Biochimica et Biophysica Acta, 604 (1980) 297–320
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BBA 85210

INTEGRATING FUNCTIONS OF BIOMEMBRANES

PROBLEMS OF LATERAL TRANSPORT OF ENERGY, METABOLITES AND ELECTRONS

VLADIMIR P. SKULACHEV

Department of Bioenergetics, A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow (U.S.S.R.)

(Received June 3rd, 1980)

Contents

I. Introduction

Usually, biological membranes are regarded as structures that separate the cytoplasm from the environment, or one intracellular compartment from the rest. Without any doubt, such a role is inherent in any biomembrane. However, the separating functions always involve some integrating effects. For instance, the cytoplasmic membrane of a bacterium not only segregates cytoplasm from the outer medium but also unites diverse intracellular contents into a common system.

Development of biochemistry and cytology has shed light on new intriguing aspects of the integrating functions of biomembranes. My objective in this paper is to formulate and consider those functions which may be important in bioenergetics.

II. Energy transmission along membranes

IIA. Electrochemical H^+ potential as a convertible form of energy

During the last decade, the so-called chemiosmotic principle of oxidative and photosynthetic phosphorylation was experimentally proved. According to this principle, introduced by Mitchell [1,2], redox chains of respiratory or photosynthetic systems generate a transmembrane difference of electrochemical H^+ potentials ($\Delta\bar{\mu}_H$) which is then utilized to form ATP by an H^+-ATP-synthetase (EC 3.6.1.3). Numerous studies inspired by Mitchell's concept have allowed the chemiosmotic principle to be extended to systems other than the ADP phosphorylation process. In particular, it was found that $\Delta\bar{\mu}_H$ can be used to support (a) reverse electron transfer in redox chains, (b) inorganic pyrophosphate synthesis, (c) uphill transport of many ions, substrates and metabolites across membranes, (d) bacterial motility and (e) regulatory heat production in warm-blooded animals (for reviews, see Refs. 2–7). All these processes do not require ATP-synthetase, being in fact alternatives to the $\Delta\bar{\mu}_H$-dependent ATP formation. It was concluded that there is not one, but two, convertible energy currencies for the cell: (1) the well known family of high-energy compounds headed by ATP, and (2) $\Delta\bar{\mu}_H$ [6]. The latter, membrane-linked, form of convertible energy consists of the electric potential difference ($\Delta\Psi$) and the H^+ concentration difference (ΔpH). Due to the low electric capacitance of biological membranous systems, $\Delta\Psi$ proves to be the primary form of generated $\Delta\bar{\mu}_H$[8,9]. In the presence of a charged penetrant, a $\Delta\Psi \rightarrow \Delta$pH transition takes place, resulting in a great increase in the amount of energy equivalents stored in the form of $\Delta\bar{\mu}_H$, since the capacitance of pH buffers in the cell is much higher than the electric capacitance of a membrane [8,9].

The suggestion has been put forward [9] that there are ion transport processes specialized in $\Delta\Psi \rightarrow \Delta$pH energy conversion. For example, in bacteria, this might be influx of K^+ which is electrophoretically accumulated in the cytoplasm. ΔpK, when formed, can play a role as a $\Delta\Psi$ buffer. ΔpNa produced by means of Na^+/H^+ antiport was suggested to be a ΔpH buffer.

IIB. Lateral $\Delta\bar{\mu}_H$ transport

IIB-1. General remarks

Membrane-linked energy, being in its electric form, seems to be very convenient for transmission along a coupling membrane. The electric conductance of the media on both

sides of the membrane is very high while that of the membrane can be extremely low. This means that $\Delta\Psi$ produced by a $\Delta\bar{\mu}_{H}$ generator in a certain area of a membrane can, in principle, be transmitted as such along the membrane and transduced into work when used in another region of the same membrane. This reasoning was extended to a hypothesis regarding membranes as electric power-transmitting cables operating at the cell level. Such a concept was put forward in 1969 and 1971 [3,10] and recently considered in connection with the possible functions of giant mitochondria in muscle [4,6,11,12] and yeast [13] (for details, see subsection IIB-5).

It should be stressed that $\Delta\Psi$, if produced by a $\Delta\bar{\mu}_{H}$ generator, cannot avoid irradiation over the membrane surface [5]. However, it does not exclude the possibility that H^+ pumped by a $\Delta\bar{\mu}_{H}$ generator may be utilized by the nearest $\Delta\bar{\mu}_{H}$ consumer without diffusion of H^+ in the solution washing the membrane surface. An extremal point of view will be that a $\Delta\bar{\mu}_{H}$ consumer such as H^+-ATP-synthetase in mitochondria uses respiratory chain-produced protons before they are diluted in the extramitochondrial water (e.g., see Ref. 14). There is no unequivocal evidence for such a 'microchemiosmotic' mechanism, but this possibility is still not excluded. In fact, one of the substrates of oxidative phosphorylation, extramitochondrial ADP, seems to be transported by ADP/ATP antiporter directly to H^+-ATP-synthetase, without release of ADP into the intramitochondrial solution [15]. On the other hand, it was found that another substrate of the same reaction, extramitochondrial inorganic phosphate, is first transported to the intramitochondrial solution and then interacts with H^+-ATP-synthetase [16].

IIB-2. $\Delta\bar{\mu}_{H}$ transport along Halobacterium membrane

Lateral $\Delta\bar{\mu}_{H}$ transmission should be an indispensable step in the utilization of light energy in *Halobacterium*. In this micro-organism, more than 50% of the cytoplasmic membrane can be occupied by oval membranous areas reaching 0.5 μm in diameter and composed of bacteriorhodopsin and phospholipids (purple sheets) [17,18]. Bacteriorhodopsin was shown to pump H^+ from the cell interior to the outer medium at the cost of light energy [19–22]. $\Delta\bar{\mu}_{H}$ generated by bacteriorhodopsin is utilized by H^+-ATP-synthetase, some metabolite transport systems, flagellar motors, etc. (see Ref. 6). All these $\Delta\bar{\mu}_{H}$ consumers must be localized in membrane regions other than the purple sheets, since bacteriorhodopsin is the only protein constituent found in the sheets [17,19]. There is no doubt that bacteriorhodopsin is connected with $\Delta\bar{\mu}_{H}$ consumers via lateral $\Delta\bar{\mu}_{H}$ transmission.

This means, in particular, that an H^+-ATP-synthetase localized in *Halobacterium* membrane must be able to utilize $\Delta\bar{\mu}_{H}$ produced in a remote region of this membrane, namely, in a purple sheet [23]. So, such an H^+-ATP-synthetase operates in a 'macrochemiosmotic' fashion. As soon as light-dependent ATP formation is observed in proteoliposomes containing bacteriorhodopsin and H^+-ATP-synthetases from mitochondria [24], chloroplasts [25] and heterotrophic bacteria [26], one may conclude that macrochemiosmotic coupling can be a mechanism of ATP production in all major types of coupling membranes. Another body of evidence in support of this conclusion is the observations on ATP synthesis driven by artificially imposed $\Delta\Psi$ and ΔpH in natural and reconstituted coupling membranes (for review, see Refs. 3 and 27). As for microchemiosmotic coupling involving no lateral $\Delta\bar{\mu}_{H}$ transmission, this can only be a second pathway of oxidative (or photosynthetic) phosphorylation, co-existing, perhaps, with the macrochemiosmotic mechanism.

IIB-3. Lateral energy transport in chlorophyll-containing membranes

It is well known that light quanta are primarily absorbed by the antenna chlorophyll. Then the chlorophyll excitation energy migrates in the plane of the membrane until it reaches a chlorophyll-electron acceptor complex defined as a photosynthetic reaction center. The amount of antenna chlorophyll is very much greater than that of the reaction center chlorophyll (for review, see Ref. 28).

In all likelihood, the excitation energy migration is supplemented with a $\Delta\bar{\mu}_H$ transmission. There are electron-microscopic indications [29] that in chloroplasts, H^+-ATP-synthetases are mainly localized in lamellar membranes, whereas photosynthetic redox chains are found both in thylakoid and lamellar membranes. Thylakoid membranes comprise the major part of the membranous material of the chloroplast. Lateral $\Delta\bar{\mu}_H$ transport seems to be the only way to actuate the H^+-ATP-synthetases of lamellar membranes at the expense of the thylakoid-generated $\Delta\bar{\mu}_H$.

IIB-4. Intercellular power transmission along cyanobacterial trichomes

Any coupling membrane carries thousands of individual $\Delta\bar{\mu}_H$ generators and consumers. All of these can be united in a common system owing to the transportability of $\Delta\bar{\mu}_H$. It seems reasonable to assume that the obvious advantages of such an energy unification are used by the cell even when $\Delta\bar{\mu}_H$ generators are localized in the same membrane region as $\Delta\bar{\mu}_H$ consumers.

