/Bacteria	Alkaliphilic, psychrophilic, thermophilic, barophilic
Barophile	> 1 atm	Aerobe/anaerobe	Archaea/Bacteria	Marine, thermophilic, psychrophilic
Psychrophile	*Topt* < 15°C	Aerobe/anaerobe	Bacteria	Marine, barophilic
Thermophile	*Topt* > 65°C	Aerobe/anaerobe	Archaea/Bacteria	Barophilic, marine, acidophilic

* Physical condition which determines whether a particular organism is classified as extremophilic.
† Classification based on the microorganisms isolated to date.
Topt—Temperature at which optimal growth occurs.

respiratory chain, thereby alkalifying the cytoplasm. Acidification of the cytoplasm is achieved by an electrogenic Na^+/H^+ antiporter, which imports two H^+ upon export of one Na^+. Since for every two H^+ taken up one Na^+ is exported, a significant uptake (or leak) of Na^+ is needed to regulate the internal pH. Evidence has been presented for an involvement of Na^+-coupled nutrient uptake in this regulation (Krulwich *et al.* 1985). The relatively large reversed ΔpH, together with a $\Delta\psi$ of normal polarity, results in a Δp that is low (-25 to -50 mV). The organism, however, can maintain a large ΔpNa and Δs to drive various membrane-linked processes. Motility (Hirota & Imae 1983; Sugiyama *et al.* 1985; Krulwich *et al.* 1990) and substrate transport (Krulwich *et al.* 1990) are coupled to the sodium motive force, but ATP synthesis, on the other hand, is H^+-coupled (Hicks & Krulwich 1990; Hoffman & Dimroth 1990). The energetics of 'oxidative phosphorylation' has still not been solved satisfactorily. Non-chemiosmotic phenomena (Guffanti *et al.* 1984; Guffanti & Krulwich 1992) and unusual H^+/ATP stoichiometries have been proposed to explain the observed Δp-driven ATP synthesis (Hoffman & Dimroth 1991; Dimroth 1992c). The vectorial energy-transducing processes in an aerobic alkaliphile are depicted in Figure 2B.

The way in which the internal pH is regulated in anaerobic alkaliphiles is far from clear but a Na^+/H^+ antiporter seems to play a crucial role in pH homeostasis in these organisms (Koyama *et al.* 1988).

Halophiles

Extreme halophilic microorganisms grow at high ionic strength. The membrane lipids of these Archaea are composed of diphytanylglycerol diether analogues of glycerophospholipids (Russell 1989; Gambacorta *et al.* 1995). The extreme halophiles contain high intracellular concentrations of Na^+ (up to 1 M) and K^+ (4 to 5 M), and their proteins seem to have adapted to this high salt concentration by having a higher fraction of acidic amino-acid residues and a more compact packing of the polypeptide chain than proteins from non-halophilic bacteria (Zaccai & Eisenberg 1991).

In the halophiles studied to date, the Δp is formed by a respiratory chain or, in some cases by the light-driven H^+ pump bacteriorhodopsin. A Na^+/H^+ antiporter is used to pump Na^+ outwards (Konishi *et al.* 1992), and solute uptake has been shown to be Na^+-coupled in several halobacterial species (Lanyi 1979). As in the obligate alkaliphiles, ATP synthesis in obligate halophiles is H^+-coupled (Mukohata & Isoyama 1987). A scheme for the energy transduction in an extreme halophile is shown in Figure 2C.

Halotolerant, Alkalitolerant and Marine Bacteria

Moderate halophiles need salt for growth and can tolerate salt up to 3.5 M. At increasing salt concentration the relative amount of negatively charged phospholipids is increased (Russell 1989). In contrast to the halophiles, halotolerant/alkalitolerant and marine bacteria keep their internal Na^+ concentration low. At neutral pH and under aerobic conditions these organisms generate a Δp by respiration, and use a secondary Na^+/H^+ antiporter to generate a Na^+ gradient. In addition, respiratory-linked primary Na^+ pumps and Na^+-extruding decarboxylases can be expressed under conditions of a low Δp (Dimorth 1992a; Tokuda 1992). The ability to switch between secondary and primary Na^+ pumps has been proposed as an adaptation to the fluctuating physical conditions in, for instance, an algal mat (see below). Solute uptake and motility have been shown to be Na^+-coupled processes in several *Vibrio* spp. (Tokuda 1992); in most cases the nature of the coupling ion of the membrane-bound ATPase has not been established. However, for the strictly anaerobic, marine bacterium *Pr. modestum*, a Na^+-coupled ATPsynthase has been demonstrated (Laubinger & Dimroth 1988a; Dimroth 1992b, c). Energy transduction in alkalitolerant/halotolerant and marine organisms is shown in Figure 2D.

