ELSEVIER

Biochimica et Biophysica Acta 1187 (1994) 216–221

BBA Biochimica et Biophysica Acta

The latest news from the sodium world

Vladimir P. Skulachev *

Department of Bioenergetics, A.N. Belozersky Institute of PhysicoChemical Biology, Moscow State University, 119899 Moscow, Russia

Received 9 March 1994

Abstract

Analysis of the latest information on the Na^+-linked energy transductions indicates that these processes are ubiquitous in living organisms. It is inferred that the existence of the Na^+ cycle side by side with the H^+ cycle plays an important role in the adaptation of the living cells to changing ambient conditions.

Key words: Sodium ion cycle; Sodium oxidase; ATPase, Na^+-; Sodium ion motor; Methanogen

1. Introduction

Early investigators of the primary sodium pumps [1–3] did not analyze systematically the question of why it is Na^+ that is expelled from the cell by these enzymes. They usually addressed themselves to a current point of view that a high concentration of Na^+ is incompatible with life. Such a statement was shaken when it was found that some methanogens contain more Na^+ in their cytosol than in the outer medium [4], and in halobacteria $[Na^+]_{in}$ can, under certain conditions, be as high as 2 M [5]. Such a fact may be considered as a precedent that at least some extremophiles can live with a high concentration of intracellcular Na^+ (this does not mean, however, the revision of the dogma that for the great majority of living cells high internal Na^+ is unfavorable).

The bioenergetic role of Na^+ was partially explained by Mitchell, who assumed that co-operation of the primary H^+ pump and Na^+/H^+ antiporter can increase the pH buffer capacity of cytosol in the bacterial cell [6]. Later, I postulated that the K^+ gradient can buffer the $\Delta\Psi$ constituent of $\Delta\bar{\mu}_{H^+}$ so that the combination of the oppositely directed Na^+ and K^+ gradients operates as a buffer for total $\Delta\bar{\mu}_{H^+}$ [7]. This concept, now experimentally proved [8–13], was supplemented in 1984 by the idea that Na^+ can effectively substitute for H^+ under low $\Delta\bar{\mu}_{H^+}$ conditions [14]. Since then, it has been found that the (i) primary sodium pumps are much more widely distributed in living organisms than was assumed previously; (ii) the pumps are, as a rule, accompanied by $\Delta\bar{\mu}_{Na^+}$ consumers capable of performing all types of the membrane-linked work and (iii) at least in some cases, the appearance of the Na^+ cycle is caused by lowering $\Delta\bar{\mu}_{H^+}$ (for reviews, see Refs. [15–17]). In this minireview, I would like to summarize the most recent observations related to this quickly growing branch of bioenergetics.

2. Na^+-motive NADH-Q reductase

The main result in this field is that this enzyme discovered in 1981–1982 by Tokuda and Unemoto in *Vibrio alginolyticus* [3,28] is, in fact, quite frequent among marine and moderately halophilic bacteria. In Tokuda's group it has been found that eight of the nine marine bacteria of genera *Vibrio, Alcaligenes, Alteromonas* and *Flavobacterium* studied show this activity [[22,29,30]. Independently, Unemoto's laboratory reported that of seven moderate halophiles, the activity in question is inherent in five Gram-negative bacteria, namely in *Deleya halophila, Halovibrio variabilis, Pseudomonas halosaccharolytica, Ps. beijerinckii* and in an unidentified halophile. On the other hand, in two cocci (*Marinococcus halophilus* and *Micrococcus varians halophilus*) the Na^+-motive NADH-Q reductase was

* Corresponding author. E-mail: libro@Genebee.msu.su. Fax: +7 095 9390338.

SSDI 0005-2728(94)00086-K

not revealed [31,32]. In our group, this activity was found in *E. coli* and halo- and alkalotolerant *Bacillus FTU* growing aerobically on succinate at low $\Delta\bar{\mu}_{H^+}$ [33]. Dimroth reported that the Na^+-motive NADH-Q reductase is present in anaerobic *Klebsiella pneumoniae* [33a].

Unfortunately, the sequence of this enzyme is still unknown. Its mechanism of action is also obscure, although some ideas on the $Q^- \cdot Na^+$ complex as the Na^+-transporting intermediate have been discussed [26,34,35].

