Coupling Membranes As Energy-transmitting Cables
I. Filamentous Mitochondria in Fibroblasts
and Mitochondrial Clusters in Cardiomyocytes

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Abstract. An hypothesis considering mitochondria as intracellular power-transmitting protonic cables was tested in human fibroblasts where mitochondria are thin and long and in rat cardiomyocytes where they show cluster organization. Mitochondria in the cell were specifically stained with fluorescent-penetrating cation ethylrhodamine, which electrophoretically accumulates in the mitochondrial matrix.

A 40-μm-long mitochondrial filament of fibroblast was illuminated by a very narrow ($\leqslant0.5$ μm) laser beam to induce local damage of the mitochondrial membranes. Such a treatment was found to induce quenching of the ethylrhodamine fluorescence in the entire filament. According to the electron microscope examination, the laser-treated filament retained its continuity after the laser illumination. Other mitochondrial filaments (some of which were localized at a distance <10 μm from the laser-treated one) remained fluorescent. In a cell where mitochondrial filaments seemed to be united in a network, laser illumination of one filament resulted in fluorescence quenching in the whole network, whereas fluorescence of small mitochondria not connected with the network was unaffected.

The illumination of cardiomyocyte was found to result in the fluorescence quenching not only in a laser-illuminated mitochondrion but also in a large cluster of organelles composed of many mitochondria. Electron microscopy showed that all the mitochondria in the cluster change from the orthodox to the condensed state. It was also found that mitochondria in the cluster are connected to one another with specific junctions. If a mitochondrion did not form junctions with a quenched cluster, its fluorescence was not decreased even when this mitochondrion was localized close to an illuminated one. The size of the mitochondrial cluster may be as long as 50 μm. The cluster is formed by branched chains of contacting mitochondria, which may be defined as *Streptio mitochondriale*. In the cardiomyocyte there are several mitochondrial clusters or, alternatively, the quenched cluster is a result of decomposition of a supercluster uniting all the mitochondria of the cell. Cluster organization of mitochondria could also be revealed when a single mitochondrion was punctured in situ with a microcapillary. The obtained data are in agreement with the idea that mitochondrial junctions are H^+ permeable so that, within the cluster, $\Delta\psi$ may be transmitted from one mitochondrion to another.

The above results are consistent with the assumption that mitochondrial filaments or networks represent a united electrical system. (Possible functions of extended mitochondrial systems are discussed.)

MEMBRANE structures containing energy-releasing and energy-consuming enzymes are defined as coupling membranes. If within one membrane there are a $\Delta\bar{\mu}H$-generating respiratory chain and an H^+–ATP synthase, respiration and phosphorylation can be coupled via $\Delta\bar{\mu}H$ (19). It is obvious that both $\Delta\psi$ and ΔpH constituents of $\Delta\bar{\mu}H$, once they are formed across it, immediately spread along the membrane. The electric conductance of the media on both sides of the coupling membrane is very high since these media are water solutions of electrolytes. At the same time, conductance of the membrane can be extremely low. This means that $\Delta\psi$, if produced by the given $\Delta\bar{\mu}H$ generator, cannot avoid fast irradiation over the membrane surface. As for ΔpH, it also must irradiate rather quickly due to the high rate of H^+ diffusion in the water and the high concentration of mobile pH buffers which cause a further increase in the rate of H^+ movement. This means that the $\Delta\bar{\mu}H$ produced by a $\Delta\bar{\mu}H$ generator 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. In 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 (24, 25); also see reference 27).

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 (7, 18, 26).

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 microplasmodesmata, the very thin and short tubules crossing the intercellular space (11). It was found that illumination of ∼5% of the trichome length with a small light beam energizes the entire trichome. $\Delta\psi$ transmission along the light beam–illuminated trichomes was detected with a classical electrophysiological technique.

In large eucaryotic cells, the role of power-transmitting structures might be performed by mitochondria assuming that some of them are sufficiently long.

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: (*a*) filamentous and (*b*) spherical or ellipsoid.

