Journal of Supramolecular Structure 13:421–446 (1980)
Membrane Transport and Neuroreceptors 179–204

Evolution of Membrane Bioenergetics

T. Hastings Wilson and Edmund C.C. Lin

Department of Physiology and Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115

One of the first problems encountered by primitive cells was that of volume regulation; the continuous entry of ions, (eg, NaCl) and water in response to the internal colloid osmotic pressure threatening to destroy the cell by lysis. We propose that to meet this environmental challenge cells evolved an ATP-driven proton extrusion system plus a membrane carrier that would exchange external protons with internal Na^+. With the appearance of the ability to generate proton gradients, additional mechanisms to harness this source of energy emerged. These would include proton-nutrient cotransport, K^+ accumulation, nucleic acid entry, and motility. A more efficient system for the uptake of certain carbohydrates by vectorial phosphorylation via the PEP-phosphotransferase system probably appeared rather early in the evolution of anaerobic bacteria.

The reversal of the proton-ATPase reaction to give net ATP synthesis became possible with the development of other types of efficient proton transporting machinery. Either light-driven bacterial rhodopsin or a redox system coupled to proton translocation would have served this function. Oxidation of one substrate coupled to the reduction of another substrate by membrane-bound enzymes evolved in such a manner that protons were extruded from the cell during the reaction. The progressive elaboration of this type of redox proton pump permitted the use of exogenous electron acceptors, such as fumarate, sulfate, and nitrate. The stepwise growth of these electron transport chains required the accretion of several flavoproteins, iron-sulfur proteins, quinones, and cytochromes. With modifications of these four basic components a chlorophyll-dependent photosynthetic system was subsequently evolved. The oxygen that was generated by this photosynthetic system from water would eventually accumulate in the atmosphere of the earth. With molecular oxygen present, the emergence of cytochrome oxidase would complete the respiratory chain.

The proton economy of membrane energetics has been retained by most present-day microorganisms, mitochondria, chloroplasts, and cells of higher plants. A secondary use of the energy stored as an electrochemical difference of Na^+ for powering membrane events probably also evolved in microorganisms. The exclusive use of the Na^+ economy is distinctive of the plasma membrane of animal cells; the Na^+-K^+ ATPase sets up an electrochemical Na^+ gradient that provides the energy for osmoregulation, Na^+-nutrient cotransport, and the action potential of excitable cells.

Key words: evolution, membrane transport, proton pumps, ATPase, oxidative phosphorylation, flavoproteins, quinone, cytochromes, photosynthesis, bacterial rhodopsin, protonmotive force

Received May 9, 1980; accepted July 28, 1980.

0091-7419/80/1304-0421$04.40

A great deal of thought has been given to the evolution of the mechanisms of bioenergetics [1–20]. We shall not review all of the various hypotheses in great detail, but shall instead attempt to bring together thoughts from several sources and to emphasize what we believe to be a reasonable series of events. This paper focuses attention on newer knowledge in membrane biology with particular emphasis on the chemiosmotic ideas of Mitchell [21, 22] and the major events in the evolution of some of the ion transporting mechanisms and other membrane mechanisms involved in energy transformations. In several cases, contemporary examples are given for the particular mechanisms under discussion.

In trying to formulate the sequence in the evolution of bioenergetics based on various types of data, we adopt the assumption that almost every invention in this long process is preserved by certain lines of organisms, either because they were channeled by specialization or because they continue to occupy ecological niches not very different from those in which the ancestral forms lived. For instance, anaerobic environments endured even after oxygen became abundant in the atmosphere, and one might therefore expect some anaerobes to refine their mechanisms for energy transduction rather than to proceed to entirely novel systems. If indeed there are relatively few "missing links," one should be able to piece together the puzzle of evolution with hints provided by present-day representatives of different classes of organisms.

Fortunately, there is an independent approach to this question of biological evolution that can be called upon to aid this inquiry. This involves the study of the macromolecular sequences with homologous functions, such as the amino acid sequences of cytochrome molecules and the nucleotide sequences of 5S RNA. The basic assumption in this approach is that cells possessing macromolecules of similar sequences are closely related, whereas those with very different patterns are more distantly related. Computer analysis based on this basic criterion has provided valuable phylogenetic information [23–27].

THE TIME SCALE IN EVOLUTION

Let us first review the currently accepted time scale for biological evolution [28–30]. The earth is generally belived to have appeared about four and a half billion years ago. Fossil evidence dates the appearance of microorganisms to over three billion years ago (Fig. 1). At that time, the atmosphere consisted of nitrogen, hydrogen, ammonia, and carbon dioxide. The oxygen concentration was exceedingly low and was due to photolysis of water. Thus, the earliest microorganisms must have been anaerobes. Fossil evidence suggests that cyanobacteria (blue-green algae) appeared almost three billion years ago and the first eukaryotes about 1.4 billion years [31]. In the absence of oxygen in the atmosphere, there was no ozone shielding of the ultraviolet radiation, which would be highly lethal to living cells. Thus, it is presumed that the various microorganisms were protected from the ultraviolet radiation by several feet of water, which reduced the intensity to tolerable levels. When molecular oxygen was being released through photosynthesis by the early cyanobacteria, the gas accumulated in the atmosphere, and ozone was in turn generated; organisms could then grow at the surface of the water due to the much lower levels of radiation. By about 0.6 billion years ago, the atmospheric oxygen concentration had risen to approximately 0.2%, which allowed the development of the mature aerobic respiratory chain. During the next 150 million years the oxygen content rose to 2%, further increasing the ozone shield, so that life was then possible on dry land for animals and plants.

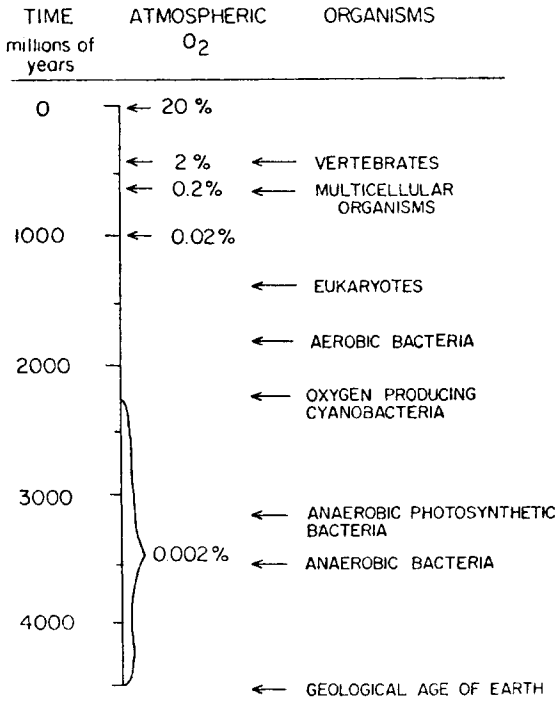

Fig. 1. Time sequence in biological evolution.

THE 'FIRST CELL'

As Haldane [32] has suggested, "The critical event that may best be called the origin of life was the enclosure of several different self-reproducing polymers within a semipermeable membrane." Whereas the appearance of the first primitive plasma membrane was an important key to future development of the cell, it posed special problems of its own (Fig. 2). The presence of nondiffusible macromolecules within the cell would result in an osmotic force (colloid osmotic pressure), which results in entry of water and salts into the cell [33–36]. When all of the ion pumps are turned off, the natural tendency for NaCl and H_2O to enter can be observed in animal cells [36]. When the normal ion pumps are permitted to function once again, the NaCl is pumped out of the cell, which shrinks back to its original volume.

The osmotic crisis of the primitive cell could, in principle, be met in one of several ways. If the membrane became completely impermeable to water and to NaCl, no increase in cell volume would occur. However, artificial membranes, as well as biological membranes, are intrinsically quite permeable to water and show a low but measureable permeability to NaCl. A second possible solution to the osmotic difficulties would be the synthesis of a rigid cell wall surrounding the plasma membrane. This particular mechanism has been widely utilized by microorganisms and plants. It seems reasonable, however, to suppose that the simple cell membrane, unprotected by rigid cell walls, existed for some period of time before the development of the complex apparatus required for cell wall biosynthesis. Without a rigid cell wall, another solution for the osmotic crisis must have been utilized: an ion pump. We speculate that to regulate cell volume even primitive cells must have been equipped with the machinery necessary for extruding the NaCl that entered by diffusion [15].

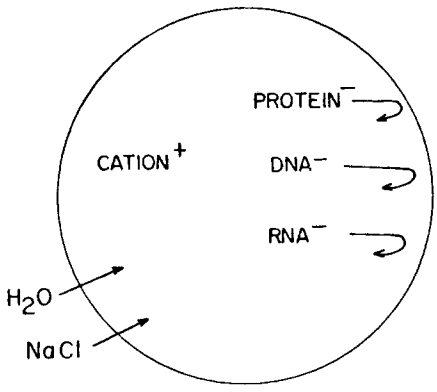

Fig. 2. Colloid osmotic problem of the "first" cell. The formation of a membrane would trap within the cell macromolecules plus the ions of the early sea, which consisted primarily of NaCl. The non-diffusable macromolecules within the cell cause the entry of water and NaCl which in turn causes continuous swelling, and if unopposed, would lead to lysis.

There is a class of microorganisms, the mycoplasmas, that lack a cell wall and must regulate their volume by ion pumps. Unfortunately, these cells have not yet been studied in detail. It seems reasonable to look among this and other primitive types of microorganisms and see if any of them possesses a mechanism that might have served this purpose. The familiar Na^+-K^+ ATPase of animal cells would be ideally suited for the purpose, but we believe it did not evolve until much later, since it has never been found in microorganisms, algae, or higher plants. On the other hand, two ion pumps that could have carried out this function together are found in almost all present-day microorganisms. This consists of an ATP-driven proton pump and an Na^+-H^+ exchange pump. As illustrated in Figure 3, an ATP-driven mechanism pumps out of the cell H^+, which reenters on a carrier in exchange for Na^+. Since most of the returning H^+ become buffered and thereby osmotically inactive, the process leads to a net loss of Na^+.

