J. Membrane Biol. 114, 97–112 (1990)

The Journal of
Membrane Biology

Topical Review

Power Transmission along Biological Membranes

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Introduction

Biological membranes are regarded as structures that separate the cytoplasm from the environment, or one intracellular compartment from the rest. No doubt such a property is inherent in any biomembrane. However, the separating functions always entail some integrating effects. For instance, the cytoplasmic membrane not only segregates the cytoplasm from the extracellular medium, but also unites diverse intracellular contents into a common system.

Both the $\Delta\Psi$[1] and ΔpH constituents of $\Delta\bar{\mu}H$, a convertible form of the membrane-linked energy, once they are formed across the membrane, immediately spread along it. Such an effect is due to the following: (i) the electric conductance of the media on both sides of the membrane is very high, since these media are water solutions of electrolytes; and (ii) conductance of the membrane may be extremely low. This is why $\Delta\Psi$, if produced by a $\Delta\bar{\mu}H$ generator, cannot avoid fast irradiation over the membrane surface. As to ΔpH, it also must irradiate rather quickly because of the high rate of H^+ diffusion in the water and the high concentration of mobile pH buffers, which cause a further increase in the H^+ movement rate. Thus, $\Delta\bar{\mu}H$ generated in a certain area of the membrane can, in principle, be transmitted as such along the membrane and transduced into work when used in another region of the same membrane. From 1969–1971 we extended this line of reasoning to the hypothesis that coupling membranes act as power-transmitting cables at the cellular (or even at supracellular) level (Skulachev, 1969, 1971). Recent progress in development of this concept is summarized below.

Key Words coupling membranes · lateral power transmission · giant mitochondria · mitochondrial reticulum · cyanobacterial membranes · intracellular electric cable

[1] *Abbreviations:* $\Delta\bar{\mu}H$ and $\Delta\bar{\mu}Na$, transmembrane electrochemical potential differences of H^+ and Na^+; $\Delta\Psi$, transmembrane electric potential difference; ΔpH, transmembrane pH difference.

Lateral Transmission of $\Delta\bar{\mu}H$ Produced by Light-Dependent Generators in Halobacteria, Cyanobacteria and Chloroplasts

Lateral $\Delta\bar{\mu}H$ transmission seems to be a step in the utilization of light energy in *Halobacterium halobium*. In this microorganism, more than 50% of the area of the cytoplasmic membrane can be occupied by purple sheets with a diameter of up to 0.5 μm. The bacteriorhodopsin-generated $\Delta\bar{\mu}H$ is utilized by $\Delta\bar{\mu}H$ consumers, which must be localized in membrane regions other than purple sheets since bacteriorhodopsin is the only sheet protein. Most probably bacteriorhodopsin is connected with $\Delta\bar{\mu}H$ consumers via lateral $\Delta\bar{\mu}H$ transmission (Skulachev, 1980, 1988).

There are at least two kinds of lateral energy transports in chloroplasts and cyanobacteria. It is well known that photons are absorbed mostly by antenna chlorophyll. Then the electron excitation energy migrates in the plane of the membrane between antenna chlorophylls until it reaches the reaction center chlorophyll. The amount of antenna chlorophyll is much higher than that of the reaction center chlorophyll (Clayton, 1980).

Another lateral transport event in the thylakoid membrane is transmission of $\Delta\bar{\mu}H$ generated in grana regions by photosystem II to stroma regions where H^+-ATP-synthase is localized (Miller & Staehelin, 1976; Staehelin & Arntsen, 1983; Allred & Staehelin, 1985).

Transcellular Power Transmission along Cyanobacterial Trichomes

In *Halobacterium* and chloroplasts, $\Delta\bar{\mu}H$ is transmitted for distances not longer than a micrometer. At the same time, transmission, e.g., of $\Delta\Psi$, can be effective even for millimeter distances, as the calculation of energy losses accompanying the transmission process has shown [the electric cable equation was used (Skulachev, 1980, 1981; Chailakhyan et al., 1982)]. The first example of such a long-distance $\Delta\bar{\mu}H$ transmission was described in our laboratory when trichomes of filamentous cyanobacteria *Phormidium uncinatum* were studied (Chailakhyan et al., 1982; Levin et al., 1982).

A cyanobacterial trichome, i.e., the linear sequence of hundreds of cells, can be several millimeters long. There are indications that the trichome-forming cells are connected with microplasmadesmata, the very thin, short tubules crossing the intercellular space (Gidding & Staehelin, 1978).

If there is a high electric conductance via the microplasmadesmata, one may assume that the electric potential difference generated across the cytoplasmic membrane near, e.g., one of the trichome ends, can be transmitted along trichome and utilized in its distal end to carry out work.

To verify this suggestion, we used light as the energy source for $\Delta\bar{\mu}H$ generation, and motility as $\Delta\bar{\mu}H$-dependent work. It was found that illumination of a trichome end (about 5% of the trichome length) with a small light beam initiated motility of the trichome when other energy sources were unavailable. Control experiments showed that at least one-third of the trichome length should be energized to allow the trichome to be motile. Thus, one may conclude that transcellular energy transmission occurred in the light-beam illuminated trichome (Chailakhyan et al., 1982). This conclusion was subsequently supported by two independent methods.

(i) $\Delta\Psi$ generation was monitored by accumulation of a fluorescent penetrating cation, ethylrhodamine. The light beam illumination was shown to induce the ethylrhodamine accumulation throughout the trichome (Severina & Skulachev, 1984).

(ii) $\Delta\Psi$ transmission along the light-beam illuminated trichomes was detected with a classical electrophysiological technique. Computer simulation of these data revealed good agreement between (a) the measured amplitude of $\Delta\Psi$, the kinetics of the $\Delta\Psi$ propagation along the trichome, etc., and (b) the same parameters calculated if one assumed that electric cable properties were inherent in the cyanobacterial trichome (Skulachev, 1980; Chailakhyan et al., 1982; Potapova et al., 1986).

The conclusion about the cable properties of cyanobacterial trichomes may be extended to the plant tissues in which the cells are connected with plasmadesmata quite permeable for ions and in particular, for H^+. A study of the possible significance of such a power transmission for the plant tissue economy seems an interesting subject for further investigation.

The Membrane Potential is Transmitted along Extended Mitochondrial Systems

The Dogma of Small Mitochondria

Translated from Greek, the word "mitochondrion" means "thread-grain." This term was introduced by cytologists who used the light microscope. The first students of mitochondria always indicated that mitochondria may exist in two basic forms: (i) filamentous and (ii) spherical or ellipsoid.

The development of electron microscope and thin section techniques changed this opinion in such a way that filamentous mitochondria came to be regarded as a very rare exception, while the spherical shape was assumed to be canonical. This change of views stemmed from the fact that single-section electron microscopy deals with a two-dimensional, rather than three-dimensional picture of the cell. A clue considered this way may be erroneously interpreted as a number of small grains if its reconstruction with the aid of many parallel sections is not carried out.

The new dogma about the shape and size of mitochondria has become especially popular among the young generation of biochemists who have never seen mitochondria under a light microscope. For this, one should possess certain essential skills since the thickness of mitochondrial filaments is usually close to the limit of resolution of light microscopy. It would be easier to take at their word electron microscopists who have evolved a new physical method of much higher resolution.

It is clear that if $\Delta\bar{\mu}H$ transmission is confined to a single spherical mitochondrion, this mechanism cannot be used to transport power for a distance comparable to the size of an eukaryotic cell. However, if mitochondria are, at least under certain conditions, filamentous and the old cytologists were right by putting the "mitos" first, then the role of mitochondria as intracellular protonic cables may be discussed as a realistic hypothesis.

Giant Mitochondria and *Reticulum Mitochondriale*

Three approaches have shaken the spherical mitochondrion dogma, namely: (i) reconstitution of three-dimensional electron-microscope pictures of