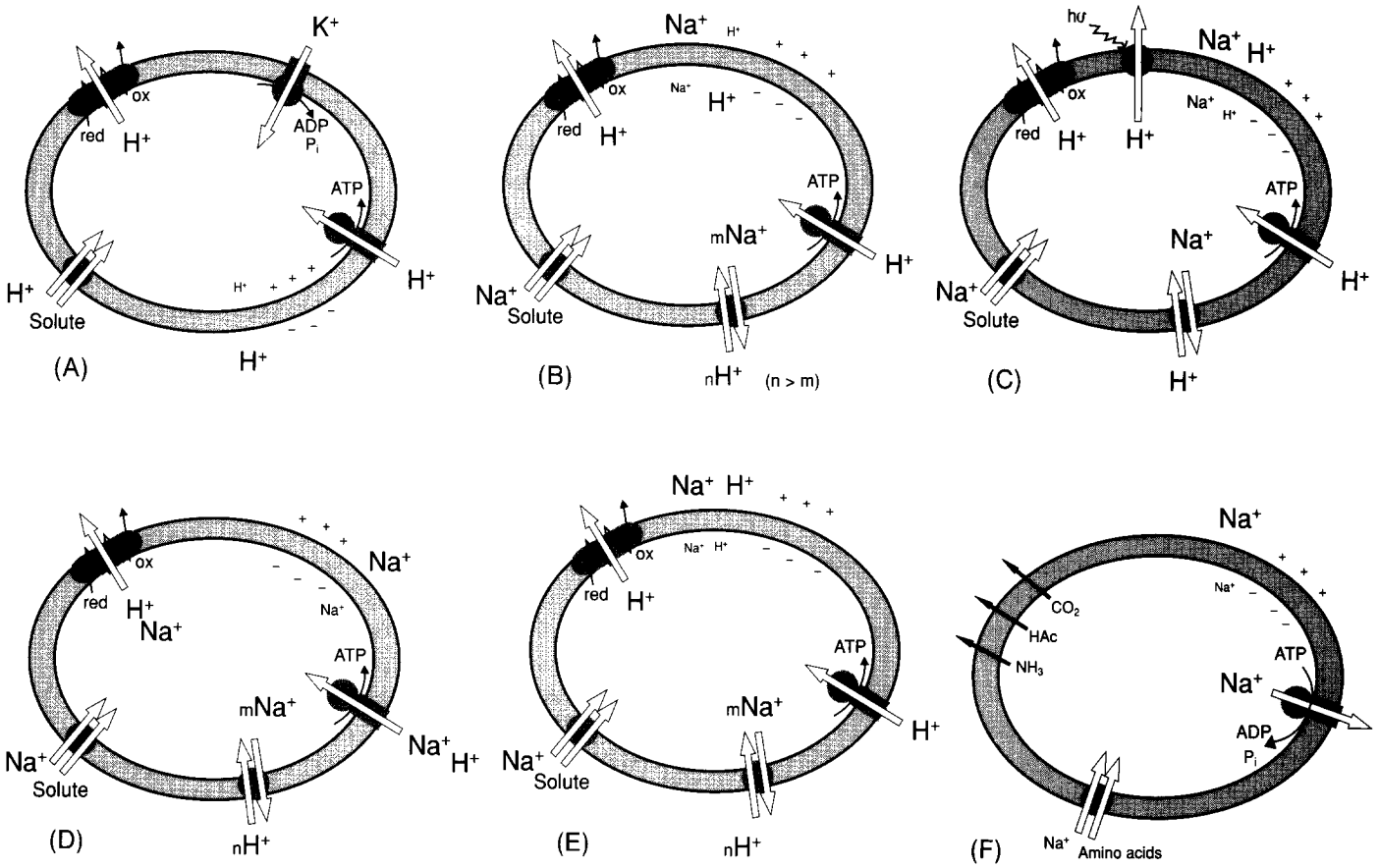

Figure 2. Schematic representation of energy-transducing processes in: (A) aerobic alkaliphiles (inside $\Delta\Psi$ positive relative to outside); (B) aerobic acidophiles (inside ΔpH acid relative to outside); (C) halophiles; (D) marine microorganisms; (E) aerobic thermophiles; and (F) the anaerobic thermophile *Clostridium fervidus*. See text for details.

Barophiles

Little is known about the physiology of barophiles. Barophiles grow optimally at high pressures, and this physical parameter can be important for psychrophiles (living in the deep ocean) and extreme thermophiles with an optimum temperature of growth $> 100°C$ (growing below the sea level). For the bacterium, NPT3, the proportion of unsaturated fatty acids is increased in order to maintain the membrane in a liquid–crystalline state at elevated pressure (DeLong & Yayanos 1985). It is not known whether H^+, Na^+ or both are used as coupling ions in energy transduction in this type of organism.

Psychrophiles

Psychrophiles have a low temperature of growth (below 20°C). The phospholipid composition of the cell membrane is such that it is in a liquid–crystalline phase at the low growth temperatures (Russell & Fukunaga 1990), thereby enabling the membrane-embedded enzymes to function properly at low temperatures. Little is known about the coupling ion(s) involved in energy-transduction, although for the marine bacterium *Vibrio* ABE-1 a Na^+-ATPase has been suggested (Harashima *et al.* 1993); this organism probably exhibits the same principles of energy transduction as mesophilic *Vibrio* spp. belonging to the class of marine bacteria.

Thermophiles

Thermophiles have an optimum temperature of growth above 65°C. The cytoplasmic membrane is in the liquid–crystalline phase at the optimum temperature of growth because it accomodates a high percentage of lipids with relatively long fatty-acid chains. Usually the membranes of thermophilic bacteria also contain a high amount of glycolipids (Russell & Fukunaga 1990). For an individual organism, an increase in growth temperature mainly affects fatty acyl chain composition; the affects on lipid content and head-group composition are relatively minor. In general, with increasing temperature of growth an increase in degree of saturation, chain length and/or *iso*-branching of the acyl chains is observed (Russell & Fukunaga 1990). Sometimes special lipids (the sterol-like hopanoids) are present in thermophiles: these may also reflect an adaptation to life at higher temperatures, by making the membrane more rigid (Ourisson *et al.* 1987). The membranes of thermophilic Archaea can contain tetraether lipids or mixtures of di- and tetra-ether lipids which stabilize the membrane and lower the

proton (ion) leakage relative to that of phospholipid bilayer membranes (Russell & Fukunaga 1990; Van De Vossenberg, S., Driessen, A.J.M. and Konings, W.N. unpublished work).

Bacillus stearothermophilus and PS3 contain a H^+-pumping respiratory chain (Fee *et al.* 1986; De Vrij *et al.* 1988). De Vrij *et al.* (1988) showed that although the membranes of *B. stearothermophilus* have a relatively high H^+ permeability at their optimal growth temperature, an active H^+-pumping respiratory chain compensates for this. As far as amino-acid transport has been studied in thermophilic bacteria, a clear preference for Na^+ as coupling ion is observed (Hirata *et al.* 1984; De Vrij *et al.* 1990; Heyne *et al.* 1991; Konings *et al.* 1992). To generate the Δs, PS3 uses a Na^+/H^+ antiporter (Goto 1980); ATP synthesis in this organism is H^+-coupled (Yoshida *et al.* 1977). Similarly, *Thermus thermophilus* and the archaeon *Sulfolobus acidocaldarius* contain a H^+-extruding respiratory chain and a V-type, H^+-coupled ATP synthase (Fee *et al.* 1986; Lübben & Schäfer 1987; Schäfer & Moll 1988; Yokoyama *et al.* 1990). Energy transduction in aerobic thermophiles is schematically shown in Figure 2E.

