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Review

The past and present of sodium energetics: May the sodium-motive force be with you

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ABSTRACT

All living cells routinely expel Na^+ ions, maintaining lower concentration of Na^+ in the cytoplasm than in the surrounding milieu. In the vast majority of bacteria, as well as in mitochondria and chloroplasts, export of Na^+ occurs at the expense of the proton-motive force. Some bacteria, however, possess primary generators of the transmembrane electrochemical gradient of Na^+ (sodium-motive force). These primary Na^+ pumps have been traditionally seen as adaptations to high external pH or to high temperature. Subsequent studies revealed, however, the mechanisms for primary sodium pumping in a variety of non-extremophiles, such as marine bacteria and certain bacterial pathogens. Further, many alkaliphiles and hyperthermophiles were shown to rely on H^+, not Na^+, as the coupling ion. We review here the recent progress in understanding the role of sodium-motive force, including (i) the conclusion on evolutionary primacy of the sodium-motive force as energy intermediate, (ii) the mechanisms, evolutionary advantages and limitations of switching from Na^+ to H^+ as the coupling ion, and (iii) the possible reasons why certain pathogenic bacteria still rely on the sodium-motive force.

1. Introduction

The transmembrane electrochemical gradient of H^+ ions (proton-motive force, or *PMF*) is the source of energy for a variety of cellular processes in most bacteria, mitochondria, and chloroplasts. A typical H^+ cycle includes the generation of *PMF* by primary transport systems (H^+ pumps) and its utilization for ATP synthesis, solute transport, motility, reverse electron transport, etc [1,2]. In bacteria, protons are expelled by the cell, so that their concentration inside is less than outside. However, maintaining high levels of *PMF* becomes increasingly difficult for the bacteria living in alkaline environments, where the external concentration of H^+ ions is low [1,3–6]. To reconcile the life at alkaline pH values with the chemiosmotic principle, Skulachev proposed that alkaliphilic bacteria could use Na^+ as a coupling ion instead of or in addition to H^+ and coined the term "Sodium World" [1]. Another reason for a switch from H^+ to Na^+ was suggested by Konings and co-workers, who observed a rapid increase of proton leakage through the membrane at elevated temperatures [7] and reasoned that hyperthermophilic bacteria would benefit from relying on the sodium-motive force (*SMF*) instead of *PMF* [8,9]. Similarly to the H^+ cycle, the Na^+ cycle would include generators of *SMF* (primary Na^+ pumps) and *SMF* consumers, such as Na^+-translocating membrane ATP synthase, Na^+-dependent membrane transporters for nutrient uptake and/or a Na^+-dependent flagellar motor for motility.

The idea that *SMF* might substitute for *PMF* has been verified by studies of such anaerobic bacteria as *Propionigenium modestum*, *Malonomonas rubra*, and *Clostridium* (renamed *Caloramator*) *fervidus*, which rely exclusively on Na^+ ions for their energy metabolism [10–12]. However, none of these bacteria was either an alkaliphile or a hyperthermophile (*C. fervidus* is a moderate thermophile growing optimally at 60 °C). Further, recent studies as well as analyses of the genomic data have questioned the very premise that alkaliphiles and/or hyperthermophiles must rely on the *SMF* to overcome their energetic difficulties. Several alkaliphiles and hyperthermophiles had no (known) primary Na^+ pumps encoded in their genomes [13–17]. In contrast, Na^+ cycling has been inferred for a number of mesophilic bacteria, including various marine strains (reviewed in [18]) and several important human pathogens [19,20]. As a result, the reasons why certain bacteria and archaea depend on the *SMF* or *PMF* for their energy metabolism became even more obscure than ever before.

Abbreviations: *PMF*, proton-motive force; *SMF*, sodium-motive force; NaNQR, Na^+-translocating NADH:ubiquinone oxidoreductase

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We review here the recent progress in understanding the role of sodium-motive force, including the conclusion that *SMF* was the initial form of membrane energy intermediate, the mechanisms and evolutionary advantages of switching from Na^+ to H^+ as the coupling ion, and the possible reasons why certain pathogenic bacteria still rely on the sodium-motive force. We try to minimize the overlap with several excellent reviews of the Na^+-dependent systems published in the past several years [21–27]. Instead, based on own attempts to understand the different facets of the Sodium World [20,28–30], we try to provide here a holistic picture of this world in its evolutionary development.

2. Structural organization of the F-type and V-type ATPases and similarity of their Na^+-binding sites

While the *PMF* and *SMF* can be generated by several different enzymes (see below), the only enzyme capable of catalyzing *PMF*- or *SMF*-energized ATP synthesis is the membrane ATP synthase. Therefore the coupling ion specificity of the ATP synthase defines whether the organism relies on a Na^+- or H^+-type membrane energetics.

