A HYPOTHESIS OF THE EVOLUTION OF BIOLOGICAL ENERGY TRANSDUCERS

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In describing biological phenomena we cannot avoid the question of the evolution of the biosphere. Therefore, elucidation of any general principle of living cell organization always stimulates interest in possible pathways of evolution of the studied system. Investigation of the mechanism of energy coupling in respiration and photosynthesis recently resulted in solving this most important problem of bioenergetics in terms of Mitchell's chemiosmotic concept [1]. It was shown that electric membrane potential plays the role of factor, coupling oxidation and phosphorylation [2–13]. This success allows the system of the intracellular energy conserving processes to be rationalized in toto. In this paper, I'll try to imagine evolutionary development of this system in the framework of the general concept of the biosphere originally postulated 50 years ago by A. I. Oparin [14].

As a starting-point in this consideration, I would like to use an assumption that the ATP-mediated coupling of energy supplying and energy-consuming processes is an ancient and the most fundamental principle of bioenergetics. (Because this function of ATP is universal for all types of living cells, ATP was recommended as a life indication for searches of living organisms outside the Earth.) Ponnamperuma and his colleagues reported several years ago about non-enzymatic ADP phosphorylation by ethylmetaphosphate as a result of the ultraviolet light treatment [15]. Ultraviolet was used also for adenine formation from NH_3 and CO_2 [15–17]. As a matter of fact, ADP is highly adaptive for utilization of energy, as it can be absorbed by the adenine 'head' of the molecule, to elongation of the pyrophosphate 'tail' [18]. In one of ADP conformations, the distance between N_6 of adenine and terminal phosphate oxygen exactly corresponds to the 3rd phosphoryl residue. Furthermore, any factor inducing the loss of coupling in the adenine part of ADP should transform N_6 amino groups from aromatic to aliphatic. This process, according to calculations of Blumenfeld and Temkin [19], is associated with the free energy change of about 10 kcal $mole^{-1}$ which agrees with the energy of the ATP terminal phosphate hydrolysis.

The study of chemical and physical properties of ADP undertaken by our group several years ago [18] revealed a number of other features of the adenine residue as an energy acceptor. However, any attempts to show a specific function of adenine moiety of ADP in the enzymatic processes of energy transduction have failed. It might be reasonable to consider the specific role of adenine not in modern organisms but in their precursors. One may assume that excitation of the adenine part of ADP

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by ultra-violet quantum in primary living systems gave rise to the 'head-to-tail' energy transfer in the ADP molecule, resulting in ATP synthesis.*

At least two factors may be responsible for the disappearance of the primary 'ultraviolet' photosynthesis: (1) Penetration of the life into the ultraviolet impermeable areas of the primary ocean and (2) formation of the ultraviolet-absorbing atmosphere. Apparently, after its disappearance two mechanisms were utilized instead of 'ultraviolet' photosynthesis: dark anaerobic reactions of glycolysis and new photosynthetic system utilizing visible light. It is the latter which is responsible now for energy accumulation in biosphere.

The numerous studies on photosynthesis have clearly demonstrated the necessity of closed membraneous structures to the light-driven ATP formation. Therefore, the problem of the origin of modern photosynthesis includes the question of how the membranes originated. This is not a very difficult question since the spontaneous reconstitution of membrane vesicles in the phospholipid solution is well known. So, it is easy to imagine formation of the membrane surrounding coacervate drops [14] containing a phospholipid material. However, one should keep in mind that phospholipids usually form multilayer micelles instead of the monomembraneous structures inherent in living cells. One feature of the membrane reconstitution process which may help to overcome this difficulty has been revealed: It was discovered in experiments with chlorophyll-containing protein complexes from photosynthetic bacteria and plant chloroplasts, that the addition of hydrophobic proteins, isolated from biological membranes, to the phospholipid solution, stimulates membrane reconstitution and induces the formation of closed monomembraneous vesicles.

In all photosynthetic organisms (with one exception, see below), the utilization of light energy is mediated by chlorophyll, and the absorption of a light quanta always gives rise to the reduction of an electron acceptor by an excited form of chlorophyll. If such an oxidoreduction occurs in the solution, or in the membrane along the membrane surface, the energy released in oxidation dissipates as heat. If, however, the oxidoreduction occurs across the membrane, a transmembraneous electric field may be generated, and the membrane may be charged like a capacitor. To do this, it is enough to place the chlorophyll and acceptor molecules on opposite sides of the membrane.

Experiments carried out by Drs L. A. Drachev and V. D. Samuilov in this laboratory revealed that, in fact, chlorophyll-protein complexes, namely photosynthetic reaction centers from a purple bacteria, *Rhodospirillum rubrum*, can generate an electric photocurrent, and this kind of activity may be demonstrated in reconstituted system. When bacteriochlorophyll reaction center complexes were isolated from *R.rubrum* chromatophores and mixed with cholate solution of soya bean phospholipids, an electric photocurrent was generated.

* The structures of natural non-adenylic nucleoside diphosphates also allow, in principal, the ultraviolet energy to be utilized to form corresponding triphosphates. Apparently, ATP was used as the most universal energy donor due to fact that adenine is the most resistant structure (among purines and pyrimidines) to the ultraviolet – induced decomposition. One may speculate that nucleoside triphosphates were chosen by evolution as substrates of the nucleic acid formation because of the simplest way of the ultraviolet energy utilization to support the synthesis of these polymers.

Dialysis of the mixture resulted in a spontaneous reconstitution of proteoliposomes containing the bacteriochlorophyll-acceptor system. Then these proteoliposomes were incorporated into the planar phospholipid membrane separating two macrovolumes of the electrolyte solutions of equal composition. Ag/AgCl electrodes, connected with a voltmeter, were immersed into solutions on both sides of the membrane. Illumination of the planar membrane by visible light induce the generation of a transmembrane electric potential difference (Figure 1). The action spectrum of the photoeffect resembled closely the absorption spectrum of the bacteriochlorophyll reaction centers (Figure 2).