Therefore, one may regard the cytoplasmic membrane of bacteria that is always competent in $\Delta\bar{\mu}_H$ formation as an energy-transmitting structure operating at the cell level. The same may be true of the outer membrane of fungae or plant cells that apparently also possess $\Delta\bar{\mu}_H$ generators (for review, see Ref. 30). Very long cells of some algae, fungi and higher plants might be nice experimental subjects for studying lateral energy transport.

In our laboratory, experiments were recently carried out demonstrating that in the multicellular prokaryotes, cyanobacteria (blue-green algae), there is a mechanism for intercellular power transmission over distances of the order of millimeters. Indications were obtained that the power is transmitted in the form of membrane potential.

A trichome of cyanobacteria represents a linear sequence of many hundreds of cells. It can be several millimeters long.

The cytoplasmic membrane of cyanobacteria belongs to the category of coupling membranes bearing $\Delta\bar{\mu}_H$. If there is an electric conductance between trichome-composing cells, one may hope that the electric potential difference generated across the cytoplasmic membrane near to one end of the trichome can be utilized in its distal part to perform work [31,32].

In the experiments described below, Chailakhian and co-workers [32] have participated. The light and the motility of trichomes were used as a source of $\Delta\bar{\mu}_H$ generation and $\Delta\bar{\mu}_H$-supported work, respectively. The latter proved to be a convenient intrinsic probe for $\Delta\bar{\mu}_H$, since it was found in this laboratory by Glagolev and co-workers that the motility of trichomes of cyanobacteria is directly supported by $\Delta\bar{\mu}_H$ like motility of flagellar bacteria (for review, see Ref. 31).

A trichome of the cyanobacterium, *Phormidium uncinatum*, was exposed to white light under conditions where light was the only energy source for motility. Then the same trichome was illuminated by a small, white-light beam forming a spot of light which covered only about 5% of the trichome length. It was shown that such partial illumination of the trichome could support motility. Moreover, the motility rate proved to be of the same order of magnitude as that under total illumination, if the light intensity in the spot

was sufficiently high. Two explanations of this fact may be considered. (i) The capacity of a 5% portion of the total amount of the trichome protonic motors is sufficient to move the organism. (ii) The power produced in the illuminated part of the trichome is transmitted from cell to cell so that all protonic motors of the body of this organism are activated.

To discriminate between these alternative possibilities, the following experiment was carried out. A viscous medium (agar) used in the above experiments was replaced by an aqueous solution to prevent cyanobacterial locomotion. Under such conditions, trichomes were motionless although protonic motors were operative. Their activity could be revealed when the rotatory movement of rings of slime excreted by cyanobacteria was observed. As the experiments showed, the light spot induced rotation of the slime rings, not only in the illuminated, but also in the non-illuminated parts of the trichome, including its distal end. Thus, the light spot activates all protonic motors of this organism and such an effect requires, of course, intercellular power transmission.

It should be stressed that the rate of power transmission in cyanobacteria occurs on a time scale of seconds. This is in agreement with calculations showing that electric transmission along a 1 mm trichome composed of several hundreds of electrically connected cells must occur within several seconds, while diffusion of any chemical compound (ATP, glucose or a chemical transmitter) requires many minutes or even hours.

In the same series of experiments, it was revealed that dicyclohexylcarbodiimide treatment does not inhibit the light spot-induced motility and hence does not prevent transmission of power. This fact also indicates that $\Delta\bar{\mu}_H$ (or one of its constituents), rather than ATP or any other chemical product of photosynthesis, is responsible for power transmission.

It should be noted that light was the only energy source for generation of $\Delta\mu_H$ in the dicylohexylcarbodiimide-treated trichomes, if the age of the organisms was about 3 h, and cyanobacteria were motionless if not illuminated with light of wavelengths actuating electron flow in the photosynthetic redox chain. Under dim blue-green illumination, trichomes were motionless. A motility could be actuated by an artificial formation of ΔpH (acidification of the medium) or of $\Delta\Psi$ (valinomycin addition to a K^+-free medium).

Experiments with valinomycin allowed us to identify the $\Delta\mu_H$ constituent responsible for power transmission. It proved to be $\Delta\Psi$. Trichomes were illuminated with non-saturating total light or a light beam forming a spot which covered about 5% of the trichome. The light in the beam was chosen of an intensity such that it gave the same motility rate as that under total illumination. Then valinomycin was added. It was found that valinomycin induced (i) a strong, but transient, decrease in the motility rate when illumination was total, and (ii) complete and irreversible inhibition of the light spot-supported motility. The difference between the two regimes of illumination was especially dramatic several minutes after valinomycin addition when the rate of motility under total illumination recovered to a level close to that before valinomycin addition, whereas the light spot failed to support any motility. The totally illuminated trichomes could be stopped by addition of nigericin after valinomycin. Without valinomycin, nigericin was ineffective. Apparently, valinomycin caused $\Delta\Psi \rightarrow \Delta$pK energy transduction accompanied by a ΔpH increase. After several minutes, such a large ΔpH was formed that it effectively replaced $\Delta\Psi$, and a $\Delta\bar{\mu}_H$ level as large as that before valinomycin addition was regenerated. If ΔpH were a transportable form of power, it would support motility not only under total, but also under partial, illumination. Since this was not the case, one can assume that $\Delta\Psi$ rather than ΔpH is the transportable $\Delta\bar{\mu}_H$ constituent.

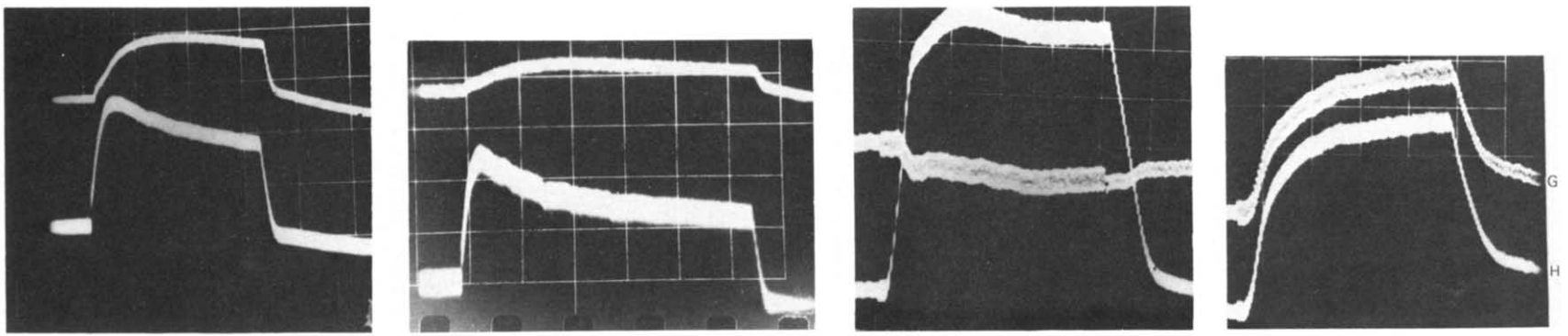

Fig. 1. Lateral transmission of an electric potential difference, measured with extracellular electrodes situated along trichomes of the cyanobacterium, *Phormidium uncinatum*. Abscissa, time (s); ordinate, voltage (mV). The distances between electrode 4 and electrodes 1, 2 and 3 were 1100, 750 and 400 μm, respectively. In Expt. 1 (curves A–D), the 100 μm-long end-part of trichomes near electrode 1 has been illuminated with a light beam. Synchronous measurements of $\Delta\Psi$ between electrodes 2 and 4 (A) and 1 and 4 (B). Synchronous $\Delta\Psi$ measurements between electrodes 3 and 4 (C), and 1 and 4 (D). In Expt. 2 (curves E–H), the 100 μm-long middle-part of trichomes near electrode 3 has been illuminated. Synchronous $\Delta\Psi$ measurements between electrodes 2 and 4 (E), and 3 and 4 (F). Curves G and H, as E and F, respectively, but after cutting trichomes in the region between electrodes 2 and 3. (From Chailakhian et al. [32].)

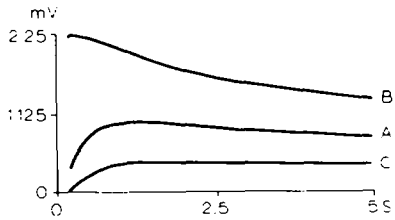

Fig. 2. Computer simulation of the process of lateral $\Delta\Psi$ transmission along cyanobacterial trichome conforming to the data of the experiment shown in Fig. 1, curves A–C. (From Chailakhian et al. [32].)