Let us return to the proton pump for closer scrutiny. We speculate that the primary driving force for membrane-related events was an ATP-driven proton pump. The primitive anaerobes probably depended upon substrate level phosphorylation for ATP production. The respiratory and photosynthetic processes were to come later. ATP was split by the ATPase and H^+ transferred from the cytoplasm to the external medium. This process gave rise to an electrochemical potential difference of protons across the membrane, which we shall refer to as protonmotive force, according to the suggestion of Mitchell [21, 22]. Protonmotive force consists of a chemical potential difference or pH gradient (inside alkaline), as well as a membrane potential $\Delta\Psi$ (inside negative). The quantitative relationship between these factors is given by the equation:

$$\Delta\bar{\mu}_{H^+} = \Delta\Psi - 60\,\Delta ph \qquad (1)$$

All present-day anaerobic microorganisms possess the reversible ATP-driven proton pump. In those anaerobes that have been studied, this mechanism is catalyzed by a complex series of polypeptides associated with an additional membrane-bound protein that acts as a pro-

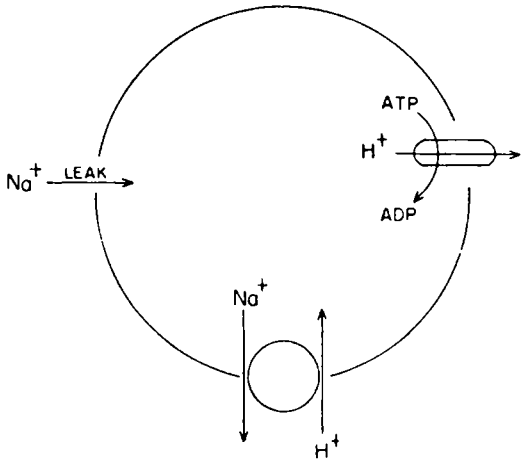

Fig. 3. Solution to the colloid osmotic problem; evolution of two carriers. This primitive sodium pump is driven by a proton gradient, which in turn results from an ATP-driven proton pump. Redrawn from Wilson and Maloney [15].

ton channel. Such a sophisticated mechanism almost certainly emerged after numerous successive refinements. The possible modes of evolution of the ATPase of mitochondria, chloroplasts, and bacteria will be discussed in a later section. A very simple proton ATPase has recently been found in Neurospora [37–39] and yeast cells [40–43]. This enzyme consists of a single polypeptide chain (functioning probably as a dimer) that can pump protons across a plasma membrane with the splitting of ATP. We propose that the first proton pump consisted of a single peptide (probably functioning as a dimer) that was a common ancestor of the Neurospora (and yeast) ATPase and the more complex enzyme found in most modern bacteria.

Several interesting features of Na^+-H^+ exchange mechanisms might be mentioned. This sodium pump is ubiquitous among microbial cells [44–50]. As indicated above, we postulate that this mechanism associated with proton pumping was responsible for volume regulation. In primitive bacteria, this exchange mechanism might have involved the entry of one H^+ and exit of one Na^+. Thus, the driving force for Na^+ extrusion would be the pH gradient across the membrane. The efficacy of this sodium pump would depend upon the pH gradient found across the plasma membrane. Under certain circumstances when the ΔpH was extremely small, Na^+ pumping would be ineffective. Hence, there must have evolved a second, more effective type of Na^+-H^+ exchange mechanism that could pump Na^+ against larger concentration gradients, although it would be energetically more expensive to operate. In this mechanism, two or more protons enter while one sodium ion is extruded. Thus two types of driving forces are harnessed: electrical and chemical gradients.

A second role for the Na^+-H^+ exchange mechanism has been suggested by Skulachev [51]. He pointed out that the Na^+ gradient is, in effect, a storage form of energy that may be converted into ΔpH when needed. During proton pumping, the $\Delta\Psi$ can store very little

energy, while the ΔpH stores far more due to buffering. The Na^+ gradient (which is in equilibrium with either ΔpH or $\bar{\mu}_{H^+}$) is a further storage form of energy.

Potassium ion would be expected to accumulate within a proton pumping cell even if the membrane were only slightly permeable to postassium. The driving force for K^+ uptake would be ΔΨ, 120 mV being sufficient to provide a ratio of K^+ (in)/K^+ (out) of 100/1. As a consequence of this accumulation, the K^+ gradient becomes a form of stored energy that is in equilibrium with ΔΨ. As ΔΨ is drained off, K^+ exits from the cell tending to buffer the membrane potential. Several specific accumulating mechanisms for K^+ were evolved, one of them to overcome the adverse effects of shrinkage due to exposure to external media of high ionic strength. Epstein showed that when E coli cells are plasmolysed in hypertonic solutions K^+ pumps are stimulated to accumulate K^+ which, in turn, leads to H_2O entry and deplasmolysis of the cell [52].

UPTAKE OF NUTRIENTS

The use of phospholipids as the matrix of the plasma membrane created another problem: the simple passive diffusion of most nutrients from the external medium would be severely restricted. Only lipid-soluble substances such as fatty acids (in their protonated form) could readily penetrate the primitive cell membrane. On the other hand, sugars, amino acids, di- and tri-carboxylic acids, and phopshorylated substances could not penetrate the membrane from the external surface at a sufficiently rapid rate to be useful in metabolism. Thus, some type of transport system must have appeared at a very early stage, as Holden has suggested [6]. Two simple transport mechanisms may be visualized: 1) a nonspecific pore (or channel) may be created in the membrane by the insertion of a transmembrane portein(s), or 2) a partially specific protein carrier may be installed in the membrane to catalyze bidirectional movement of small molecules. These proteins might arise de novo as relatively low molecular weight hydrophobic polypeptides, which provided the membrane with a slight increase in permeability to a variety of small molecules. Modifications of these polypeptides that allowed a greater entry rate of existing nutrients would give selective advantage. Alternatively, pre-existing transmembrane proteins (such as the proton pump or Na^+-H^+ exchange carrier) might have been precursors for other membrane carriers. In either case, the substrate would move acorss the membrane without the expenditure of energy. These systems have the advantages of simplicity and the lack of energy requirement. Their disadvantages are that a relatively high external substrate concentration would be required and that with low specificity the loss of metabolic intermediates would be unavoidable. Progressive increase in specificity of channels or carriers would restrict the uptake of irrelevant compounds as well as impede escape of intermediate metabolites.

Examples of channels in present-day organisms include the Na^+-channel of nerve and muscle, the glycerol facilitator of E coli [55–58], the porin channels, and the maltose channels (lamda receptor) in the outer cell membrane of Gram-negative microorganisms. Examples of carriers mediating facilitated diffusion in present-day organisms include glucose, glycerol, and anion carriers of the red blood cell, glucose carriers of most animal cells [53]; and sugar carriers in yeast [54].

As the concentration of nutrients in the environment fell, there developed increasing selection pressure to invent scavenging mechanisms for uptake. One of these processes involved the cotransport of nutrients and cations [59]. By this system, nutrients at low external concentration may be accumulated by the cell to internal concentrations

far higher than those in the environment. The driving force for this pump is energy stored as an electrochemical potential difference of a specific cation. Microorganisms frequently use the electrochemical potential difference of H^+ for their transport system, while animal cells use Na^+ gradients. There is obligatory coupling between H^+ entry and nutrient entry. Thus, protons enter the cell via the carrier down an electrochemical gradient, dragging nutrients into the cell (Fig. 4).

The best known mechanism of this type in microorganisms is the lactose-H^+ cotransport system of E coli. Two types of experiments support the view that there is obligatory coupling between the sugar and the cation. The addition of lactose to energy-depleted cells resulted in proton entry into the cell and an alkalinization of the external medium [60]. In a second type of experiment, it was shown that when energy-depleted cells were incubated in the presence of lactose or lactose analogs, substrate accumulation occurred if an inwardly directed proton-motive force, either in the form of a membrane potential or a pH gradient (outside acid), was provided [61–63].

A different mechanism has evolved for the uptake of certain sugars and polyhydric alcohols. The phosphenolpyruvate-dependent phosphotransferase system [64] translocates a variety of carbohydrates from the external medium and phosphorylates them in the same process. In view of the fact that the sugar must be phosphorylated as an obligatory intermediate for its metabolism, this mechanism may be viewed as energetically most economical, since no additional energy is used for the transport event. Saier [65] suggested that substitutions of hydrophilic amino acids by hydrophobic ones might have enabled a primitive hexokinase to become associated with the membrane. Further mutations might have led to a transmembrane location, thus providing "access" for glucose from the medium. (In this regard, it may be of interest to note that certain specific membrane proteins of the phosphotransferase system exhibit the ability to catalyze facilitated diffusion of the substrate under nonphosphorylating conditions [66, 67].) The culmination of such an evolutionary sequence resulted in the ability of the cell to capture carbohydrates from the medium and to deliver them in phosphorylated form into the cytoplasm. The prototype translocation system would later give rise to a family of protein complexes with

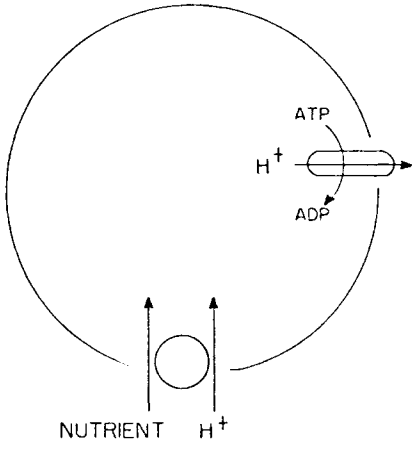

Fig. 4. Nutrient-proton cotransport system.

different substrate specificities. The energy donor might have shifted from ATP to phosphoenolpyruvate. It is possible to visualize other primeval enzymes that might have been converted into membrane carriers in a stepwise manner as described above.