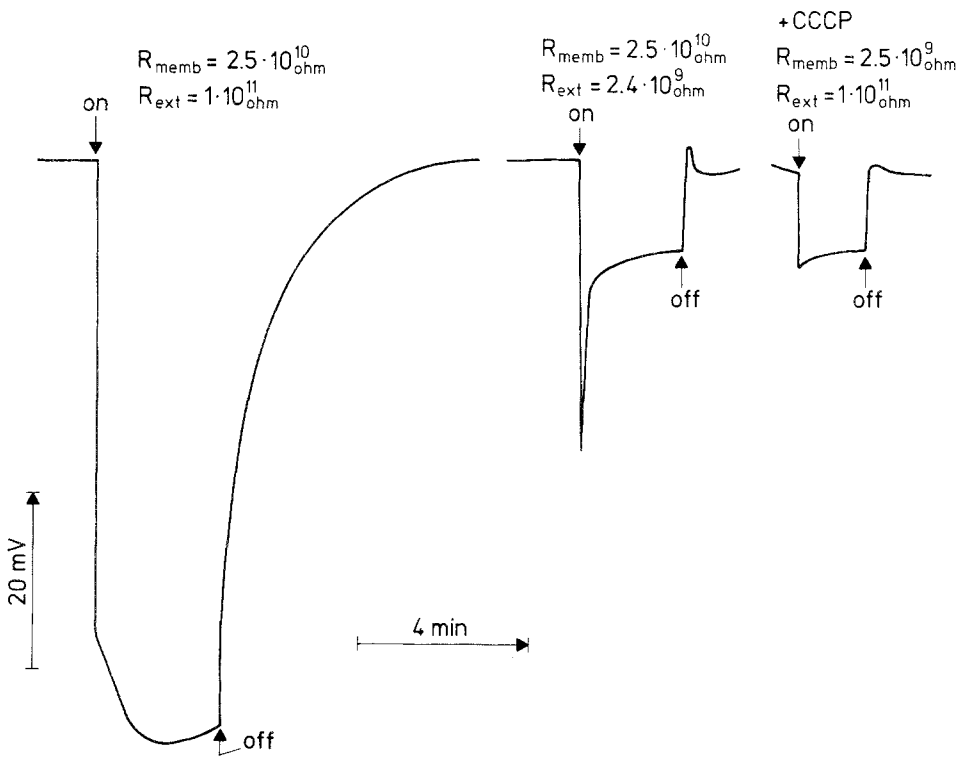

Fig. 1. Electric generation by the bacteriochlorophyll proteoliposomes associated with planar azolectin membrane. $R_{memb.}$ – resistance of planar membrane, $R_{ext.}$ – external resistance. Incubation mixture: 0.2 M sucrose, 0.05 M Tris–HCl (pH 7.4), 5 mM $MgSO_4$, 30 mM $CaCl_2$ and, in one of compartments proteoliposomes containing bacteriochlorophyll reaction center complexes (1.2×10^{-7} M bacteriochlorophyll). Addition: 5×10^{-7} M trichlorocarbonylcyanidephenylhydrazone (CCCP)

These data show that a photoelectric battery can be reconstituted in a rather simple mixture containing chlorophyll-protein complex and phospholipid. It is interesting that such a reconstituted system can utilize the energy accumulated in the electric form, to carry out one of the types of work inherent in a living system; namely, the uphill transport of ions. The following experiment illustrates this statement: A suspension of the bacteriochlorophyll proteoliposomes was pre-equilibrated with synthetic penetrating anion, phenyl dicarbaundecaborane (PCB^-). Then the light source was switched on. The light-induced extrusion of PCB^- from

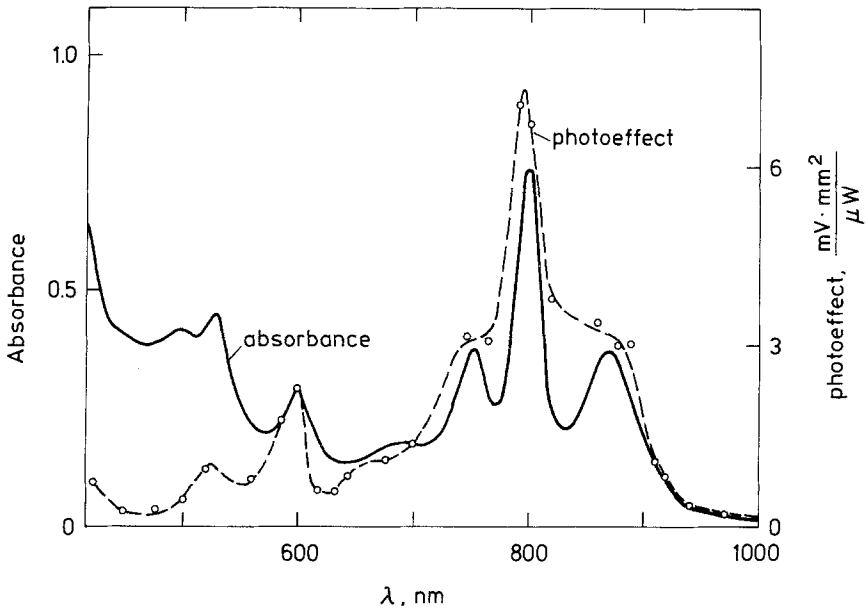

Fig. 2. Action spectrum of the bacteriochlorophyll – mediated photoeffect. Incubation mixture: 0.2 sucrose, 0.05 M Tris – HCl (pH 7.5), 5 mM $MgSO_4$, 30 mM $CaCl_2$, 5×10^{-4} M TMPD and bacteriochlorophyll proteoliposomes (1.2×10^{-7} M bacteriochlorophyll).

proteoliposomes becomes evident. This effect can be simply explained by electrophoresis of PCB^- anions from minus inside proteoliposomes to plus outside.

One can speculate that light-supported uphill ion translocation was the first kind of useful work mediated by a chlorophyll system of a primitive living cell.

For the membrane containing chlorophyll reaction centers to perform such a task, only a penetrating ion is necessary. Non-penetrants may be involved in electrophoresis by way of simple valinomycin-like ionophores, which increase specific membrane permeability to the ion being transported.

Unfortunately, the operation of this light-dependent pump is limited by the pools of chlorophyll and primary electron acceptor, oxidizing excited chlorophyll. Regenerations of reduced chlorophyll and oxidized acceptor are necessary to maintain the activity of the system for a long time.

Thus it may be done by the return of reducing equivalent from the acceptor to oxidized chlorophyll. This process should represent transmembrane movement of an electroneutral component (but not of electrons) to avoid discharge of the membrane charged by the light-induced oxidoreduction. The problem might be solved if a hydrogen atom-carrier could play the role of a shuttle transferring reducing equivalents through the membrane. The function of the shuttle can be performed by a quinone, coenzyme *Q*, for example.

In our experiments with the bacteriochlorophyll proteoliposomes, the addition of coenzyme *Q*, or of some artificial hydrogen atom carriers, was found to strongly stimulate the photoelectric response. Without added hydrogen carriers, the response was apparently mediated by endogeneous coenzyme *Q* of the reaction center preparation.