The next series of experiments performed in this group by Chailakhian and co-workers [32] allowed direct evidence of electric potential transmission along trichomes to be obtained. To this end, a technique for electric current and potential measurement with external (extracellular) electrodes has been applied.

Phormidium trichome was put into a groove in a plastic plate. The groove was filled with distilled water and four electrodes were placed close to the trichome: electrodes 1 and 4 in the vicinity of its opposite ends whereas the other two (2 and 3) were at a distance of 1/3 and 2/3 of the trichome length, respectively. When a small end portion of the trichome, situated, for example, close to electrode 1, was illuminated with a light beam, a potential difference between electrodes was found to arise as if there were positive charge extrusion in the illuminated part, the trichome being a unitary electric system with a high electric conductance between cells. In some cases, $\Delta\Psi$, which developed on a time scale of seconds, reached 10 mV.

$\Delta\Psi$ proved to be greater, and increased faster, when measured between electrodes 1 and 4 situated near opposite trichome ends than between a middle electrode and electrode 4 (Fig. 1, curves A–D). Computer simulation of these data carried out in this group by Kara-Ivanov and co-workers [32] revealed good agreement between (i) the measured amplitudes and kinetics of $\Delta\Psi$ and (ii) those calculated assuming that electric cable properties are inherent in cyanobacterial trichomes (Fig. 2).

Theoretical curves were obtained for a model of a non-inductional infinite cable, at a point in which a potential difference, V_m, is generated. Calculation of the potential difference, V_i, for an isolated cable at a given electrode was performed according to Eqn. 1:

$$V_i(t) = V \cdot \left[\phi\left(\frac{x_r}{2} \cdot \sqrt{\frac{RC}{t}}\right) - \phi\left(\frac{x_i}{2} \cdot \sqrt{\frac{RC}{t}}\right)\right] \tag{1}$$

where x_i and x_r are the distances of a generator from the measuring electrode and the reference electrode, respectively; t is the time after switching on the generator; R and C are the longitudinal resistance and capacitance of a 1 cm long trichome, respectively (the RC value was calculated from the experiment of Fig. 1, curves A–C) and $\phi_{(y)}$ is the error function found from Eqn. 2:

$$\phi_{(y)} = \frac{2}{\sqrt{\pi}} \cdot \int_0^y e^{-y^2} \cdot dy \tag{2}$$

V_i was obtained for a trichome, of which the specific membrane resistance (R_m) was assumed to be $4 \cdot 10^7\ \Omega \cdot cm^2$. To this end, Eqn. 3 was used:

$$V_i^1(t) = V_i(t) + a \cdot \int_0^t V_i(\tau) \cdot e^{a\tau} \cdot d\tau \tag{3}$$

where $a = 1/R'C$ and R' is the membrane resistance of a 1 cm long trichome.

The cutting of trichomes between the illuminated end-part and a middle electrode was found to prevent completely the light-induced generation of $\Delta\Psi$ between the middle electrode and the electrode situated close to the dark trichome end. $\Delta\Psi$ appeared again when the light spot was shifted to the previously dark end.

Thus, if the middle-part of the trichome, instead of its end, was illuminated with a light spot, trichome cutting could reverse the direction of light response. In Fig. 1, curves E–H, the light spot was situated near electrode 3. Rather a large potential difference between electrodes 3 and 4 developed (curve F). Simultaneous $\Delta\Psi$ measurement between electrodes 2 and 4 revealed a small $\Delta\Psi$ of the opposite direction (curve E). This $\Delta\Psi$ changed its sign, and its amplitude greatly increased, after the trichome between electrodes 2 and 3 was cut (curve G). Such an effect can be predicted if we consider cyanobacterial trichome as an electric cable. Indeed, H^+ extrusion from trichomes by photosynthetic $\Delta\bar{\mu}_H$ generators in the illuminated trichome part must result in currents (i) between electrodes 3 and 2, and (ii) between electrodes 3 and 4. These two currents are of opposite directions, and the overall current between electrodes 2 and 4 is in fact the difference of currents i and ii. After cutting trichomes between electrodes 2 and 3, current i disappears. Now, only current ii contributes to the overall current between electrodes 2 and 4. This must result in the formation of a $\Delta\Psi$ of the same direction and magnitude as that between electrodes 3 and 4. Thus, all the data of this experimental series confirm the concept of power transmission in the form of $\Delta\Psi$ spreading along cyanobacterial trichome.

Trichomes of cyanobacteria do not seem to be too lengthy to make electric power transmission ineffective. Energy losses accompanying $\Delta\Psi$ transport along the trichome membrane were calculated by Kara-Ivanov and co-workers [32] with the electric cable equation used previously, conformably to nerves.

The distribution of electric potential (V) along the long axis of trichome (x) was calculated according to Eqn. 4:

$$V = V_o \cdot \exp\left(-\frac{x}{\lambda}\right) \tag{4}$$

where V_o is the transmembrane electric potential difference in the illuminated region of a trichome, and λ is the length constant, calculated using Eqn. 5:

$$\lambda = \sqrt{\frac{R_m}{R_i}} \cdot \sqrt{\frac{d_1 \cdot d_2^2}{2(l_1 + d_1)(l_1 d_2^2 + l_2 d_1^2)}} \cdot (l_1 + l_2) \tag{5}$$

where R_m and R_i are the specific resistances of 1 cm^2 of the membrane and of 1 cm^3 of the cytoplasm, l and d are the length and radius of the cyanobacterial cell (subscript 1) or of the intercellular conducting channels (subscript 2), respectively.

Exact values of some of these parameters are obscure. However, by varying them within reasonable limits, we can estimate the probable energy losses accompanying lateral power transmission. For example, assuming $R_m = 4 \cdot 10^7\ \Omega \cdot cm^2$, $R_i = 50\ \Omega \cdot cm$, $l_1 = 2\ \mu m$, $l_2 = 0.1\ \mu m$, $d_1 = 3\ \mu m$ and $d_2 = 0.1\ \mu m$, we obtain $\lambda = 11$ cm, so that energy losses, i.e., $1 - V/V_o = 1 - \exp(-x/\lambda)$, prove to be less than 5% and about 37% for 0.5 and 5 mm distances, respectively (Fig. 3).

In conclusion of this section, the data of the above experiments on cyanobacteria can be considered as a precedent demonstrating an intercellular power transmission in the form of membrane potential.

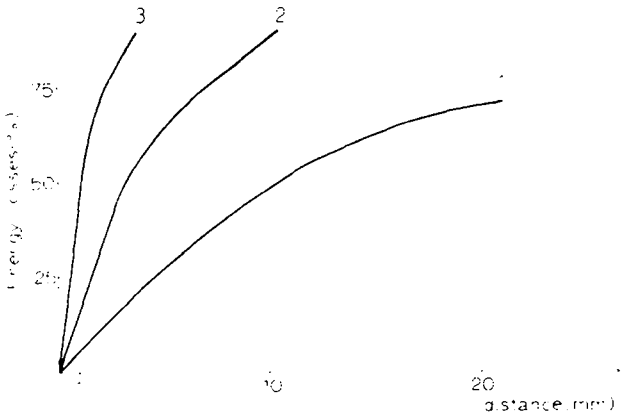

Fig. 3. Energy losses accompanying lateral $\Delta\Psi$ transmission, as a function of the distance. Membrane resistances for curves 1, 2 and 3 were assumed to be $4 \cdot 10^7$, $4 \cdot 10^6$ and $4 \cdot 10^5$ $\Omega \cdot cm^2$, respectively. (From Chailakhian et al. [32].)

IIB-5. Membranes of giant mitochondria as routes for $\Delta\bar{\mu}_H$ transport

One would think that if $\Delta\bar{\mu}_H$ transport were confined to a single mitochondrion, it could not be used to transfer energy over distances comparable with those existing in the cell, since mitochondria are believed to be small, spherical corpuscules dispersed in the cytoplasm. This point of view is based on light-microscopic studies which seemed to be supported by electron micrographs of random sections across cells and tissues.

However, both the mentioned techniques are hardly adequate in searching for possible membranous connections between mitochondria.

Systematic analysis of serial sections of some unicellular organisms has provided evidence for the existence of giant branched mitochondrial structures of approximately the same size as the cell itself. For example, in one of the cells of a flagellate (*Polytomella agilis*), Burton and Moore [34] observed a single mitochondrion which looks like a hollow perforated sphere arranged near the outer cell membrane (see also, Refs. 35 and 36).

A single giant mitochondrion was observed in cells of yeasts [13,37–39], the unicellular alga, *Micromonas* [40], *Leishmania donovani* [41], *Chlorella* [42] and a water mold [43]. Several huge branched mitochondria were revealed in *Chlamydomonas* [44–47], *Euglena gracilis* [48–50], *Trypanosoma* [51–53] and some fungi [54]. Thread-like, 10-μm long mitochondria were found in exocrine cells of pancreas [55]. Apparently, similar mitochondria form the chondriom of spermatozoon [55–57]. In liver cells, Brandt et al. [58] described two types of mitochondria, small spherical and large branched, resembling giant mitochondria of unicellular organisms.