The acetogenic, anaerobic *Clostridium thermoautotrophicum* contains a H^+-extruding electron transfer chain (Hugenholtz *et al.* 1987), a H^+-coupled ATP synthase (Ivey & Ljungdahl 1986) and Δp-driven amino-acid uptake systems (Hugenholtz & Ljungdahl 1990). In the related *C. thermoaceticum*, electrogenic Na^+/H^+ antiport activity, which allows the cell to generate a Δs, has been demonstrated (Terraciano *et al.* 1987).

Clostridium fervidus is an anaerobic, peptidolytic thermophile (Speelmans *et al.* 1989) that generates a Δs by using a F/V-type Na^+-extruding ATPase (Speelmans *et al.* 1993a). In this bacterium, all amino-acid uptake systems studied to date are Na^+-coupled (Speelmans *et al.* 1989, 1993b). Surprisingly, however, Na^+/H^+ antiport activity could not be detected in the organism (Speelmans *et al.* 1993a). Since other H^+-pumping mechanisms are also lacking, the organism is unable to regulate its internal pH, and, consequently, is only able to grow in a narrow pH range (Speelmans *et al.* 1993a). As yet, *C. fervidus* is the only species for which there is solid evidence that Na^+/H^+ antiport activity is absent. The energy-transducing processes in this anaerobic thermophile are depicted in Figure 2F.

Advantages and Disadvantages of Na^+-coupled Energy Transduction in Extremophiles

Extremophiles other than Thermophiles

The possibility of switching between primary and secondary H^+ and Na^+ transport allows marine bacteria to adapt readily to conditions fluctuating in pH and salinity. In a marine algal mat the pH is neutral in the morning, and alkaline in the evening (Skulachev 1987). At alkaline pH, the Δp is low and lowering of the internal Na^+ concentration via a secondary Na^+/H^+ antiporter will not be very effective. Furthermore, if H^+ (or H_3O^+) and Na^+ (or Li^+) compete for a similar cationic binding site on transport enzymes and the pH is high ($[H^+] < 10^{-8}$), the enzymes may not be specific enough for H^+ (or H_3O^+) and may use Na^+ instead (at 0.5 M). This hypothesis implies that the use of a primary Na^+ pump at alkaline and high salt conditions has distinct advantages over a primary H^+ pump.

The secondary ΔpNa-generating system in extreme alkaliphiles is essential for adaptation at high pH (moderate salinity, non-marine). The low Δp (-25 to -50 mV) is due to the electrogenic Na^+/H^+ antiporter activity, which generates a reversed ΔpH (up to three pH units). The Na^+/H^+ antiporter has a crucial role in the homeostasis of the cytoplasmic pH and allows the cells to face extracellular pH values as high as 11 (Ivey *et al.* 1992). Since Δp is low and Δs is high in the alkaliphiles the use of Na^+ as coupling ion in, for instance, solute transport would be favourable (as large concentration gradients can be maintained).

Extreme halophiles are adapted to high intracellular Na^+ concentrations (up to 1 M) (Grant 1990). The organisms use a primary H^+ pump and use a Δp-dependent Na^+/H^+ antiporter to extrude Na^+ against a high extracellular Na^+ concentration (3.5 M NaCl). The Na^+ gradient (Na^+_{in}/Na^+_{out}) which can be generated by the Na^+/H^+ antiporter (Konishi *et al.* 1992) in the extreme halophiles is less than 10-fold ($\Delta pNa < -60$ mV) and, therefore, the use of H^+ as dominant coupling ion in energy transduction could be advantageous.

A primary Na^+ pump can generate a Δs which allows anaerobic organisms to cope with a low Δp, provided membrane-bound chemiosmotic reactions can use Na^+ as coupling ion. Since Na^+/H^+ antiporters require a high Δp to maintain a large Na^+ concentration gradient, this form of Na^+ export may not be sufficient for ΔpNa generation and/or Na^+ homeostasis at low Δp. Furthermore, the absence of a ΔpH allows a more efficient excretion of acidic end-products by passive efflux and/or H^+-symport mechanisms. If the excretion of acidic end-products together with protons has to proceed against a large ΔpH, the end-product concentrations inside the cell may increase to inhibitory levels.

Thermophiles

Thermophilic bacteria have to invest more energy to maintain a Δp than mesophiles, due to the relatively high ion (proton) permeability of the membrane at the optimal growth temperatures (Kuhn *et al.* 1980; De Vrij *et al.* 1988). Especially in thermophilic anaerobes, this may cause problems due to the limited amount of ATP that can be

supplied by the fermentative pathways. The permeability of the cytoplasmic membrane for Na^+ ions is much less than for H^+ ions. The permeability coefficient for Na^+ is approx. 10^{-14} cm/s at 25°C, whereas the permeability coefficients for H^+ vary between 10^{-4} and 10^{-8} cm/s at 25°C (Gennis 1989). Between 18 and 38°C, the rate of Na^+ influx was found to be 7 to 8 orders of magnitude lower than the rate of H^+ influx into liposomes (Speelmans 1993). At increasing temperatures, both the Na^+ and H^+ fluxes into the liposomes increase, but the absolute H^+ flux increases by much more than the Na^+ flux (Speelmans 1993). On the basis of these differences in ion permeability, the use of a ΔpNa is of considerable bioenergetic advantage for microorganisms, and in particular for thermophiles.