3. Na^+-motive terminal oxidases

This activity was disclosed in our group in 1988–1991 when the *Bacillus FTU* cells and their everted vesicles were studied [36–38]. Later we found a similar activity in *E. coli* [33]. In both cases, the Na^+-motive oxidase has been shown to be induced in cells growing at low $\Delta\bar{\mu}_{H^+}$ conditions. In *E. coli* and *Bacillus FTU,* these oxidases proved to be much less sensitive to cyanide than the H^+-motive oxidases which are present in the same bacteria growing at high $\Delta\bar{\mu}_{H^+}$. In *Bacillus FTU,* an *o*-type oxidase was shown to be responsible for the Na^+ pumping, whereas the alternative oxidase of the aa_3-type pumped H^+.

In *E. coli*, there is no aa_3 oxidase. Instead, a *d*-type oxidase can operate as an alternative to the *o*-type oxidase. Surprisingly it was shown that it is the *E. coli d*, rather than *o*, oxidase that functions as a Na^+ pump. As to the *o*-type oxidase, it plays in *E. coli* the role that in *Bacillus FTU* is performed by cytochrome aa_3 i.e., the pumping of H^+ The similarity between the *Bacillus FTU o* oxidase and the *E. coli d* oxidase and their difference from the *Bacillus FTU* aa_3 oxidase and the *E. coli o* oxidase were found to consist not only in the nature of the pumped ion and the cyanide sensitivity but also in some other properties such as the rate of CO recombination, affinity for the redox mediators, etc. [39–40a].

Strong evidence that it is cytochrome *d* that is involved in the Na^+ pumping in *E. coli* have recently been obtained in experiments with mutants deficient in cytochrome *d* or, alternatively, in cytochrome *o*. It was found that the cytochrome-*d*-deficient mutant (i) could not grow in the presence of an uncoupler, (ii) showed a lower growth rate under alkaline conditions, (iii) under these conditions, could not transport Na^+ when succinate or TMPD and ascorbate were used as a substrate whereas NADH oxidation was coupled to the Na^+ pumping. On the other hand, the cytochrome *o*-deficient mutant could grow with an uncoupler or at high pH and transported Na just as the wild-type strain possessing both *d* and *o* oxidases. The Na^+ transport was stimulated in vitro by uncouplers. The wild strain

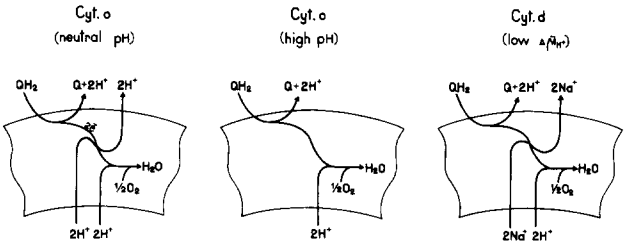

Fig. 1. Possible mechanisms of ion pumping by *E. coli* cytochromes *o* and *d*.

was found to synthesize much more cytochrome *d* when growing with an uncoupler [41].

Our findings are in agreement with those reported by Verkhovskaya et al. in Wikström's group [44], where the H^+ pumping by *E. coli* cytochromes *o* and *d* was studied. It was found that at neutral pH, the $H^+/\bar{e}$ ratio is equal to 2 for cytochrome *o* and 1 for cytochrome *d* [42–44]. At higher pH in vitro, the ratio decreased to 1 even for cytochrome *o* [44].

Fig. 1 summarizes the data of Wikström and ourselves. It is assumed that at any pH cytochrome *o* and cytochrome *d* operate as the electron-transporting Mitchellian 'half-loop' forming $\Delta\bar{\mu}_{H^+}$ as a result of (i) QH_2 oxidation to Q and $2H^+_{out}$ and (ii) H_2O formation from $1/2\ O_2$ and $^2H^+_{in}$ [6,45]. Moreover, at neutral pH, cytochrome *o* transports two more H^+_{in} per QH_2 oxidized by the H^+-pump mechanism originally discovered by Wikström in studies of mitochondrial cytochrome aa_3 [47,48]. At high pH, cytochrome *o* fails to transport 'the Wikströmian protons'. This may well be a result of adaptation to the alkaline medium when the problem of unfavorable alkaline shift of PH_{in} arises and the Na^+ cycle becomes operative. Under these conditions, cytochrome *d* is induced. It operates, according to the scheme, like cytochrome *o* at neutral pH but 'the Wikströmian protons' are replaced by sodium ions.