As shown in Fig. 1, ATP synthases are rotary molecular machines that exist in two distinct types, namely, the F-type that is present in bacteria, some archaea, and eukaryotic organelles [30–35] and the V-type that unifies ATPases of archaea and some bacteria [30,36–41] with the eukaryotic V-type ATPases, which routinely hydrolyze ATP to acidify certain cellular compartments, in particular, the vacuoles [37,40,42]. Some authors classify the simpler, prokaryotic V-type ATPases into a separate subgroup of A-type (from archaeal) ATPases/ATP synthases [39,43,44]. Others, instead, prefer to speak about prokaryotic and eukaryotic V-type ATPases [30,37,38,40,41].

All F/V-ATPases have a mushroom-like structure, with the hexameric head part (F_1 or V_1, respectively) protruding ~100 Å from the membrane and carrying ATP/ADP-binding catalytic sites (see Fig. 1 and Refs. [39–41,45,46]). The F/V-type ATPases are rotary dynamo machines in which the central stalk together with the membrane oligomeric ring is thought to slide along the interface with the stator membrane subunits bound via the peripheral stalk to the catalytic hexamer (see Fig. 1 and [31,34,47,48]). This sliding movement is coupled to the transmembrane ion transfer [1,49–52].

While sharing a common overall scaffold (Fig. 1), F-type and V-type ATPases differ in many structural and functional features (see Fig. 1 and [40,42,46,53]). In phylogenetic trees, bacterial, archaeal and eukaryotic V-type ATP synthases/ATPases invariably cluster together and separately from the F-type ATPases [54–56].

Proton-translocating and Na^+-translocating forms are found among both F- and V-type ATPases. In the absence of Na^+ ions, Na^+-translocating ATPases of F- and V-types could translocate protons [57,58]. In contrast, H^+-translocating ATPases are apparently incapable of translocating Na^+ ions [59]. This asymmetry is most likely due to the higher coordination number of Na^+, which requires six ligands to draw it from water and to keep it in the non-polar inner space of the membrane [60]. Comparative studies of the membrane rotor subunits c of the Na^+-translocating and H^+-translocating ATPases identified several residues that were involved in Na^+ binding and served as principal determinants of the coupling ion specificity [59,61–63]. However, the exact modes of Na^+ ion binding in the F- and V-ATPases remained obscure until the structures of the membrane-spanning, rotating oligomers of the Na^+-translocating ATP synthases of the F- and V-type were resolved [64,65]. These structures revealed the same overall configuration of the Na^+-binding sites, including conservation of all key ligands (Fig. 2). The minor difference between the two binding sites turned out to be the presence of a Gln residue that could bind the Na^+ ion directly in the c/K subunit of a V-type ATPase [65], whereas the corresponding Thr residue in the *c* subunit of the F-type ATPase apparently coordinates the Na^+ ion via an additional water molecule (Thomas Meier, personal communication). As seen in Fig. 2, the structural conservation even extends to the non-ligating Tyr residue that is important for stabilization of the principal, Na^+-binding Glu residue [58].

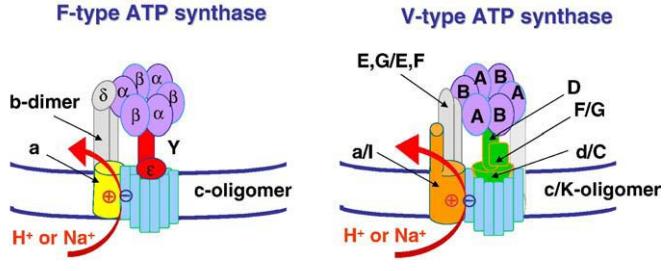

Fig. 1. Similar and distinct features in the organization of prokaryotic F- and V-type ATPases. Homologous subunits in the two types of ATPases are indicated by identical colors and shapes, whereas analogous by evolutionarily unrelated subunits of the central stalk are shown by different colors and shapes. Subunits that show structural analogy but do not appear to be homologous are shown by different but similar colors. The dual notation of some V-ATPase subunits (e.g., c/K) reflects their designations in eukaryotic and prokaryotic V-ATPases, respectively. For further details, see Ref. [53].