One of the most interesting subjects to study in intracellular energy transport is a muscle cell. Multinuclear muscle cells (fibers) are very large. Their energy requirements are extremely high. In a hard-working muscle, gradients of oxygen and respiratory substrates between the periphery and the core of the cell should arise and this can limit the scope of the work performed. $\Delta\bar{\mu}_H$ transport from the cell edge to its core along mitochondrial membranes might facilitate energy delivery to the inner parts of the muscle fiber. If this is the case, the dimensions of muscle mitochondria should be especially large [12].

In insect flight-muscle, slab-like mitochondria of about the same length as the muscle fiber radius, i.e., 10 μm, have been described [59]. The first indications of the existence

of a mitochondrial system penetrating muscle fibres of mammalians were obtained by Bubenzer [60,61] and Gauthier and Padykula [62,63]. The authors studied random sections of rat diaphragm-muscle [60,62] and of Musculi semitendinosus [59].

In this laboratory, Bakeeva et al. [11,12] undertook a systematic investigation of serial sections of rat diaphragm. It was shown that in this tissue, mitochondrial material is organized into networks transpiercing the I band regions of the muscle near the Z-discs. Each network forms tubules, oriented perpendicular to its plane, and branches, connect-

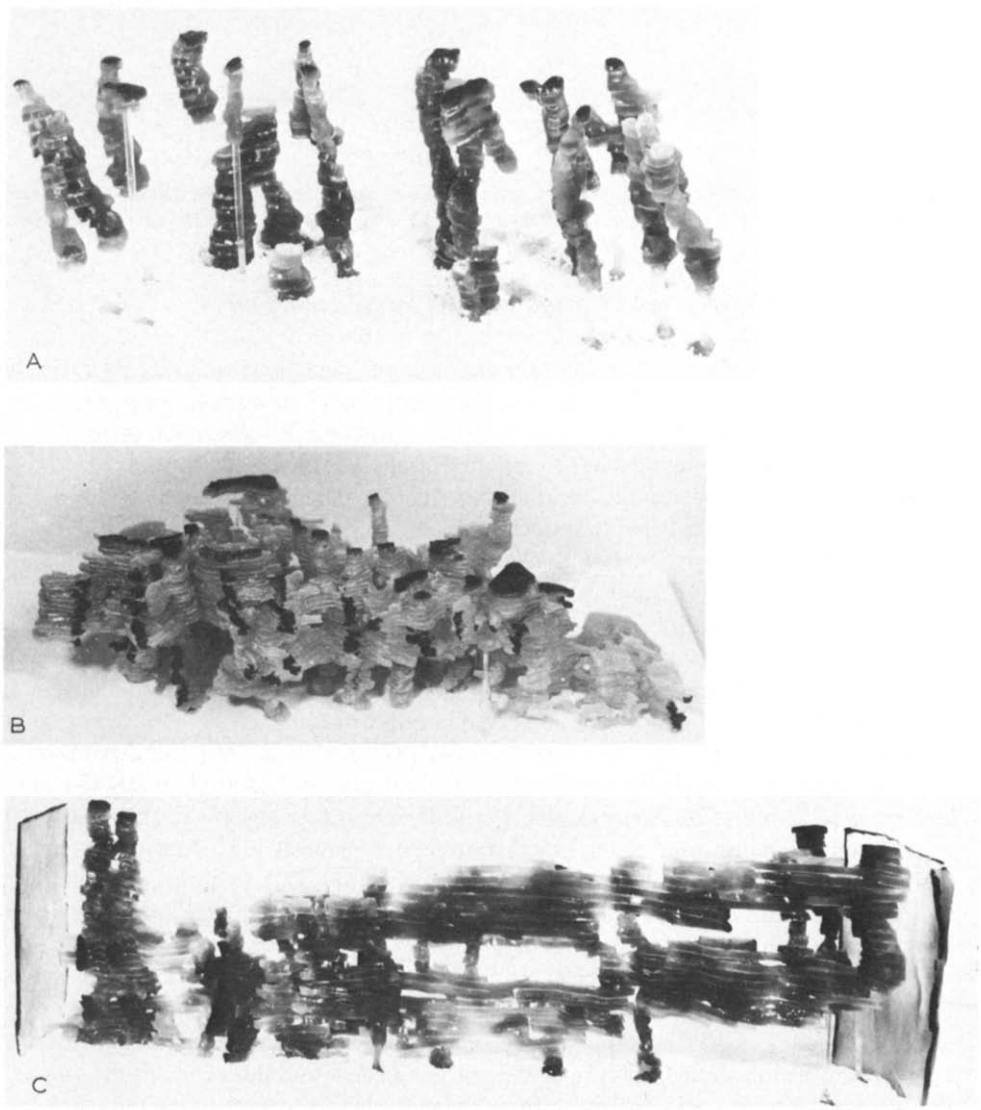

Fig. 4. Three-dimensional reconstitution of a part of the mitochondrial system of rat diaphragm-muscle. A, embryo, B and C, adult animal. In C, a mitochondrial profile crossing a muscle cell parallel to a Z-disc is shown; plates at the peripheries of the model represent parts of the outer membrane of the muscle cell. The cuts in mitochondrial branches are blackened. (From Bakeeva et al. [12].)

ing the network with mitochondrial clusters in the fiber periphery. Such a system, defined as mitochondrial reticulum, is found to be characteristic of the diaphragm of adult animals. It is absent from the diaphragm of rat embryos and new-born rats [12]. Further study revealed the time course of the post-embryonal development of the mitochondrial framework in diaphragm. Its formation was shown to be completed during the first two post-natal months.

In Fig. 4, one can see results of three-dimensional reconstitution of mitochondrial systems in rat diaphragm in embryo (A) and adult animal (B and C). As is clear from Fig. 4C, mitochondrial material traverses the cell body, stretching from one edge of the cell to the other. The mitochondrial cristae in the construction shown in Fig. 4C were found to be oriented mainly parallel to the long axis of the tubule, which must be favorable for any lateral transport processes if carried out by mitochondrial reticulum.

It seems remarkable that there is no mitochondrial reticulum in the embryonic diaphragm, i.e., a muscle which does not perform mechanical work, and that it develops later, when the main tissue function appears. Formation of mitochondrial reticulum during ontogenesis may be compared with the phenomenon of the end-to-end aggregation of mitochondria accompanying conversion of spermatide to spermatozoon [55-57]. Moreover, this type of aggregation was described as taking place in different tissues under conditions unfavorable for cell energetics (e.g., hypoxia, hyperthyroid state, etc. [64-72]). This phenomenon may be accounted for as an attempt by the cell to unite the activity of individual organelles when their uncoordinated functioning proves insufficient for maintaining a necessary rate of energy production. Under normal conditions, this system may be important to such tissue as muscle that must be always ready to manifest its maximal working capacity [12].

An important question is the structure of mitochondrial reticulum. It may be organized in two manners: a giant mitochondrion is really a single organelle surrounded by continuous outer and inner membranes, or alternatively, there is an assembly of many end-to-end associated mitochondria. It was found that sometimes the reticulum-forming tubules are crossed by dark partitions built up of four (two outer and two inner) membranes, the intermembrane space being filled with an osmiophilic material. Apparently, these partitions represent junctions of two branches of mitochondrial reticulum. In some cases, it was shown that both these branches belong to one and the same mitochondrion.

In a special experiment, we followed a mitochondrial profile without crossing a junction site, assuming that in this case we were dealing with a single mitochondrion in the sense that at least its outer membrane should be continuous. It was found that one could cross the whole muscle cell body, moving along a tubule of mitochondrial reticulum oriented parallel to a Z-disc (see Fig. 4C and Ref. 12).

The nature of mitochondrial junctions is not clear. They are absent from embryonic diaphragm and appear simultaneously with post-embryonal development of mitochondrial reticulum. An intriguing speculation about their function is that they play a role in the elimination of damaged parts of reticuli or in any other cases where $\Delta\mu_H$ transport via a given mitochondrial branch is prevented. To do so, it may be sufficient to disrupt the junction and separate this branch from an adjacent one. Such a situation was described for conductive gap junctions of animal cell (see subsection IIC-2). Conductance through the gap junctions was found to be switched off in the case of, for example, swelling of cells or an increase in the intracellular Ca^{2+} level.

Certainly, we have no information about the conductance of mitochondrial junctions. If they are impermeable for H^+, the energy transport along membranes of giant mito-

chondria may be described as a mixed relay in which energy is transmitted over the major part of a distance (between two junctions) as $\Delta\bar{\mu}_H$, whereas the junction site is overcome by ATP diffusion via the cytoplasm region near the junction [12].