The next problem to be solved is the maintenance of uphill ion transport during the night period. This energy-linked function would have been performed, like other endergonic biochemical processes, at the expense of ATP energy. The question was to couple ATP hydrolysis with the membrane charging. Existence of the electrogenic ATPases in biomembranes was postulated by Peter Mitchell in 1966 [1]. Some pieces of indirect evidence confirming this hypothesis were obtained in several laboratories. Recently we succeeded in directly demonstrating the generation of an electric current by a membraneous ATPase; namely, ATPase complex from beef heart mitochondria [13]. To this end, we used the proteoliposome-planar membrane system which was studied in the above experiments with bacteriochlorophyll. The data, presented in Figure 3, show that the addition of ATP

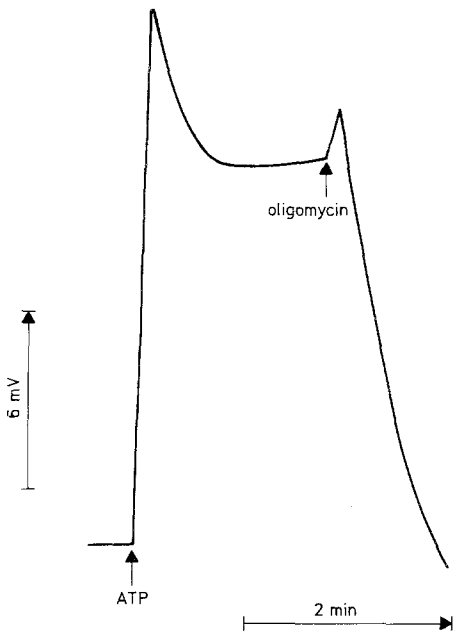

Fig. 3. Electric generation by ATPase proteoliposomes associated with planar azolectin membrane. Incubation mixture: 0.2 M sucrose, 0.05 M Tris-HCl (pH 7.3), 5 mM $MgSO_4$, 30 mM $CaCl_2$ and in one of compartments proteoliposomes containing oligomycin – sensitive ATPase complex from beef heart mitochondria (0.5 mg protein ml^{-1}). Additions: 1 mM ATP and oligomycin (6.6 $\mu g ml^{-1}$).

to this system results in the formation of an electric potential across the planar membrane, which is sensitive to specific inhibitor of mitrochondrial ATPase, oligomycin.

How ATPase charges the membrane, is still unknown. It is clear however that a mechanism other than electron transfer should be used in this case since an electron transfer chain is not required for ATPase system. Recently P. Mitchell [20] and independently I. A. Koslov of my group proposed the following scheme of the electrogenic ATPase: They postulated that ATP hydrolysis is organized in such a way that two H^+ ions formed when reaction products (ADP and phosphate) are deprotonated, are released into extracellular space, while ADP and phosphate

anions are released into the cell interior. As a result, hydrolysis of one ATP molecule is accompanied by translocation of two H^+ ions from intra- to extracellular space (H^+-ATPase system). Electrogenic ATPase mechanism can maintain membrane potential in the dark if ATP, inorganic polyphosphate or any other compounds which can give ATP, were formed and stored during the light period.

A very interesting feature of the ion transport ATPase reactions is their reversibility. It was found that K^+, Na^+-ATPase of outer membrane of animal cells and Ca^{2+}-ATPase of the muscle sarcoplasmic reticulum synthesize ATP from ADP and phosphate when a downhill ion transport takes place. This property was demonstrated also when studying the H^+-ATPase of a photosynthetic membrane. In 1966 Jagendorf and Uribe observed dark ATP formation coupled with the downhill H^+ influx into chloroplasts. The process was sensitive to inhibitors of the chloroplast ATPase. Inhibitors of photosynthetic redox chain were without effect.

Assuming that ATPase, localized in the photosynthetic membrane, is reversible, we come to the most important conclusion that the light-induced electron transport can be coupled with ADP phosphorylation via an electrochemical potential of H^+ ions ($\bar{\mu}_H$) as was proposed originally by Mitchell [1]. Indeed, proton gradient formed by the redox cycle can, in principle, be discharged via H^+-ATPase channel, and this downhill proton movement can give ATP due to reversal of the H^+-ATPase reaction. According to this scheme, light induces the cyclic flow of H^+ ions across the membrane which leads to the only result: ATP formation. One may speculate that this type of the energy coupling was responsible for ADP phosphorylation supported by visible light in a primitive precursor of modern cyclic bacterial photosynthesis.

Further progress in the development of the photosynthetic apparatus has resulted in the increased effectiveness of light energy utilization. In the primitive version of the photosynthetic redox cycle, one quantum induces translocation of one charge across the membrane. Apparently, the same situation takes place in our reconstituted system – proteoliposomes with bacteriochlorophyll reaction center complexes. In modern photosynthetic bacteria, the redox cycle includes (besides chlorophyll, primary acceptor and ubiquinone) some additional electron carriers, namely, cytochromes of *b* and *c* types. In this cytochrome system, one more mechanism of the membrane charging is organized. Reducing equivalents, returning from primary acceptor to oxidized chlorophyll, are transported via cytochrome system actualizing acytochrome-linked membrane-charging mechanism. As a result, one quantum induces transport of two charges across the membrane.

The question about the molecular organization of the second membrane-charging site of the photosynthetic redox cycle remains unanswered. Mitchell [1] postulated rather complicated scheme including a redox 'loop': antiport of electron and hydrogen atom (like the cycle 'chlorophyll-primary acceptor-coenzyme *Q*'). According to hypothesis developed by Konstantinov and myself, a cytochrome oxidoreduction may result directly in the uphill translocation of H^+ through the membrane (redox proton pump). Besides ATP synthesis and ion transport, the photosynthetic apparatus of purple and green bacteria performs one more function; it regenerates NAD(P)H from $NAD(P)^+$. To this end, two more charge transport sites are organized: one between NADH and cytochrome *b* (NADH-dehydrogenase)

and the other between NADPH and NAD^+ (transhydrogenase). These systems act as catalysts reducing equivalent transfer from donors of redox potential about zero to NAD^+ and further to $NADP^+$ (redox potential $-0.3V$). Electron transfer against gradient of redox potential is carried out at the expense of the energy of the electrochemical H^+ gradient formed by light-dependent electron transfer or ATP hydrolysis. The mechanism of the membrane charging by NADH dehydrogenase may be of the same nature as that by cytochromes (a redox loop or proton pump). As to transhydrogenase, experimental data indicate that it should be a proton pump but not a loop. Thus, formation of a set of electric generators in the membrane of photosynthetic bacteria was completed (Figure 4). It includes the bacteriochlorophyll electron pump, apparently three redox proton pumps and the hydrolytic proton pump of H^+–ATPase. (There are some indications to the existence of one more hydrolytic proton pump in photosynthetic bacteria, namely H^+ – inorganic pyrophosphatase. It is possible that the pyrophosphatase pump was an evolutionary precursor of H^+–ATPase.)

The great discovery of biological evolution consists in organization of a mechanism of $NADP^+$ reduction by electrons of water, which provides an inexhaustible source of reducing equivalents for biosyntheses. The mechanism in question is the noncyclic redox chain of chloroplasts of green plants. This chain (Figure 5) may be considered as an open modification of the redox cycle of photosynthetic bacteria. It includes (1) photosystem I corresponding to bacteriochlorophyll–primary acceptor complex of bacterial cycle, (2) cytochromes of *b* and *c* types and, apparently, a cytochrome-linked proton pump, (3) plastoquinone (*PQ*) instead of coenzyme *Q* in bacteria, (4) photosystem II, reducing plastoquinone by electrons of a donor (D_{II}), (5) the system catalyzing D_{II} reduction by water, and (6) additional redox chain, reducing $NADP^+$ by primary electron acceptor of the photosystem I via ferredoxin and a flavoprotein. In the same chloroplast membrane, H^+–ATPase, resembling closely corresponding enzyme from bacteria, is localized.