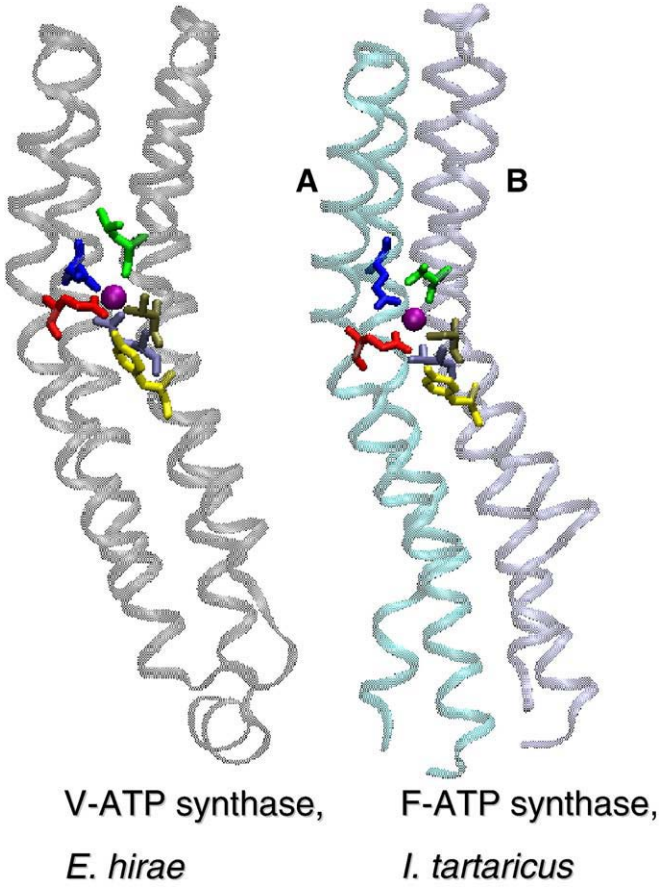

Fig. 2. Similar structural organization of membrane rotor subunits and their Na^+-binding sites in the F- and V-type Na^+-translocating ATP synthases. The structures were drawn by using the VMD software package [123]. Left panel, a single K subunit of the Na^+-translocating V-type ATP synthase of *Enterococcus hirae* (Protein Data Bank entry 2BL2 [65]); right panel, two c subunits, A (cyan) and B (ice-blue), of the Na^+-translocating F-type ATP synthase of *Ilyobacter tartaricus* (Protein Data Bank entry 1YCE [64]). In both structures, the Na^+ ion is shown as a purple ball, amino acid residues that coordinate the Na^+ ion are shown in stick representation and colored. The principal Na^+-coordinating Glu residue (Glu65A in *I. tartaricus* and Glu139 in *E. hirae*) is colored red; other Na^+ ligands are colored as follows: Gln32A in *I. tartaricus* and Gln110 in *E. hirae*, blue; Ser66B in *I. tartaricus* and Thr64 in *E. hirae*, tan; Val63B in *I. tartaricus* and Leu61 in *E. hirae* (which coordinate Na^+ with their backbone carbonyls), green. One more bond is provided by a grey-colored Gln65 in *E. hirae* and, via a water molecule, by a Thr67B in *I. tartaricus* (T. Meier, personal communication). The remaining sixth bond is provided, most likely, by the unseen water molecules as discussed in more detail elsewhere [30]. The Tyr residue (Tyr70B in *I. tartaricus* and Tyr68 in *E. hirae*) that is important for stabilization of the principal Na^+-binding Glu residue [58] is colored yellow.

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3. Ancestral status of the Na^+ bioenergetics

Deciphering of binding modes of Na^+ ions in the F-type and V-type ATPases made it possible to address the question of the cation specificity of the common ancestor of these two types of membrane ATPase. The nearly identical arrangement of the Na^+-binding sites in F-type and V-type c subunits (Fig. 2) strongly suggests that the common ancestor of V-type and F-type ATPases also was a Na^+-translocating enzyme. In the phylogenetic tree of the F/V-ATPases, as shown elsewhere [30], Na^+-translocating ATPases did not form a clade within either the F or the V branch but instead comprised three distinct lineages among V-ATPases and at least three lineages among F-ATPases with the same set of Na^+ ligands conserved in each of these clades. Independent emergence of nearly identical sets of Na^+ ligands in several distinct lineages does not look plausible, leading to the conclusion that the common ancestor of V- and F-ATPases had a Na^+-binding site [30]. This would mean that the ability to translocate Na^+ was independently lost in multiple lineages of F-type and V-type ATPases, yielding H^+-translocating enzymes. Multiple instances of ligand loss in the absence of purifying selection are far more plausible than multiple instances of ligand gain and are consistent with the conserved sequence patterns in the vicinity of the H^+-binding Glu residue in the c subunits of various bacterial phyla [30]. As discussed above, this also indicates that the primordial organism that harbored this ancestral enzyme possessed a Na^+-based membrane energetics.

The possibility that sodium bioenergetics antedated proton bioenergetics has been considered previously [1,20,66] but has not drawn much attention, and proton-dependent energy metabolism is widely believed to be ancestral [67]. The primacy of proton bioenergetics is attractive considering the intrinsic chemical coupling between protonation/deprotonation events and redox reactions, in particular, those of water and diverse quinones [68–70]. However, the complexity of the proton-tight membranes serves as a powerful argument against proton energetics being the ancestral state. Indeed, the transition to proton energetics could not have happened until the cellular membranes became impermeable for protons. As argued elsewhere [30,71,72] and discussed in some detail below, organization of such membranes is anything but trivial and their origin must have involved several distinct evolutionary steps.