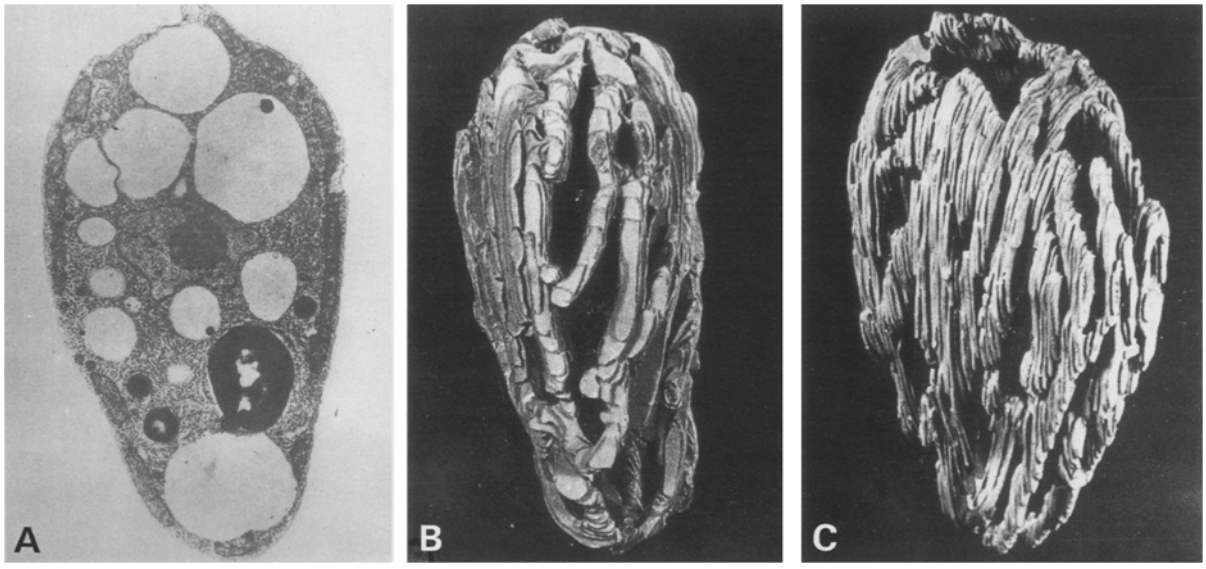

Fig. 1. Single mitochondrion in the cell of a flagellate, *Polytomella angilis*. (*A*) Single section. The picture may be erroneously interpreted as one indicating the presence of several, small, roundish or ellipsoid mitochondria. (*B,C*) Front and side views of the model of the single giant mitochondrion, reconstituted from serial sections (Reprinted from Burton & Moore, 1974, *J. Cell Biol.*)

the whole cell with the aid of serial thin sections, (ii) high-voltage electron microscopy allowing to increase the thickness of the studied preparation, and (iii) the staining of mitochondria with fluorescent penetrating cations, the technique making it possible to return to the studies of mitochondria in the living cell by means of a light microscope.

The serial section method has been applied first of all in studies on unicellular eukaryotes. Here very large and complicated mitochondrial structures were detected. For example, in a flagellate, *Polytomella agilis*, Burton and Moore (1974) described a single(!) mitochondrion which looked like a hollow, perforated sphere arranged immediately below the outer cell membrane so that the cytoplasm and the nucleus proved to be packed into a mitochondrial "stringbag" (Fig. 1).

A single giant mitochondrion was described in some yeast cells, the unicellular alga, protozoa and fungi. Thread-like, 10-μm-long mitochondria were found in exocrine cells of pancreas. A chain of the long end-to-end-arranged mitochondria were observed in spermatozoa (for refs., *see* Skulachev, 1988). In the liver cell, Brandt et al. (1974) found mitochondria of two types, the small spherical and the large branched ones. A similar picture was revealed in ascites tumor cells and in an algal cell, *Polytoma papillatum*. In the latter case, 228 consecutive sections were analyzed. It was found that there are 246 separate small mitochondria, two large and branched ones and a very large one forming a perforated hollow sphere (Gaffal & Schneider, 1980).

Muscle tissue seems to be one of the most interesting objects for studying intracellular power transmission. Multinuclear muscle cells (fibers) are very large. Their energy requirements are extremely high. In a hard-working muscle, gradients of oxygen and substrates between the periphery and the core of the cell should arise, the effect limiting the scope of the work performed. $\Delta\bar{\mu}H$ transmission from the muscle cell edge to its core along mitochondrial membranes might solve this problem. If this is the case, the dimensions of muscle mitochondria should be particularly large.

Indeed, slab-like mitochondria of about the same length as the muscle fiber radius, i.e., 10 μm, have been described in insect flight-muscle.

The first indications of the existence of a mitochondrial system penetrating the muscle fibers of higher animals were obtained in the 1960s by Bubenzer (1966, 1967), Gauthier and Padykula (1966), Gauthier (1969), and Ogata and Murata (1969). The authors studied random sections of rat diaphragm muscle, of *Musculi semitendinosus* and human intercostal muscle, respectively.

In our laboratory, Bakeeva, Skulachev and Chentsov (1977) and Bakeeva, Chentsov and Skulachev (1978) undertook a systematic investigation of serial section of rat diaphragm. It was shown that in this tissue the mitochondrial material is organized into networks piercing the I-band regions of the muscle near the Z-discs. The networks are connected with column-like mitochondria oriented perpendicular to their plane, i.e., parallel to myofibrils. Moreover, there are branches, arranged parallel to

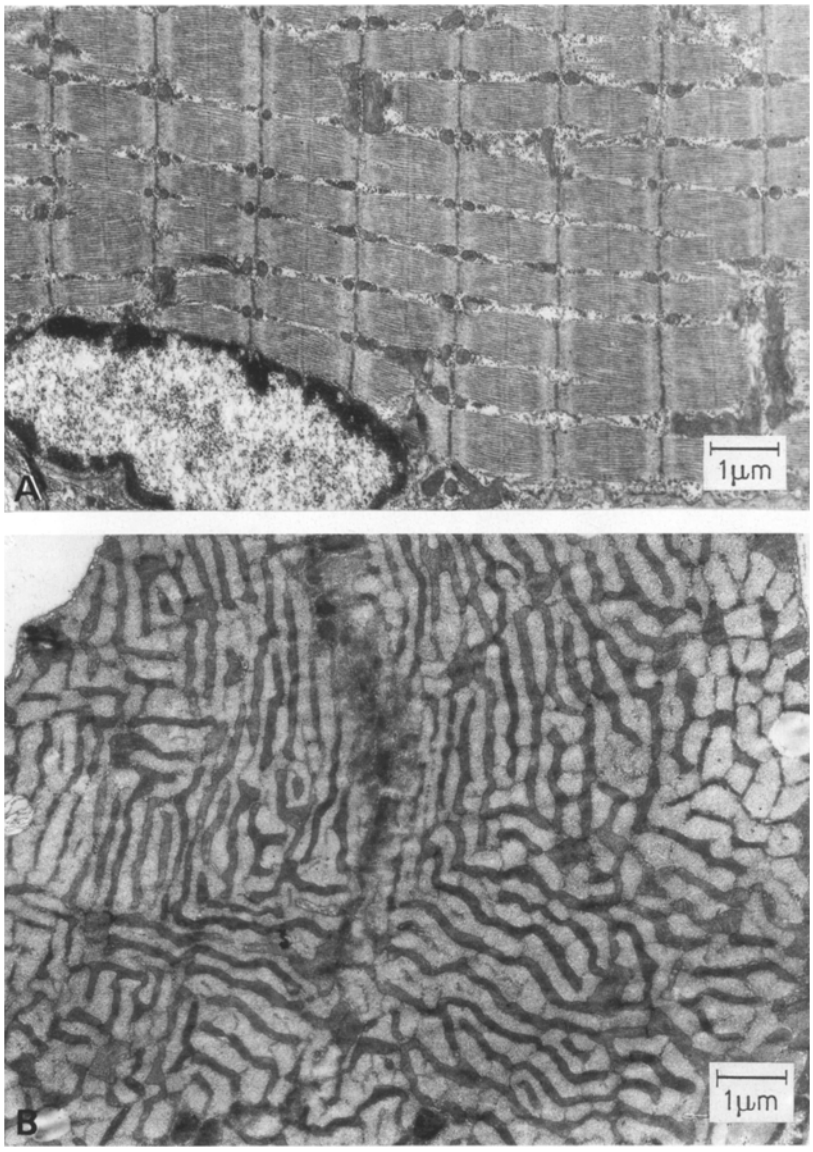

Fig. 2. Mitochondrial reticulum in the diaphragm of a 2-month-old rat. (*A*) Longitudinal section. (*B*) Transverse section of the isotropic region (Reprinted from Bakeeva et al., 1981, *Eur. J. Cell Biol.*)

Z-discs, connecting the networks with mitochondrial clusters at the fiber periphery (Fig. 2). Such a system, defined as mitochondrial reticulum (*Reticulum mitochondriale*), is found to be characteristic of the diaphragm of adult animals. It is absent from the diaphragm of rat embryos and new-born rats. Further study (Bakeeva, Chentsov & Skulachev, 1981, 1983) revealed the time course of the post-natal development of the mitochondrial framework in the diaphragm. Its formation was shown to be completed during the first two months after birth.

It seems remarkable that there is no mitochondrial reticulum in the embryonic diaphragm, i.e., an idle muscle which performs no mechanical work, and that it develops later when the main tissue function appears. The formation of a mitochondrial reticulum during muscle ontogenesis may be compared to the phenomenon of the end-to-end aggregation of mitochondria accompanying the conversion of a spermatid to a spermatozoon (Pratt, 1968; Bacetti & Afzelius, 1976). Moreover, this type of aggregation was described as taking place in different tissues under the conditions of energy deficiency such as hypoxia, hyperthyroidism, hard work, etc. (for refs., *see* Skulachev, 1988). This effect may be accounted for as an attempt by the cell to unite the activity of individual organelles when their uncoordinated functioning becomes insufficient for maintaining the necessary rate of energy production. Under normal conditions, this system may be important to a tissue such as the muscle, which should be always ready for hard work.