Two essential results of non-cyclic photosynthesis are important for this consideration: (I) the accumulation of molecular oxygen in the atmosphere and (2) the formation of large amounts of organic compounds synthesized from CO_2 and H_2O at the expense of light energy. Thus conditions, favourable for development of aerobic heterotrophic organisms, have been created. This event was followed by formation of special mechanism for the oxidation of organic compounds by molecular oxygen. Such a mechanism includes a very complex set of catabolic enzymes responsible for (1) decomposition of various biopolymers down to carboxylic acids of low molecular weight, (2) interconversion of carboxylic acid via Krebs cycle accompanied by $NAD(P)^+$ and flavoprotein reduction, and (3) oxidation of NAD(P)H and reduced flavoproteins by oxygen via respiratory chain. The latter may be considered as slightly modified redox system of photosynthetic bacteria. It should be stressed that respiratory chain of many aerobic heterotrophic bacteria and mitochondria differs from bacterial photosynthetic chain only in one point: in respiratory chain, bacteriochlorophyll – primary acceptor complex is substituted by cytochrome *a*-cytochrome a_3 complex (Figure 6). All other steps of two chains are similar (transhydrogenase, NADH-dehydrogenase, coenzyme *Q*, cytochromes *b* and *c* types). In both types of membranes, H^+–ATPase was found; it is of interest that

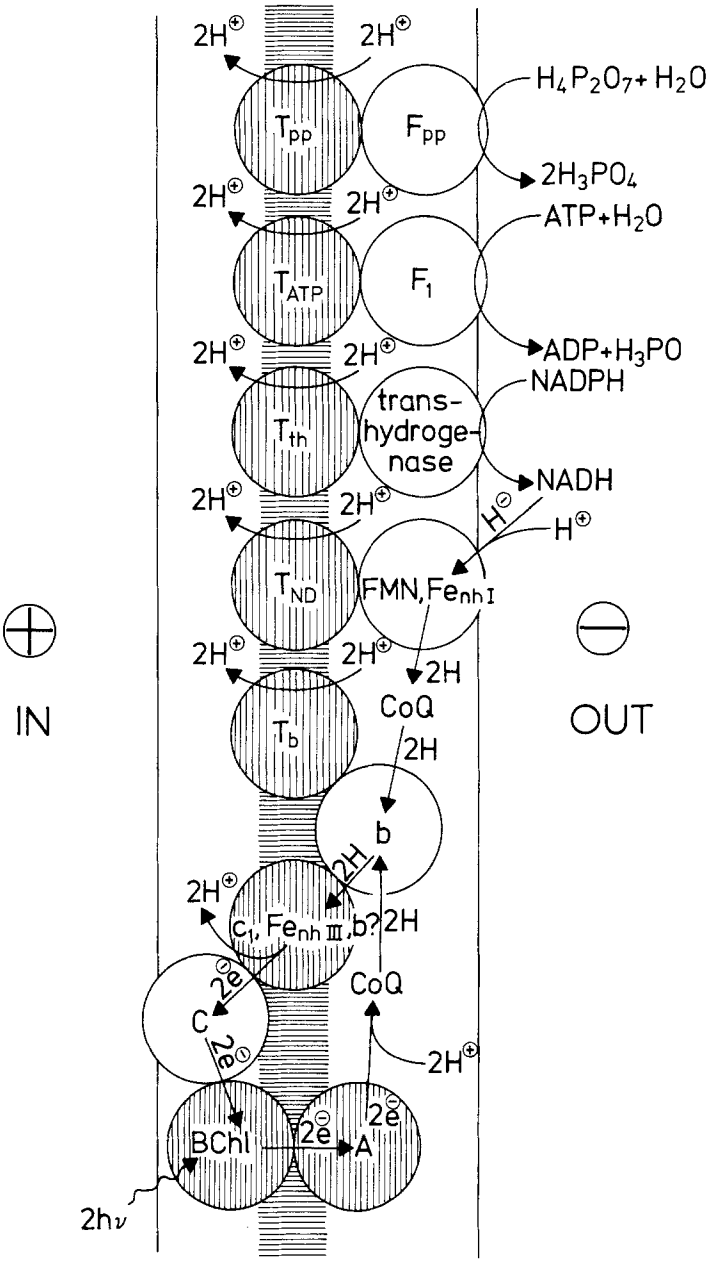

Fig. 4. Energy transducers in the membrane of chromatophores of photosynthetic bacteria. F_{pp} and F_1 – catalytic components of membraneous inorganic pyrophosphatase and ATPase, respectively; FMN, $Fe_{nh}I$ – catalytic components of NADH dehydrogenase; *b*, c_1, *c*, – cytochromes; $FeIII_{nh}$-non-heme iron protein participating in the cytochrome *b* oxidation; BChl – bacteriochlorophyll; *A* – primary electron acceptor reduced by excited BChl; T_{pp}, T_{ATP}, T_{ND}, and T_b – H^+ – translocating components of inorganic pyrophosphatase, ATPase, NADH-dehydrogenase, and cytochrome *b*, respectively. It is assumed that membrane charging is a result of the transmembraneous transfer of electrons (BChl–A system) or of protons (pyrophosphatase, ATPase, NADH dehydrogenase, cytochrome *b*).