4. Emergence of membrane bioenergetics in the Sodium World

It is conceivable that the functional evolution of the F/V-ATPases was determined by the evolution of the membranes and not the other way around [30], so that the tentative evolutionary scenario has to consider parallel, coupled changes of membrane enzymes and membranes per se. One such evolutionary reconstruction suggested that F/V-ATPases evolved from ATP-dependent RNA/protein translocases that functioned within primordial membranes, which were permeable for both H^+ and Na^+ but not for RNA or protein [53]. The ancient translocase could have used a ring of small membrane subunits to form a membrane channel and could employ Na^+ ions to crosslink and stabilize them (as in *Ilyobacter tartaricus* [64], see Fig. 2), preventing eventual destruction of the channel by the translocated polymer. This suggestion is supported by experiments demonstrating a dramatic decrease in the stability of the c-oligomers of Na^+-translocating F-ATPases from *I. tartaricus* and *P. modestum* in the absence of Na^+ [73]. Thus, even if primordial membranes were leaky for both Na^+ and H^+, there could have been a mechanistic demand for Na^+-binding and, accordingly, selection for the corresponding set of amino acid ligands.

The next stage of evolution could be envisaged as selection for tighter membranes that would maintain the ionic homeostasis of the evolving cells, and concomitantly, would create the opportunity for the utilization of ion gradients. As suggested by Skulachev [1], sodium-tight membranes could precede proton-tight membranes as structurally less demanding (see also below). With sodium-impermeable membranes, a mechanism for pumping Na^+ out of the cell, in response to the increasing salinity of the primordial ocean, would provide a clear evolutionary advantage, driving the transition from an ATP-dependent protein translocase to an ion-translocating membrane ATPase (see [53] for details). The common ancestor of the F- and V-ATPases, thanks to its rotating scaffold, would then be able to translocate Na^+ ions in both directions, depending on the magnitude of the *SMF*. Upon further increase in the external salinity, reversal of the rotation could result in the Na^+-driven synthesis of ATP by this primordial rotary machine.

The transition from an ATPase to an ATP synthase could only have happened if other primary Na^+ pumps were available for maintaining the Na^+ gradient. Several classes of such pumps have been characterized. The first one includes Na^+-transporting oxaloacetate decarboxylase, described in 1980 by Dimroth [74], and similar biotin-dependent membrane-bound enzymes that couple export of Na^+ ions to the decarboxylation of malonate, methylmalonyl-CoA, or glutaconyl-CoA (reviewed in [27]). The second class of primary Na^+ pumps is represented by the Na^+-translocating N^5-methyltetrahydromethanopterin: coenzyme M methyltransferase [75], detected so far only in methanogenic archaea (reviewed in [76]). One more class of primary Na^+ pumps includes Na^+-transporting ATPases that belong to the ABC (ATP binding cassette)-type or P-type ATPase families. An ABC-type Na^+-transporting ATPase has been described in *Bacillus subtilis* [77], while a P-type Na^+-transporting ATPase has been reported in another member of Bacillaceae, the alkaliphilic bacterium *Exiguobacterium aurantiacum* [78]. Close homologs of these enzymes are found in a variety of other bacteria, suggesting that ATP-dependent export of Na^+ might be widespread in the microbial world. The next class of primary Na^+ pumps is represented by the Na^+-translocating NADH:ubiquinone oxidoreductase (NaNQR), a respiratory Na^+ pump, first described in a marine bacterium *Vibrio alginolyticus* by Tokuda and Unemoto [79], and a closely related RNF enzyme, originally identified by its role in *Rhodobacter* nitrogen fixation [80] and proposed to function as a Na^+-translocating ferredoxin:NAD oxidoreductase [23].[1]

A recent work by Malinen et al. [90] identified an entirely new, fifth, class of primary Na^+ pumps. The membrane-bound inorganic pyrophosphatase (PPase) has been long known to be an energy-linked enzyme, capable of coupling pyrophosphate hydrolysis to the transfer of H^+ ions across the membrane. Previous studies of the membrane-bound PPase from *Thermotoga maritima* showed that its activity was Na^+-dependent [91], suggesting that this enzyme might translocate Na^+ ions. A direct study of ion translocation by *T. maritima* PPase expressed in *E. coli* and characterized in sub-bacterial membrane vesicles proved it to be an electrogenic Na^+ pump extruding sodium ions from the cytoplasm at the expense of pyrophosphate hydrolysis. The ability to translocate Na^+ ions was also demonstrated for the closely related PPase enzymes from the moderately thermophilic bacterium *Moorella thermoacetica* and the mesophilic archaeon *Methanosarcina mazei* [90]. Given that close homologs of these PPases are found in a wide variety of bacteria and archaea, generation of the *SMF* at the expense of pyrophosphate may turn out to be a very common mechanism of membrane energy transformation.