In the thermophilic anaerobe, *C. fervidus* the yield of metabolic energy per molecule of substrate is much less than in aerobes. Furthermore, ΔpH dissipating end-products (CO_2, NH_3 and acetic acid) are formed. Such a fermentative organism has to develop special mechanisms in order to cope with life at high temperature. The H^+ permeability of liposomes prepared from *C. fervidus* lipids does not differ significantly from that of liposomes prepared from *E. coli* phosphatidylethanolamine/egg phosphatidylcholine (Speelmans 1993). Hence, at its growth temperature (68°C) *C. fervidus* will be confronted with higher rates of passive H^+ diffusion across the membrane than mesophilic bacteria. Therefore, in thermophilic anaerobes, a relatively higher fraction of the metabolic energy formed needs to be spent on maintaining a high Δp than in aerobic thermophiles or mesophilic anaerobes. Since Na^+ is less membrane permeable than, H^+ less metabolic energy has to be invested to maintain high driving forces for energy-transducing processes when Na^+ is used as coupling ion. This advantage will only be valid if no H^+ cycling is present at the same time. This turns out to be the case in *C. fervidus*. The organism, however, has to pay a price for using Na^+ as the sole energy-coupling ion, since the pH range in which growth is possible becomes narrow. When no mechanisms are available for pH homeostasis, and the external pH changes as a result of bacterial metabolism (or otherwise) and the buffering capacity of the intracellular milieu is exceeded, the activity of pH-sensitive metabolic processes will decrease and ultimately affect growth. By fermenting amino acids *C. fervidus* produces both acidic and alkaline end-products, and this may keep the external pH relatively constant even at high cell densities. Furthermore, the organism can survive less favourable circumstances (e.g. a low or high pH) by forming spores.

Concluding Remarks

In recent years a wealth of data has become available about the bioenergetics of some extremophiles, such as extreme halophiles, aerobic alkaliphiles and aerobic thermophiles. The information, however, is less complete for energy-transducing processes in other types of extremophiles, such as acidophiles, barophiles, psychrophiles and various types of anaerobic extremophiles.

From the information currently available it can be concluded that prokaryotes have very versatile Na^+- and H^+-extrusion systems and very versatile ways to cope with high concentrations of internal Na^+, varying concentrations of H^+ or combinations of these parameters. In extremophiles in particular, growing under circumstances considered to be a stress condition for 'normal' bacteria, and often dealing with a low proton motive force, the role of Na^+ in energy transduction is crucial. The most extreme representative is the anaerobic thermophile *Clostridium fervidus*, the energy-transducing processes of which are completely dependent on Na^+ as coupling ion.

References

Abee, T. & Konings, W.N. 1990 The important role of internal pH in energy transduction in bacteria. *Trends in Biomembranes and Bioenergetics* **1**, 75–91.

Apel, W.A., Dugan, P.R. & Turtle, J.N. 1980 Adenosine 5'-triphosphate formation in *Thiobacillus ferrooxidans* vesicles by H^+ ion gradients comparable to those of environmental conditions. *Journal of Bacteriology* **142**, 295–301.

Atsumi, T., McCarter, L. & Imae, Y. 1992 Polar and lateral flagellar motors of marine *Vibrio* are driven by different ion-motive forces. *Nature* **355**, 182–184.

Avetisyan, A.V., Bogachev, A.V., Murtasina, R.K. & Skulachev, V.P. 1992 Involvement of a *d*-type oxidase in the Na^+-motive respiratory chain of *Escherichia coli* growing under low $\Delta\mu_H{}^+$ conditions. *FEBS Letters* **306**, 199–202.

Avetisyan, A.V., Dibrov, P.A., Semeykina, A.L., Skulachev, V.P. & Sokolov, M.V. 1991 Adaption of *Bacillus* FTU and *Escherichia coli* to alkaline conditions: the Na^+-motive respiration. *Biochimica et Biophysica Acta* **1048**, 95–104.

Bakker, E.P. 1990 The role of alkali-cation transport in energy coupling of neutrophilic and acidophilic bacteria: an assesment of methods and concepts. *FEMS Microbiological Reviews* **75**, 319–334.

Becher, B., Müller, V. & Gottschalk, G. 1992 The methyltetrahydromethanopterin:coenzyme M methyltransferase of *Methanosarcina* strain Gö1 is a primary sodium pump. *FEMS Microbiological Letters* **91**, 239–244.

Bogachev, A.V. & Skulachev, V.P. 1993 The role of protonic and sodium potentials in the motility of *E. coli* and *Bacillus* FTU. *Biochimica et Biophysica Acta* **1142**, 321–326.

Booth, I.R. 1985 Regulation of cytoplasmic pH in bacteria. *Microbiological Reviews* **49**, 395–398.

Boyer, P.D. 1988 Bioenergetic coupling to the proton motive force: Should we be considering hydronium ion coordination and not group protonation? *Trends in Biochemical Science* **13**, 5–7.

Castle, A.M., Macnab, R.M. & Schulman, R.T.G. 1986a Measurements of intracellular sodium concentration and sodium transport in *Escherichia coli* by $^{23}Na^+$ NMR. *Journal of Biological Chemistry* **261**, 3288–3294.

Castle, A.M., Macnab, R.M. & Schulman, R.T.G. 1986b Coupling between the sodium and the proton gradient in respiring

Escherichia coli cells measured by $^{23}Na^+$ NMR. *Journal of Biological Chemistry* **261**, 7797–7806.

Clejan, S. & Krulwich, T.A. 1988 Permeability studies of lipid vesicles from alkalophilic *Bacillus firmus* showing opposing effects of membrane isoprenoid and diacylglycerol fractions and suggesting a possible basis for obligate alkalophily. *Biochimica et Biophysica Acta* **946**, 40–48.

DeLong, E.F. & Yayanos, A.A. 1985 Adaption of the membrane lipids of a deep-sea bacterium to changes in hydrostatic pressure, *Science* **228**, 1101–1103.