Further indication of the functional significance of the mitochondrial reticulum in the muscle was

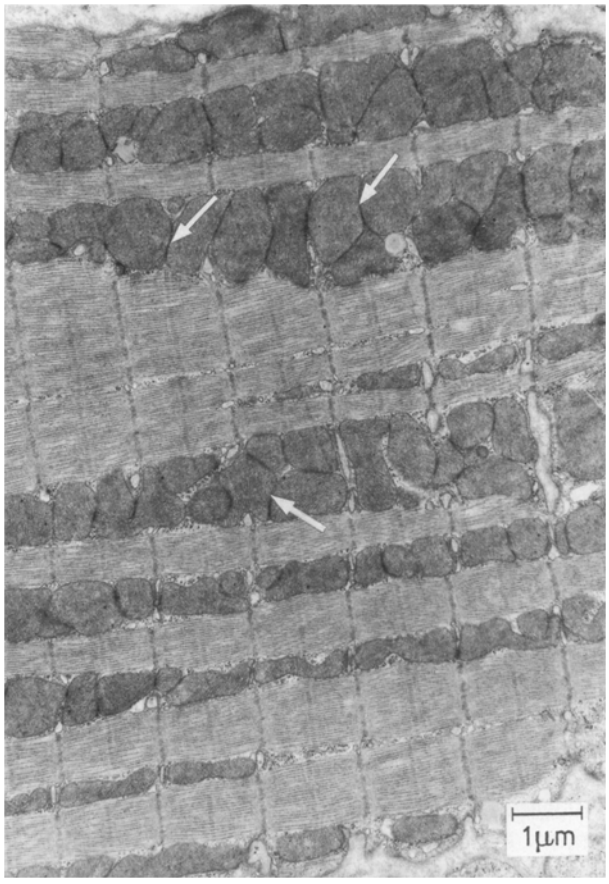

Fig. 3. Longitudinal section of a myocardiocyte. Arrows indicate some of the mitochondrial junctions (Reprinted from Bakeeva et al., 1983, *J. Mol. Cell. Cardiol.*)

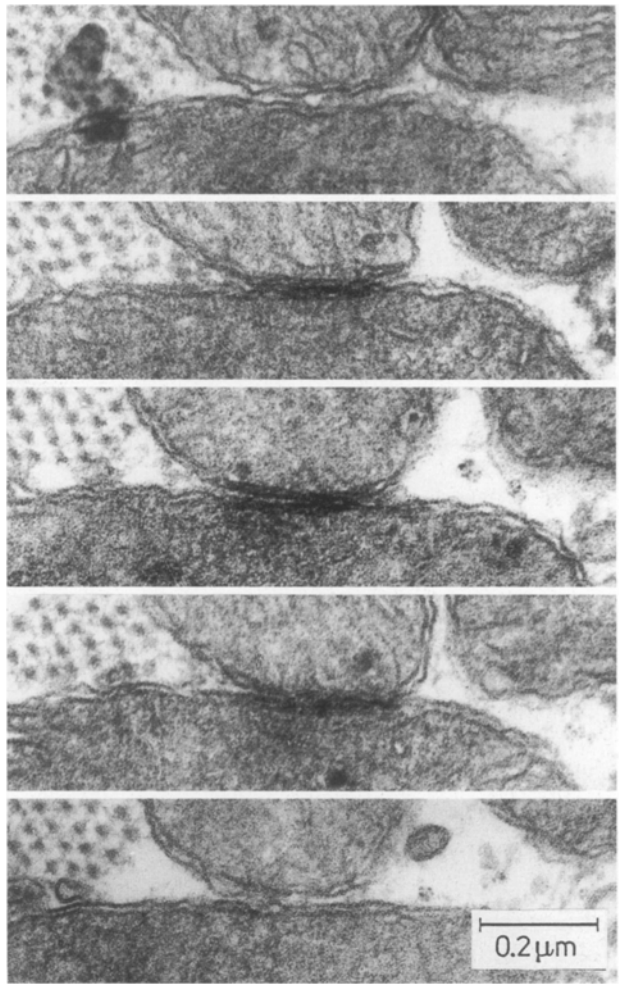

Fig. 4. Serial sections of a mitochondrial junction region in a myocardiocyte (Reprinted from Bakeeva et al., 1983, *J. Mol. Cell. Cardiol.*)

obtained when it was shown that in the skeletal muscle tissue, such a structure is specific for red, rather than white, fibers where the contribution of respiration to the energy production is especially large (Ogata & Yamasaki, 1985).

In principle, the mitochondrial reticulum may be organized in two cardinally different modes: as a giant organelle surrounded by continuous outer and inner membranes or, alternatively, as an assembly of many end-to-end associated mitochondria. An intermediate version provides for the case when the common outer membrane covers many mitoplasts, each of them being surrounded by its own inner membrane.

It was found that sometimes two reticulum-forming mitochondrial filaments form dark partitions built of four membranes, the intermembrane space being filled with osmiophilic material. Apparently these partitions represent junctions of two branches of the mitochondrial reticulum. This structure, discovered in our laboratory by Bakeeva, Chentsov and Skulachev (1978) in diaphragm, was later studied in detail by the same group in heart muscle since here mitochondrial junctions proved to be especially numerous (Bakeeva et al., 1983, 1985).

It was found that in this tissue, mitochondria also form a three-dimensional system, but instead of a thin, filamentous mitochondrial network found in diaphragm and skeletal muscle, the heart has a multitude of thick, poorly branched organelles. However, all of them are coupled to each other by numerous junctions (Fig. 3). The junction zone was found to represent a disk with a diameter of 0.1 to 1.0 μm. In this zone, membranes and intermembrane spaces are of higher density. Two outer membranes of contacting mitochondria appear to maximally approach each other in a manner similar to that observed in tight junctions of the outer cell membranes (Fig. 4). Each mitochondrion was shown to be connected to its neighbors by several such junctions. They were found in myocardiocytes with both contracted and relaxed myofibrils. At the

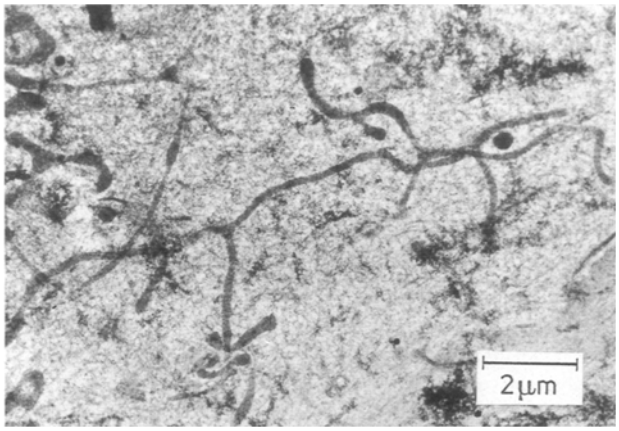

Fig. 5. High-voltage electron micrograph of filamentous mitochondria in kidney culture (PtK_1) cells (*Photograph by* I.A. Vorobjev)

same time, we could not detect mitochondrial contacts in the hearts of 3-day-old rats. Recently mitochondrial contacts were described in the heart muscle of an invertebrate (Nylund et al., 1986).

An even more complicated junction structure has been found in the zone of intercellular gap junction (nexus). Occasionally, one can see that two mitochondria belonging to two neighboring cells are in close contact with the outer cell membranes. As a result, a junction composed of six membranes is formed (two cellular and four mitochondrial membranes) (Bakeeva et al., 1983).

Contacts of mitochondrial and outer cell membranes were detected, not only in heart but also in some other tissues. As it was shown by Mashansky and his colleagues (1984), mitochondria in neurons of telencephalon can form contacts (similar to those described above), not only with other mitochondria but also with the outer cell membrane in its specialized areas, i.e., near the node of Ranvier.

Filamentous Mitochondria

If the studied tissue does not have such a high electron density as muscle, the real shape and size of mitochondria can be easily determined by high-voltage electron microscopy. Investigations of this kind revealed long filamentous mitochondria in diverse cell types. An example of this picture is shown in Fig. 5.

Two limitations of this method should be noted, however. (i) It is impossible to observe mitochondrial junctions using this technique, and so one cannot answer the question whether it is really a single lengthy mitochondrion or several end-to-end joined organelles. (ii) Like any other electron microscope method, it deals with the fixed material, and thus does not make it possible to follow directly the functioning of mitochondria.

To overcome the latter limitation, we decided to return to light microscopy. Here functional analysis is quite possible, but the great disadvantage is low contrast which is insufficient to see with certainty thin mitochondrial filaments and networks. However, such a limitation is absent if we deal with light emission instead of light absorption. If the light emission is sufficiently strong, the light source will be seen in the dark even if it cannot be observed as a light-absorbing body.