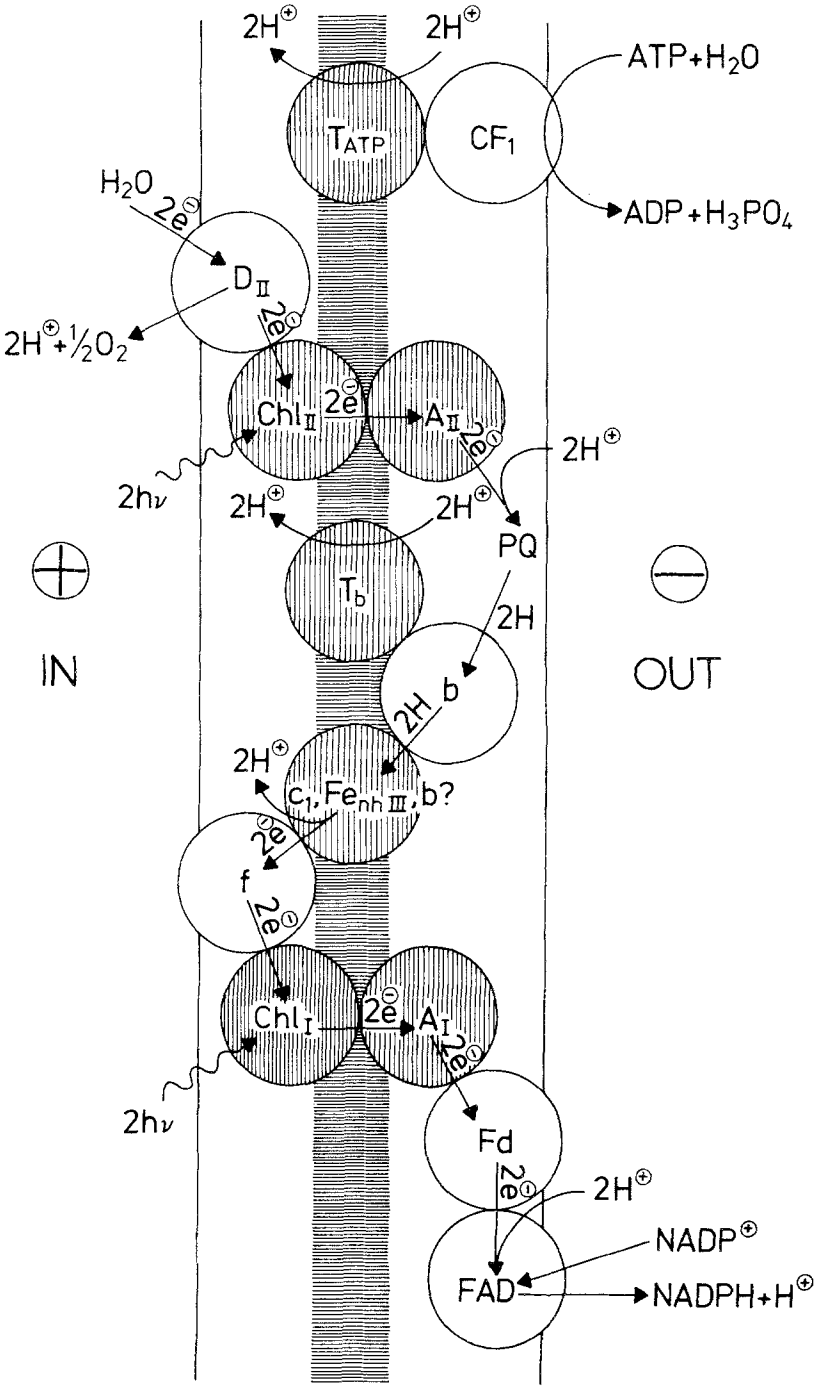

Fig. 5. Energy transducers in the chloroplast membrane. CF_1-catalytic component of the chloroplast ATPase; Chl_I and Chl_{II} – chlorophylls of the first and second photosystems; A_I and A_{II} – primary electron acceptors of these systems; D_{II} – electron donor of the second system; *PQ* – plastoquinone; *f* – the chloroplast cytochrome of the *c* type; *Fd* – ferredoxin. For other abbreviations, see Figure 4.

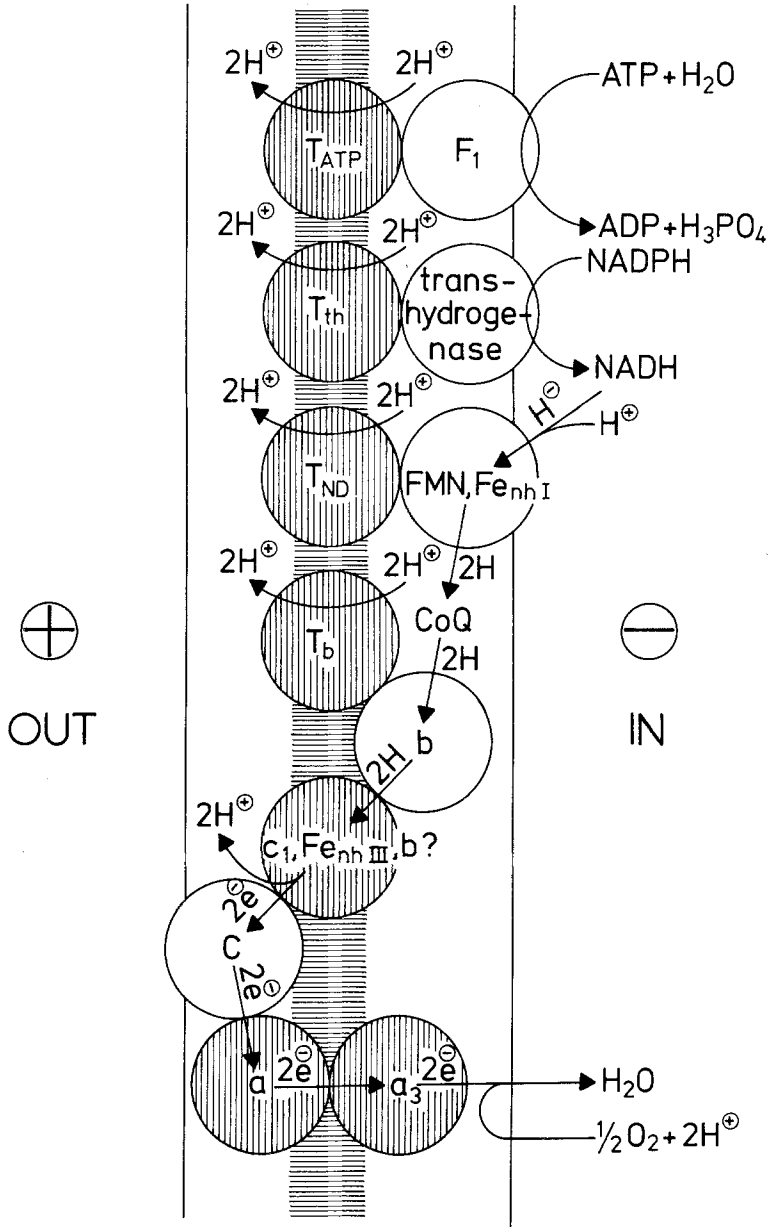

Fig. 6. Energy transducers of the mitochondrial membrane. a and a_3 – cytochromes. For other abbreviations see Figure 4.

terminal part of respiratory chain and corresponding segment of the photosynthetic system demonstrate, beside obvious structural differences, some features of functional resemblance: both systems charge membrane by means of transmembrane electron movement; both systems may be spontaneously reconstituted from the solution of corresponding proteins and phospholipids, and resulting proteoliposomes generate an electric current supported by an external energy source (light or oxidation of an electron donor by oxygen). Above we demonstrated the electric responses of the bacteriochlorophyll proteoliposomes. Figure 7 shows similar response of the cytochrome oxidase proteoliposomes. One