Since not a single 'free' (i.e., not connected with the mitochondrial reticulum) mitochondrion was found in the parts studied of the adult rat diaphragms, we concluded that the mitochondrial framework in this tissue forms a united membranous system which may be used to transmit power [12].

IIB-6. Possible mechanisms of lateral $\Delta\bar{\mu}_H$ transmission

If $\Delta\bar{\mu}_H$ is in the form of $\Delta\Psi$, energy is transmitted along the membrane due to diffusion of mobile ions on both sides of the membrane. For intracellular organelles, this should be mainly K^+ and Cl^-, the most common ions of protoplasm. In the case of outer cell membranes, it should be Na^+ and Cl^- outside the cell, K^+ and Cl^- in the cell interior. At the same time, $\Delta\bar{\mu}_H$ generators and consumers deal with H^+, of which the concentration in biological system is much lower than that of the above-mentioned ions. This may result in a situation where $\Delta\Psi$ transmission gives rise to the formation of local pH gradients.

For example, let us assume that it is a peripheral part of the mitochondrial reticulum in a hard-working muscle that produces $\Delta\Psi$ due to respiration-driven H^+ extrusion from the matrix to extramitochondrial space. $\Delta\Psi$, when formed, is transmitted to the core region of the fiber deprived of oxygen and/or respiratory substrates. This transmission occurs by means of K^+ and Cl^- diffusion in cytoplasm and mitochondrial matrix along a tubule of mitochondrial reticulum. In the core part of the fiber, $\Delta\Psi$ is used to form ATP by H^+-ATP-synthetase, which is accompanied by H^+ influx from cytoplasm to the matrix space. As soon as K^+ and Cl^-, but not H^+ and OH^-, are transported between the peripheral and core parts of the mitochondrial reticuli, the matrix space must be made alkaline in the peripheral part and acidified in the core part. The pH gradient formed along the reticulum membrane can be abolished by subsequent diffusion of H_3O^+, OH^- and pH buffers, H_3O^+ moving from the core to the peripheral regions of the mitochondrial reticulum. This mechanism proves to be the only one if $\Delta\bar{\mu}_H$ is produced in the form of ΔpH. In such a case, lateral movement of K^+, Cl^-, etc., is not involved.

Perhaps, there is a special mechanism facilitating lateral movement of H^+. It might consist of, for example, proton translocation along the membrane surface via membrane-bound water molecules forming an ice-like structure. This can, in principle, result in a great increase in the H^+ transmission rate, since proton conductivity in ice is much higher than in water. In this connection, it will be of importance to study changes in cellular energetics, induced by agents disrupting bound water layers at membrane surfaces.

IIB-7. Hypothesis on taxis of membrane enzymes to their substrates

If there are gradients of O_2 as well as of ADP and P_i between the peripheral and core regions of a muscle fiber, it seems reasonable that respiratory enzymes should be concentrated in the periphery and ATP-synthetase in the core. Such a concentration of enzymes might be achieved by lateral diffusion of mitochondrial membrane enzymes, directed from the substrate-poor to substrate-rich parts of the cell. The advantages of the mechanism in question are obvious but how can it be organized? In this context, I would like to consider the following two points.

(1) When localization of factor F_1 in chloroplast membranes was studied [29], it was revealed that an even distribution of knob-like F_1 structures on the thylakoid and lamella

membranes takes place only after a pretreatment inducing swelling of thylakoid membranes. In a non-treated preparation, the knobs were found on lamellae and on the surfaces of thylakoid membranes facing the chloroplast matrix. There were no knobs on the thylakoid membrane surfaces facing interthylakoid spaces, apparently since the distance between thylakoid membranes proves to be shorter than the height of the knob. After swelling, this distance increases and factors F_1 diffuse along membranes from lamellae to thylakoid membranes down an F_1 concentration gradient. As a result, the amount of knobs was found to increase in thylakoid membranes and decrease in lamellae [29]. So, we can conclude that H^+-ATP-synthetase is mobile in the plane of the membrane. Lateral mobility of a respiratory chain enzyme, mitochondrial cytochrome oxidase, was recently demonstrated by Höchli and Hackenbrock [73].

(2) There are some indications that, besides a knob-like conformation, factor F_1 can be immersed into the membrane (for review, see Ref. 27). In the protruded (knob-like) state, the membrane part of H^+-ATP-synthetase (factor F_0) was estimated to comprise only 15% of the synthetase molecule [26]. Immersion of a larger part of the complex into the membrane should decrease the lateral mobility of H^+-ATP-synthetase. If such immersion is induced by addition of ADP and P_i, H^+-ATP-synthetase must be immobilized in the membrane by its substrates.

Summarizing the two above points, one may speculate that H^+-ATP-synthetase, being in the protruded conformation, scans the membrane by means of lateral diffusion in search of its hydrophilic substrates, ADP and P_i. Combining with ADP and P_i, factor F_1 sinks into the membrane in search of membrane potential required to form ATP. Immersion immobilizes H^+-ATP-synthetase, so that this enzyme accumulates in the parts of a giant mitochondrion that cross ADP- and P_i-rich regions of the cell, e.g., in the internal parts of the muscle fiber.

Using the same reasoning, one may suggest that the respiratory chain enzyme complexes have a taxis to O_2 and respiratory substrates, being therefore concentrated in the fiber periphery.

It is clear that substrate taxis of enzymes, should it exist, must be inherent in membrane-linked, but not in soluble, enzyme systems.

An example of a 'mutual taxis' of two membranes proteins, resulting in both of them being immobilized, was reported by Kämpe and Peterson [74]. These authors found that attachment of a T-killer cell to a target cell is accompanied by a gathering of transplantation antigens of the former in the region of contact of two cells (see also, Ref. 75). It was also shown that the T-killer cell membrane proteins responsible for interaction with transplantation antigens also gather in the same region. The mechanism of this phenomenon includes (1) lateral diffusion of antigens and antigen-binding proteins along outer cell membranes of the target and killer cells, respectively, and (2) formation of a motionless complex of two kinds of proteins, anchored to membranes of two different cells.

IIC. $\Delta\bar{\mu}_{Na}$ as a transportable energy form linked with the outer cell membrane

IIC-1. Bacterial membranes

In bacteria, certain kinds of osmotic work were found to be supported by the electrochemical potential difference of Na^+ ($\Delta\bar{\mu}_{Na}$) generated across the cytoplasmic membrane owing to H^+/Na^+ antiport, as discovered by West and Mitchell [76] (see also, Refs. 77 and 78). The most impressive example of this phenomenon has been reported by Lanyi and co-workers [79–82] who studied amino acid transport in *Halobacterium*. In particular,

they found that 19 amino acids are accumulated inside the *Halobacterium halobium* cell by means of symport with Na^+ [82]. Apparently, Na^+-dependent symports are less important for bacteria other than extreme halophils. Nevertheless, some of these can also absorb certain metabolites together with Na^+ [83–86].

Of course, $\Delta\bar{\mu}_{Na}$ can hardly be regarded as a convertible form of membrane-linked energy. Even in *H. halobium*, uphill transport of some metabolites seems to be the only type of work that can be directly supported by $\Delta\bar{\mu}_{Na}$. The role of $\Delta\bar{\mu}_H$ undoubtedly proves to be much more diverse. However, $\Delta\bar{\mu}_{Na}$ can spread along the membrane like $\Delta\bar{\mu}_H$. Therefore, $\Delta\bar{\mu}_{Na}$ may play the role of a transportable energy form parallel with $\Delta\bar{\mu}_H$.

IIC-2. Outer membrane of animal cells

In the animal cell, $\Delta\bar{\mu}_H$ production is localized in the inner mitochondrial membrane, whereas the outer membrane of the cell is specialized in two other functions; osmotic work and signal transmission. Many processes of the uphill transport through the animal cell outer membrane are organized as a metabolite-Na^+ symport (for reviews, see Refs. 87–89). Certainly, Na^+ can diffuse along the outer membrane, this fact being sufficient to conclude that $\Delta\bar{\mu}_{Na}$ should be defined as a transportable energy component. Lateral $\Delta\bar{\mu}_{Na}$ transport may be very important for those animal cells as well as plant cells which have a large size, especially for long cells, the opposite parts of which exist under entirely different conditions.