De Vrij, W., Bulthuis, R.A. & Konings, W.N. 1988 Comparative study of the energy transducing properties of cytoplasmic membranes from a mesophilic and thermophilic *Bacillus* species. *Journal of Bacteriology* **170**, 2359–2366.

De Vrij, W., Speelmans, G., Heyne, R.I.R. & Konings, W.N. 1990 Energy transduction and amino acid transport in thermophilic aerobic and fermentative bacteria. *FEMS Microbiological Reviews* **75**, 183–200.

Dibrov, P. 1991 The role of sodium ion transport in *Escherichia coli* energetics. *Biochimica et Biophysica Acta* **1056**, 209–224.

Dimroth, P. 1980 A new sodium transport system energized by the decarboxylation of oxaloacetate. *FEBS Letters* **122**, 234–236.

Dimroth, P. 1987 Sodium ion transport: decarboxylases and other aspects of sodium ions cycling in bacteria. *Microbiological Reviews* **51**, 320–340.

Dimroth, P. 1991 Na^+ coupled alternate to H^+-coupled primary transport systems in bacteria. *BioEssays* **13**, 463–468.

Dimroth, P. 1992a Na^+ extrusion coupled to decarboxylation reactions. In *Alkali Cation Transport Systems in Prokaryotes*, ed Bakker, E.P. pp. 77–100. Boca Raton, FL: CRC Press.

Dimroth, P. 1992b The Na^+-translocating ATP-synthase from *Propionigenium modestum*. In *Alkali Cation Transport Systems in Prokaryotes*, ed Bakker, E.P. pp 139–154. Boca Raton, FL: CRC Press.

Dimroth, P. 1992c The ATPases of *Propionigenium modestum* and *Bacillus alcalophilus*. Strategies for ATP synthesis under low energy conditions. *Biochimica et Biophysica Acta* **1101**, 236–239.

Dmitriev, O., Deckers-Hebestreit, G. & Altendorf, K. 1993 ATP synthesis energized by ΔpNa and $\Delta\psi$ in proteoliposomes containing the F_0F_1-ATPase from *Propionigenium modestum*. *Journal of Biological Chemistry* **268**, 14776–14781.

Dmitriev, O.Y., Krasnoselskaya, I.A., Papa, S. & Skulachev, V.P. 1991 F_0F_1-ATPase from *Vibrio alginolyticus*. Subunit composition and proton pumping activity. *FEBS Letters* **284**, 273–276.

Dunkley Jr, E.A., Guffanti, A.A., Clejan, S. & Krulwich, T.A. 1991 Facultative alkaliphiles lack fatty acid desaturase activity and lose the ability to grow at near-neutral pH when supplemented with an unsaturated fatty acid. *Journal of Bacteriology* **173**, 1331–1334.

Efiok, B.J.S. & Webster, D.A. 1990 A cytochrome that can pump sodium ion. *Biochemical and Biophysical Research Communications* **173**, 370–375.

Fee, J.A., Kuila, D., Mather, M.W. & Yoshida, T. 1986 Respiratory proteins from extremely thermophilic, aerobic bacteria. *Biochimica et Biophysica Acta* **853**, 153–185.

Gambacorta, A., Gliozzi, A. & De Rosa, M. 1995 Archaeal lipids and their biotechnological applications. *World Journal of Microbiology and Biotechnology* **11**, 114–130.

Gennis, R.B. 1989 Interaction of small molecules with membranes: partitioning, permeability, and electrical effects. In *Biomembranes: Molecular Structure and Function*, ed Gennis, R.B. pp. 235–269. New York: Springer-Verlag.

Goto, K. 1980 A stable Na^+/H^+ antiporter of thermophilic bacterium PS3. *Journal of Bioenergetics and Biomembranes* **12**, 297–308.

Grant, W.D. 1990 General view of halophiles. In *Superbugs. Microorganisms in Extreme Environments*, eds Horikoshi, K. & Grant, W.D. pp. 37–50. Berlin: Springer-Verlag.

Guffanti, A.A. & Krulwich, T.A. 1992 Features of apparent non-chemiosmotic energization of oxidative phosphorylation by alkaliphilic *Bacillus firmus* OF4. *Journal of Biological Chemistry* **267**, 9580–9588.

Guffanti, A.A., Mann, M., Sherman, T. L. & Krulwich, T.A. 1984 Patterns of electrochemical proton gradient formation by membrane vesicles from an obligately acidophilic bacterium. *Journal of Bacteriology* **159**, 448–452.

Hama, H. & Wilson, T.H. 1993 Cation-coupling in chimeric melibiose carriers derived from *Escherichia coli* and *Klebsiella pneumoniae*. The amino-terminal portion is crucial for Na^+ recognition in melibiose transport. *Journal of Biological Chemistry* **268**, 10060–10065.

Harashima. A., Takada, Y., Fukunaga, N. & Sasaki, S. 1993 Membrane-bound ATPase of a psychrophilic marine bacterium, *Vibro* sp. strain ABE-1. *Bioscience and Biotechnological Biochemistry* **57**, 973–977.

Harold, F.M. & Van Brunt, J. 1977 Circulation of H^+ and K^+ across the plasma membrane is not obligatory for bacterial growth. *Science* **197**, 372–373.

Hellingwerf, K.J. & Konings, W.N. 1985 The energy flow in bacteria: the main free energy intermediates and their regulatory role. *Advances in Microbiological Physiology* **26**, 125–154.

Heise, R., Müller, V. & Gottschalk, G. 1989 Sodium dependence of acetate formation by the acetogenic bacterium *Acetobacterium woodii*. *Journal of Bacteriology* **171**, 5473.

Heise, R., Müller, V. & Gottschalk, G. 1993 Acetogenesis and ATP synthesis in *Acetobacterium woodii* are coupled via a transmembrane primary sodium ion gradient. *FEMS Microbiological Letters* **112**, 261–268.

Heyne, R.I.R., De Vrij, W., Crielaard, W. & Konings, W.N. 1991 Sodium ion-dependent amino acid transport in membrane vesicles of *Bacillus stearothermophilus*. *Journal of Bacteriology* **173**, 791–800.