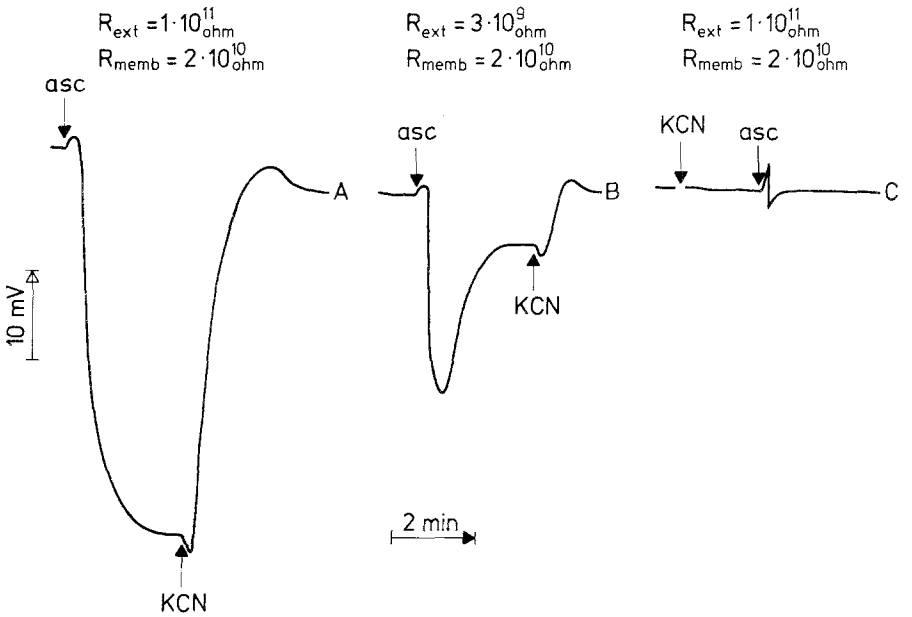

Fig. 7. Electric generation by cytochrome oxidase proteolisposomes associated with planar azolectin membrane. Incubation mixture: 0.3 M sucrose, 5 mM Tris-citrate (pH 7.2), 30 mM $CaCl_2$, 5 mM $MgSO_4$, 1×10^{-4} M cytochrome *c*, and in one of compartments, proteoliposomes containing cytochrome oxidase from beef heart mitochondria (0.3 mg protein ml^{-1}). Additions: 10 mM ascorbate and 1 mM NaCN.

can see that addition of an electron donor, ascorbate, to the system 'proteoliposomes-planar membrane' results in generating electric potential across the planar membrane. The process was completely inhibited by cyanide.

Originally, the respiratory chain probably appeared as an additional mechanism of energy conservation in some photosynthetic bacteria. Further specialization of these organisms in the heterotrophic, light-independent way of life resulted in the disappearance of the photosynthetic apparatus; the respiratory chain replaced it as the only system of electron transfer phosphorylation.

In the kingdom of bacteria, one can find many combinations of photosynthetic, respiratory and glycolytic types of energetics. One striking example is halophilic bacteria. These microorganisms transform their energetics from an aerobic heterotrophic type to a quite unusual photosynthetic mechanism when the bacterial culture reaches exponential growth phase. According to Stoeckenius,

Oesterhelt and associates [21–24], *Halobacterium halobium* membranes contain bacteriorhodopsin, which closely resembles rhodopsin, the visual chromoprotein of higher animals. This protein is composed of an opsin-like polypeptide of 20000 molecular weight forming Schiff base with retinal. Hypotonic disruption of the bacterial cells gave the mixture of membrane fragments, from which a fraction of purple round or oval sheets 0.5μ in diameter and 50Å thick was isolated. These purple membranes were composed of bacteriorhodopsin as a single protein component (75% of dry weight) and an ether of phosphoglycerol and dihydrophytol (25%). Some observations indicating that bacteriorhodopsin carries out light energy conversion into a utilizable form have been made: (a) Illumination of the bacteria increases the intracellular ATP level and decreases respiration rate, the effect being sensitive to uncouplers of oxidative and photosynthetic phosphorylation. (b) Reconstitution of proteoliposomes containing bacteriorhodopsin and the mitochondrial oligomycin-sensitive ATPase results in the system capable of the light-supported ADP phosphorylation by inorganic phosphate [24].

Considering these data in terms of the chemiosmotic theory of energy coupling, one may suggest that bacteriorhodopsin mediates light-dependent formation of a transmembrane electrochemical potential of H^+ ions. Indeed, illumination of bacterial cells, as well as of proteoliposomes, was shown to induce some changes in pH of the incubation medium. These changes were accelerated by valinomicin and inhibited by uncouplers [23, 24]. Studies have directly demonstrated that bacteriorhodopsin incorporated into planar phospholipid membrane functions as a photoelectric generator. The data of a typical experiment of this kind, carried out in our group by L. A. Drachev, A. D. Kaulen, S. A. Ostroumov and A. Yu. Semenov, are shown in Figure 8. One can see that illumination induces generation

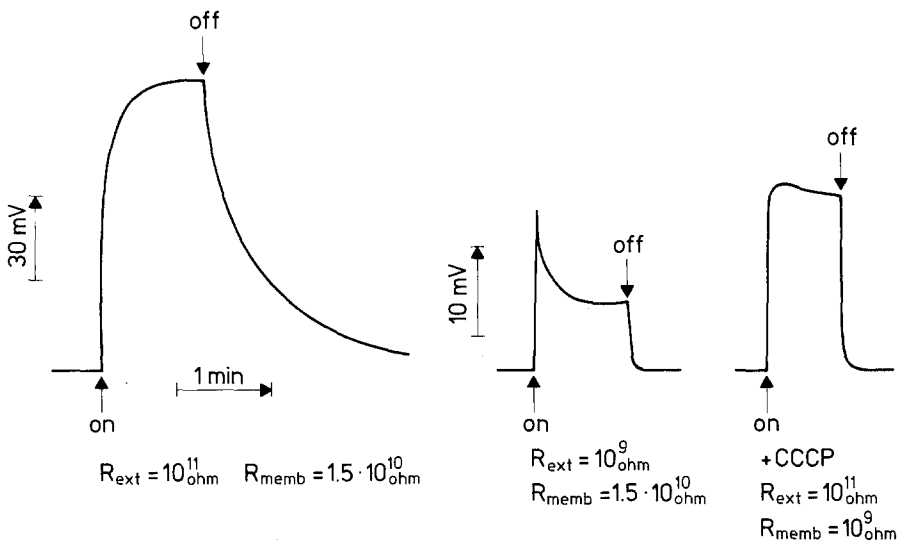

Fig. 8. Electric generation by bacteriorhodopsin proteoliposomes associated with planar azolection membrane. Incubation mixture: 0.2 M sucrose, 0.05 M Tris–HCl (pH 7.2), 30 mM $CaCl_2$ and in one of compartments, proteoliphosomes containing bacteriorhodopsin from *H. halobium*. Addition: 3×10^{-7} M CCCP.