In some cases, $\Delta\bar{\mu}_{Na}$ may apparently be used for energy exchange between cells connected via ion-permeable junctions [9]. In many types of animal cells, the so-called gap junctions have been described (for review, see Refs. 90 and 91). In a gap junction, outer membranes of two adjacent cells are kept at some distance from each other by special structures forming bridges which cross both membranes and the intermembrane gap. Inside a bridge structure, there is a channel permeable to small molecules, and in particular to K^+ and Na^+. This means that $\Delta\bar{\mu}_{Na}$, if formed in a cell connected with other cells of the tissue by gap junctions, should be a property of all connected cells. In other words, all $\Delta\bar{\mu}_{Na}$ generators (i.e., ($Na^+ + K^+$)-ATPases) of the cell palisade prove to be united into a common system, producing energy in the form of an Na^+/K^+ gradient *. By regulating the amount of gap junctions (or their permeability), one can control the energy flow in different parts of the tissue.

III. Lateral transport of lipid-soluble substrates

IIIA. Comparison of the diffusion processes in three-dimensional space of cytosol and two-dimensional space of a membrane

In Section II of this paper, the possible role of the lateral transport of $\Delta\bar{\mu}_H$ and $\Delta\bar{\mu}_{Na}$ in cellular energetics was considered. It has been stressed that both processes must take

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* It should be noted that the concentration gradient of K^+, also produced by ($Na^+ + K^+$)-ATPase, is never used to support the osmotic work of the animal cell outer membrane. The reason for this is most probably the fact that ($Na^+ + K^+$)-ATPase exchanges three intercellular Na^+ for two extracellular K^+ per one ATP hydrolyzed. Thus, $\Delta\Psi$ arises and the cell interior is charged negatively, which is favorable for Na^+ influx down an Na^+ gradient and unfavorable for K^+ efflux down a K^+ concentration gradient. In other words, two constituents of $\Delta\bar{\mu}_{Na}$, i.e., electric and concentration gradients, are unidirectional, whereas those of $\Delta\bar{\mu}_K$ prove to be of opposite directions. (About the possible biological significance of this fact, see Ref. 9.)

place owing to the physical properties of the membranes which produce $\Delta\mu_H$ and $\Delta\mu_{Na}$. So, the only problem to solve was whether the cell uses the mobile character of transmembrane ion gradients.

In this section, I would like to apply the same approach to components that, as distinct from ion gradients, can exist not only in connection with membranes but also independently of any membranous structures.

Let us consider a substrate that diffuses into the cell from the extracellular space, and a substrate-utilizing enzyme localized in an intracellular membrane. There can be two different routes for the substrate to reach the enzyme: diffusion of the free substrate through cytosol and diffusion of the membrane-bound substrate in the membrane plane.

As Adam and Delbrück [92] noted, diffusion along membranes permits a much faster transfer of a component from cytosol to a small target on the membrane than free diffusion in cytosol. Sumper and Träuble [93] calculated that there is quite a wide range of parameters of a target and a cell for which lateral diffusion in the two-dimensional plane of the membrane proves to be more favorable than diffusion in the three-dimensional cytosol.

It is generally believed that the viscosity of the membrane is much greater than that of cytosol, and in this respect the cytosol route should have an advantage over the membrane route. However, one must take into account that cytosol is not an aqueous solution of low molecular weight compounds but rather a colloidal suspension. So, diffusion, via liquid regions of a membrane might be not much slower than via cytosol. According to Keith and Snipes [94], the viscosity of cytosol can be even greater than that of membranes. These authors have found that a spin-labelled synthetic compound of low molecular weight, of which the partition coefficient in a lipid/water system is close to unity, moves preferentially in the lipid phase of various types of cells.

IIIB. Transport of fatty acids: Problem of membrane asymmetry

Apparently, fatty acids may examplify a substrate which has a high affinity to membranes bearing the enzyme system responsible for the metabolism of this substrate.

The lateral diffusion mechanism must be especially effective if the substrate of a given membrane-linked enzyme is concentrated not in any lipid structure but mostly in that membrane where the enzyme is localized. If the enzyme in question is arranged at one of the membrane surfaces, the substrate should be only at this surface.

For fatty acids, the problem seems to be solved in the following way. (1) Free fatty acids as well as their CoA and carnitine esters, are amphipathic compounds that tend to be in lipid/water interfaces. Hence, they should be concentrated at membrane surfaces rather than in intramembrane regions or in intracellular lipid droplets. (2) The fatty acid derivative which is transported across membranes is a fatty acyl carnitine, a compound moving to the mitochondrial matrix in a $\Delta\bar{\mu}_H$-dependent fashion (this is presumably due to the fact that the protonated fatty acyl carnitine traverses the inner mitochondrial membrane as a penetrating cation [95]). Thus, the fatty acyl carnitine can specifically saturate the inner surface of the inner mitochondrial membrane where β-oxidation of fatty acids takes place.

The fatty acyl carnitine may serve as a component that is suitable not only for transmembrane but also for lateral transport of fatty acids. A compound that can perform the latter (but not the former) function is fatty acyl CoA, as was suggested by Sumper and Träuble [93]. (For lateral transport of steroids, see Refs. 96 and 97.)

IIIC. Problem of lateral transport of molecular oxygen

Considering this question, we should take into account the following facts.

(1) The solubility of molecular oxygen in lipid is about 5-times greater than in water. This difference may be even greater if we compare lipid with cytosol. In fact, O_2 cannot be dissolved in bound water taking part in the hydration of cytosol solutes.

(2) The main oxygen-utilizing enzyme, cytochrome oxidase, is localized in the inner mitochondrial membrane. It must result in formation of local low O_2 concentration regions in this membrane due to O_2 consumption by cytochrome oxidase.

(3) The rate of O_2 diffusion in certain types of membranes at 37°C is as high as in blood plasma [98]. Cholesterol, strongly decreasing this rate [98], is localized in membranes other than the inner mitochondrial one.

(4) In muscles, the inner mitochondrial membranes comprise a very large portion of the total membranous material of the cell [12].

(5) In spite of the above-mentioned fact (4), even the inner mitochondrial membranes occupy only a small portion of the cell volume.

The observations stated in the first four items favor diffusion of O_2 in the two-dimensional space of the inner mitochondrial membrane to its target, cytochrome oxidase. However, the last item proves to be unfavorable to such a lateral transport process.

An experimental study seems to be necessary to reveal the proportions of two- and three-dimensional O_2 transport inside cells of different types. Nevertheless, lateral diffusion of O_2 along mitochondrial membrane should be taken into account as a possible intracellular mechanism of O_2 transport in the tissues containing a large amount of mitochondrial material, especially if it is organized as giant mitochondria [12].

IIID. Cooperation of several lateral transport processes

When studying rat diaphragm-muscle, we found [12] that fat droplets inside the muscle cells are always covered by mitochondrial material connected with networks of mitochondrial reticulum. Previously, a close contact of mitochondria and fat droplets was observed in liver and pancreas by de Robertis et al. [99]. These authors mentioned that there is only one (inner) mitochondrial membrane between a fat droplet and mitochondrial matrix as if the droplet were localized in the intermembrane space of the mitochondrion.

Since fatty acids are the main oxidizable substrate for skeletal muscles, one may suggest that, during hard muscle work, an intracellular gradient of respiratory substrates can

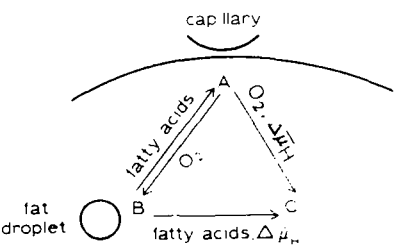

Fig. 5. Probable directions of lateral transport of $\Delta\bar{\mu}_H$, O_2 and fatty acids in mitochondrial reticulum of skeletal muscles (for explanation, see text).

arise, the maximal substrate concentration being close to the fat droplets (Fig. 5, point B). As for O_2, its maximal concentration must be close to those parts of the cell which are near the capillary (Fig. 5, point A). Respectively, the highest ADP and phosphate levels must be at a maximal distance from the capillary and fat droplet (Fig. 5, point C). A hypothesis which seems to be attractive is that lateral movement of $\Delta\mu_{H^+}$, of fatty acids (acyls) and, maybe, of O_2 along the membranes of the mitochondrial reticulum, results in equilibration and buffering of all these components of the cellular energy system. Such a process should be facilitated if (1) in the quantitative respect, inner mitochondrial membrane is the main membranous component of the tissue, and (2) the mitochondrial cristae are arranged mostly parallel to the long axis of mitochondrial profiles. Both these features are inherent in mammalian skeletal muscle [12].

IV. Lateral transport of electrons

IVA. Features of proteins specialized in lateral transport

In the previous sections, I considered the possible role of lateral transport of the components inseparably linked with a membrane ($\Delta\bar{\mu}_{H^+}$ and $\Delta\bar{\mu}_{Na^+}$) or possessing a high affinity to a membrane (e.g., fatty acids). Now I shall try to extend such a concept to components having none of these two properties.

For a substance of low membrane affinity, the only way to make lateral transport possible is to combine it with a mobile membrane constituent which is capable of lateral diffusion. To have a high specificity, such a lateral carrier should be, most probably, a protein.