Thus the problem was how to obtain light emission from mitochondria. To do this, we applied the same approach introduced earlier in our group to detect $\Delta\Psi$ formation across the mitochondrial membrane, i.e., synthetic penetrating cations. The only difference was that this time, fluorescent cations were used. The idea was that fluorescent cations, electrophoretically moving into mitochondria, may be accumulated in the matrix space. A concentration of the cation inside may be 10^4 higher than that outside mitochondria provided that $\Delta\bar{\mu}H$ is in the form of $\Delta\Psi$. If one succeeds in finding such a cation, invisible mitochondrial filaments may be seen under the fluorescent microscope. In search of penetrating fluorescent cations, we turned our attention to rhodamines. These compounds are (i) positively charged, (ii) rather hydrophobic and (iii) have a high quantum yield of fluorescence. Moreover, rhodamine derivatives were known specifically to stain mitochondria in the living cell. This was shown as early as 1941 by Johannes (1941), who used light transmission microscopy.

As it was found in our group by Dr. I.I. Severina (Murvanidze, Severina & Skulachev, 1981; Severina & Skulachev, 1984), ethyl-substituted rhodamine added to solutions washing the planar phospholipid membrane generates a diffusion potential predicted by the Nernst equation. This means that ethylrhodamine may be regarded as a penetrating cation.

Independently, Dr. L.B. Chen and coworkers had found that the treatment of cultured fibroblasts with a commercially available sample of rhodamine-B-conjugated IgG antibody resulted in the staining of "snake-like structures" inside the cell, which later were identified as mitochondrial ones (Walsh, Jen & Chen, 1979). Further study revealed that the staining was caused by contamination of some free rhodamine derivatives in this sample (Chen et al., 1982). Systematic investigation of rhodamines 3B, 6G and 123 and other fluorescent hydrophobic cations, such as cyanine dyes and safranine O, showed that they are selectively accumulated in energized

mitochondria inside the cell so that mitochondria become fluorescent. Discharging $\Delta\Psi$ by uncouplers or by valinomycin + K^+, one could abolish mitochondrial fluorescence, while nigericin, converting ΔpH into $\Delta\Psi$, increased it. Not only fibroblasts but also primary cultures of bladder epithelium and of myocardiocytes were found to respond in such a way. Methylrhodamine (the other name is rhodamine 123) proved to be especially useful because of the low toxic side-effects upon the cell (Johnson, Walsh & Chen, 1980; Johnson et al., 1981; Johnson, Summerhayes & Chen, 1982). Later rhodamine 123 was employed in several studies on mitochondria in animal and plant cells (for reviews, *see* Summerhayes et al., 1982; Bereiter-Hahn et al., 1983; Skulachev, 1988).

The main result of the fluorescent studies of mitochondria in the living cell is that rather frequently they have the form of filaments, tens of micrometers in length. The filaments may be so long as to connect the cell core and the cell periphery, or even to cross the cell from edge to edge. In some cases, mitochondrial networks were detected. A typical case in point is that two mitochondrial populations, i.e., (i) filamentous or networking-forming and (ii) small spherical, oval or rod-like organelles, co-exist in one and the same cell.

Some drugs and other in vivo treatments were shown to provoke fragmentation of long mitochondria in the cell (*see*, e.g., Vorobjev & Zorov, 1983). In fact, fluorescent studies of mitochondria have confirmed the data obtained by traditional methods, indicating that thread–grain transition is a typical feature inherent in these organelles.

Mitochondria as Intracellular Proton Cables: Verification of the Hypothesis

If a mitochondrial filament represents the continuum of both inner and outer membranes, $\Delta\bar{\mu}H$ will be transmitted throughout the entire filament length. Yet, if the mitochondrial filament is an assembly of many small contacting end-to-end mitochondria or mitoplasts, two possibilities should be considered. (i) The filament is similar to a cyanobacterial trichome where the constituents (individual cells) are connected to each other by junctions of high electric conductance. (ii) There is no electric contact of adjacent mitochondria in the filament.

In a preceding section, we described the intermitochondrial junctions which clearly represent specific structures responsible for contact of adjacent mitochondria. It remained obscure, however, whether the electric resistance of the junctions is as high as in other regions of the inner mitochondrial membrane or as low as in cyanobacterial microplasmadesmata or gap junctions of two animal outer cell membranes. The latter version is identical to the mitochondrial continuum in the sense of $\Delta\bar{\mu}H$ transmission. The former possibility requires an exchange of energy equivalents other than $\Delta\bar{\mu}H$ between neighboring mitochondria to be postulated.

An obvious prediction of the hypothesis, considering a filamentous mitochondrion as cable, is that the whole filament must be de-energized when any of its part becomes leaky. This prediction was recently verified in our group. Combining a laser and a fluorescent microscope, Zorov in our laboratory succeeded in illuminating a single mitochondrial filament in a human fibroblast cell stained with ethylrhodamine. A very narrow laser beam (the diameter of the light spot was commensurable with the thickness of the mitochondrial filaments) was used to cause a local damage of the filament. As seen in Fig. 6, the laser treatment resulted in the disappearance of the rhodamine fluorescence in the entire 40 μm filament. It is essential that (i) other filaments remain fluorescent so that the laser effect is not the result of the nonspecific damage of the cell and (ii) the illuminated filament retains its continuity when scrutinized under a phase-contrast or electron microscope. In fact, no detectable traces of laser-induced damage were found. It was also shown that the above effect was not due to a displacement of the illuminated mitochondrial filament (Drachev & Zorov, 1986; Bakeeva et al., 1986; Amchenkova et al., 1986, 1988). As one can see in Fig. 6*E*, some cristae in the filamentous mitochondrion look like partitions crossing the tubule interior. Therefore one may speculate that a mitochondrial filament is composed of several mitoplasts surrounded by a common outer mitochondrial membrane. One cannot, however, exclude the alternative possibility, i.e., there is an aperture in the partition that is not seen in the given cross section. In any case, these partitions cannot prevent the laser-induced local membrane discharging from being irradiated along the mitochondrial filament.

In some human fibroblast cells, the majority of mitochondria seemed to represent a network of interconnected filaments. In this case, laser irradiation of one of the branches resulted in a $\Delta\Psi$ collapse not only in this branch but also in other parts of the mitochondrial system. Short mitochondria, which were localized in the same cell but were out of the network, retained fluorescence after the laser irradiation (Amchenkova et al., 1988).

Figure 7 shows the results of a similar study on myocardiocytes. In the culture of myocardial cells of 3-day-old rats, the main portion of the mitochondrial material was shown to represent large (several μm long) spherical or ellipsoid mitochondria. More-