One more possibility is that in cytochrome *d*, like in cytochrome *o*, the pump can, under certain conditions, be decoupled from the electron transfer.

An indication that a terminal oxidase can operate as a Na^+ pump was also published by Efiok and Webster, who studied *Vitreoscilla* [49,50]. The enzyme responsible for this activity was found to be similar to *Bacillus FTU* cytochrome *o* rather than to *E. coli* cytochrome *d*. The Na^+-motive oxidase was quite recently described in *Bacillus halodurans* (formerly *Bacillus alcalophilus halodurans*) by Azarkina in our group [46].

4. The Na^+-motive and Na^+-driven reactions in methanogenesis

It has been found that methane formation by methanogenic bacteria includes energy-producing H^+-

motive and Na^+-motive steps and an energy-consuming Na^+-driven step. For instance, methanol disproportionation to CH_4 and CO_2 is described by reactions (1) and (2):

$$CH_3OH \longrightarrow CH_3\text{-S-CoM} \xrightarrow[\Delta\bar{\mu}_{H^+}]{} CH_4 \quad (1)$$

$$CH_3OH \longrightarrow CH_3\text{-S-CoM} \xrightarrow[\Delta\bar{\mu}_{Na^+}]{} CH_2{=}TMPT \longrightarrow CHO\text{-MFR} \xrightarrow[\Delta\bar{\mu}_{Na^+}]{} CO_2 \quad (2)$$

where TMPT stands for tetrahydromethanopterin and MFR for methanofuran [51].

Reaction (1) is exergonic and pumps H^+ from the cell. As for reaction (2), its initial step is endergonic being driven by a downhill influx of Na^+ to the cell, whereas its final step is exergonic and expells Na^+ from the cell in an uphill fashion.

The combination of the H^+- and Na^+-cycles is also inherent in some other types of methanogenesis [51–55] as well as acetogenesis [56].

Mechanisms of the Na^+-coupled reactions in methano- and acetogenesis remain unclear. In some of them, Na^+ translocation is coupled to the electron transfer [51,54], whereas in one case (methyl-THMP:CoM methyltransferase reaction) it is the transfer of the methyl group that is coupled to the transmembrane movement of Na^+ [53].

5. Na^+-motive decarboxylations

The first reaction of this type was described in 1980 by Dimroth. This was oxaloacetate decarboxylation in *Klebsiella pneumoniae* [57]. The enzyme catalyzing such a process was shown to be composed of 3 subunits, the largest (α) being peripheral. The other two subunits (β and γ) are integrated into the membrane. The α subunit contains the biotin prostetic group responsible for catalysis. As to the β and γ subunits, they are somehow involved in the Na^+ translocation through the membrane hydrophobic barrier. The β and γ subunits seem to contain 6 and 1 transmembrane α-helices, respectively. Sequences of the subunits have already been published. The enzyme was reconstituted into proteoliposomes competent in the Na^+ pumping (reviewed in Refs. [17,26,58]). Recently, it has been found that at high $\Delta\bar{\mu}_{Na^+}$, decarboxylase begins to operate as a Na^+ channel (carrier). This avidin-sensitive process requires carboxylation/decarboxylation of the biotin group whereas oxaloacetate/pyruvate interconversion appears to be not necessary [59]. A similar enzyme was found in *Salmonella typhimurium* [60]. In anaerobic *Acidomonas fermentans, Peptococcus aerogenes, Clostridium symbiosum and Fusobacterium nucleatum*, the Na^+-motive glutaconyl-CoA decarboxylase was described by Buckel [61]. Dimroth reported on the Na^+-motive methylmalonyl-CoA decarboxylase from *Veilonella alcalensis* and *Propionigenum modestum* [17].