Out of the five classes of primary sodium pumps, at least two, namely the Na^+-pumps related to the Na^+-transporting decarboxylases [27,57] and the Na^+-transporting pyrophosphatase [90], are present both in bacteria and archaea and thus appear to antedate the divergence of the three domains of life. It would be reasonable to

[1] There have been reports of Na^+-translocating terminal oxidases in a variety of bacteria, including *Escherichia coli*, *Vibrio alginolyticus*, *Bacillus halodurans*, and *Vitreoscilla* sp. [81–85]. However, most of those studies were conducted on whole cells and the ability of any terminal oxidase to transport Na^+ ions still remains to be confirmed with a purified enzyme preparation. One more enzyme, formyl-methanofuran dehydrogenase of *Methanothermobacter thermautotrophicus* and other methanogenic archaea, can translocate sodium ions [86] but normally works in the reverse direction, using the *PMF* or *SMF* to generate an electron donor for CO_2 reduction [87–89].

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assume that the plethora of Na^+-coupled secondary membrane transporters, encoded in various bacteria and archaea, also originate from the Sodium World. Many of these Na^+-dependent transporters do not have proton-driven counterparts, so substrate transport into the cell essentially depends on a system of Na^+/H^+ exchangers transmitting part of the *PMF* into *SMF* even in the organisms with proton energetics (reviewed in [92,93]).

Taken together, ancient Na^+ pumps, the Na^+-driven ATP synthase, and Na^+-driven secondary transporters could complete the first, sodium-dependent energy cycle in the primitive cell membrane, marking the onset of the membrane bioenergetics.

5. Evolutionary advantages and constraints of switching from Na^+ to H^+ energetics

As argued by Haines, proton impermeability of the membrane is achieved by tighter packing of atoms in the middle of the bilayer, between its two leaflets [71]. Representatives of the three domains of life utilize distinct solutions to make their membranes tighter to protons [26,30,71,72], which additionally buttresses the suggestion on the independent transition from the sodium to proton energetics in different lineages. Anyway, the majority of bacteria, archaea and eukaryotes all found ways to make the membrane proton-tight (with a notable exception of animal plasma membranes that remained "sodium membranes" [1]).

As briefly noted above, proton energetics is chemically more advantageous. The use of H^+ as a coupling ion offers the benefit of direct mechanistic linkage of scalar redox reactions to vectorial translocation of proton across the membrane, resulting in the generation of the *PMF*. Thus, a plethora of primary redox-driven H^+ translocators with different characteristic mid-point potentials became available for the organization of orderly electron-transfer chains with multiple coupling sites, sequentially covering the redox span of about 1.2 eV from organic substrates to oxygen [1,2]. In contrast, the (known) redox-driven Na^+ pumps operate over a much smaller redox gap, namely from NADH to quinone in case of the NQR and from ferredoxin to NAD^+ in case of RNF. Besides, the number of required coordinating ligands for the proton is either one (for H^+) or three (if H_3O^+ is translocated [94,95]), compared to as many as six for the Na^+ ion [60]. Not surprisingly, after membranes became proton-tight, the more robust and versatile proton-transporting devices spread all over the microbial world. Why then proton energetics did not completely replace the sodium energetics? Why do some organisms still rely upon sodium energetics?

In the historical perspective, Na^+ ion cycle was originally proposed as an adaptive mechanism to solve the bioenergetic problems arising in organisms populating the habitats where generation and maintenance of sufficiently high *PMF* seems to be impossible, most importantly, in extreme alkaliphiles [1,3–5]. In addition, Na^+ ion cycle has been suggested as a solution for extreme thermophiles. Indeed, prokaryotic growth at high temperatures is limited by the increased permeability of the cytoplasmic membrane for ions resulting in low levels of the total *PMF* [7]. Remarkably, membrane permeability for Na^+ ions is several orders of magnitude lower than for protons [7,26], due to the fundamentally different mechanisms of H^+ and Na^+ transfer across the membrane [30,71,96–99].

An important consequence of the structural characterization of the Na^+-binding sites in the c subunits of F-type and V-type ATPases [64,65] was that it finally allowed unequivocal assignment of the cation specificity for the membrane ATPases encoded in numerous sequenced genomes [30] and, accordingly, a large-scale check of the correlations between the growth conditions of various organisms and the types of membrane energetics they employ. These assignments, while confirming many earlier observations, brought some surprising results.

The original concept of the Na^+ ion cycle as an adaptation to alkaline environments proved to be correct only for a limited number of organisms. While some alkaliphiles have a fully functioning Na^+ cycle, i.e. encode one or more primary Na^+ pumps and have a Na^+-dependent F- or V-type ATP synthase, others can grow at pH 10 and above using H^+ as the coupling ion (Table 1). These data clearly show that Na^+ cycle is not necessary for survival in alkaline environments, confirming previous results from the Krulwich group (reviewed in [24,100–102]), as well as reports by others [17,103].[2]