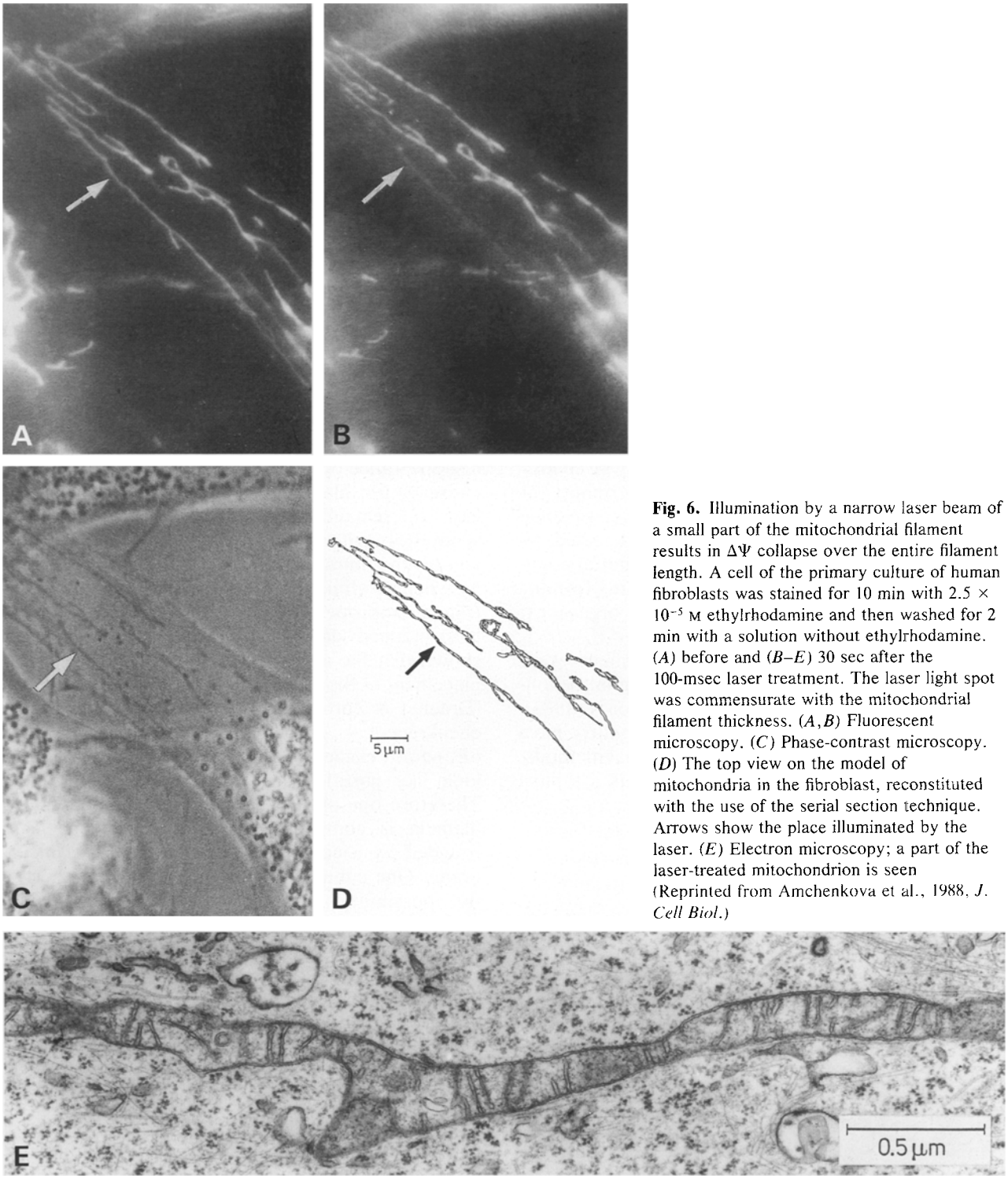

Fig. 6. Illumination by a narrow laser beam of a small part of the mitochondrial filament results in $\Delta\Psi$ collapse over the entire filament length. A cell of the primary culture of human fibroblasts was stained for 10 min with 2.5×10^{-5} M ethylrhodamine and then washed for 2 min with a solution without ethylrhodamine. (*A*) before and (*B–E*) 30 sec after the 100-msec laser treatment. The laser light spot was commensurate with the mitochondrial filament thickness. (*A,B*) Fluorescent microscopy. (*C*) Phase-contrast microscopy. (*D*) The top view on the model of mitochondria in the fibroblast, reconstituted with the use of the serial section technique. Arrows show the place illuminated by the laser. (*E*) Electron microscopy; a part of the laser-treated mitochondrion is seen (Reprinted from Amchenkova et al., 1988, *J. Cell Biol.*)

over, there were rather long, thin filaments that often were in contact with large mitochondrial bodies. It was found that the illumination of a single mitochondrion gives rise to a quenching of a cluster composed of many mitochondria. In the experiment shown in Fig. 7*A–E*, this cluster includes mitochondrion N1 which was illuminated by laser, its neighbors (mitochondria N2 and 3) as well as mitochondria N7–10, 14–19, 23 and 24. Note that some mitochondria, being very close to the illuminated

one (e.g., N4), are fluorescent, whereas other mitochondria localized at a distance as long as 18 μm from it (N14, 15), are nevertheless completely quenched. Electron microscopic analysis revealed that mitochondria, which become quenched after the laser treatment, are connected with the illuminated mitochondria by intermitochondrial contacts, whereas those retaining fluorescence are not (Fig. 7*D*, *E*).

As one can see from Fig. 7*D*, the quenced mitochondria can be easily recognized in an electron micrograph: they look much darker than those which were fluorescing when studied with a fluorescent microscope. Control experiments showed that the addition of protonophorous uncouplers transforms the entire mitochondrial population in the cell to this dark state characterized by a very condensed matrix space.

Figure 7*F–I* demonstrates that there are thin filamentous mitochondria between two clusters of large mitochondria quenched due to illumination of one of them. Electron microscopy revealed junctions connecting large mitochondria and filaments (not shown in the figure).

To exclude the possibility that de-energizing is a consequence of lateral diffusion of some chemical photoproducts which may be formed under illumination of an ethylrhodamine-stained mitochondrion, we used, in certain experiments, another way of damaging a mitochondrion inside the cell. A large ellipsoid mitochondrion in a cardiomyocyte was punctured with a glass microcapillary (~0.2 μm diameter). This was found to result in an effect similar to that in laser experiments, i.e., fast (1–2 sec) quencing of a mitochondrial cluster, while other mitochondria continue to fluoresce (Amchenkova et al., 1986, 1988).

The simplest explanation of these data is the following: In a myocardiocyte, there are several mitochondrial clusters (*Streptio mitochondriale*) formed by many mitochondria joined by mitochondrial junctions. These junctions are of high electric conductance, so the cluster is de-energized when at least one of the cluster-composing mitochondria becomes leaky (Fig. 8). Thus, functionally mitochondrial junctions seem to resemble the microplasmadesmata of cyanobacterial trichomes.

How Lateral $\Delta\bar{\mu}H$ Transmission is Organized

If $\Delta\bar{\mu}H$ is in the form of $\Delta\Psi$, the energy is transmitted along the membrane due to the diffusion of mobile ions on both sides of the membrane. For intracellular organelles, this should be mainly K^+, Cl^- and charged metabolites most of which are, at neutral pH, anions of weak acids. In the case of outer cell membranes, it should be Na^+ and Cl^- outside and K^+, metabolites and Cl^- inside. At the same time, $\Delta\bar{\mu}H$ generators and consumers deal with H^+, of which the concentration in the biological system is significantly lower than that of the above-mentioned ions. This may result in a situation where the long-distance $\Delta\Psi$ transmission causes the formation of local pH gradients along, e.g., mitochondrial filaments.

Such an effect is illustrated in Fig. 9. In this figure, lateral power transmission along the main axis of a filamentous mitochondrion is considered. It is assumed that $\Delta\bar{\mu}H$ is generated by respiration occurring close to that end of the filament which is near the cell border where the concentrations of respiratory substrate and O_2 must be maximal. Initially, $\Delta\bar{\mu}H$ is presented by $\Delta\Psi$, which is transmitted to the opposite end of the filament. It is utilized there to form ATP by H^+-ATP-synthase. Thus a pH difference between the two opposite ends of the filament appears, the matrix space being alkalinized in the respiring part and acidified in the ATP-synthesizing part of the mitochondrion. pH changes outside the mitochondrion should be considerably smaller than in the matrix because of the volume difference between the cytosol and matrix spaces. Formation of the lateral pH gradient causes the movement of H^+ along the filament. H^+ flow in the water phase is accelerated by mobile pH buffers of which the concentration is high both inside and outside the mitochondria. In fact, amount of mobile pH buffers composing 10–20% of the salt concentration in the cell is very much higher than that of H^+ and OH^-. Certainly it is still lower than concentration of monovalent metal cations and Cl^-, but one should take into account that it is a gradient of H^+, not of K^+, Na^+, Cl^-, etc., that is formed due to operation of H^+ pumps. It is not excluded, moreover, that equilibration of lateral H^+ gradients may be facilitated by H^+ conductance of the membrane/water interface as it was assumed for a model system by Prats, Tocanne and Teissie (1985) and Sakurai and Kawamura (1987).

To organize the long-distance power transmission in the mitochondrial clusters (e.g., in the heart muscle *Streptio mitochondriale, see above*), the intermitochondrial junctions must be H^+ permeable. One of possible mechanisms of such kind is illustrated in Fig. 10. It is assumed that in the junction region, channel-forming proteins of the outer and inner membranes are in contact so that an H^+-permeable pore crossing four membranes is organized. The proteins in question may be porins for the outer membranes and, e.g., F_1-deprided parts of H^+-ATP-synthase (i.e., factors F_α) for the inner membrane.