The functional activity of hypothetical proteins specialized in lateral transport [100] cannot be revealed by the conventional methods of membranology such as the measurement of diffusion of transported components across artificial membranes containing a studied carrier. The proteins in question must have the following features: (1) they must bind a transported component specifically and in a reversible manner; (2) they must be anchored in the membrane, being mobile in its plane; (3) they must not penetrate the membrane. One may look for potential lateral carriers among proteins of unknown function that display these properties. NADH-cytochrome b_5 oxidoreductase flavoprotein (fp_5) and cytochrome b_5 may exemplify proteins of these features.

IVB. Mystery of the cytochrome b_5 pathway

In certain types of cells and tissues there is a redox chain that comes to an end, failing to reach oxygen: NADH → fp_5 → cytochrome b_5.

In principle, cytochrome c might function as a link between cytochrome b_5 and cytochrome oxidase. However, cytochrome b_5 and cytochrome c are localized in different compartments: the former in microsomes and in the outer mitochondrial membrane, the latter in the inner mitochondrial membrane. Such a compartmentalization can explain why, for example, in aerobic liver cells, cytochrome b_5 is completely reduced [101]. Despite the absence of an active oxidant, the reducing capacity of the fp_5-cytochrome b_5 chain is extremely high. Among the redox enzymes, fp_5 belongs to those that have the highest turnover number [102]. In liver, there is more cytochrome b_5 than any cytochrome of the inner mitochondrial membrane [10].

Strittmatter et al. [103–105] demonstrated lateral diffusion of both fp_5 and cyto-

chrome b_5 in the plane of the membrane. In particular, it was found that microsomal membrane can bind large amounts of added cytochrome b_5, so that the cytochrome b_5 : fp_5 ratio becomes as high as 100. The rate of cytochrome b_5 reduction by fp_5 per molecule of cytochrome b_5 was found to be much higher in the cytochrome b_5-loaded microsomal membranes than in those containing a normal amount of this cytochrome. A similar relationship was shown in experiments with microsomes binding an excess of fp_5.

Molecules of fp_5 and cytochrome b_5 are composed of two unequal parts: the larger hydrophilic part, containing flavin or the heme group responsible for the catalytic function, and the smaller, hydrophobic part, requiring the protein to be anchored to the membrane [105,106]. Being like a float, fp_5 and cytochrome b_5 should move along the membrane, meeting with relative ease.

It should be noted that these features, i.e., the float-like structure and a very high mobility in the plane of the membrane, are far from being a common property of membrane-linked enzymes.

One should bear in mind that a lipid bilayer occupies a small part of the inner mitochondrial membrane (the lipid content is only 25--30% [107]) and a large part of the outer mitochondrial membrane and microsomal membrane (the lipid content is 80--85% [107,108]). Float-like proteins should be more free to move around on the plane of, for example, the outer rather than the inner mitochondrial membrane, since the former represents, in fact, the fluid phospholipid bilayer with infrequent protein inclusions.

Taking into account all these observations, we have put forward the suggestion that fp_5 and cytochrome b_5 are lateral carriers of reducing equivalents, i.e., H atoms and electrons, respectively [100]. Since fp_5 has a redox potential close to that of NADH (about 0.3 V), it can serve as a component equilibrating reducing equivalents at the NADH level in different parts of the cell. As for cytochrome b_5, it might perform a similar function for reducing equivalents at zero redox potential level.

IVC. Intermembrane electron transfer without water-soluble carriers

In certain states of the cell, electrons from mitochondrial cytochrome b_5 can reduce cytochrome *c* oxidase via cytochrome *c* desorbed into the intermembrane space of mitochondria. Such cytochrome *c* desorption seems to take place, for example, during urgent heat production in the liver of warm-blooded animals exposed to cold [109]. A question arises as to whether the microsomal pool of cytochrome b_5 could be linked with the respiratory system of mitochondria.

Analysis of this problem has led us to the discovery of a phenomenon defined as intermembrane electron transport [110–113]. It was shown that rat liver mitochondria, microsomes, and proteoliposomes containing cytochrome b_5 as the only protein, can exchange electrons without any added water-soluble electron carriers. Some results of these experiments are given in Fig. 6.

One can see that microsomes mediate proteoliposomal cytochrome b_5 reduction by NADH (Fig. 6A). If microsomal fp_5 was inhibited by mersalyl treatment, a small amount of untreated microsomes was shown to reduce cytochrome b_5 in mersalyl-treated microsomes (Fig. 6B). Intact rat liver mitochondria were found to substitute for untreated microsomes (Fig. 6C). In Fig. 6D, electron transport from untreated microsomes to mersalyl-inhibited mitochondria is shown. It can be seen that addition of microsomes to such mitochondria (curve 2) initiates measurable respiration with NADH as substrate even without added cytochrome *c*. The respiration rate in the mixed system could be increased by cytochrome *c* addition (curve 3).