Table 1
Utilization of Na^+ and H^+ cycle by alkaliphilic bacteria and archaea

Organism	Growth at pH		Na^+ pumps[a]	ATPase ion specificity[b]
	Optimum	Maximum		
Archaea				
Natronomonas pharaonis	8.5	11.0	–	H^+
Bacteria				
Alkalilimnicola ehrlichei	9.3	10.2	–	H^+
Alkaliphilus metalliredigens	9.6	11.0	OAD (2x) PP, RNF	Na^+
Bacillus halodurans	9.0	10.8	–	H^+
Bacillus clausii	9.0	10.5	–	H^+
Clostridium paradoxum	9.3	10.2	nd	**Na^+**
Vibrio cholerae	7.6	9.6	NQR, OAD, RNF	**H^+**
Vibrio parahaemolyticus	7.8–8.6	11.0	NQR, OAD, RNF	H^+
Vibrio vulnificus	7.8	10.0	NQR, OAD, RNF	H^+
Yersinia enterocolitica	7.4	10.0	NQR, RNF	H^+

[a] The Na^+ pumps encoded in completely sequenced genomes are abbreviated as follows: NQR, Na^+-translocating NADH:quinone oxidoreductase; OAD, Na^+-translocating oxaloacetate decarboxylase (*A. metalliredigens* carries two *oad* operons); PP, Na^+-translocating pyrophosphatase; RNF, putative Na^+-translocating ferredoxin:NAD^+ oxidoreductase. A dash indicates absence of the encoded Na^+ pumps, nd — no data (*Clostridium paradoxum* genome has not been sequenced).

[b] Cation specificity of the respective ion-translocating ATPases has been predicted based on the presence or absence of the complete set of Na^+ ligands (see Fig. 1). Experimentally characterized ion selectivity is indicated by bold typeface.

How can alkaliphilic, proton-dependent prokaryotes manage to energize ATP synthesis in the conditions of high pH? The first reports of proton energetics in extreme alkaliphiles already asked for consideration of so-called local coupling mechanisms [6]. One group of such hypothetical mechanisms implies that the effective concentration of protons on the external surface of the bacterial cell membrane is higher than that in the bulk; a rapid lateral H^+ movement between the respiratory H^+ pump and the ATP synthase could then be able to support ATP synthesis [6,105–107]. This mechanism might rely on a particular trait of the membrane/water interface: the negatively charged membrane surface is separated from the bulk water phase by an electrostatic barrier. For protons, its height could be estimated as about 0.12 eV, which is high enough to keep the pH value at the external surface of metabolizing alkaliphiles neutral even if the surrounding medium is strongly alkaline [29,108,109]. Structural analysis of prokaryotic proton pumps has revealed that their periplasmic surface is covered by Asp and Glu residues that should facilitate proton transfer along the membrane/water interface [29]. Further on, a direct intramembrane transfer of H^+ from respiratory complex to F_O portion of the ATP synthase was suggested to occur via protein–protein interaction [6], a mechanism that echoes the original hypothesis by R.J.P. Williams [105,110]. A recent paper from the Krulwich group shows a direct interaction between cytochrome caa_3 and the F-type ATP synthase in liposomes made from the alkaliphile *Bacillus pseudofirmus* [111]. The two mechanisms of local coupling, in fact, are not contradictory. The already noted structural analysis of prokaryotic proton pumps has also revealed a buried plexus of Arg and Lys residues beyond the surface layer of acidic side chains. These buried bases should operate as proton buffers — proton sponges (see

[2] It should be noted that no genome sequences of obligate alkaliphilic bacteria (defined by Horikoshi as organisms that require pH 9 or more for their growth and have an optimal growth pH of around 10 [104]) are available at this time.

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[29] and references therein). Proton transfer between the sponges of two neighboring enzymes is well imaginable. The potential importance of such proton transfer is supported by the finding that replacement of a single Lys residue in the F_O part of the alkaliphilic ATP synthase of *B. pseudofirmus* by Gly leads to the loss of the non-fermentative, respiration-driven growth at high pH [112].

As follows from Table 2, the idea that sodium cycle was indispensable for growth at high temperatures also did not prove to be valid. Certain bacterial and archaeal hyperthermophiles (defined as organisms that grow optimally at or above 77 °C) encode primary Na^+ pumps and have a Na^+-dependent ATP synthase, indicating that they do rely on the Na^+ ion cycle for their energy metabolism (see also [113]). Nevertheless, a significant number of hyperthermophilic bacteria and archaea do not encode any (known) primary Na^+ pumps and have H^+-translocating ATP synthases (Table 2). Thus, a functional Na^+ cycle is not necessary for survival at high temperature either. How then would bacterial cells overcome massive transmembrane proton leakage at high temperatures? Or, rather, which factors define whether a particular group of organisms uses H^+ or Na^+ as the coupling ion?