Hicks, D.B. & Krulwich, T.A. 1990 Purification and reconstitution of the F_1F_0 ATP synthase from alkaliphilic *Bacillus firmus* OF4. *Journal of Biological Chemistry* **265**, 20547–20554.

Higgins, C.F. & Booth, I. 1987 In *Homeostatic Mechanisms in Microorganisms*, eds Whittenbury, R., Gould, G.W., Ganks, J.W. & Board, R.G. pp. 29–40. Bath, UK: Bath University Press.

Hilpert, W. & Dimroth, P. 1991 On the mechanism of sodium ion translocation by methylmalonyl-CoA decarboxylase from *Veillonella alcalescens*. *European Journal of Biochemistry* **195**, 79–86.

Hirata, H., Kambe, T. & Kagawa, Y. 1984 A purified alanine carrier composed of a single polypeptide from the thermophilic bacterium PS3 driven by either proton or sodium ion gradient. *Journal of Biological Chemistry* **259**, 10653–10656.

Hirota, N. & Imae, Y. 1983 Na^+-driven flagellar motors of an alkalophilic *Bacillus* strain YN-1. *Journal of Biological Chemistry* **258**, 10577–10581.

Hoffmann, A. & Dimroth, P. 1990 The ATPase of *Bacillus alcalophilus* — purification and properties of the enzyme. *European Journal of Biochemistry* **194**, 423–430.

Hoffmann, A. & Dimroth, P. 1991 The electrochemical proton potential of *Bacillus alcalophilus*. *European Journal of Biochemistry* **201**, 467–474.

Hugenholtz, J., Ivey, D.M. & Ljungdahl, L. 1987 Carbon-mononox-

ide driven electron transport in *Clostridium thermoautotrophicum*. *Journal of Bacteriology* **169**, 5845–5847.

Hugenholtz, J. & Ljungdahl, L. 1990 Amino acid transport in membrane vesicles of *Clostridium thermoautotrophicum*. *FEMS Microbiological Letters* **69**, 117–122.

Inatomi, K. 1986 Characterization and purification of the membrane-bound ATPase of the archaebacterium *Methanosarcina barkeri*. *Journal of Bacteriology* **167**, 839–841.

Ivey, D.M., Guffanti, A.A. & Krulwich, T.A. 1992 The Na^+ cycle in alkaliphilic *Bacillus* species. In *Alkali Cation Transport Systems in Prokaryotes*, ed Bakker, E.P. pp. 101–124. Boca Raton, FL: CRC Press.

Ivey, D.M. & Ljungdahl, L.G. 1986 Purification and characterization of the F_1-ATPase from *Clostridium thermoaceticum*. *Journal of Bacteriology* **165**, 252–257.

Kaback, H.R. 1990 Active transport: membrane vesicles, bioenergetics, molecules, and mechanisms. In *Bacterial Energetics* ed Krulwich, T.A. pp. 151–202. San Diego: Academic Press.

Kaesler, B. & Schönheit, P. 1989 The sodium cycle in methanogenesis. CO_2 reduction to the formaldehyde level in methanogenic bacteria is driven by a primary electrochemical potential of sodium generated by formaldehyde reduction to methane gas. *European Journal of Biochemistry* **186**, 309–316.

Kakinuma, Y. & Igarashi, K. 1990 Some features of the *Streptococcus faecalis* Na^+-ATPase resemble those of the vacuolar-type ATPases. *FEBS Letters* **271**, 97–101.

Khan, S. 1990 Motility. In *Bacterial Energetics*, ed Krulwich, T.A. pp. 301–344. San Diego: Academic Press.

Kinosita, N., Unemoto, T. & Kobayashi, H. 1984 Proton motive force is not obligatory for growth of *Escherichia coli*. *Journal of Bacteriology* **160**, 1074–1077.

Kluge, C. & Dimroth, P. 1992 Studies on Na^+ and H^+ translocation through the F_o part of the Na^+-translocating F_1F_o-ATPase from *Propionigenium modestum*: discovery of a membrane potential dependent step. *Biochemistry* **31**, 12665–12672.

Kobayashi, H., Suzuki, T., Kinoshita, N. & Unemoto, T. 1984 Amplification of the *Streptococcus faecalis* proton-translocating ATPase by a decrease in cytoplasmic pH. *Journal of Bacteriology* **158**, 1157–1160.

Konings, W.N., Tolner, B., Speelmans, G., Elferink, M.G.L., De Wit, J.G. & Driessen, A.J.M. 1992 Energy transduction and transport processes in thermophilic bacteria. *Journal of Bioenergetics and Biomembranes* **24**, 601–609.

Konishi, T., Murakami, N. & Miyazawa, Y. 1992 $\Delta\mu_{H^+}/\Delta\mu_{Na^+}$ exchange ratio in the halobacterial Na^+/H^+-antiporter determined by a simultaneous measurement of ΔpH, $\Delta\psi$ and ΔpNa. *Biochimica et Biophysica Acta* **1140**, 1–5.

Koyama, N., Niimura, Y. & Kozaki, M. 1988 Bioenergetic properties of a facultative anaerobic alkalophile. *FEMS Microbiological Letters* **49**, 123–126.

Kroll, R.G. & Booth, I.R. 1981 The role of potassium transport in the generation of a pH gradient in *Escherichia coli*. *Biochemical Journal*, 691–698.

Krulwich, T.A., Federbusch, J.G. & Guffanti, A.A. 1985 Presence of a non-metabolizable solute that is translocated with Na^+ enhances Na^+-dependent pH homeostasis in an alkalophilic *Bacillus*. *Journal of Biological Chemistry* **260**, 4055–4058.

Krulwich, T.A., Guffanti, A.A. & Seto-Young, D. 1990 pH homeostasis and bioenergetic work in alkalophiles. *FEMS Microbiological Reviews* **75**, 271–278.