The exploitation of the cation-driven mechanism for transporting lactose by some bacterial species and the use of the phosphotransferase mechanism for uptake of lactose by other species permitted a comparative study. The relative advantages of the two strategies of transport were evaluated by examining the growth abilities on several galactosides. Streptococcus lactis and Staphylococcus aureus, which utilize a phosphotransferase system show a more efficient scavenging ability for lactose than E coli and Klebsiella aerogenes, which utilize the proton-sugar cotransport system (Table I). The threshold for growth of the former two species is 1 μM, whereas that for the latter two species is 10 times higher. On the other hand, the organisms with the cotransport mechanism have a broader specificity that allows at least two additional galactosides to be utilized for growth. In keeping with the hypothesis that the proton driven lactose transport was selected for its versatility, a detoxification reaction catalyzed by thiogalactoside transacetylase apparently coevolved to disencumber the cell of nonutilizable galactosides that are unavoidably accumulated [69]. Thus, depending upon the environmental conditons, different transport systems may be favored.

ENERGY REQUIREMENT FOR DNA TRANSFER

The transfer of DNA molecules from one cell to another played a central role in the evolutionary process. Methods available to prokaryotes include transformation, conjugation, and phage-mediated transduction. These processes apparently require energy in the form of ion gradients. In 1976, Grinius postulated [71] that protonmotive force drives the uptake of DNA. Experimental verification of this hypothesis has come from several sources. The uptake of free DNA (transformation) by Bacillus subtilis is drastically reduced by lowering the protonmotive force [72, 73]. Likewise, a protonmotive force appears to be necessary for DNA transfer in E coli by conjugation [74]. Protonmotive force is also necessary for DNA transfer by phage T4 [75–77].

PROTON PUMPS NOT DRIVEN BY ATP

The earliest cells were capable of producing high energy phosphate in only one manner: substrate level phosphorylation. This is extremely inefficient. It should be recalled that ,whereas the converions of glucose to lactate in the Embden-Meyerhoff pathway yields only two molecules of ATP from ADP, 36 ATP molecules can be generated when the complete respiratory chain is available. The evolution of mechanisms that could transport protons from the interior of the cell across the membrane to the external medium was a crucial process, since it would provide the energy for ATP synthesis by reversing the proton ATPase. Two classes of such mechanisms are well known: oxidation-reduction reactions coupled to proton pumping and light-driven proton pumping dependent on rhodopsin or chlorophyll. There have been diverse views on the order of emergence of components responsible for chlorophyll-dependent photosynthesis and anaerobic electron transport system.

As suggested by Calvin [7], Egami [11], Hall [9] and others [10, 19], it seems reasonable that individual steps in anaerobic oxidation reduction reactions developed prior to photosynthesis. This postulate is supported by data on nucleotide and amino acid se-

quence, which led Schwartz and Dayhoff [26] to conlude that "... many components of the respiratory chain predate oxygen-releasing photosynthesis." We belive that anaerobic respiratory pathways developed by the accretion of one component at a time, resulting in the construction of complexes that can couple a variety of redox reactions to proton pumping. In this process emerged flavoproteins, iron-sulfur compounds, quinones, and cytochromes. The next major event was the evolution of the chlorophyll molecules from the porphyrins, which were already exploited for energy transduction. And finally, the chlorophyll was coupled to preexisting cytochromes, iron-sulphur proteins, and quinones to give the first simple type of bacterial photosynthesis. This was followed by oxygen-liberating photosynthetic processes. The accumulation of atmospheric oxygen allowed the completion of the respiratory chain with the appearance of cytochrome oxidase. Several of these steps will be considered in sequence.

EVOLUTION OF REDOX PROTON PUMPS

An enormous step forward in the evolution of bioenergetics occurred when cells learned to couple oxidation reduction mechanisms to proton pumping. We postulate that a soluble enzyme catalyzing a redox reaction became associated with the plasma membrane. Several mechanisms can be suggested to account for such an event. For example, the gene coding for the redox enzyme might become fused with a duplicated DNA segment of the ATPase gene coding for the transmembrane proton channel. The hybrid protein would thus acquire two functional domains at once. Alternatively, a redox enzyme and a transmembrane protein might have coevolved to give a new enzyme complex. In either case,

TABLE I. Scavenging Power Versus Versatility of the Vectorial Phosphorylating and Concentrative Mechanisms for the Uptake of β-Galactosides*

	Threshold for growth (μM)		
Bacteria	D-galactosyl-β-1,4-D-glucose	D-galactosyl-β-1,4-D-fructose	D-galactosyl-β-1,3-D-arabinose
Escherichia coli (K12)	10	10	10
Klebsiella aerogenes (13882)	10	10	10
Streptococcus lactis (7963)	1	No growth	No growth
Staphylococcus aureus (655)	1	No growth	No growth

Taken from Andrews and Lin [68].

*Gram-negative organisms (E coli and K aerogenes) have both an outer and an inner phospholipid membrane, whereas Gram-positive organisms (S lactis and S aureus) have only an inner plasma membrane. The presence of the outer membrane, however, should not significantly influence the scavenging power of the cell for a β-galactoside, since nutrients with molecular weights up to 600 can pass freely through the nonspecific aqueous channel constituted by the porin proteins. This belief is supported by the observation that the half-saturation constant K_m for growth on glucose (entering via the phosphotransferase system) is 1 μM in the case of E coli [70]. The threshold glucose concentration for growth should be even lower.

the consequence would be the coupling of the oxidation of an internal substrate to the ejection of protons. This redox pump would allow the energy released in the redox step to be converted into an electrochemical potential difference for protons (Fig. 5). When the protonmotive force across the membrane reached a critical level (200–240 mV), the reaction catalyzed by the preexisting proton ATPase would be reversed to give ATP.

REVERSAL OF THE H^+-ATPase FUNCTION (ATP SYNTHESIS)

Mitchell [79] has pointed out the evolutionary significance of the reversal of direction of the proton ATPase. "It is an evolutionarily atrractive proposition that the proton-translocating oxidation loop system and the reversible proton-translocation ATPase may have arisen separately as alternatives for generating the pH difference and membrane potential required for nutrient uptake and ionic regulation via porter systems in primitive prokaryotic cells, and that the accidental occurrence of both systems in the same cell may then have provided the means of storing the free energy of oxidoreduction in ATP synthesized by the reversal of the ATPase, or in some other anhydride, such as pyrophosphate, produced by a similar mechanism." While both redox and ATP-driven pro-

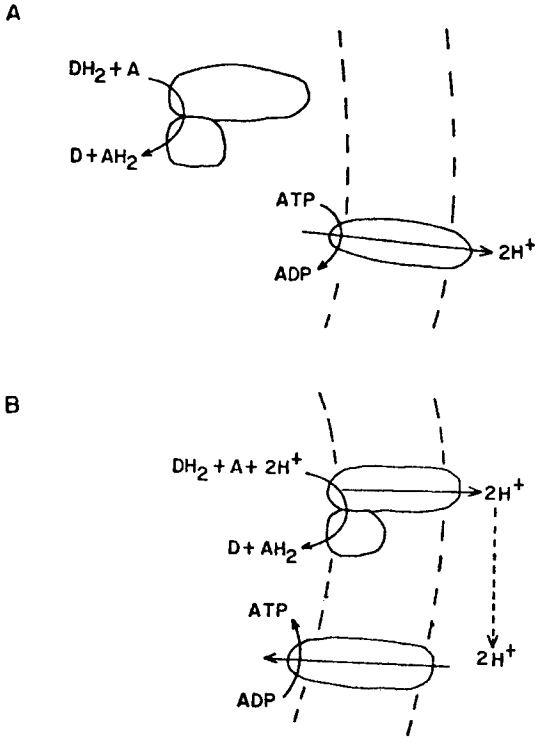

Fig. 5. The redox-proton pump. In A) the oxidation reduction reaction is catabolyzed by a soluble enzyme. In B) the enzyme complex is embedded in the membrane and becomes capable of proton pumping [78]. The protonmotive force reverses the direction of the ATPase giving ATP synthesis.

ton pumps would provide an equally effective source of energy for primitive membrane-related functions, we belive that a proton ATPase (involving only a single peptide) represents a simpler process than the redox loop, which would require two or more mutually adapted proteins. Thus, we postulate that the ATPase predated the redox proton pumps and that such redox mechanisms evolved in a cell already possessing an ATPase.

A key feature of our proposed scheme is the antecedence of a proton ATPase and the capacity of this ATPase to be reversed in direction. Although the proton ATPase of Neurospora and yeast, which we take as a prototype of the original proton pump, have not yet been studied in detail for this reversibility, there is ample evidence that the corresponding enzymes of mitochondria [80], chloroplasts [81], and microorganisms [82] do function in a reversible manner. For example, the proton ATPase of the anaerobe Streptococcus lactis is used physiologically for proton pumping at the expense of ATP, but it can be readily reversed by providing artificial protonmotive force that is inwardly directed [83].