Comparative analysis of microbial genomes clearly demonstrates that the distribution of the Na^+ cycle in hyperthermophilic species is rather patchy and does not correlate with the optimal growth temperature (Table 2). However, there is a clear correlation between the H^+ cycle and utilization of oxygen: every archaeal and bacterial hyperthermophile capable of growing in aerobic or microaerophilic conditions has an H^+-translocating ATP synthase, even if it encodes a potential Na^+ pump and uses *SMF* to energize substrate symports and/or motility. Hyperthermophilic anaerobes that utilize such high-potential alternative electron acceptors as nitrate, sulfate, or sulfite also tend to have an H^+-translocating ATP synthase (Table 2). The Na^+-translocating ATP synthase, and, accordingly, the full Na^+ cycle are found primarily in anaerobic hyperthermophiles that grow by fermentation. We think that this trend, as well as the distribution of the Na^+ cycle in analyzed genomes in general, could be explained by the following simple considerations:

(1) The H^+ cycle represents a relatively recent evolutionary acquisition that spread only after proton-tight membranes appeared, as discussed in detail previously [30]. Importantly, the proton-tightness of modern membranes is not absolute: even with all the improvements their conductivity to protons is by orders of magnitude higher than to Na^+ ions [71,96–99]. Accordingly, the organisms that rely on proton energetics have to cope with a notable leakage of H^+ ions that should dramatically increase with temperature [7,26] and with the rise in the external pH. Although psychrophilic and mesophilic bacteria, as well as archaea, are able to adjust the lipid composition of their membranes in order to limit the H^+ permeability (so-called "homeo-proton permeability adaptation"), the capacity of such adjustment is limited [26,71,114,115].

(2) As discussed above, the H^+ energetics is, generally, lucrative; that is why it is employed by mitochondria, chloroplasts, and the vast majority of modern microorganisms. Using a respiratory chain and the H^+ cycle, it becomes possible to gain plenty of free energy upon oxidation of external substrates — but only if the high-potential electron acceptors, such as oxygen, nitrate, sulfate, or sulfite, and respective oxidoreductases are available. Otherwise, the energy gain from proton energetics becomes no better than that from sodium energetics, which appears to operate over small redox gaps, as discussed above.

(3) Therefore, whether an organism relies on Na^+ or H^+ as the coupling ion, seems to depend on a trade-off between amount of potentially available free energy and the intensity of ion leakage across the coupling membrane. Under mesophilic conditions, when proton leaks appear to be moderate, prokaryotes routinely select the more rewarding proton energetics. Only in some obligate anaerobes, whose energy budget is tight and cannot cover the losses caused by proton leaks, Na^+ energetics may become the favored one. At high temperature and/or high pH, when proton leaks are expected to be large, the sodium energetics becomes more advantageous than under mesophilic conditions, so that obligate anaerobes routinely exploit the sodium cycle. Those anaerobic organisms that, in addition, are capable of reducing sulfur (or polysulfide) make a threshold case — some of them use sodium energetics while *Thermofilum pendens* and possibly others have an H^+-ATPase (see Table 2). This distribution reflects the fact that the ability to reduce sulfur seems to give only a marginal advantage as compared to fermentation alone. Indeed, the energy gain from sulfur reduction by hydrogen has been estimated as 30 kJ/mol at pH 7.0 as compared to >150 kJ/mol for the reduction of sulfate, sulfite or thiosulfate [116], not to mention oxygen. Accordingly, extremophiles that have ample supply of powerful electron acceptors, be it oxygen, nitrate or sulfate, are less energy-limited and can rely on proton energetics even while paying a heavy price of elevated proton leakage at high temperature or at high pH (see Table 2). The need to "pay" for increased proton leaks might explain a sharp increase in the H^+-extruding respiration in some thermophiles in response to the elevated temperatures [117], and the three-fold increase of the cytochrome oxidase content in *B. pseudofirmus* grown at pH 10.5 [118].

Table 2
Utilization of Na^+ and H^+ cycle by hyperthermophilic bacteria and archaea