Krulwich, T.A. & Ivey, D.M. 1990 Bioenergetics in extreme environments. In *Bacterial Energetics*, ed Krulwich, T.A. pp. 417–447. San Diego: Academic Press.

Krumholz, L.R., Esser, U. & Simoni, R.D. 1990 Characterization of the H^+ pumping F_1F_o ATPase of *Vibrio alginolyticus*. *Journal of Bacteriology* **172**, 6809–6817.

Kuhn, H.J., Cometta, S. & Fiechter, A. 1980 Effects of growth temperature on maximal specific growth rate, yield, maintenance and death rate in glucose-limited continuous culture of the thermophilic *Bacillus stearothermophilus*. *European Journal of Applied Microbiology and Biotechnology* **10**, 303–315.

Lanyi, J.K. 1979 The role of Na^+ in transport processes in bacterial membranes. *Biochimica et Biophysica Acta* **559**, 377–397.

Laubinger, W., Deckers-Hebestreit, G., Altendorf, K. & Dimroth, P. 1990 A hybrid adenosine triphosphatase composed of F_1 of *Escherichia coli* and F_o of *Propionigenium modestum* is a functional sodium pump. *Biochemistry* **29**, 5458–5463.

Laubinger, W. & Dimroth, P. 1987 Characterization of the Na^+-stimulated ATPase of *Propionigenium modestum* as an enzyme of the F_1F_o type. *European Journal of Biochemistry* **168**, 475–480.

Laubinger, W. & Dimroth, P. 1988a Characterization of the ATP synthase of *Propionogenium modestum* as a primary sodium pump. *Biochemistry* **27**, 7531–7537.

Laubinger, W. & Dimroth, P. 1988b The sodium translocating adenosine trophosphatase of *Propionigenium modestum* pumps protons at low sodium concentrations. *Biochemistry* **28**, 7194–7198.

Lübben, M. & Schäfer, G. 1987 A plasma-membrane associated ATPase from the thermoacidophilic archaebacterium *Sulfolobus acidocaldarius*. *European Journal of Biochemistry* **164**, 533–540.

Maloy, S.R. 1990 Sodium-coupled transport. In *The Bacteria*, Vol. 12, pp. 203–224.

Matin, A. 1990a Bioenergetic parameters and transport in obligate acidophiles. *Biochimica et Biophysica Acta* **1018**, 267–270.

Matin, A. 1990b Keeping a neutral cytoplasma; the bioenergetics of obligate acidophiles. *FEMS Microbiological Reviews* **75**, 307–318.

Mitchell, P. 1961 Coupling of phosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism. *Nature [London]* **191**, 144–148.

Mitchell, P. 1968 *Chemiosmotic coupling and energy transduction*. Bodmin, UK: Glynn Research.

Mitchell, P. 1976 *Journal of Theoretical Biology* **62**, 327–367.

Moll, R. & Schäfer, G. 1988 Chemiosmotic H^+ cycling across the plasma membrane of the thermoacidophilic archaebacterium *Sulfolobus acidocaldarius*. *FEBS Letters* **232**, 359–363.

Müller, V. & Gottschalk, G. 1992 Na^+ translocation in the course of methanogenesis from methanol or formaldehyde. In *Alkali Cation Transport Systems in Prokaryotes*, ed Bakker, E.P. pp. 155–178. Boca Raton, FL: CRC Press.

Mukohata, Y. & Isoyama, M. 1987 The proton-translocating ATP synthase in *Halobacterium halobium* differs from F_1F_o-ATPase/synthase. *Journal of Biochemistry* **102**, 797–802.

Muntyan, M.S. & Skripnikova, E.V. 1993 Two types of terminal oxidase in alkalotolerant *Bacillus* FTU. *Biochimica et Biophysica Acta* **1143**, 142–146.

Nakamura, T., Tokuda, H. & Unemoto, T. 1984 K^+/H^+ antiporter functions as a regulator of cytoplasmic pH in a marine bacterium *Vibrio alginolyticus*. *Biochemical and Biophysical Research Communications* **776**, 330–336.

Ourisson, G., Rohmer, M. & Poralla, H. 1987 Prokaryotic hopanonoids and other polyterpenoid sterol surrogates. *Annual Reviews in Microbiology* **41**, 301–335.

Padan, E. & Schuldiner, S. 1992 Na^+ transport systems in prokaryotes. In *Alkali Cation Transport Systems in Prokaryotes*, ed Bakker, E.P. pp. 3–24. Boca Raton, FL: CRC Press.

Poolman, B. 1993 Energy transduction in lactic acid bacteria. *FEMS Microbiological Reviews* **12**, 125–148.

Poolman, B. & Konings, W.N. 1993 Secondary solute transport in bacteria. *Biochimica et Biophysica Acta* **1183**, 5–39.

Poolman, B., Molenaar, D. & Konings, W.N. 1994 Diversity of transport mechanisms in bacteria. In *Handbook of Biomembranes. Vol II. Structural and Functional Aspects* ed Shinitzky, M. pp. 263–313. Rehovot, Israel: Balaban.

Pourcher, T., Zani, M.-L. & LeBlanc, G. 1993 Mutagenesis of acidic residues in putative membrane-spanning segments of the melibiose carrier of *Escherichia coli*. I. Effect on Na^+-dependent transport and binding properties. *Journal of Biological Chemistry* **268**, 3209–3215.

Russell, N.J. 1989 Adaptic modifications in membranes of halotolerant and halophilic microorganisms. *Journal of Bioenergetics and Biomembranes* **21**, 93–113.

Russell, N.J. & Fukunaga, N. 1990 A comparison of thermal adaptation of membrane lipids in psychrophilic and thermophilic bacteria. *FEMS Microbiological Reviews* **75**, 171–182.

Schäfer, G. & Moll, R. 1988 Chemiosmotic H^+ cycling across the plasma membrane of the thermoacidophilic archaebacterium, *Sulfolobus acidocaldarius*. *FEBS Letters* **232**, 359–363.