ELABORATION OF ELECTRON TRANSPORT CHAINS

In a cell that has invented a redox pump, a terminal electron receptor must be available for the redox reaction to continue (see Table II). It is possible that initially Fe^{3+} complexes served this purpose. The resulting Fe^{2+} complex might then be photooxidized to regenerate the oxidized form. Even simple ferrous and ferrocyanide ions can be oxidized under the influence of low wavelength ultraviolet light [90]. However, due to poor solubility, it is not liekly that ferric ions in aqueous solution played a biological role in the evolution of the redox chain. This problem could have been overcome by

TABLE II. Redox Potential of Electron Donors and Acceptors

System	E'_o (mV)
Pyruvate/acetate + CO_2	−700
$CO_2{}^+/HCO_2H$	−420
$H^+/1/2\ H_2$ (1 atm)	−420
Ferredoxin Fe^{3+}/Fe^{2+} (Clostridium pasteurianum)	−420
Cytochrome *b* (at least five forms in E coli)	−340 to +220 mV
$NAD^+/NADH$	−320
$FAD/FADH_2$	−220
Cytochrome c_3 Fe^{3+}/Fe^{2+} (Desulfovribrio desulfuricans)	−205
$SO_4{}^=/HS^-$	−200
Pyruvate/lactate	−190
Dihydroxyacetone-P/glycerol-3-P	−190
Menaquinone ox/red (MK)	− 74
Rubredoxin Fe^{3+}/Fe^{2+} (Desulfovribrio desulfuricans)	− 60
Fumarate/succinate	+ 31
Ubiquinone ox/red (Q)	+ 100
Cytochrome *a* Fe^{3+}/Fe^{2+}	+ 290
Ferricyanide/ferrocyanide	+ 360
$NO_3{}^-/NO_2{}^-$	+ 420
Fe^{3+}/Fe^{2}(aqueous)	+ 770
$1/2\ O_2/H_2O$	+ 820

Adopted from references 84–89.

the ability of the organisms to use iron-sulfur compounds. Another advantageous property of these compounds is that they adsorb a broad range of visible light, and therefore the ferrous ion might be readily reconverted to the ferric state by photo-oxidation. Moreover, clustering of the iron-sulfur pairs might greatly facilitate the production of two transitional hydrogen atoms in close proximity so that they can combine to form hydrogen gas in the reaction:

$$2\,e^- + 2\,H^+ \rightarrow H_2. \quad (2)$$

In time, an iron-sulfur complex might have become associated with a specific protein to accept more effectively the electrons released from the redox-proton pump (Fig. 6). As the atmospheric pressure of hydrogen gas gradually decreased, iron-sulfur proteins with lower Fe^{3+}/Fe^{2+} redox potentials might have evolved to become ferredoxins and iron-sulfur hydrogenases. (For reviews of the properties of iron-sulfur proteins, see [91, 92]). This would allow reaction 2 to proceed effectively towards the right both kinetically and thermodynamically. The disposal of electrons by combination with protons to give hydrogen gas would then dispense with the need for photo-oxidation of the iron centers.

A clue to the importance of iron-sulfur complexes in the early development of redox proton pumps is provided by Methanobacterium thermoautotrophicum, which like other methanogenic bacteria, is devoid of cytochromes, menaquinones, or ubiquinones. This anaerobe uses CO_2 as the sole source of energy and carbon in the presence of hydrogen gas. There is no known mechanism in this reaction that would provide substrate level phosphorylation; thus, a redox-proton pump must be responsible for energy generation. About 95% of the CO_2 consumed can be accounted for by the reaction [85, 93, 94]:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (3)$$

Figure 7 depicts schematically the generation of protonmotive force by this reaction. The actual mechanisms, as well as the stoichiometry of the process, await investigation.

The acquisition of a membrane system capable of ejecting protons not only contributes to the ATP pool, but also spares organic compounds such as pyruvate as internal electron acceptors. The carbon compounds thus saved can be diverted to serve as structural material for biosynthetic intermediates or for energy generation at the level of substrate phosphorylation.

Gest suggested another evolutionary pathway for diverting the reducing equivalents arising from glycosis: the use of fumarate as a hydrogen sink [95]. As shown in Figure 8, instead of reducing all of the pyruvate coming from glycolysis to lactate, half of the pyruvate is carboxylated to oxaloacetate, which then accepts two hydrogens from NADH to give malate. The malate is in turn converted to fumarate. It is suggested that the primitive fumarate reductase started as a soluble cytoplasmic enzyme. This type of enzyme was found in Veillonella alcalescens (Micrococcus lactilyticus), a strict anaerobe [96–98]. Although the free energy released from the coupling of NADH oxidation to fumarate reduction is more than sufficient to generate an ATP, the soluble enzyme in this organism seems merely to play a role as a hydrogen disposal system [99].

Further evolution of fumarate reductase resulted in its association with the NADH dehydrogenase complex in the membrane (Fig. 8C). This would now give rise to proton pumping when fumarate is converted to succinate. An example of such a redox-proton pump is found in the anaerobe Streptococcus faecalis, which is devoid of cytochromes. The NADH oxidation coupled to fumarate reduction was shown by Faust and Vandemark to generate high energy phosphate [100]. This energy yielding reaction appears to be medi-

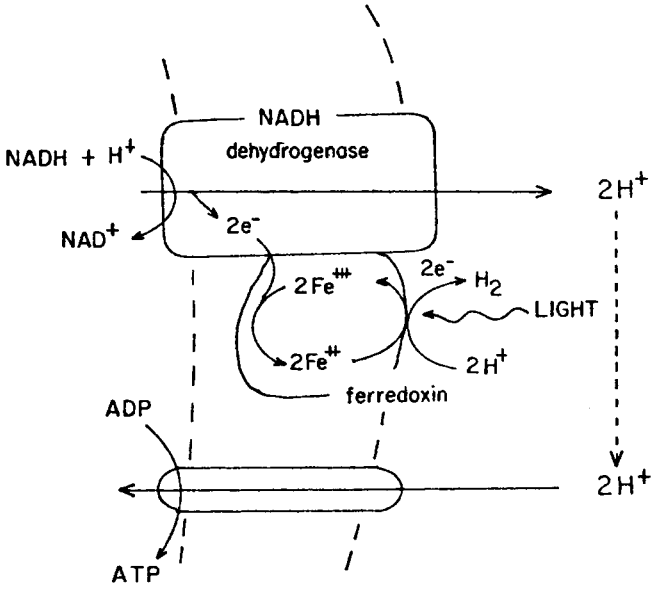

Fig. 6. The role of an iron-sulfur protein in the redox proton pump.

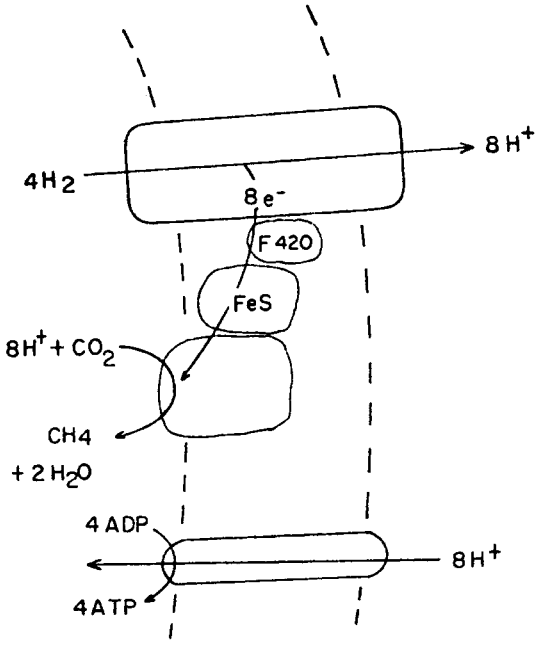

Fig. 7. Redox-proton pump with hydrogen as donor and CO_2 as electron acceptor [85, 93, 94]. F_{420} is a flavinoid compound and FeS deontes an iron sulfur protein.

ated only by ferredoxin and a quinone. The harnessing of the redox reaction to a proton pump explains why fumarate not only reduced the production of lactate and ethanol during glucose fermentation, but also increased the growth yield (70%) beyond what can be accounted for by the small increment in acetate excretion. This excretion is a measure of pyruvate cleavage by the phosphoroclastic reaction that yields ATP [101–102].

The cytochromes probably evolved as a new class of electron carriers following the advent of the redox-proton pump involving ferredoxin and flavins. The heme proteins with different redox potentials were able to greatly increase the spectrum of substrates that could be coupled to various terminal electron acceptors, either as additional sites for proton ejection or by acting as carriers to facilitate the transmission of the electrons.

The *b*-type cytochromes, electron carrier proteins with noncovalently bound protoheme IX as a prosthetic group, might have made their debut in improved redox chains with fumarate as the hydrogen acceptor [102–104]. Perhaps the intercalation of the heme

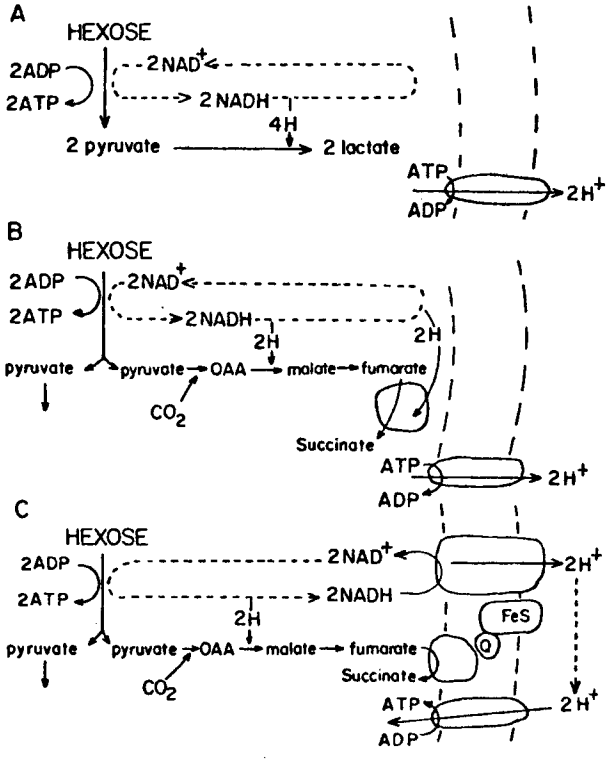

Fig. 8. The role of fumarate reductase in the evolution of a redox-proton pump. A) Glycolysis is by homolactic fermentation, and the ATP generated from glycolysis is used to drive the proton pump. B) The removal of hydrogens by reducing oxaloacetate (OAA) and fumarate allows the cell to save one pyruvate for biosynthesis. The soluble fumarate reductase merely catalyzes the regeneration of NAD^+ from NADH to promote glycolysis. C) Fumarate reductase becomes embedded in the membrane and is coupled to NADH dehydrogenase with ferredoxin and a quinone as intermediate electron carriers. This results in a redox proton pump, the operation of which can reverse the ATPase reaction. Modified from Gest [95].

carrier increases the effectiveness of the proton pumping. A present-day example of a fumarate reductase redox pump that requires a cytochrome *b* is the E coli system with glycerol 3-phosphate as the hydrogen donor (Fig. 9). Membrane preparation of anaerobically grown E coli can couple the oxidation of glycerol 3-phosphate to the reduction of fumarate and the generation of ATP [105, 106]. Furthermore, the mechanisms for ATP synthesis involves proton pumping [107].