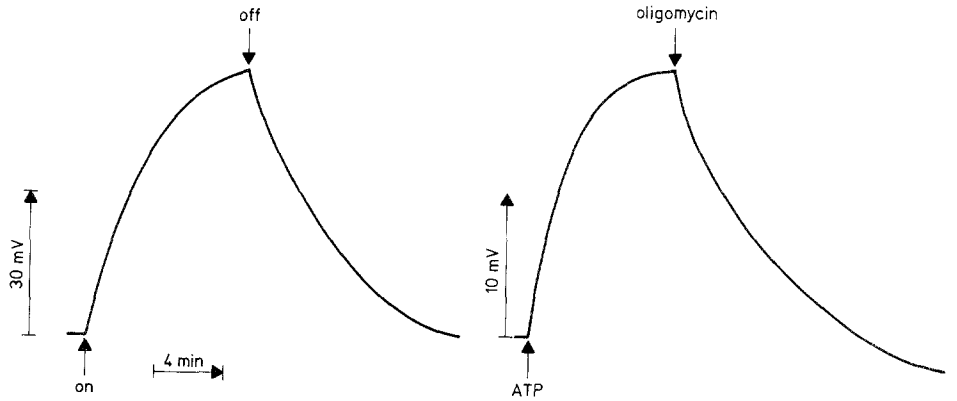

Fig. 9. Electric generation by bacteriorhodopsin + ATPase proteoliposomes associated with planar phospholipid membrane. Incubation mixture: 0.3 M sucrose, 5 mM Tris-citrate (pH 7.2), 30 mM $CaCl_2$, 5 mM $MgSO_4$ and in one of compartments, proteoliposomes containing *H. halobium* bacteriorhodopsin and beef heart mitochondrial ATPase (0.3 mg protein ml^{-1}). Additions: 2.5 mM ATP and oligomycin (30 $\mu g ml^{-1}$).

of electric potential difference of about 100 mV. In Figure 9 responses of proteoliposomes reconstituted from bacteriorhodopsin, mitochondrial H^+-ATPase and soya bean phospholipid are given. It should be noted that electric potential differences generated by light and ATP are of the same direction. It means that membrane potential generated by bacteriorhodopsin can be used as a driving force in ATP synthesis by reversible H^+-ATPase.

Apparently, bacteriorhodopsin photosynthesis is a result of adaptation of heterotrophin aerobic organism to the existence at low oxygen concentration. *H. halobium* has a complete respiratory chain with three sites of the membrane charging as it was shown in this group by A. A. Jasaitis and J. P. Kadziauscas. Transition of this microorganism from heterotrophic to phototrophic pattern of energetics is connected, most probably, with a change in O_2 level. Oxygen concentration, being initially low due to high salt concentration in the medium, decreases further because of respiratory activity of dividing *H. halobium* cells, whose amount increases, the process being accompanied by fast increase in bacteriorhodopsin content in the bacterial membrane.

The remarkable similarity of bacteriorhodopsin and animal rhodopsin in molecular weight, amino acid composition, and light-induced changes in properties of retinal-protein complex can hardly be a simple coincidence. Apparently, we should look for a common function, and possibly, for common origin of these proteins despite the fact of the great distance between the positions of halobacteria and higher animals in the evolutionary system. In fact, bacteriorhodopsin and animal rhodopsin are membraneous proteins; the former utilizes light energy for H^+ translocation; the latter utilizes light energy to change membrane properties which results in excitation of visual cell.

The bacteriorhodopsin-mediated proton translocation should include two events: reversible protonation of bacteriorhodopsin and the channeling of H^+ ions across the membrane. One may assume that animal rhodopsin retained only one of these

functions, namely, the transmembrane channelling of ions which is no longer controlled by protonation-deprotonation reaction. If so, the light might induce a conformation change in rhodopsin resulting in formation of a through ion-conducting channel in the membrane of photoreceptor discs of retina cell. The efflux of some ions (e.g. Ca^{2+}) via this channel may initiate a long chain of subsequent events which results in visual excitation.

The last problem which will be considered in this paper is the function of intracellular organelles and the outer membrane of animal cell. The appearance of eucaryotic cells meant cells had developed intracellular specializations for different metabolic functions. Energy production, formerly associated with the cell membrane or its derivatives in procaryotic cells, was transferred to the membranes of specialized organelles, mitochondria and chloroplasts, which apparently included a stage of symbiosis of eucaryotic and procaryotic cells. In procaryotic organisms, the cell membrane functions in energy production, and is responsible for the transport of extracellular compounds from the medium into the cell. The latter function is supported by the same driving force as ATP synthesis, i.e. electrochemical potential of H^+ ions produced by a redox chain. The fact that it is hydrogen ion whose gradient is formed by redox chain is apparently a result of the chemical mechanism of the primary evolutionary reaction of the membraneous energy conservation organized as a light-induced $\bar{e}/H$ antiport.

There are several independent lines of evidence that in mitochondria and chloroplast, as well as in bacterial membranes $\Delta\bar{\mu}_H$ (or one of its constituents, $\Delta\psi$ or ΔpH) is the driving force for ATP synthesis and for the uphill transport of different compounds (for review, see [1, 7, 25, 26]). To maintain $\Delta\bar{\mu}_H$, the membranes in question must have very low H^+ permeability and high electric resistance. It is not easy, considering the fact that hydrophobic phospholipid bilayer of the membranes is interrupted by membraneous proteins of redox chain carriers, translocases, etc. It is not surprising, therefore, that any changes in the native membrane structure result in the uncoupling of oxidation and phosphorylation and inhibition of transport processes.

Low H^+ permeability and high electric resistance, should it have been inherent in membranes liberated from the function of carrying out oxidative phosphorylation, is but an anachronism. This would have been especially dangerous in the case of animal cells having no thick cell wall which could protect outer membrane against the influence of the extracellular factors. Hence, any increase in the functional reliability of the outer membrane of animal cells would be very important. This problem was probably solved by substitution of $\Delta\bar{\mu}_H$ by $\Delta\bar{\mu}_{Na}$ as a driving force for transport processes.

There are indications of symport of Na^+ and an extracellular compound being a mechanism of several transport processes in animal cell membranes (for review, see [20, 27]). Such a symport was postulated to be catalyzed by a carrier binding both Na^+ and a compound which should be accumulated in the cell. The ternary complex composed of a carrier, Na^+ and a transported compound moves into the cell down Na^+ gradient, the free carrier returning down the carrier gradient. Na^+ is pumped out by the Na^+, K^+-ATPase. *In toto*, this mechanism resembles the symport of H^+ and lactose into cells of *E. coli* [28, 29] or of H^+ and fatty acyl

(with carnitine as the carrier) into mitochondria [25]. Systems of this type can utilize both electric ($\Delta\psi$) and concentration (ΔpH or ΔpNa) components of $\Delta\bar{\mu}_{H}$ ($\Delta\bar{\mu}_{Na}$). It is remarkable that high electric resistance of the membrane is not obligatory for the ΔpNa-supported transport process if the membrane permeability for Na^+ is low.