6. The happy family of Na^+-ATPases

This is a rather large group of enzymes. Its members are found in all kingdoms of living organisms so that Na^+-ATPases may be called 'the happy family' as was done previously by Saraste regarding cytochrome oxidase [62]. Besides the well-known Na^+/K^+-ATPase from the animal plasma membrane, the ATP-driven Na^+ pumps have been described in several bacterial genera and recently in plant species. Among them are representatives of all three types of ATPase, i.e., F, V and P types (reviewed in Ref. 26).

The animal Na^+/K^+-ATPase is the best example of a Na^+ pump of the P-type (reviewed in Ref. [26]). Currently, a similar enzyme has been discovered by Wada et al. [63,64] in the plasma membrane of the marine alga raphidophycean biflagellate *Heterosigma akashiwo*. This 140 kDa vanadate-sensitive Na^+ pump belongs to the P-type ATPases. In the presence of Na^+ it forms a phosphorylated intermediate which is hydrolyzed in the K^+-dependent manner. The protein possesses an immunologically identical epitope to the animal Na^+/K^+-ATPase [64].

The ATP-dependent transport of Na^+, activated by uncoupler and resistant to amiloride, was quite recently described by Balnokin and Popova in everted vesicles of the plasmalemma of marine eukaryotic microalga *Platymonas viridis*. The authors concluded that these cells possess an electrogenic Na^+-ATPase [64a].

As to the F-type Na^+ ATPase, the *P. modestum* enzyme seems to be the most elaborate example. It is very similar to bacterial H^+-ATPase (ATP-synthases) both in the sequence and subunit composition. In fact, this is a typical F_0F_1 complex. According to Dimroth [65], active chimeric enzymes can be obtained combining *E. coli* F_0 and *P. modestum* F_1 and vice versa, the ion specificity being determined by the F_0 part. Earlier it was shown by the same group that the ion specificity of native *P. modestum* Na^+-ATPase is not absolute. When Na^+ is absent, H^+ is pumped [66]. Apparently, both Na^+ and H^+ compete for one and the same carboxylate of Glu-65 in the *c*-subunit of the ATPase. DCCD attacking this carboxylate in its protonated form was shown to be much less effective in the presence of Na^+ [67].

The physiological function of the *P. modestum* enzyme consists in the ATP synthesis at the expense of $\Delta\bar{\mu}_{Na^+}$ which is produced by the Na^+-motive methylmalonyl CoA-decarboxylase [17]. In our laboratory it

has been found that the *E. coli* F_0F_1 ATPase becomes competent in the $\Delta\bar{\mu}_{Na^+}$-driven ATP synthesis when the cell grows under low $\Delta\bar{\mu}_{H^+}$ conditions, i.e., in the presence of an uncoupler or at high pH. The everted membrane vesicles from these cells were shown to catalyze oxidative phosphorylation in the presence of protonophorous uncouplers. The ATP synthesis was completely inhibited by Na^+ ionophore ETH 157, monensin, the artificially-imposed reverse Na^+ gradient, as well as by the F_0 inhibitors, venturicydin and DCCD, the F_1 inhibitor, aurovertin, or *unc*-mutation (deletion in the F_0F_1 operon). It was suggested that a post-translational modification of the F_0F_1 complex is responsible for the switch from H^+ to Na^+ under unfavorable conditions [68].

Electroneutral Na^+/K^+-ATPase of the V-type was described by Kakinuma in *Enterococcus hirae* (formerly *Streptococcus faecalis*) [69]. The enzyme is sensitive, like V-ATPases, to nitrate and *N*-ethylmaleimide and resistant to vanadate. It is composed of detachable and membrane-embedded sectors (presumably V_1 and V_0). The sequence of the largest subunit in such a detachable sector is similar to that in V_1 [70].

A nitrate-sensitive, vanadate-resistant Na^+-ATPase has been discovered recently by Koning's group in thermophilic anaerobic *Clostridium fervidus* [71]. There is evidence for Na^+-ATPase (synthase) in *Vibrio alginolyticus, Exiguobacterium aurantiacum, Mycoplasma, Acholeplasma*, methanogens [71a] (for review, see Ref. [26]), *Vitreoscilla* [72], *Acetobacterium* [73], anaerobic alkalophile *Amphibacillus* [74].