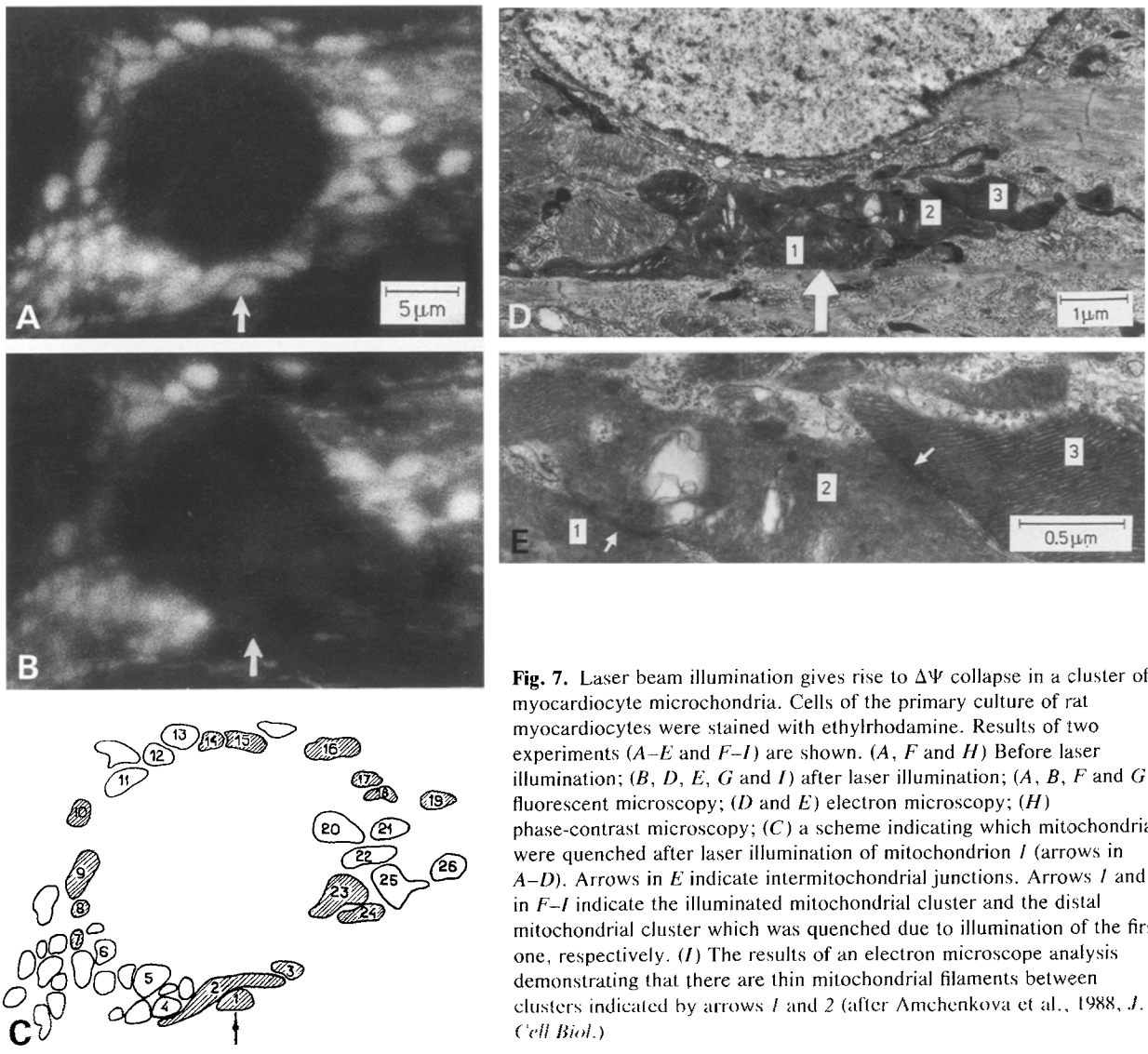

Fig. 7. Laser beam illumination gives rise to $\Delta\Psi$ collapse in a cluster of myocardiocyte microchondria. Cells of the primary culture of rat myocardiocytes were stained with ethylrhodamine. Results of two experiments (*A–E* and *F–I*) are shown. (*A*, *F* and *H*) Before laser illumination; (*B*, *D*, *E*, *G* and *I*) after laser illumination; (*A*, *B*, *F* and *G*) fluorescent microscopy; (*D* and *E*) electron microscopy; (*H*) phase-contrast microscopy; (*C*) a scheme indicating which mitochondria were quenched after laser illumination of mitochondrion *1* (arrows in *A–D*). Arrows in *E* indicate intermitochondrial junctions. Arrows *1* and *2* in *F–I* indicate the illuminated mitochondrial cluster and the distal mitochondrial cluster which was quenched due to illumination of the first one, respectively. (*I*) The results of an electron microscope analysis demonstrating that there are thin mitochondrial filaments between clusters indicated by arrows *1* and *2* (after Amchenkova et al., 1988, *J. Cell Biol.*)

Instead of F_o, so-called anion channel of mitochondria might be involved. This mechanism transports anions and cations of low molecular weight being slightly more permeable for anions. It is activated in the inner mitochondrial membrane under certain conditions. Even a small potential difference across the membrane inactivates the anion channel (Colombini, 1987; Sorgato, Keller & Stuhmer, 1987; Tedeschi & Kinnally, 1987). $\Delta\Psi$ dependence was also shown for porin (Roos, Benz & Brdiczka, 1982). One may speculate that such $\Delta\Psi$ dependence allows the junction conductance to be switched off when one of the mitochondria in cluster becomes leaky for a long time.

In this context, it should be mentioned that power transmission via cyanobacterial microplasmadesmata can be switched off when a trichome-composing cell is damaged. This was recently found in our group by Severina et al. (1988). The same effect was described for plasmadesmata that are responsible for the cell-to-cell connections in hyphae of *Neurospora crassa* (Potapova et al., 1988). As it was assumed by McGillviray and Gow (1987), transcellular current along hyphae is due to the flux of H^+ ions. It was also shown that the plasma membrane of the hyphal apical cell is at least partially energized by H^+-ATPases localized in the plasma membranes of the other cells. This includes lateral transcellular $\Delta\bar{\mu}H$ transmission along plasma membranes via plasmadesmata (Potapova et al., 1988).

Assuming that mitochondrial junctions can be reversibly closed or disrupted, one may explain the data by Ferguson-Miller, Hochman and Schindler (1986) on aligned mitochondria in 3T3 cells, stained with rhodamine 123. The authors performed patterned bleach of the stained cells, but found no re-