The E coli system displayed an interesting property of requiring cytochrome *b* only for the proton pumping process. In the case of a mutant unable to synthesize heme, the addition of fumarate to the medium permitted anaerobic growth on glycerol but at a rate slower than that observed with the wild-type strain. Moreover, the growth yield of the mutant was smaller [108]. In the presence of fumarate, glycerol 3-phosphate stimulated the anaerobic uptake of proline by membrane vesicles prepared from heme-containing cells but not from heme-deficient cells, despite the ability of these cells to couple the dehydrogenation of glycerol 3-phosphate to the reduction of fumarate [109]. Thus, in the absence of cytochrome *b*, the fumarate reductase system appears to act only as a hydrogen sink without proton pumping, as in the case of Veillonella alcalescens. The disposal of the hydrogen would permit glycerol 3-phosphate to be converted to dihydroxyacetone phosphate, which can be effectively used in the glycolytic pathway.

The *c*-type cytochromes, electron carrier proteins with covalently attached protoporphyrin IX as the prosthetic group, might have first evolved in sulfate-reducing bacteria [11, 89]. The strict anaerobes Desulfovibrio are known to possess ferredoxins, quinones, *b* cytochromes, and *c* cytochromes. These organisms can carry out an eight electron reduction of sulfate to sulfide. Sulfate, being a poor oxidant, requires an ATP for initiation

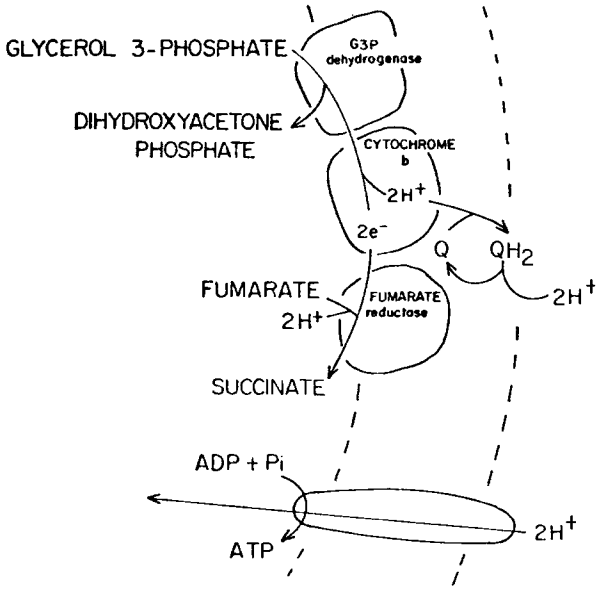

Fig. 9. A cytochrome b associated with a redox proton pump. This kind of mechanism is widely distributed in anaerobic, aerobic, and facultative bacteria.

into the electron transport process. However, the role of the key heme carrier, cytochrome c_3, with a highly negative midpotential, has not yet been clarified in this group of organisms. The sulfate-reducing bacteria are believed to have been in existence for more than two billion years [110, 111].

Nitrate reduction systems could have evolved only after the atmosphere ceased to be highly reducing, since nitrate is readily reduced by hydrogen gas. There is considerable discussion [2, 4, 5, 9, 13] as to whether nitrate respiration preceded or followed the emergence of photosynthesis and aerobic respiration.

Figure 10 depicts in a schematic way several anaerobic redox chains are found in E coli. Although the detailed mechanisms have not yet been worked out, it is likely that all the branches in the network depend on similar basic components: flavins, molybdenum, selenium, iron-sulfur centers, and *b* cytochromes.

The electron donor and acceptor branches show Fe/S proteins and *b* cytochromes symmetrically placed on either side of the quinone. Quinone molecules link the primary dehydrogenase limbs with the terminal electron transport chains. Each dehydrogenase path consists of a proximal electron acceptor (MO or FAD), a high-potential (relatively negative) Fe/S center, and a high-potential *b* cytochrome; each terminal chain contains a low potential (relatively positive) *b* cytochrome, and a low-potential Fe/S center. It seems that by providing specific components for each limb, the electron carriers can be made to interact more readily along a series of cascading redox potentials with large drops only at steps where the coupling system drives protons against an energy gradient.

From the design of branches radiating from the quinone in Figure 10, one would predict that the genes coding for the protein elements of each limb evolved into a regulon that is inducible by the particular primary donor or terminal acceptor. For example, glycerol 3-phosphate might induce its chain consisting of a dehydrogenase, an Fe/S protein, and a cytochrome *b*, while fumarate would induce its chain consisting of reductase, Fe/S, and cytochrome *b*. A second control, dependent on the respiratory state of the cell is superimposed on the specific substrate control, permitting expression of these pathways only under the appropriate redox conditions. This higher order regulation is probably also responsible for the change in relative contents of ubuiquinone and menaquinone when growth is shifted from aerobic to anaerobic conditions. There is a hierarchy of induction in this higher order regulatory mechanism analogous to that of catabolite repression in the metabolism of carbon source, whereby the most efficient, ie, redox positive, electron acceptors repress less efficient pathways. Thus, the availability of molecular oxygen as an acceptor prevents the induction of the nitrate reductase and fumarate reductase systems, and the availability of nitrate as an acceptor bars the induction of the fumarate system.

A variety of redox proton pumps were probably evolved in early anaerobic microorganisms before the atmosphere was enriched with molecular oxygen as a result of microbial photosynthesis. The cytochromes of the *a* type, with heme A (in which an extended farnesyl ethyl group is attached to one pyrrole ring) as a prosthetic group, were then invented to utilize oxygen as the terminal electron acceptor [112]. With such a strong acceptor, three proton ejection sites became possible in the elongated electron transport chain.

It is striking that in the evolution of the energy transducing system associated with membranes, a pivotal role has been played by the element iron, the atomic properties of which make it uniquely suited for catalyzing one electron redox reactions. Calvin [113] pointed out many years ago that the intrinsic properties of the ions Fe^{2+} and Fe^{3+} can be selectively enhanced by certain ligands to suit particular situations. For instance, the

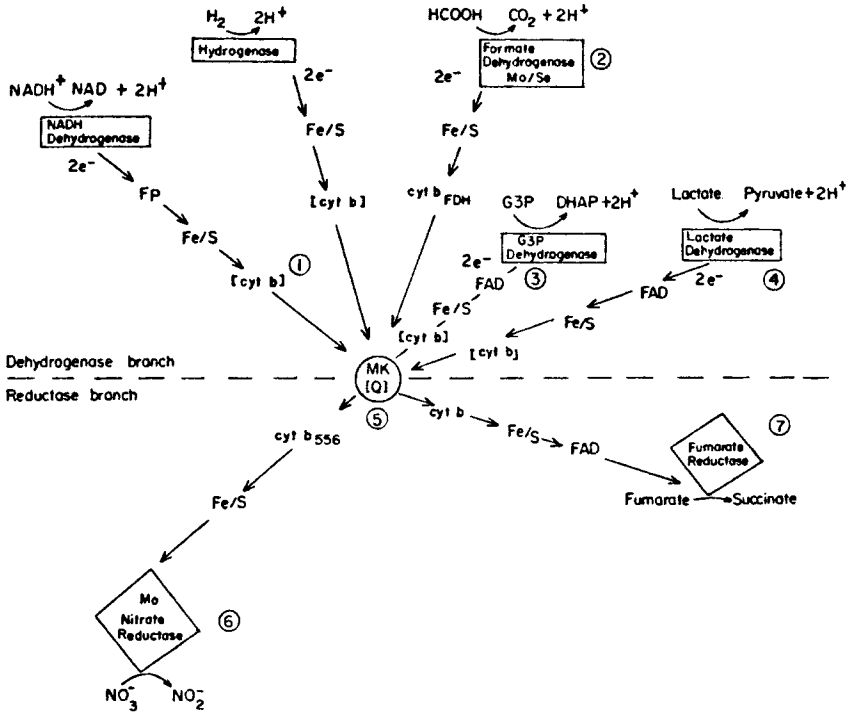

Fig. 10. Organization of anaerobic electron transport. 1) High potential *b* cytochromes in brackets are postulated by analogy to the formate dehydrogenase-fumarate reductase pathway in Vibrio succinogenes and the formate dehydrogenase-nitrate reductase pathway in E coli. 2) The formate dehydrogenase limb has been shown to be a complex constituted with three kinds of subunits and contains Mo/Se, Fe/S, and cyt *b*. 3) Maximal activity of anaerobic G3P dehydrogenase (distinct from an aerobic enzyme) was observed when both FAD and FMN were present. 4) In analogy to the G3P system, it is possible that there may be an anaerobic lactate dehydrogenase which is distinct from an aerobic enzyme. 5) aerobic schemes place ubiquinone at several loci within the chain, shuttling between quinol, semiquinone, and quinone forms. For clarity of presentation, the aerobic path has been eliminated (dehydrogenase components would also change). 6) the nitrate reductase limb has been shown to made of three subunits and contains Mo, Fe/S, and cytochrome b_{556}. 7) fumarate reductase uses FAD as a prosthetic group.