7. Na^+, solute-symporters

Na^+, solute-symporters are quite typical for marine bacteria as well as for the animal plasma membrane (reviewed in Refs. [18,26,75,76]). In some bacteria, this seems to be the main (*V. alginolyticus* [32]) or even the sole (*Clostridium fervidus* [71,77]) mechanism of the uphill import of metabolites into the cell.

There is an example in which the efflux of an end-product of metabolism in simport with Na^+ is employed to generate $\Delta\bar{\mu}_{Na^+}$. In *Selenomonas ruminantium*, fermentation results in succinate^{2-} which is exported with $3Na^+$, so that $\Delta\bar{\mu}_{Na^+}$ is generated composed of $\Delta\Psi$ (the interior negative) and ΔpNa (low $[Na^+]$ inside) [78].

8. Why living cells employ the sodium cycle?

For a bacterium growing at low $\Delta\bar{\mu}_{H^+}$ the reason why the Na^+ cycle substitutes for the H^+-cycle seems quite obvious: this is a way to survive under unfavorable conditions [14]. In those bacteria where the Na^+ cycle is inducible, the $\Delta\bar{\mu}_{H^+}$ level is apparently monitored by a special receptor of the protonmotive force (we called it 'protometer') which sends a signal to systems responsible for switching of the energetics from H^+ to Na^+ when $\Delta\bar{\mu}_{H^+}$ is lowered [18]. There are at least three examples when mechanistically different effects, resulting in a $\Delta\bar{\mu}_{H^+}$ decrease, were found to induce the Na^+ cycle. In our laboratory, it was found that the Na^+-motive respiratory chain of *Bacillus FTU* is induced under alkaline conditions or, at neutral pH, by adding to the grow medium a protonophorous uncoupler or low concentration of cyanide which specifically inhibits the H^+ motive oxidase [79]. In *E. coli*, the Na^+ cycle was induced either by an uncoupler or by high pH [33]. The induction requires Arc A and Arc B proteins [80], a regulatory system which is known to be involved in control of expression of several catabolic enzymes [81].

In Kakinuma's group the Na^+/K^+-ATPase of anaerobic *E. hirae* was shown to be induced by uncouplers, high pH or mutation in the H^+-ATPase. Mutant deficient in the Na^+/K^+-ATPase could not grow at high pH (for reviews, see Refs. 69,26).

The reason to employ the Na^+ cycle in *Cl. fervidus* is thermophilism. It was found that at high temperature the H^+ conductance of the bacterial membrane is much higher than the Na^+ conductance [71,77].

Induction of the Na^+ cycle enzymes in *E. coli* and *E. hirae* was shown to require Na^+ [26,80]. There is an example when a decrease in $[Na^+]_{out}$ results in the opposite switch, i.e., from the Na^+ to H^+ cycle. Imae and co-workers [82] showed that marine *Vibrio parahaemolyticus* produces two different types of flagella, namely a single polar flagellum to swim in liquid medium or numerous lateral flagella for swarming over viscous surfaces. The first type of flagella is driven by the Na^+ motor [83], the second by the H^+ motor [82]. The lateral flagella are induced by growth under viscous conditions [82]. One might speculate that the switch from Na^+ to H^+ is due to a decrease in $[Na^+]_{out}$ when the bacterium changes habitat from seawater to the surface of a substrate outside the sea.

Such a regulation is absent from *E. coli* and *Bacillus FTU*. According to our data, switching the energetics from H^+ to Na^+ when $\Delta\bar{\mu}_{H^+}$ is lowered does not cause any change in the flagellar motor which in *E. coli* is always $\Delta\bar{\mu}_{H^+}$-driven, whereas in *Bacillus FTU* it is always $\Delta\bar{\mu}_{Na^+}$-driven. As a result, *E. coli* and *Bacillus FTU* are motionless when $\Delta\bar{\mu}_{H^+}$ and $\Delta\bar{\mu}_{Na^+}$ are low, respectively [84].