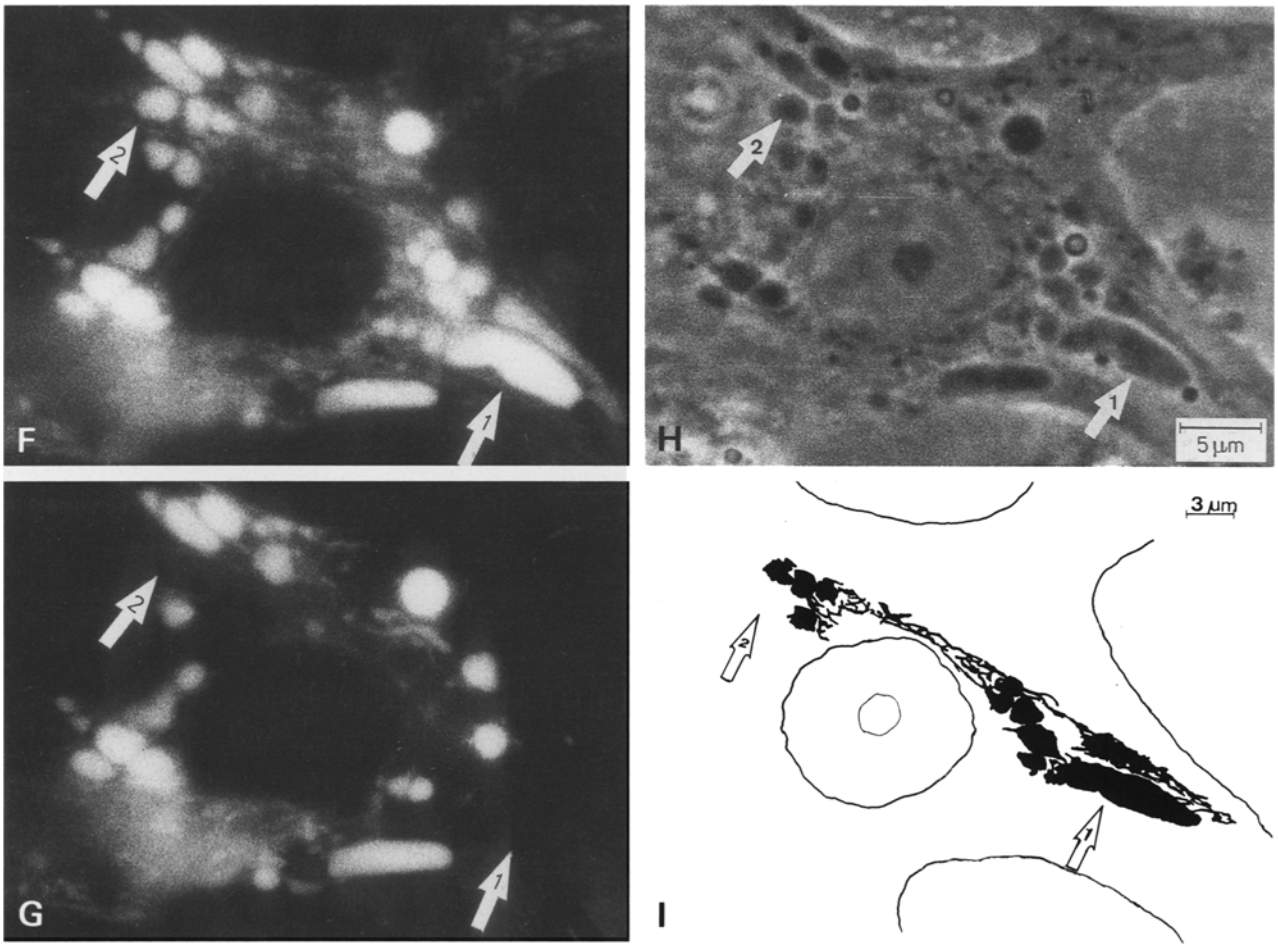

Fig. 7. *Continued*

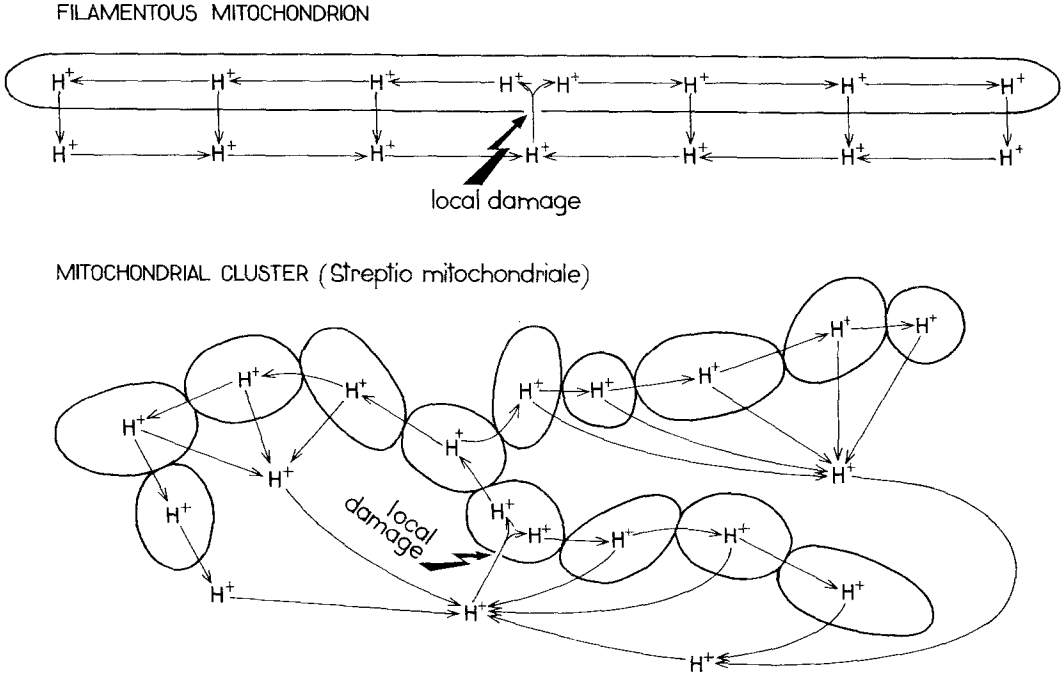

Fig. 8. De-energization of filamentous mitochondrion or mitochondrial cluster, induced by local damage of the membrane. In the latter case, it is assumed that the mitochondrial junctions are H^+ permeable

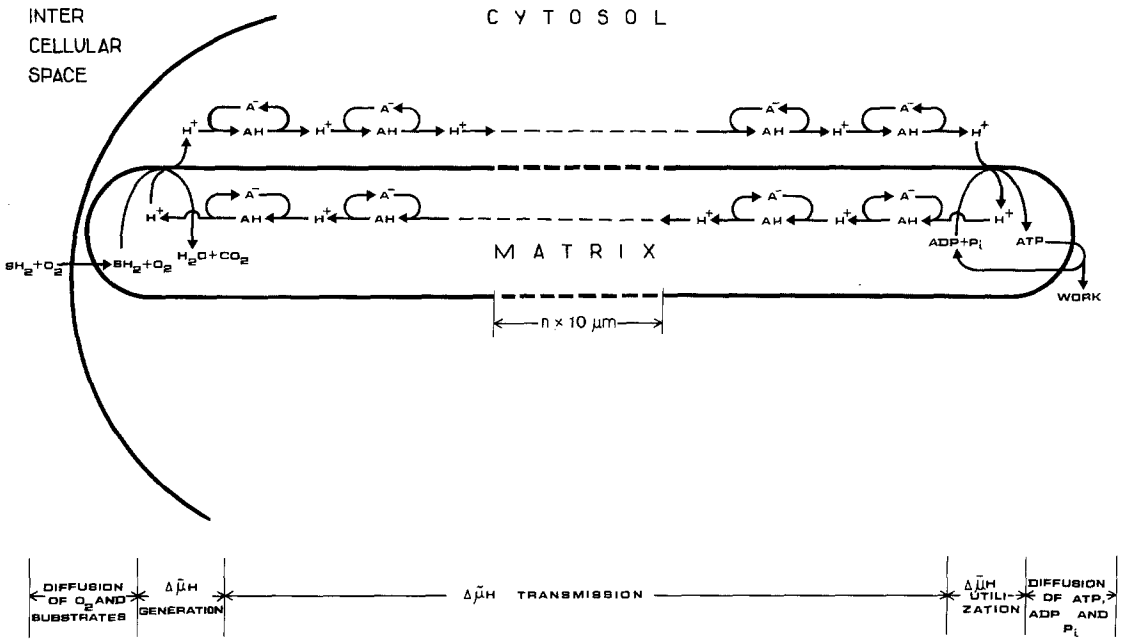

Fig. 9. Power transmission along the mitochondrial filament from the cell periphery to the cell core. SH_2, respiratory substrate; AH and A^-, protonated and deprotonated pH buffers facilitating transport of H^+ along the mitochondrial filament (from Skulachev, 1988). A situation is considered when intracellular gradients of O_2 (and/or respiratory substrates), and of ADP and phosphate arise, $[O_2]$ and $[SH_2]$ being maximal in the peripheral regions of the cell, whereas [ADP] and $[P_i]$ being maximal in the cell core. In this case, "peripheral" respiration is assumed to contribute to ATP synthesis, not only in the peripheral regions but also in the cell core

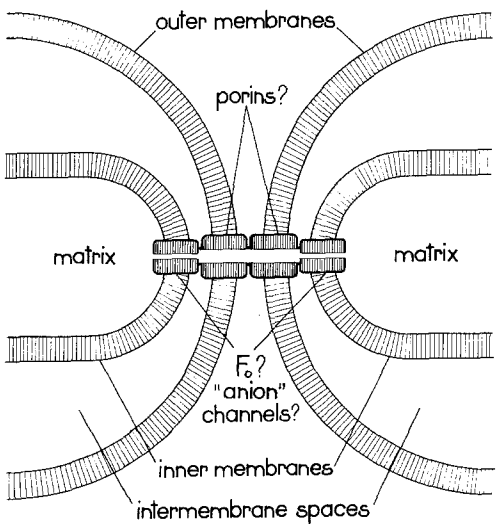

Fig. 10. Possible structure of the mitochondrial junction. It is suggested that the junction structure is composed of porins of the outer membrane and of some channel-forming proteins of the inner membrane, such as F_1-deprived membrane sector of H^+-ATP-synthase (F_o), "anion" channel, etc.

covery. The control experiment with rhodamine-stained giant mitochondria showed that rhodamine 123, when bleached, recovered very rapidly. Maybe the bleaching resulted in damage of the bleached mitochondria with their subsequent isolation from the intact ones. In the laser-treated cardiomyocytes, such an isolation apparently does not occur or occurs too slowly in comparison with fast damage induced by the laser illumination of one of the cluster-forming mitochondria. At the same time, it is yet impossible to exclude that the above-described mitochondrial clusters were formed as a result of partial decomposition of a united system of mitochondria in the cardiomyocite. In other words, it might be that before the laser treatment, all the mitochondria in the cell are connected with junctions, i.e., there is one huge mitochondrial supercluster. An alternative point of view may be that several octopus-like clusters are coexistent in one and the same cell. In this case, the laser treatment simply visualizes pre-existing clusterization.

Possible Function of Lateral Power Transmission Process in Mitochondria

The operation of a filamentous mitochondrion or mitochondrial cluster as a proton-conducting cable allows the long-distance diffusion of metabolites, ATP, ADP and phosphate to be replaced with H^+ movement (Skulachev, 1980). Such a substitution may (i) accelerate the power transmission and (ii) direct the delivery of energy to a certain intracellular area.

On a cellular scale, the movement of a substance along the mitochondrial filament may be approximated as one-dimensional diffusion. This means that the replacement of, e.g., ATP diffusion in the cytosol with the diffusion of H^+ and pH buffers along filamentous mitochondria is equivalent to the replacement of a process occurring in the three-dimensional space with that in the one-dimensional space. Such an effect undoubtedly saves time when energy should be delivered to a certain place. This reasoning makes it clear why filamentous mitochondria and mitochondrial reticula often appear under conditions of hard work or energy deficiency.

That mitochondrial filaments are not randomly arranged in the cytoplasm is indicated by several observations:

(i) They are usually oriented parallel to the longest axis of the cell and stretch from the cell periphery to the nucleus (*see*, e.g., Fig. 6).

(ii) This direction usually coincides with that of the main bundles of microtubules. There are special cross-bridges between mitochondria and microtubules (Smith, Jarlfors & Cayer, 1977).

(iii) There is a general rule formulated by Bereiter-Hahn and Fuhrmann (1983) that mitochondria are always localized in the endoplasmic reticulum-containing regions of the cytoplasm.

Lateral Transmission of $\Delta\bar{\mu}$Na

The outer membrane of the animal cell utilizes Na^+ rather than H^+ as the coupling ion. Many processes of the uphill transport through the animal cell outer membrane are organized as a metabolite-Na^+ symport (Hoshi & Himukai, 1982; Kanner, 1983; West, 1983). 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 that 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 (Skulachev, 1988). In many types of animal cells, the so-called gap junctions have been described (Bennett, 1973; Loewenstein, 1973; Hertzberg & Johnson, 1988). In 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 (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. Recently Potapova et al. (1990) have found in our laboratory that power transmission via gap junctions is effective in energization of a cell by another one. Two cell cultures greatly differing in sensitivity of Na^+/K^+-ATPase to ouabain were mixed and, after formation of gap junctions, treated with such a ouabain concentration that specifically inhibited only one cell population. Microelectrode measurement showed that the membrane potential maintained, not only in the ouabain-resistant but also in the ouabain-sensitive cells. Under the same conditions, the sensitive cell kept without resistant ones failed to maintain the membrane potential.