Symbols and abbreviations: F_p stands for flavoprotein, Fe/s for nonheme iron-sulfur center or protein, G3P for glycerol 3-phosphate, DHAP for dihydroxyacetone phosphate, MK for menaquinone, and Q for ubiquinone.

ability of iron to catalyze the decomposition of hydrogen peroxide is enhanced by several orders of magnitude when the metal ion is complexed with porphyrins. The combination of a heme with an apoprotein can enhance such catalytic activity by another several orders of magnitude.

EVOLUTION OF PHOTOSYNTHESIS

It is highly probable that chlorophyll evolved after heme had appeared. With the acquisition of a chlorophyll system, it was possible for the first time to trap light energy

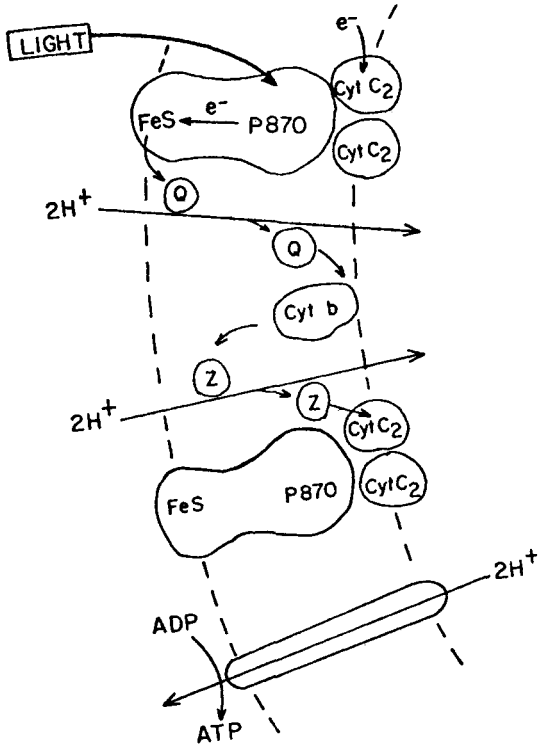

Fig. 11. Light-driven proton pump in chromatophores of Rhodopseudomonas spheroides. Redrawn from Crofts et al., [114] and Prince et al [115].

and use it for proton pumping across the membrane. This reaction utilized the pre-existing iron-sulfur proteins, cytochromes *b* and *c*, and quinones for bacterial photosynthesis [114–118] (Fig. 11). Direct evidence for a light-induced membrane potential (presumably by proton pumping) in such systems came from studies in the laboratory of Skulachev on the reconstitution of the bacteriochlorophyll reaction centers of Rhodospirillum rubrum in liposomes [119]. These vesicles were then introduced to one side of a planar artificial membrane by fusion. Upon illumination, a large membrane potential developed across the fused membrane (presumably as a result of proton pumping).

With the evolution of an additional segment of the photosynthetic chain in cyanobacteria (blue-green algae), it was possible to liberate oxygen from water, as well as to generate a protonmotive force. Figure 12 shows the intermediate steps that are now recognized in the photosynthetic process of chloroplasts [120].

BACTERIAL RHODOPSIN

Bacteriorhodopsin [121] was an excellent mechanism for harvesting the energy from light to provide proton transport across the plasma membrane. While this mechanism must have conferred selective advatnage to certain prokaryotes, nonetheless it apparently was not in the mainstream of evolution of light-trapping mechanisms, for threre is no

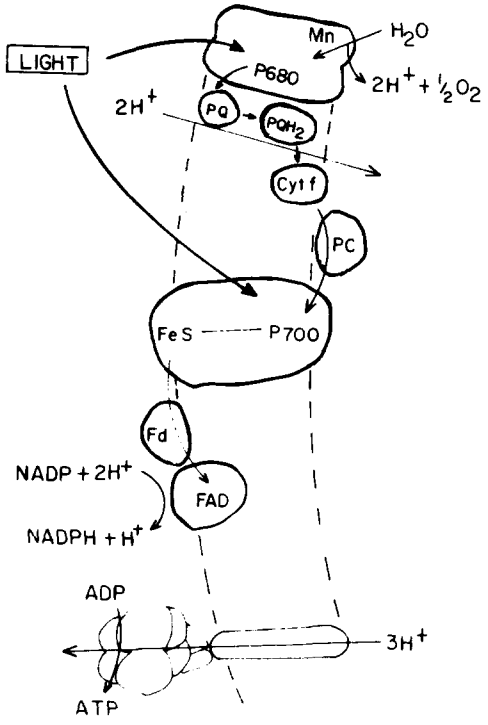

Fig. 12. Photosynthesis in chloroplasts. Redrawn from Hinkle and McCarty [120].

evidence that bacterial rhodopsin was ever incorporated into any of the more complex examples of photosynthetic mechanisms. It seems to us most likely, therefore, that bacterial rhodopsin later diverged to give rise to light recognition elements in phototactic. phototropic, and visual systems, thereby departing from the mainstream of evolutionary events in bioenergetics.

PLANTS

The unicellular photosynthetic plant Chlorella possesses proton pumps and proton-nutrient cotransport systems [122, 123]. Even though there are relatively few specific data on the primary ion pumps of the plasma membrane of higher plants, it is probable that the proton economy is conserved. The Na^+-K^+ ATPase of animal cells has not been found although a K^+-stimulated ATPase has been studied in higher plants by Hodges [124]. Consistent with the hypothesis of a proton economy is the finding of a proton-sucrose cotransport in Ricinus cotyledons [125] and in leaf discs of Beta vulgaris [126, 127].

EVOLUTION OF THE SODIUM POTASSIUM ATPase

The evolution of animal cells removed restrictive features of the outer cell wall, which made possible new types of motility such as ameboid movement. With the loss of the protective rigid cell wall, the problem of colloid osmotic swelling reasserted itself. It was

presumably in response to this osmotic problem that led to the evolution of the sodium-potassium ATPase. Associated with the splitting of one ATP molecule, three Na^+ are extruded and two K^+ accumulated. The net result is the exchange of two internal Na^+ for two external K^+ plus the extrusion of one NaCl. This process allows the cell to pump out the NaCl (and water) that enters under the influence of colloid osmotic forces. The Na^+-K^+ ATPase is simpler and perhaps more efficient than the combination of proton pump and Na^+-H^+ exchange mechanism found in microorganisms. The new sodium economy of animal cells freed the cell from the pH gradients (inside alkaline) required by more primitive organisms. In fact, most animal cells show a reversed pH gradient (inside slightly more acidic than the external environment).

The original animal cells presumably evolved some 600 million years ago in a sea. At that time, cells were probably in approximate osmotic equilibrium with their environment but pumped out the NaCl and H_2O that continually leaked in under the colloid osmotic forces. The reason for the value of 300 mOsm for the osmotic strength of body fluids of many animal species is not entirely clear. Although Macallum [128] has postulated that animal cells evolved in an ocean with an ionic content corresponding to 300 mOsm, the actual salinity of sea water at that time was probably much higher [129]. Perhaps animals evolved in estuaries where fresh water mixed with sea water. Multicellular organisms later acquired general types of insulation against osmotic forces in the environment so that they could move into regions of altered ionic strength. Skin, cuticle, and scales represented different attempts to protect the developing animals from environmental factors. Some animal cells living frequently in hypotonic media, such as amoeba, developed a very special type of osmoregulatory equipment, the contractile vacuole (a combined sodium transport system and mechanical pumping device).

With the appearance of skin and other surface protections, the internal milieu became independent of the external environment, and the internal ionic pattern (including an ionic strength of 300 mOsm) apparently drifted very little. Individual cells within the animal body were bathed in extracellular fluid the composition of which was carefully regulated. Such cells were not faced with large osmotic gradients but required an Na^+ pump to counteract colloid osmotic forces across the membrane.

With the evolution of the Na^+-K^+ ATPase, the animal cell gave up its previous proton economy for a sodium economy for its plasma membrane. One obvious consequence of this change in allegiance from one cation to the other was the necessity of converting H^+-nutrient cotransport systems to Na^+-nutrient mechanisms. All of the nutrient-cation transport systems that have been described in animal cells utilize Na^+ (Fig. 13). Some microorganisms probably had already learned to use Na^+ for nutrient cotransport systems long before animal cells emerged. We find a number of present-day microorganisms, especially those living in high salt concentrations, that utilize Na^+ gradients for driving the uptake of sugars and amino acids [130, 131]. A few Na^+-nutrient cotransport systems are also found in such organisms as E coli (transport of glutamic acid [132] and melibiose [133]). Of particular interest from an evolutionary point of view is the E coli transport system for melibiose. The transport of this compound can be driven by H^+ if Na^+ is absent. However, in the presence of both ions, Na^+ is preferred [134, 135]. Thus we may have an example of an intermediate step in the conversion of the proton economy to the sodium economy.

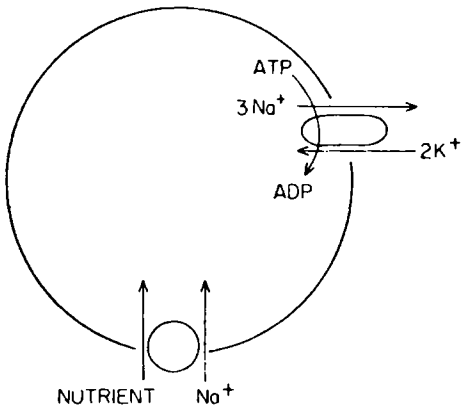

Fig. 13. The sodium economy of animal cells. An ATP-driven Na^+-K^+ pump evolved only in animal cells. The $\bar{\mu}_{Na^+}$ drives Na^+-nutrient cotransport.