There are some cases when reasons to use the Na^+-cycle instead of the H^+-cycle are not obvious. For instance, it is rather difficult to explain in the functional terms why all the membranous energy-coupled decarboxylases pump Na^+, not H^+, or why some of the energy-transducing steps of methanogenesis are H^+-

linked whereas others are Na^+-linked. Perhaps, these questions cannot be settled now, just as the question of why glycolytic phosphorylation is of the substrate type whereas respiratory chain phosphorylation is of the chemiosmotic type. The H^+ cycle and the Na^+ cycle look like two kinds of the chemiosmotic mechanism of energy transduction of ubiquitous distribution among living cells. The existence of such parallel pathways should greatly stabilize the bioenergetic system. It seems quite natural that, e.g., the Na^+ energetics substitutes for the H^+ energetics when the latter appears to be inefficient. However, in certain chemiosmotic reactions only one of these two possibilities is realized. Thus, the question of why it is Na^+, not H^+ that is pumped by decarboxylases may be answered in a very simple way: because the Na^+-motive decarboxylases were invented by evolution while the H^+-motive decarboxylases were not.

References

[1] Skou, J.C. (1960) Biochim. Biophys. Acta 42, 6–23.
[2] Dimroth, P. (1980) FEBS Lett. 122, 234–236.
[3] Tokuda, H. and Unemoto, T. (1982) J. Biol. Chem. 257, 10007–10014.
[4] Sprott, G.D. and Jarrell, K.F. (1981) Can. J. Microbiol.27, 444–451.
[5] Ginzburg, M., Sachs, L. and Ginzburg, B.Z. (1970) J. Gen. Physiol. 55, 187–207.
[6] Mitchell, P. (1968) Chemiosmotic coupling and energy transduction. Glynn Research, Bodmin.
[7] Skulachev, V.P. (1978) FEBS Lett. 87, 171–179.
[8] Wagner, G., Hartmann, R. and Oesterhelt, D. (1978) Eur. J. Biochem. 89, 169–179.
[9] Skulachev, V.P. (1980) Can. J. Biochem. 58, 161–175.
[10] Arshavsky, V. Yu., Baryshev, V.A., Brown, I.I., Glagolev, A.N. and Skulachev, V.P. (1981) FEBS Lett. 133, 22–26.
[11] Brown, I.I., Galperin, M. Yu., Glagolev, A.N. and Skulachev, V.P. (1983) Eur. J. Biochem. 134, 345–349.
[12] Michels, M. and Bakker, E.P. (1985) J. Bacteriol. 161, 231–237.
[13] Drachev, A.L., Markin, V.S. and Skulachev, V.P. (1985) Biochim. Biophys. Acta 811, 197–215. 1, 453–477 (Russ.).
[14] Skulachev, V.P. (1984) Trends Biochem. Sci. 9, 483–485.
[15] Skulachev, V.P. (1985) Eur. J. Biochem. 155, 199–208.
[16] Skulachev, V.P. (1987) in Ion Transport in Prokaryotes (Rosen, B.P. and Silver, S., eds.), pp. 131–164, Academic Press, San Diego,
[17] Dimroth, P. (1987) Microbiol. Rev. 51, 320–340.
[18] Skulachev, V.P. (1988) Membrane Bioenergetics, Springer, Berlin.
[19] Skulachev, V.P. (1986) Biol. Membrany 3, 5–25 (Russ.).
[21] Skulachev, V.P. (1989) J. Bioenerg. Biomembr. 21, 635–647.
[22] Tokuda, H. (1989) J. Bioenerg. Biomembr. 21, 693–704.
[23] Unemoto, T. and Hayashi, M. (1989) J. Bioenerg. Biomembr. 21, 649–662.
[24] Skulachev, V.P. (1991) Biosci. Reports 11, 387–444.
[25] Skulachev, V.P. (1992) Eur. J. Biochem. 208, 203–209.
[26] Skulachev, V.P. (1992) In: Molecular Mechanisms in Bioenergetics (Ernster, L., ed.), Elsevier, Amsterdam.