Other Possible Functions of Extended Membranous Systems: Lateral Transport of Ca^{2+}, Fatty Acids, Oxygen, Reducing Equivalents and Enzymes

It seems obvious that extended membranous structures may, in principle, be applied to transport components other than $\Delta\Psi$, ΔpH or ΔpNa. For example, the mitochondrial filaments might be the routes for any substance that is concentrated in a mitochondrial compartment, including the membranes. For instance, if Ca^{2+} ions are transported from the intercellular space to cytosol at the cell periphery, they may be accumulated in the matrix space of mitochondrial filaments by means of Ca^{2+} uniporter, diffuse along the filament as in a tube and be released to cytosol in the cell core if the mitochondrial $Ca^{2+}/2H^+$ antiporter is activated in this region of the filament (Petrunyaka, 1983; Skulachev, 1988).

The same logic may be applied to transport of any other solute that can be reversibly accumulated in the matrix.

Lateral transport of the membrane-linked compounds may be another variation of this theme. It is generally believed that transports occurring in a membrane must be slower than in the water phase of the cell since the viscosity of the membrane is much greater than that of water, and in this respect the water route should have an advantage over the membrane route for any amphiphilic compound. However, one must take into account that the cytosol and the mitochondrial matrix are not aqueous solutions of low molecular mass compounds, but

rather colloid solutions which are crossed by non-soluble structures, namely, by cytoskeleton and/or membranes. So, diffusion via liquid regions of the membrane might be not much slower than via cytosol provided that phospholipids, rather than large protein clusters, occupy a major part of membrane area. Such a condition is fulfilled, e.g., for the outer mitochondrial membrane.

It is not surprising therefore that a spin-labeled synthetic compound of low molecular mass, of which the partition coefficient in a lipid/water system is close to 1, was shown to move preferentially in the lipid phase of various types of cells (Keith & Snipes, 1974). Such preference must be even more pronounced for substances for which the lipid/water distribution coefficient is higher than 1, such as free fatty acids and their carnitine and CoA esters. For the latter, the role in the lateral transport of fatty acyls was postulated by Sumper and Träuble (1973). Fatty acyl carnitine seems adapted for this function even better since its hydrophilic moiety is much smaller and the concentration is much higher than those of fatty acyl CoA.

When studying rat diaphragm muscle, we found (Bakeeva et al., 1977) that fat droplets inside the muscle cell are always covered by the mitochondrial material connected to the mitochondrial reticulum. A close contact of mitochondria and fat droplets in liver and pancreas cells was observed by De Robertis, Nowinski and Saez (1976). The authors mentioned that there is only one (inner) mitochondrial membrane between a fat droplet and the matrix as if the droplet were localized in the intermembrane space of a mitochondrion. Urgent mobilization of neutral lipids in brown fat at cold-induced stress was found to be accompanied by such a massive release of free fatty acids that they seem to form a continuum extending from a fat droplet to the inner mitochondrial membrane (Blanchette-Mackie & Scow, 1982).

One may suggest that membranes can be employed as a route for lipid-soluble metabolic regulators such as diacylglycerol.

The lateral transport of molecular oxygen poses an intriguing problem. There are at least four effects favorable for the use of the inner mitochondrial membrane as a route for the intracellular oxygen transport:

(i) Solubility of O_2 in lipid is about fivefold higher than in water. This difference may be even greater if we compare lipid with cytosol. In fact, O_2 cannot be dissolved in the bound water hydrating cytosol solutes.

(ii) The main oxygen-utilizing enzyme, cytochrome oxidase, localized in the inner mitochondrial membrane, has K_m for oxygen very much lower than the oxygen concentration in water under standard conditions. It must result in the formation of a local low O_2 concentration area in the inner membrane.

(iii) The rate of O_2 diffusion in hydrocarbons proved to be between 10- and 100-fold more rapid than could be predicted from macroscopic viscosity data, being as high as in water (Subczynski & Hyde, 1984). The oxygen diffusion rate in certain biomembranes at 37°C was found to be of the same order of magnitude as in the blood plasma (Fischkoff & Vanderkooi, 1975). This process was strongly decelerated by cholesterol which is localized in membranes other than the inner mitochondrial one.

(iv) In such tissues as muscles, the inner mitochondrial membrane comprises the bulk of the total membrane material of the cell (Bakeeva et al., 1977).

However, in spite of point *iv*, the inner mitochondrial membranes, even in the heart muscle, occupy only a small portion of the cell volume. Further studies are required to find out whether the flow of O_2 along membranes can effectively compete with that through cytosol.

In the endoplasmic reticulum and in the outer mitochondrial membrane of many tissues (liver, kidney, brain, leukocytes, etc.) there is a very active redox chain that does not reach the oxygen:

$$\text{NADH} \rightarrow f_{P_5} \rightarrow \text{cytochrome } b_5$$

where f_{P_5} stands for NADH-cytochrome b_5 reductase flavoprotein (Skulachev, 1969, 1988). Both f_{P_5} and cytochrome b_5 are composed of two unequal parts: the larger, hydrophilic, containing a flavin or heme group, and the smaller, hydrophobic part, requiring the protein to be anchored to the membrane. Like floats, f_{P_5} and cytochrome b_5 can move along the membrane encountering relatively weak resistance. The high lateral mobility of these two proteins was shown by Strittmatter et al. (Rogers & Strittmater, 1974*a*,*b*; Strittmater, Rogers & Spatz, 1972).

Taking into account these facts, we suggested that f_{P_5} and cytochrome b_5 are the lateral carriers of reducing equivalents, i.e., H atoms and electrons, respectively (Archakov, Karyakin & Skulachev, 1975*a*). Since the f_{P_5} redox potential is close to its reductant, NADH, i.e., about −0.3 V, it can serve as a component equilibrating the reducing equivalent (H) at the NADH level in different parts of the cell. As for cytochrome b_5, it may perform a similar function for electrons at the zero redox potential level.

Intermembrane electron transfer may be one more function of cytochrome b_5. It was found that

rat liver endoplasmic membrane vesicles or mitochondria can reduce cytochrome b_5 in proteoliposomes containing cytochrome b_5 as the only protein species. NADH was used as the reductant. Repeated washing of endoplasmic reticulum vesicles failed to diminish this effect. Thus, it was concluded that f_{P_5} and/or cytochrome b_5 are competent in intermembrane electron transfer without any water-soluble carriers involved. Our experiments showed that such a property is inherent in cytochrome b_5, not f_{P_5} (Archakov, Karyakin & Skulachev, 1973, 1974, 1975*a*,*b*).

Intermembrane electron transfer between mitochondria and the endoplasmic reticulum allows combining one-dimensional diffusion of cytochrome b_5 along mitochondrial filaments with its two-dimensional diffusion in the plane of endoplasmic reticulum cisterns. This way, one may apparently achieve more rapid equilibration of the redox potential in different areas of the cell than by means of three-dimensional diffusion of metabolites or coenzymes.

Lateral transport of enzymes, in principle, allow their functionally favorable redistribution inside the cell to be achieved.

Let us assume that formation of complex of a membrane enzyme with its substrate or with another enzyme causes a decrease in the lateral motility of this enzyme. This will inevitably give rise to increase in concentration of the given enzyme in those regions of the cell where concentration of the substrate or the enzyme partner is maximal. This way, taxis of the enzyme to the place where it is operating might occur. In this connection, one may mention an indication of a supercomplex formation by respiratory chain complex I, III and IV (Ferguson-Miller et al., 1986). Lateral mobility of mitochondrial cytochrome oxidase (complex IV) and of chloroplast F_oF_1 complex, were demonstrated by Hochli and Hackenbrock (1979) and by Miller and Staehelin (1976), respectively.

Summary

Hypothesis on long-distance power transmission along extended energy-transducing membranes (Skulachev, 1969, 1971, 1980), has been experimentally proven in four different systems, namely,

(i) trichomes of filamentous cyanobacterium *Phormidium uncinatum;*

(ii) filamentous mitochondria and mitochondrial network in fibroblasts;

(iii) clusters of roundish heart muscle mitochondria interconnected with mitochondrial junctions;

(iv) mixed animal cell cultures interconnected with gap junctions.

In all cases, energy was shown to be transmitted in the form of a transmembrane electric potential difference. The transmission occurred for distances as long as several tens of micrometers. Since the (a) $\Delta\bar{\mu}$H-bearing cytoplasmic membrane of cyanobacteria and the inner mitochondrial membrane and (b) $\Delta\bar{\mu}$Na-bearing outer animal cell membrane were found to be competent in such an effect, one may assume that the power transmission is a fundamental function of extended membrane systems. This mechanism can be used at the intracellular level (mitochondrial) as well as at the supracellular level (cytoplasmic and outer cell membranes).

Studies on the possible involvement of membranes in lateral transport of oxygen, ions, fatty acids and membrane proteins seem to hold good promise.

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Received 15 December 1988; revised 31 July 1989