ANIMAL CELLS RETAINED ATP-DRIVEN H^+ PUMPS

While Na^+-driven pumps had become predominant in the plasma membrane of animal cells, ATP-driven proton pumps were retained for specialized functions. The gastric mucosa utilizes an ATP-driven H^+-K^+ pump for the production of HCl [136]. In addition, lysozomes [137] and chromaffin granules [138] utilize proton pumps for the purpose of acidfication of the vesicular contents.

EVOLUTION OF ATPases

We propose that from the original proton ATPase of the primitive cell there evolved a wide variety of ATPases used in the active transport of different cations. In Table III are collected data on 7 ATPases that have been studied in recent years. They each have a phosphorylated intermediate with the exception of the mitochondrial ATPase, which has an ATP bound α-subunit. The second common feature is that they each have a polypeptide chain of the order of magnitude of 100,000 daltons. The exception to this latter rule is the proton ATPase of mitochondria, which has six or seven separate polypeptides. It is obviously far more complex. Guidotti [141] points out that the sum of α and β subunits, required for ATP splitting, give a combined molecular weight of slightly more than 100,000 daltons. Comparing the α subunit of the simpler ATPases with the α and β subunits of the mitochondrial ATPase he argues that ". . . the polypeptides with a molecular weight of 100,000 can be split by trypsin into approximately half molecules that resemble the size of α and β polypeptides of the H^+ pump." Thus, it might be concluded that the mitochondrial ATPase is a very sophisticated piece of machinery whose α and β chains were derived from a precursor ATPase. The γ, δ and ϵ subunits are regulatory or structural. The F_0 portion of the ATPase consists of 3 polypeptides found in the plasma membrane: one is the proton channel, another forms a ring at the inner surface, and the third is required for attachment to the remaining portion of the complex.

TABLE III. Properties of Ion Pumps

	H^+ ATPase (Neurospora)[a]	H^+-K^+ ATPase (stomach)[b]	Na^+-K^+ ATPase (animals)[c]	K^+ ATPase (E coli)[d]	Ca^{++} ATPase (muscle)[c]	Ca^{++} ATPase (red cell)[e]	H^+ ATPase (F_1-bacteria)[f]
Mol wt of native enzyme	192,000	400,000	300,000	274,000	200,000	150,000	386,000
Component peptides	96,000	100,000 (α) 100,000 (β)	100,000 (α) 50,000 (β)	90,000 (α) 47,000 (β)	100,000 (α)		56,000 (α) 53,000 (β) 32,000 (ϑ) 16,000 (δ)
Probable structure	α_2	$\alpha_2\beta_2$	$\alpha_2\beta_2$	$\alpha_2\beta_2$	α_2		$\alpha_3\beta_3\vartheta\delta\epsilon$
Phosphorylated intermediate	+	+	+	+	+	+	bound ATP

[a]Bowman et al [139]; [b]Saccomani et al [140]; [c]Guidotti [141]; [d]Epstein and Laimins [142]; [e]Knauf et al [143]; [f]Kagawa [144].

An interesting study by Bastide et al [145] showed that there are genuine similarities between the amino acid sequence immediately adjacent to the phosphate binding site of the Ca^{++}-ATPase of sarcoplasmic reticulum and that of the Na^{+}-K^{+}-ATPase. We presume that, when the complete amino acid sequences are known in all of the ATPases, widespread homologies will be found among them.

ORIGIN OF CHLOROPLASTS AND MITOCHONDRIA

Considerable evidence has been advanced to support the view that mitochondria and chloroplasts are descended from intracellular prokaryotic symbionts [26, 146]. Both kinds of organelles retained some of their genes and the machinery for their expression. These intracellular organelles have retained the proton economy of their forebearers. Mitochondria contain the complete respiratory chain consisting of the "coupling sites" for proton pumping plus an ATPase driven by protonmotive force. A variety of other proton driven reactions, ie, H^{+}-substrate cotransport and H^{+}-substrate exchange, have been clearly demonstrated in mitochondria. The contemporary Paracoccus denitrificans shows a remarkably similar respiratory chain to that of mitochondria [147] and, therefore, is believed to share a common ancestor with them.

Chloroplasts are belived to be descended from photosynthetic bacteria that were associated with eukaryotic cells as symbionts. Protons driven into the granna of the chloroplast producing a pH of approximately four units (inside acid) which, in turn, drives ATP synthesis via the H^{+} ATPase.

ACKNOWLEDGMENTS

We thank D.R. Kuritzkes for suggesting the scheme presented in Figure 17 and for contributing to the discussion of the divergence of electron transport chains. This research was supported by Public Health Service grants AM-05736 and GM-11983 from the National Institutes of Health and by grants from the National Science Foundation (PCM-78-00859 and PCM-76-81070).

REFERENCES

1. Calvin M: Am Sci 44:248, 1956.
2. Ishimoto M, Egami F: In Oparin AI, Pasynskii AG, Braunshtein AE, Pavlovskaya TE (eds): "The Origin of Life on the Earth." New York: Pergamon, 1959, pp 555–561.
3. Wald G: PNAS Centennial: First Scientific Session 52:595, 1964.
4. Klein RM, Cronquist A: Q Rev Biol 42:108. 1967.
5. De Ley J: In Dobzhansky T, Hecht MK, Steere WC (eds): "Evolutionary Biology." New York: Plenum, 1968, pp 103–156.
6. Holden JT: J Theor Biol 21:97, 1968.
7. Calvin M: "Chemical Evolution." New York: Oxford Univ, 1969.
8. Gutknect J: Am Zoologist 10:347, 1970.
9. Hall JB: J Theor Biol 30:429, 1971.
10. Horvath RS: J Theor Biol 47:361, 1974.
11. Egami F: Orig Life 5:405, 1974.
12. Gaffron H: In Kasha M, Pullman B (eds): "Horizons in Biochemistry." New York: Academic, 1962, pp 59–89.

13. Broda E: "The Evolution of the Bioenergetic Process." England: Pergamon, 1975.
14. Smith FA, Raven JA: In Lüttge U, Pitman MG (eds): "Encyclopedia of Plant Physiology." Berlin: Springer-Verlag, 1976, pp 317–346.
15. Wilson TH, Maloney PC: Fed Proc 35:2174, 1976.
16. Raven JA, Smith FA: J Theor Biol 57:301, 1976.
17. Harold F: In Gunsalus IC, Ornston LN, Sokatch JR (eds): "The Bacteria." Vol 6, New York: Academic, 1978, pp 463–521.
18. Olson JM: Evol Biol 11:1, 1978.
19. Gray CT, Gest H: Science 148:186, 1965.
20. Jasaitis AA: "Assembly of Prebiological and Biological Structures." Proc Internat Symp Moscow (in press).
21. Mitchell P: Nature (London) 191:144, 1961.
22. Mitchell P: Biol Rev 41:445, 1966.
23. Woese CR, Pribula CD, Fox GE, Zablen LB: J Mol Evol 5:35, 1975.
24. Pribula CD, Fox GE, Woese CR: FEBS Letts 64:350, 1976.
25. Dayhoff MO: "Atlas of Protein Sequence and Structure." Vol 5, supp 3, Washington DC: Natl Biomed Res Found, 1978.
26. Schwartz RM, Dayhoff MD: Science 199:395, 1978.
27. Dickerson RD: Sci Am 242:137, 1980.
28. Berkner LV, Marschall LC: Discuss Faraday Soc 37:122, 1964.
29. Cloud PE Jr: Science 160:729, 1968.
30. Schopf JW: Ann Rev Earth Plan Sci 3:213, 1975.
31. Schopf JW, Oehler DZ: Science 193:47, 1976.
32. Haldane JBS: New Biol 16:12, 1954.
33. Wilson TH: Science 120:104, 1954.
34. Leaf A: Biochem J 62:241, 1956.
35. Tosteson DC: In Hoffman JF (ed): "The Cellular Functions of Membrane Transport." Englewood Cliffs: Prentice-Hall, 1964, pp 3–22.
36. Macknight ADC, Leaf A: Physiol Rev 57:510, 1977.
37. Scarborough GA: J Biol Chem 250:1106, 1975.
38. Scarborough GA: Proc Natl Acad Sci USA 73:1485, 1976.
39. Bowman BJ, Blasco F, Allen KE, Slayman OW: In Dainty J (ed): "Plant Membrane Transport." Amsterdam: Elsevier, 1980, pp 195–205.
40. Delhez J, Dufour J-P, Thines D, Goffeau A: Eur J Biochem 79:319, 1977.
41. Dufour J-P, Goffeau A: J Biol CHem 253:7026, 1978.
42. Serrano R: Mol and Cell Biochem 22:51, 1978.
43. Willsky GR: J Biol Chem 254:3326, 1979.
44. Harold FM, Papineau D: J Membr Biol 8:45, 1972.
45. West IC, Mitchell P: Biochem J 144:87, 1974.
46. Tokuda H, Kaback HR: Biochemistry 16:2130, 1978.
47. Schuldiner S, Fishkes H: Biochemistry 17:706, 1978.
48. Lanyi JK, MacDonald RE: Biochemistry 15:4608, 1976.
49. Brey RN, Beck JC, Rosen BP: Biochem Biophys Res Commun 83:1588, 1978.
50. Beck JC, Rosen BP: Arch Biochem 194:208, 1979.
51. Skulachev VP: FEBS Lett 87:171, 1978.
52. Epstein W, Laimins L: TIBS 5:21, 1980.
53. Cereijido M, Rotunno CA: "Introduction to the Study of Biological Membranes." New York: Gordon and Breach, 1970, pp 82–89.
54. Cirillo VP: Annu Rev Microbiol 15:197, 1961.
55. Sanno Y, Wilson TH, Lin ECC: Biochem Biophys Res Commun 32:344, 1968.
56. Richev DP, Lin ECC: J Bacteriol 112:784, 1972.
57. Alemohammad MM, Knowles CJ: J Gen Microbiol 82:125, 1974.
58. Heller K, Lin ECC, Wilson TH: Unpublished observations.
59. Crane RK: Rev Physiol Biochem Pharmacol 78:99, 1977.
60. West IC: Biochem Biophys Res Commun 41:655, 1970.
61. Kashket ER, Wilson TH: Biochem Biophys Res Commun 49:615, 1972.