[27] Padan, E. and Schuldiner, S. (1993) In: Alkali Cation Transport Systems in Prokaryotes (Bakker, E.P., ed.), CRC Press, Boca Raton.
[28] Tokuda, H. and Unemoto, T. (1981) Biochem. Biophys. Res. Commun. 102, 265–271.
[29] Tokuda, H. and Kogure, K. (1989) J. Gen. Microbiol. 135, 703–709.
[30] Kogure, K. and Tokuda, H. (1989)FEBS Lett. 256, 147–149.
[31] Unemoto, T., Akagawa, A., Mizugati, M. and Hayashi, M. (1992) J. Gen. Microbiol. 138, 1999–2005.
[32] Unemoto, T. and Hayashi, M. (1993) J. Bioenerg. Biomembr.25, 385–391.
[33] Avetisyan, A.V., Dibrov, P.A., Skulachev, V.P. and Sokolov, M.V. (1991) Biochim. Biophys. Acta 1098, 95–104.
[33a] Dimroth, P. and Thomer, A. (1989) Arch. Microbiol. 151, 439–444.
[34] Ken-Dror, S., Lanyi, J.K., Schobert, B., Silver, B. and Avi-Dor, Y. (1986) Arch. Biochem. Biophys. 244, 766–772.
[35] Rich, P. (1991) Biosci. Rep. 11, 539–571.
[36] Verkhovskaya, M.L., Semeykina, A.L. and Skulachev, V.P. (1988) Dokl. Acad. Nauk SSSR 303, 1501–1503 (Russ.).
[37] Semeykina, A.L., Skulachev, V.P., Verkhovskaya, M.L., Bulygina, E.S. and Chumakov, K.M. (1989) Eur. J. Biochem. 183, 671–678.
[38] Kostyrko, V.A., Semeykina, A.L., Skulachev, V.P., Smirnova, I.A., Vaghina, M.L. and Verkhovskaya, M.L. (1991) Eur. J. Biochem. 198, 527–534.
[39] Muntyan, M.S., Bloch, D.A., Drachev, L.A. and Skulachev, V.P. (1993) FEBS Lett. 327, 347–350.
[40] Muntyan, M.S., Bloch, D.A., Ustyan, V.S. and Drachev, L.A. (1993) FEBS Lett. 327, 351–354.
[40] a. Muntyan, M.S. and Skripnikova, E.V. (1993) Biochim. Biophys. Acta 1143, 142–146
[41] Avetisyan, A.V., Bogachev, A.V., Murtasina, R.A. and Skulachev, V.P. (1992) FEBS Lett. 306, 199–202.
[42] Puustinen, A., Finel, M., Virkki, M. and Wikström, M. (1989) FEBS Lett. 249, 163–167.
[43] Puustinen, A., Finel, M., Haltia, T., Gennis, R.B. and Wikström, M. (1991) Biochemistry 30, 3936–3942.
[44] Verkhovskaya, M., Verkhovsky, M. and Wikström, M. (1992) J. Biol. Chem. 267, 14559–14562.
[45] Mitchell, P. (1966) Biol. Rev. 41, 445–502.
[46] Azarkina, N.V. (1994) Biochemistry (Moscow) (accepted).
[47] Wikström, M. (1977) Nature 266, 271–273.
[48] Wikström, M. and Casey, R. (1985) FEBS Lett. 183, 293–298.
[49] Efiok, B.J.S. and Webster, D.A. (1990) Biochem. Biophys. Res. Commun. 173, 370–375.
[50] Efiok, B.J.S. and Webster, D.A. (1990) Biochemistry 29, 4734–4739.
[51] Schönheit, P. (1993) In: The Biochemistry of Archaea pp. 113–172. (Archaebacteria) (Kates, M., Kushner, D.J. and Matheson, A.T., eds.), pp. 113–172, Elsevier, Amsterdam,
[52] Blaut, M., Müller, V. and Gottschalk, G. (1992) J. Bioenerg. Biomembr. 24, 529–546.
[53] Becher, B., Müller, V. and Gottschalk, G. (1992) FEMS Microbiol.
[53] Becher, B., Muller, V. and Gottschalk, G. (1992) FEMS Microbiol. Lett. 91, 239–244
[54] Kaesler, B. and Schonheit, P. (1989) Eur. J. Biochem. 184, 309–316.
[55] Muller, V., Winner, C. and Gottschalk, G. (1988) Eur. J. Biochem. 178, 519–525.
[56] Heise, R., Muller, V. and Gottschalk, G. (1989) J. Bacteriol. 171, 5473–5478.
[57] Dimroth, P. (1980) FEBS Lett. 122, 234–236.
[58] Dimroth, P. (1990) Philos. Trans.R. Soc. London, B 326, 465–477.