	$T°_{opt}$	Na^+ pumps[a]	ATPase ion specificity[a]	O_2 tolerance, e^- acceptor
Archaea				
Aeropyrum pernix	95 °C	–	H^+	Aerobe
Archaeoglobus fulgidus	85 °C	OAD	H^+	Anaerobe, sulfate
Hyperthermus butylicus	106 °C	–	H^+	Anaerobe, sulfur
Ignicoccus hospitalis	90 °C	–	H^+	Anaerobe, sulfur
Methanocaldococcus jannaschii	85 °C	MTase	Na^+	Anaerobe, CO_2
Methanopyrus kandleri	98 °C	MTase	Na^+	Anaerobe, CO_2
Nanoarchaeum equitans	90 °C	–	Na^+	Anaerobe
Pyrobaculum aerophilum	100 °C	PP	H^+	Aerobe, O_2 or nitrate
Pyrobaculum arsenaticum	95 °C	PP	H^+	Anaerobe, arsenate
Pyrobaculum calidifontis	95 °C	PP	H^+	Aerobe, O_2 or nitrate
Pyrobaculum islandicum	100 °C	PP	H^+	Anaerobe, sulfite
Pyrococcus abyssi	96 °C	MCD	Na^+	Anaerobe, sulfur
Pyrococcus furiosus	100 °C	MCD	**Na^+**	Anaerobe, sulfur
Pyrococcus horikoshii	98 °C	MCD	Na^+	Anaerobe, sulfur
Sulfolobus solfataricus	87 °C	–	H^+	Aerobe
Sulfolobus tokodaii	80 °C	–	H^+	Aerobe
Thermococcus kodakarensis	95 °C	MCD	Na^+	Anaerobe, sulfur
Thermofilum pendens	90 °C	–	H^+	Anaerobe, sulfur
Bacteria				
Aquifex aeolicus	95 °C	–	H^+	Aerobe
Thermoanaerobacter tengcongensis	75 °C	MCD, PP	H^+	Anaerobe, sulfur, thiosulfate
Thermotoga maritima	80 °C	OAD, PP, RNF	Na^+	Anaerobe, sulfur
Thermotoga petrophila	80 °C	OAD, PP, RNF	Na^+	Anaerobe, sulfur
Thermus thermophilus	75 °C	–	H^+	Aerobe

[a] Abbreviations and symbols as in Table 1; MCD, Na^+-translocating methylmalonyl-CoA decarboxylase; MTase, Na^+-translocating N^5-methyltetrahydromethanopterin:coenzyme M methyltransferase.

6. Why certain pathogenic bacteria still rely on the sodium-motive force?

Existence of pathogenic microorganisms that fully depend on sodium energetics [20] finds its explanation if we take into account the anaerobic conditions inside animal body and the absence of

alternative electron acceptors such as nitrate, sulfate, or sulfite. However, many bacterial pathogens encode primary Na^+ pumps, such as NQR, RNF, and/or oxaloacetate decarboxylase, even if their ATP synthases are H^+-dependent [19,20,63,119]. This intriguing property could be rationalized by taking into account the bioenergetic challenges confronting pathogenic bacteria at certain stages of their life cycles.

For example, in the case of human pathogen *Vibrio cholerae*, preservation of primary Na^+ pumps, together with extended system of Na^+/H^+ antiport and a plethora of *SMF* consumers, could be related to the estuarine environment that *V. cholerae* inhabits during the free-living part of its life cycle. Estuaries show large fluctuations in physical parameters including pH (reaching up to 9.5) and salinity [120]. Na^+-based energetics could be critical for survival of this pathogen during periods of alkaline swings that occur in estuaria. Remarkably, secretion of the cholera toxin during the colonization of the human gut by *V. cholerae* results in elevated levels of Na^+ in alkaline intestinal lumen, almost mimicking the estuarian conditions. Cholera toxin-mediated sodium enrichment of the lumen environment is probably an adaptive mechanism that boosts the efficiency of Na^+ circulation in *V. cholerae* colonizing the host [121].

Obligate intracellular parasites with a complete Na^+ cycle, such as the *Chlamydia* species that possess all key elements of the Na^+-cycle, including a respiratory Na^+ pump (NaNQR), Na^+-specific ATP synthase, a Na^+/H^+ antiporter, and Na^+-substrate symporters [20,63], might have additional reasons for reliance on the *SMF*. These organisms proliferate in the homeostatic, nutrient-rich cytoplasm of an infected cell that has relatively low Na^+ concentration and near-neutral pH, next to the mitochondria and chloroplasts that exclusively utilize the H^+ gradient. A recently suggested model presents a possible mechanism by which *Chlamydia* manipulate ion homeostasis of the host cell in such a way that the Na^+ cycle could become important to their survival [122]. It posits that, after exhausting the energy resources of the host cell, proliferating *Chlamydia* switch to amino acid fermentation, which results in chlamydial microenvironment becoming relatively alkaline and sodium-rich. Under these conditions, the *SMF* becomes the preferred source of energy for nutrient uptake by chlamydial cells (see [122] for detailed discussion).

7. Conclusions

Recent results, coming from structural characterization of membrane ATPases, experimental studies, and comparative genome analyses, provide strong evidence that, rather than being an exotic adaptation to extreme conditions, the sodium-motive force was an ancestral, albeit not very efficient, mode of membrane energy metabolism. In most ecological niches, the availability of usable terminal electron acceptors, such as oxygen, nitrate, or sulfate, led to gradual replacement of the Na^+ ion cycle with the far more efficient proton cycle. In some sense, the Na^+ cycle is an evolutionary relic that has been preserved by natural selection only in a limited set of fermentative organisms, which, incidentally, includes some important human pathogens. A much wider group of organisms retained one or more primary Na^+ pumps that are utilized under conditions of lowered *PMF* (e.g., anaerobiosis) or high salinity (e.g., in marine bacteria). The traces of Na^+-based energetics are still seen in the universal distribution of Na^+ gradients and Na^+-dependent systems of solute transport in virtually all known cell types.

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