Schönheit, P. 1992 The role of Na^+ in the first step of CO_2 reduction to methane in methanogenic bacteria. In *Alkali Cation Transport Systems in Prokaryotes*, ed Bakker, E.P. pp. 179–202. Boca Raton, FL: CRC Press.

Schuldiner, S. & Padan, E. 1992 Na^+/H^+ antiporters in *Escherichia coli*. In *Alkali Cation Transport Systems in Prokaryotes*, ed Bakker, E.P. pp. 25–52. Boca Raton, FL: CRC Press.

Semeykina, A.L. & Skulachev, V.P. 1992 Appearance of the Na^+-motive terminal oxidase in *Bacillus* FTU grown under three different conditions lowering the $\Delta\mu_H{}^+$ level. *FEBS Letters* **296**, 77–81.

Siebers, A. & Altendorf, K. 1992 K^+-translocating Kdp-ATPases and other bacterial P-type ATPases. In *Alkali Cation Transport Systems in Prokaryotes*, ed Bakker, E.P pp. 225–252. Boca Raton, FL: CRC Press.

Skulachev, V.P. 1985 Membrane-linked energy transductions: H^+ is not unique as a coupling ion. *European Journal of Biochemistry* **151**, 199–208.

Skulachev, V.P. 1987 Bacterial sodium transport: bioenergetic functions of sodium ions. In *Ion Transport in Prokaryotes*, eds Rosen, B.P. & Silver, S. pp. 131–164. New York: Academic Press.

Skulachev, V.P. 1989 The sodium cycle: a novel type of bacterial bioenergetics. *Journal of Bioenergetics and Biomembranes* **21**, 635–648.

Sone, N. 1990 Respiration-driven proton pumps. In *Bacterial Energetics*, ed Krulwich, T.A. pp. 1–32. San Diego: Academic Press.

Speelmans, G. 1993 Na^+ and energy transduction in the thermophile *Clostridium fervidus*. PhD thesis. University of Groningen, The Netherlands.

Speelmans, G., De Vrij, W. & Konings, W.N. 1989 Characterization of amino acid transport in membrane vesicles from the thermophilic fermentative bacterium *Clostridium fervidus*. *Journal of Bacteriology* **171**, 3788–3795.

Speelmans, G., Poolman, B., Abee, T. & Konings, W.N. 1993a Energy transduction in the thermophilic anaerobe *Clostridium fervidus* is exclusively coupled to sodium ions *Proceedings of the National Academy of Sciences of the United States of America* **90**, 7975–7979.

Speelmans, G., Poolman, B. & Konings, W.N. 1993b Amino acid transport in the thermophilic anaerobe *Clostridium fervidus* is driven by an electrochemical sodium gradient. *Journal of Bacteriology* **175**, 2060–2066.

Sugiyama, S.H., Matsukura, H. & Imae, Y. 1985 Relationship between Na^+-dependent flagellar rotation and amino acid transport in alkalophilic *Bacillus*. *FEBS Letters* **182**, 265–268.

Takase, K., Yamato, I. & Kakinuma, Y. 1993 Cloning and sequence of the genes encoding for the A and B subunits of the vacuolar-type Na^+-ATPase from *Enterococcus hirae*. The existence of vacuolar- and F_0F_1-type ATPases in one bacterial cell. *Journal of Biological Chemistry* **268**, 11610–11616.

Terraciano, J.S., Schreurs, W.J.A. & Kashket, E.R. 1987 Membrane H^+ conductance of *Clostridium thermoaceticum* and *Clostridium acetobutylicum*: Evidence for electrogenic Na^+/H^+ antiport in *Clostridium thermoaceticum*. *Applied and Environmental Microbiology* **53**, 782–786.

Tokuda, H. 1992 The Na^+ cycle in *Vibrio alginolyticus*. In *Alkali Cation Transport Systems in Prokaryotes*, ed Bakker E.P. pp. 125–138. Boca Raton, FL: CRC Press.

Trumpower, B.L. 1990 The protonmotive Q cycle. *Journal of Biological Chemistry* **265**, 11409–11412.

Tsuchiya, T. & Wilson, T.H. 1978 Cation-sugar cotransport in the melibiose transport protein of *Escherichia coli*. *Membrane Biochemistry* **2**, 63–79.

Unemoto, T., Akagawa, A., Mizugaki, M. & Hayashi, M. 1992 Distribution of Na^+-dependent respiration and respiration-driven electrogenic Na^+ pump in moderately halophilic bacteria. *Journal of General Microbiology* **138**, 1999–2005.

Unemoto, T. & Hayashi, M. 1993 Na^+-translocating NADH-quinone reductase of marine and halophilic bacteria. *Journal of Bioenergetics and Biomembranes* **25**, 385–392.

Walderhaugh, M.O., Dosch, D.C. & Epstein, W. 1987 Potassium transport in bacteria. In *Ion Transport in Prokaryotes*, eds Rosen, B.P. & Silver, S. pp. 85–130. New York: Academic Press.

Yokoyama, K., Oshimo, T. & Yoshida, M. 1990 *Thermus thermophilus* membrane-associated ATPase. Indication of a eubacterial V-type ATPase. *Journal of Biological Chemistry* **265**, 21946–21950.

Yoshida, M., Sone, N., Hirata, H. & Kagawa, Y. 1977 Reconstitution of thermostable ATPase capable of energy coupling from its purified subunits. *Proceedings of the National Academy of Sciences of the United States of America* **74**, 936–940.

Zaccai, G. & Eisenberg, H. 1991 A model for the stabilization of halophilic proteins. In *Life under Extreme Conditions. Biochemical Adaptations*, ed Di Prisco, G. pp. 125–141. Berlin: Springer-Verlag.

Zani, M.-L., Pourcher, T. & LeBlanc, G. 1993 Mutagenesis of acidic residues in putative membrane-spanning segments of the melibiose carrier of *Escherichia coli*. II. Effect on cationic selectivity and coupling properties. *Journal of Biological Chemistry* **268**, 3216–3221.