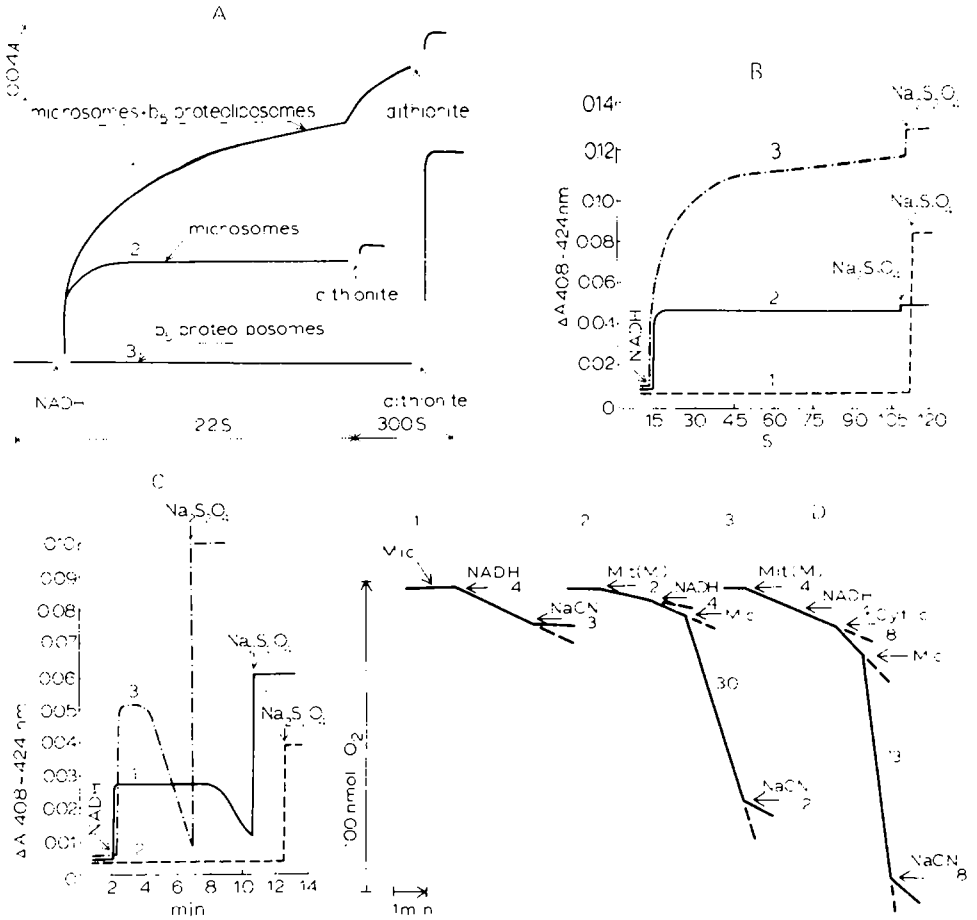

Fig. 6. Intermembrane electron transport without added water-soluble electron carriers. A, reduction of proteoliposomal cytochrome b_5 by microsomes. Incubation mixture (3 ml) contained 0.05 M Tris-HCl, pH 7.4, 33 mM glucose, glucose oxidase (50 units), catalase (500 units); proteoliposomes (2.1 mg/ml) obtained from lecithin liposomes by treatment with cytochrome b_5 (1 nmol cytochrome b_5/mg phospholipid) (curve 1); rat liver microsomes (2.1 mg protein/ml) (curve 2); proteoliposomes (2.1 mg/ml) and microsomes (2.1 mg protein/ml) (curve 3). Reaction was initiated by addition of $1.5 \cdot 10^{-5}$ M NADH. B, reduction of cytochrome b_5 in the mersalyl-treated rat liver microsomes by untreated microsomes. 3 ml of incubation mixture contained 50 mM Tris-HCl, pH 7.4, 33 mM glucose, glucose oxidase (50 units), catalase (500 units); microsomes (3 mg protein) treated with mersalyl (curve 1); untreated microsomes (1.5 mg protein) (curve 2); 3 mg protein of treated microsomes and 1.5 mg protein of untreated microsomes (curve 3). Addition: $1 \cdot 10^{-5}$ M NADH. C, reduction of cytochrome b_5 in mersalyl-treated microsomes by intact mitochondria. Incubation mixture (3 ml) contained 50 mM Tris-HCl, pH 7.4, $5 \cdot 10^{-6}$ M rotenone; rat liver mitochondria (4 mg protein) (curve 1); mersalyl-treated rat liver microsomes (3 mg protein) (curve 2); mitochondria (4 mg protein) and mersalyl-treated microsomes (3 mg protein) (curve 3). Reaction was initiated by adding $1 \cdot 10^{-5}$ M NADH. D, added NADH oxidation by O_2, catalyzed by mersalyl-treated mitochondria and untreated microsomes. Incubation mixture: 50 mM Tris-HCl, pH 7.4. Additions: 1 mM NADH, 1 mM NACN, $1 \cdot 10^{-5}$ M cytochrome c (Cyt. c), rat liver microsomes (Mic) (1 mg protein) (curve 1); rat liver mitochondria (Mit) treated with mersalyl (8 mg protein) and untreated microsomes (1 mg protein) (curve 2); mitochondria treated with mersalyl (1 mg protein) and untreated microsomes (0.05 mg protein) (curve 3). Figures above the curves show the rate of O_2 uptake in nmol $\cdot$ mg^{-1} $\cdot$ min^{-1}. (From Archakov et al. [112] and Karyakin [113].)

The ability of cytochrome b_5-containing membranes to transport electrons between membranes could not be decreased by several washings of membranous preparations, this fact indicates that water-soluble endogenous electron carriers are not involved. The rate of the intermembrane electron exchange was not increased by a 10 min pre-incubation of microsomes with proteoliposomes, or of mersalyl-treated and intact microsomes, etc. This means that migration of fp_5 or cytochrome b_5 between membranous vesicles (see Refs. 114 and 115) proved to be too slow to contribute to the intermembrane cytochrome b_5 reduction which is completed within 30–60 s after mixing two portions of membranes.

It was found that incubation of microsomes with pronase at 4°C completely abolishes their ability to reduce cytochrome b_5 in the mersalyl-treated microsomes. Under the conditions used, pronase was shown to destroy completely all microsomal cytochrome b_5, the pool of fp_5 being retained at quite a measurable level. So, intermembrane electron transport seems to be carried out by interactions between cytochromes b_5 anchored to two different membranes, rather than between fp_5 and cytochrome b_5 or between two fp_5 pools. The ability of fp_5 and cytochrome b_5 to diffuse along membranes, and of cytochrome b_5 to transport electrons from one membrane to another may alllow these two enzymes to decrease intracellular gradients of reducing equivalents –0.3 V and zero redox potential, respectively. Assuming the fp_5-cytochrome b_5 system possesses this function, one can explain why its activity and content can reach very high levels. In particular, it must prove quite necessary if two membranes come into contact with only small portions of their areas and for a short time. At any moment of time, the main part of the cytochrome b_5 pool is, apparently, not involved in the electron transfer. These cytochrome b_5 molecules diffuse on the plane of the microsomal or outer mitochondrial membrane by means of Brownian motion, scanning this membrane and searching for oxidants localized in the same or in an adjacent membrane. In the contact region, in which the intermembrane distance proves to be no larger than double the diameter of hydrophilic catalytic parts of cytochrome b_5, two enzyme molecules situated at two different membranes react with each other. It is important that electron transfer from fp_5 to cytochrome b_5 is irreversible due to a large difference in the redox potentials of these two enzymes. This means that in the region of contact of the two membranes, all cytochromes b_5 of the more 'oxidized' membrane will be completely reduced by the enzymes belonging to the more 'reduced' membrane.

The above reasoning accounts for such facts as a very high level of cytochrome b_5 reduction in the tissue, localization of catalytic sites of cytochrome b_5 at the hydrophilic head-piece of the enzyme, putative dead-end character of fp_5-cytochrome b_5 redox chain, etc.

However, several important questions remain unanswered. Among these is the cytochrome b_5 distribution between tissues. This was found in liver, brain, kidney and leukocytes, but not in muscles (see Ref. 10). This very fact has presented us with the problem of whether the latter tissue possesses a system for lateral and intermembrane electron transport. Maybe, in muscles, of which the metabolism is not as diverse as, for example, in liver, there is no need for lateral electron transport, or it is carried out by a simpler system including not two but a single enzyme, e.g., an NADH-oxidizing flavoprotein. It should be stressed that rotenone-insensitive NADH-cytochrome *c* oxidoreductases of an unknown function were found in many types of biomembranes. The inner membrane of animal mitochondria seems to be the only well documented exclusion from this rule [10, 116]. A study of the ability of such systems to carry out lateral electron transport seems to be desirable.

The problem of a natural acceptor for the fp_5-cytochrome b_5 system is another important question. One of the possibilities was mentioned above in connection with heat regulation. This is cytochrome *c* desorbed into mitochondrial intermembrane space. In this way, the redox activities of membranes other than the inner membrane of mitochondria might be linked with cytochrome oxidase. So, the initial and middle segments of the respiratory chain that are slower and much more vulnerable to different injuring actions than cytochrome oxidase may be shunted.

In the normal state, oxidants other than cytochrome *c* may be responsible for cytochrome b_5 oxidation. Among these, one should mention fatty acid desaturation, regeneration of hemoglobin from methemoglobin, some cytochrome *P*-450-linked processes, etc. (for review, see Ref. 108).

Besides, the possibility cannot be excluded that cytochrome b_5 may be oxidized directly by O_2 under some in vivo conditions, as suggested by Liberman [117] who postulated cytochrome b_5 as playing a key role in a hypothetical system integrating different intra- and extracellular regulatory effects (the so-called 'molecular computer' of the cell). In this context, the intriguing observation of the action of very low (physiological) concentrations of some hormones on NADH dehydrogenase of animal cell plasma membrane may be quoted [118–120].

V. Conclusion

I have summarized above some lines of evidence supporting the point of view that biomembranes not only divide but also integrate biological systems. I tried to accentuate the possible role of membranes as routes for the transport of energy or related components, i.e., reducing equivalents, respiratory substrates and O_2.

It was shown that there are firmly established examples of short-distance lateral energy transmission along biomembranes. This is migration of excitation energy along the photosynthetic membranes via chlorophyll antenna, and $\Delta\mu_H$ transfer from bacteriorhodopsin sheets to regions of *H. halobium* membrane where H^+-ATP-synthetase must be localized.

In this laboratory, power transmission in the form of $\Delta\Psi$ over distances as large as several millimeters was demonstrated. This was carried out in experiments with a multicellular prokaryote, the cyanobacterium, *Phormidium*.

A hypothesis has been formulated that inner membranes of giant mitochondria crossing such large cells as muscle fibers from one of its edges to the other may function as $\Delta\mu_H$ conductors. In the same membrane, lateral transports of fatty acids, the major oxidizable substrate of the muscle, and, maybe, of molecular oxygen were suggested to occur. In this way, formation of intracellular gradients of energy, reductant and oxidant might be prevented.

Finally, the problem of lateral and intermembrane electron transport was discussed. It was speculated that float-like proteins, such as NADH-cytochrome b_5 oxidoreductase (fp_5) and cytochrome b_5, play the role of mobile electron carriers capable of lateral transport of reducing equivalents −0.3 V and zero redox potential, respectively. Moreover, cytochrome b_5 belonging to two different membranes can exchange electrons with no water-soluble electron carrier involved. In this way, intermembrane electron transport may be organized.

One of my main objectives in the paper has been to indicate a unique way that may be used by living systems in their struggle against the diffusion limitations of biochemical processes. In fact, an enzymatic reaction cannot be further accelerated if its rate is limited

by diffusion of substrates, and no improvements in the catalytic properties of an enzyme can overcome this barrier. However, if we put both the enzyme and the substrate onto or into the membrane in such a way that they retain their mobility, we substitute two-dimensional for three-dimensional diffusion. The profit of such a manipulation proves so great that it might more than compensate for some decrease in the rate of enzyme and substrate motion due to binding with a membrane. A search for possible mechanisms specialized in lateral transport seems a promising new field of membranology.

Acknowledgements

I am very grateful to Drs. A.I. Archakov, A.N. Glagolev, A.A. Konstantinov and I.A. Kozlov for useful discussions, to Mrs. T.N. Konstantinova and Miss N.M. Goreyshina for help in the manuscript preparation and to Miss T.I. Kheifets for helping to correct the English version of the paper.

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