Na^+ has an advantage over H^+ in that it is the most common cation in the environment. Besides, the amount of substances binding Na^+ is rather low, so the buffer capacity of the extra- and intracellular medium for Na^+ is much lower than for H^+. As a result, it is much easier to obtain a high gradient of Na^+, than of H^+, pumping Na^+, instead of H^+, out of the cell.

It was impossible to reduce the concentration of Na^+ in the cell without substituting it by another cation. This cation proved to be K^+, the second wide-spread monovalent cation in the environment. Therefore Na^+ pump could have been organized as a Na^+, K^+-antiport system with ATP as energy source.

Thus a comparative review of biological energy transducers can be summarized by suggesting that the primary mechanism for the accumulation of usable energy was the excitation of the adenine part of ADP by ultraviolet light, followed by ADP phosphorylation with inorganic phosphate. It is proposed that such a primitive 'ultraviolet photosynthesis' was substituted by a new photosynthetic system utilizing visible light to synthesize ATP. To do this, a chromophore other than adenine was necessary. To the role of such a chromophore, chlorophyll system, converting light energy into electric form, was developed. This type of energy transduction requires membrane structures which can be formed spontaneously from phospholipids. Attachment of chlorophyll and an electron acceptor to the opposite sides of the phospholipid membrane is necessary for transmembrane electron flow from the light-excited chlorophyll to the acceptor. This process should result in electric charging of the membrane like a capacitor. Reconstitution of such a photoelectric battery was demonstrated experimentally when studying bacteriochlorophyll-protein complexes from a purple bacterium. Proteoliposomes reconstituted from these complexes and phospholipids were found to perform an osmotic work, i.e. light-dependent uphill transport of penetrating ions.

To carry out osmotic work in the dark period, the primitive chlorophyll-containing cell is postulated to include an electrogenic ATPase in its membrane. If it was reversible ATPase charging the membrane by the uphill H^+ transport, light could drive ATP synthesis coupled with discharge of the chlorophyll-mediated electrochemical potential of H^+ ions (Mitchellian chemiosmotic energy coupling).

This basic mechanism was, apparently, modified into cyclic photophosphorylation of modern purple and green bacteria, and later, to non-cyclic photosynthetic redox chain and respiratory chain phosphorylations. Bacteriorhodopsin light-dependent proton pump is considered as a component of an 'evolutionary secondary photosynthesis' developed in aerobic heterotrophic halophilic bacteria adapted to oxygen-deficient conditions.

In animal cells, the functions of osmotic work and ATP synthesis were separated

between outer cell membrane and mitochondria. Electrochemical potential of Na^+, instead of H^+, is used as a driving force of osmotic work of the outer animal cell membrane.

References

1. Mitchell, P.: 1966, *Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation*, Glynn Research, Bodmin.
2. Mitchell, P. and Moyle, J.: 1969, *Europ. J. Biochem.* **7**, 471–484.
3. Liberman, E. A., Topali, V. P., Tsofina, L. M., Jasaitis, A. A., and Skulachev, V. P.: 1969, *Nature* **222**, 1076–1078.
4. Grinius, L. L., Jasaitis, A. A., Kadziauskas, J. P., Liberman, E. A., Skulachev, V. P., Topali, V. P., Tsofina, L. M., and Vladimirova, M. A.: 1970, *Biochem. Biophys. Acta* **216**, 1–12.
5. Bakeeva, L. E., Grinius, L. L., Jasaitis, A. A., Kuliene, V. V., Levitsky, D. O., Liberman, E. A., Severina, I. I., and Skulachev, V. P.: 1970, *Biochim. Biophys. Acta* **216**, 13–21.
6. Isaev, P. I., Liberman, E. A., Samuilov, V. D., Skulachev, V. P., and Tsofina, L. M.: 1970, *Biochim. Biophys. Acta* **216**, 22–29.
7. Liberman, E. A. and Skulachev, V. P.: 1970, *Biochim. Biophys. Acta* **216**, 30–42.
8. Junge, W. and Witt, H. T.: 1968, *Z. Naturforsch.* **23b**, 244.
9. Witt, H. T.: 1972, *J. Bioenerg.* **3**, 47–54.
10. Jackson, J. B. and Crofts, A. R.: 1969, *FEBS Letters* **5**, 185.
11. Kayushin, L. P. and Skulachev, V. P.: 1974, *FEBS Letters* **39**, 39–42.
12. Drachev, L. A., Kaulen, A. D., Ostroumov, S. A., and Skulachev, V. P.: 1974, *FEBS Letters* **39**, 43–45.
13. Drachev, L. A., Jasaitis, A. A., Kaulen, A. D., Kondrashin, A. A., Liberman, E. A., Nemeček, I. B., Ostroumov, S. A., Semenov, A. Ya., and Skulachev, V. P.: 1974, *Nature* **249**, 321–324.
14. Oparin, A. I.: 1957, *The Origin of Life on Earth*, Publishing House of U.S.S.R., Academy of Sci., Moscow.
15. Ponnamperuma, C., Sagan, C., and Mariner, B.: 1963, *Nature* **199**, 222.
16. Ponnamperuma, C.: 1966, *In Origin of Prebiological Systems*, Moscow, Mir Publishing House, p. 224.
17. Sagan, C.: 1966, *Ibid.*, p. 211.
18. Shulachev, V. P.: 1969, *Energy Accumulation in the Cell*, Moscow, Nauka Publishing House.
19. Blumenfeld, L. A. and Temkin, M. I.: 1962, *Biofizika, U.S.S.R.* **7**, 731.
20. Mitchell, P.: 1970, *Symp. Sci. General Microbiol.* **20**, 121–166.
21. Oesterhelt, D. and Stoeckenius, W.: 1971, *Nature New Biol.* **233**, 149–152.
22. Blaurock, A. E. and Stoeckenius, W.: 1971, *Nature New Biol.* **233**, 152–154.
23. Oesterhelt, D. and Stoeckenius, W.: 1973, *Proc. Natl. Acad. Sci. U.S.* **70**, 2853
24. Racker, E. and Stoeckenius, W.: 1974, *J. Biol. Chem.* **249**, 662.
25. Skulachev, V. P.: 1972, *Energy Transformation in Biomembranes*, Nauka Press, Moscow.
26. Skulachev, V. P.: 1972, *FEBS Symp.* **28**, 371–385.
27. Kotyk, A.: 1973, *Biochim. Biophys. Acta Rev. Biomembranes* **300**, 183–203.
28. West, I. C.: 1970, *Biochim. Biophys. Res. Communs.* **41**, 655–661.
29. West, I. C. and Mitchell, P.: 1972, *J. Bioenerg.* **3**, 445–462.