62. Hirata H, Altendorf K, Harold FM: Proc Natl Acad Sci USA 70:1804, 1973.
63. Flagg JL, Wilson TH: J Membr Biol 31:233, 1977.
64. Kundig W, Ghosh S, Roseman S: Proc Natl Acad Sci USA 52:1067, 1964.
65. Saier MH Jr: Bacteriol Rev 41:856, 1977.
66. Solomon E, Miyal K, Lin ECC: J Bacteriol 114:723, 1973.
67. Kornberg HL, Riordan C: J Gen Microbiol 94:75, 1976.
68. Andrews KJ, Lin ECC: Fed Proc 35:2185, 1976.
69. Andrews KJ, Lin ECC: J Bacteriol 128:510, 1976.
70. von Meyenburg K: J Bacteriol 107:878, 1971.
71. Grinius L: Biokhimiya 41:1539, 1976.
72. Griniuviene BB, Grinius LL, Kiasinyte RJ, Chaustova LP, Jasaitiis AA: Biokhimiia 43:1539, 1979.
73. Chaustova LP, Grinius LL, Griniuviene BB, Jasaitis AA, Kadziauskas JP, Kiausinyte RJ: Eur J Biochem 103:349, 1980.
74. Grinius L, Berzinskiene J: FEBS Lett 72:151, 1976.
75. Kalasauskaite E, Grinius L: FEBS Lett 99:287, 1979.
76. Labedan B, Goldberg EB: Proc Natl Acad Sci USA 76:4669, 1979.
77. Labedan B, Heller KB, Jasaitis AA, Wilson TH, Goldberg EB: Biochem Biophys Res Commun 93:625, 1980.
78. Haddock BA, Jones CW: Bacteriol Rev 41:47, 1977.
79. Mitchell P: Soc Gen Microbiol Symp 20:121, 1970.
80. Reid RA, Moyle J, Mitchell P: Nature 212:257, 1966.
81. Jagendorf AT, Uribe E: Proc Natl Acad Sci USA 55:170, 1966.
82. Maloney PC, Kashket E, Wilson TH:Proc Natl Acad Sci USA 71:3896, 1974.
83. Maloney PC, Wilson TH: Membrane Biol 25:285, 1975.
84. Loach PA: In Fasman GD (ed): "Handbook of Biochemistry and Molecular Biology." Vol 1, Cleveland: CRC, 1976, pp 122–130.
85. Thauer RK, Jungermann K, Decker K: Bacteriol Rev 41:100, 1977.
86. White A, Handler P, Smith EL, Hill R, Lehman IR: "Principles of Biochemistry." New York: McGraw-Hill, 1978, p 275.
87. Deeb SS, Hager IP: J Biol Chem 239:1024, 1964.
88. Hendler RW, Towne DW, Shrager RI: Biochim Biophys Acta 376:42, 1975.
89. Dickerson RE, Timkovich R: In Boyer PD (ed): "The Enzymes." Vol 11, New York: Academic, 1975, pp 397–547.
90. Calvert JG, Pitts JN Jr: "Photochemistry." New York: John Wiley and Sons, 1966, pp 271.
91. Orme-Johnson WH: Annu Rev Biochem 42:159, 1973.
92. Palmer G: In Boyer PD (ed): "The Enzymes." Vol 12, New York: Academic, 1975, pp 1–56.
93. Balch WE, Fox GE, Magrum LJ, Woese CR, Wolfe RS: Microbil Rev 43:260, 1979.
94. Daniels L, Fulton G, Spencer RW, Orme-Johnson WH: J Bacteriol 141:694, 1980.
95. Gest H: FEMS Microbiol Lett 7:73, 1980.
96. Peck HD Jr, Smith OH, Gest H: Biochim Biophys Acta 25:142, 1957.
97. Warringa MGPJ, Guiditta A: J Biol Chem 230:111, 1958.
98. Warringa MGPJ, Smith OH, Guiditta A, Singer TP: J Biol Chem 230:97, 1959.
99. Konings WN, Boonstra J, De Vries W: J Bacteriol 122:245, 1975.
100. Faust PJ, Vandemark PJ: Arch Biochem Biophys 137:392, 1970.
101. Deibel RH, Kevetkas MJ: J Bacteriol 88:858, 1964.
102. Kroger A: In Haddock BA, Hamilton WA (eds): "Microbial Energetics." Cambridge: Cambridge University, 1977, pp 61–93.
103. Kroger A: Biochim Biophys Acta 505:129, 1978.
104. Hagihira B, Sato N, and Yamanaka T: In Boyer PD (ed): "The Enzymes." Vol 11, New York: Academic, 1975, pp 549–593.
105. Miki K, Lin ECC: J Bacteriol 114:767, 1973.
106. Miki K, Lin ECC: J Bacteriol 124:1282, 1975.
107. Miki K, Wilson TH: Biochem Biophys Res Commun 83:1570, 1978.
108. Singh AP, Bragg PD: Biochim Biophys Acta 396:299, 1975.
109. Singh AP, Bragg PD: Biochim Biophys Acta 423:450, 1976.

110. Thode HG, Macnamara J, Fleming WH: Geochim Cosmochim Acta 3:235, 1953.
111. Postgate JR: Proc Roy Soc Lond Series B 171:67, 1968.
112. Jurtshuk P Jr: Mueller TJ, Acord WC: Critical Reviews in Microbiology 3:399
113. Calvin M: Science 1170, 1959.
114. Crofts AR, Evans EH, Cogdell RS: Ann NY Acad Sci 227:227, 1974.
115. Prince RC, Baccarini-Melandri A, Hauska GA, Melandri BA, Crofts RA: Biochim Biophys Acta 387:212, 1975.
116. Olson JM: Science 168:438, 1970.
117. Crofts AR, Wood PM: Curr Topics Bioenerg 7:175, 1978.
118. Dutton PL, Prince RC: In Gunsalus IC, Ornston LN, Sokatch JR (eds): "The Bacteria," Vol 6, 1978, pp 523–584.
119. Barsky EL, Dancshazy Z, Drachev LA, Il'ina MD, Jasaitis AA, Kondrashin AA, Samuilov VD, Skulachev VP: J Biol Chem 251:7066, 1976.
120. Hinkle PC, McCarty RE: Sci Am 238:104, 1978.
121. Stoeckenius W, Lozier RH, Bogomolni RA: Biochem Biophys Acta 505:215, 1979.
122. Komor E, Tanner W: Cur J Biochem 44:219, 1974.
123. Schwab WGW, Komor E: FEBS Lett 87:157, 1978.
124. Hodges TK: In Lüttge U, Pitman MG (eds): "Encyclopedia of Plant Physiology-Transport in Plants II." New Series, vol 2, part A, New York: Springer-Verlag, 1976, pp 260–283.
125. Hutchings VM: Planta Med 138:237, 1978.
126. Giaguinta R: Plant Physiol 59:750, 1977.
127. Malek F, Baker DA: Planta Med 135:297, 1977.
128. Macallum AB: Physiol Rev 6:316, 1926.
129. Conway EJ: Proc R Ir Acad [B], 48; No 9:161, 1943.
130. Drapeau GR, MacLeod RA: Biochem Biophys Res Commun 12:111, 1963.
131. MacDonald RE, Lanyi JK: Biochemistry 14:2882, 1975.
132. Frank L, Hopkins I: J Bacteriol 100:329, 1969.
133. Tsuchiya T, Raven J, Wilson TH: Biochem Biophys Res Commun 76:26, 1977.
134. Tsuchiya T, Wilson TH: Membr Biochem 2:63, 1978.
135. Tsuchiya T, Takeda K, Wilson TH: Membr Biochem 3:131, 1980.
136. Lee J, Simpson G, Scholes P: Biochem Biophys Res Commun 60:825, 1974.
137. Mego JL, Farb RM, Barnes J: Biochem J 128:763, 1972.
138. Bashford CL, Radda GK, Ritchie GA: FEBS Lett 50:21, 1975.
139. Bowman BJ, Blasco F, Slayman CW: In Dutton PL, Leigh JS, Scarpa A (eds): "Frontiers of Biological Energetics." Vol 1, New York: Academic, 1978, pp 525–533.
140. Saccomani G, Dailey DW, Sachs G: J Biol Chem 254:2821, 1979.
141. Guidotti G: In Shmitt FO, Worden FG (eds): "The Neurosciences 4th Study Program." Cambridge: MIT, 1979, pp 831–840.
142. Epstein W, Laimins L: TIBS 5:21, 1980.
143. Knauf PA, Fulgencio P, Hoffman JF: J Gen Physiol 63:324, 1974.
144. Kagawa Y: Biochim Biophys Acta 505:45, 1978.
145. Bastide F, Meissner G, Fleischer S, Post RL: J Biol Chem 248:8385, 1973.
146. Margulis L: "The Origin of Eukaryote Cells." New Haven: Yale University, 1970.
147. John P, Whatley FR: Nature 254:495, 1975.