[59] Dimroth, P. and Thomer, A. (1993) Biochemistry 32, 1734–1739.
[60] Wilfling, K. and Dimroth, P. (1989) Arch. Microbiol. 152, 584–588.
[61] Buckel, W. (1986) Methods Enzymol. 125, 547–558.
[62] Saraste, M., Holm, L., Lemieux, L., Lubben, M. and van der Oost, J. (1991) Biochem. Soc. Trans. 19, 608–612.
[63] Wada, M., Satoh, S., Kasamo, K. and Fujii, T. (1989) Plant Cell Physiol.30, 923–928.
[64] Wada, M., Urayama, O., Satoh, S., Hara, Y., Ikawa, Y. and Fujii, T. (1992) FEBS Lett. 309, 272–274.
[64] a. Balnokin, Yu.V. and Popova, L.G. (1994) FEBS Lett. (accepted).
[65] Kaim, G. and Dimroth, P. (1993) Eur. J. Biochem. 218, 937–944.
[66] Laubinger, W. and Dimroth, P. (1989) Biochemistry 28, 7194–7198.
[67] Kluge, C. and Dimroth, P. (1993) Biochemistry 32, 10378–10386.
[68] Avetisyan, A.V., Bogachev, A.V., Murtasina, R.A. and Skulachev, V.P. (1993) FEBS Lett. 317, 267–270.
[69] Kakinuma, Y. (1993) in Alkali Cation Transport Systems in Prokaryotes (Bakker, E.P., ed.), pp. 277–290, CRC Press, Boca Raton.
[70] Takase, K., Yamato, I. amd Kakinuma, Y. (1993) J. Biol. Chem. 268,
[71] Speelmans, G., Poolman, B., Abee, T. and Konings, W.N. (1993) Proc. Natl. Acad. Sci.USA 90, 7975–7979.
[71] a. Smigan, P., Majernik, A. and Greksak, M. (1994) FEBS Lett. (accepted).
[72] Efiok, B.J.S. and Webster, D.A. (1992) Arch. Biochem. Biophys. 292, 102–106.
[73] Heise, R., Müller, V. and Gottschalk, G. (1992) Eur. J. Biochem. 206, 553–557.
[74] Koyama, N. (1993) Eur. J. Biochem. 217, 435–439
[75] Imae, Y. (1991) in New Era of Bioenergetics (Mukohata, Y., ed.), Academic Press, Tokyo, pp. 197–221.
[76] Poolman, B. and Konings, W.N. (1993) Biochim. Biophys. Acta 1183, 5–39.
[77] Speelmans, G., Poolman, B. and Konings, W.N. (1993) J. Bacteriol. 175, 2060–2066.
[78] Michel, T.A. and Macy, J.M. (1990) J. Bacteriol. 172, 1430–1435.
[79] Semeykina, A.L. and Skulachev, V.P. (1991) FEBS Lett. 296, 77–81.
[80] Bogachev, A.V., Murtasina, R.A. and Skulachev, V.P. (1993) FEBS Lett. 336, 75–78.
[81] Iuchi, S., Chepuri, V., Fu, H.-A., Gennis, R.B. and Lin, E.C.C. (1990) J. Bacteriol. 172,6020–6025.
[82] Atsumi, T., McCarter, L. and Imae, Y. (1992) Nature 355, 182–184.
[83] Chernyak, B.V., Dibrov, P.A., Glagolev, A.N., Sherman, M. Yu. and Skulachev, V.P. (1983) FEBS Lett. 164, 38–42.
[84] Bogachev, A.V., Murtasina, R.A., Shestopalov, A.I. and Skulachev, V.P. (1993) Biochim. Biophys. Acta 1142, 321–326.