The development of the 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 picture of the cell. A clue considered this way may be erroneously interpreted as a number of small grains if its reconstitution with the aid of many parallel sections is not carried out.

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 eucaryotic 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.

Three approaches have shaken the spherical mitochondrial dogma, namely (*a*) reconstitution of three-dimensional electron microscope pictures of the whole cell with the aid of serial ultrathin sections, (*b*) high-voltage electron microscopy increasing the thickness of the studied preparation, and (*c*) the staining of mitochondria with fluorescent penetrating cations, the technique making it possible to return to the studies of living cell by means of a light microscope.

The serial section method has been applied first of all in studies on unicellular eucaryotes. Here very large and complicated mitochondrial structures were detected.

A single giant mitochondrion was described in some yeast cells and in unicellular algae. 10 μm that were thread-like were found in exocrine cells of a pancreas. A train of the long end-to-end–arranged mitochondria were described in spermatozoa, (reviewed in reference 25).

In our group, it was demonstrated (1, 2) that the mitochondrial material in diaphragm muscle is organized into net-

works transpiercing the I band regions of the muscle near the Z disks. The networks are connected with columns oriented perpendicular to their plane; i.e., parallel to myofibrils. Such a system was defined as mitochondrial reticulum (*Reticulum mitochondriale*).

Any electron microscopic methods deal with the fixed material and thus do not make it possible to follow directly the functioning of mitochondria. To overcome the latter limitation, we decided to return to light microscopy using fluorescent cations penetrating mitochondrial membranes. A concentration of such a cation inside may be 10^4 higher than outside mitochondria, provided that $\Delta\bar{\mu}H$ is in the form of $\Delta\psi$. If one succeeds in finding such a cation, even very thin mitochondrial filaments may be seen under a fluorescent microscope. In search of penetrating fluorescent cations, we turned our attention to rhodamines. These compounds are positively charged, rather hydrophobic, and have a high quantum yield of fluorescence. Moreover, rhodamine derivatives were known to specifically stain mitochondria in the living cell. This was shown as early as 1941 by H. Johannes who used light microscopy (12).

As it was found in our group by Dr. I. I. Severina in 1981 (20, 22), ethyl-substituted rhodamine added to solutions that washed the planar phospholipid membrane generates a diffusion potential predicted by the Nernst equation. This means that ethylrhodamine may be regarded as a penetrating cation. In other experiments carried out in our group, ethylrhodamine was employed for detecting a mitochondrial reticulum in the diaphragm muscle. Later we used the same cation to stain mitochondria in a cell culture (30). In 1979 Walsh et al. (31) 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 being mitochondrial. Further study revealed that the staining was caused by contaminations of some free rhodamine derivatives in this sample (8, 14). Systematic investigation of rhodamine 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. By discharging $\Delta\bar{\mu}H$ with uncouplers or $\Delta\psi$ with valinomycin and K^+ one could abolish mitochondrial fluorescence, while nigericin, converting ΔpH into $\Delta\psi$, increased it (8).

To verify the hypothesis of cable function of coupling membrane, we combined in the present study, ethylrhodamine probe and the laser microbeam method of Berns (6). The latter method allows selective damage of an intracellular target, still retaining intact other parts of a cell because of the very small size of the light spot ($\leqslant 0.5$ μm) (6). The result of electrophoretic accumulation of ethylrhodamine in energized mitochondria is that the concentration of the dye in these organelles becomes many times higher than in cytoplasm (19). Aqueous solutions of ethylrhodamine show a maximum absorption at 507 nm. At 488 nm (the wavelength of the argon laser operating by one-mode manner), the ethylrhodamine absorption is ∼50% of the maximal. Therefore the laser light is apparently trapped mostly by the ethylrhodamine molecules, an effect that must increase probability of specific damage of mitochondria.

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 parts becomes leaky.

In cardiomyocytes, mitochondria are of quite another structure. They are mostly grains rather than threads so that long-distance power transmission at first glance seems impossible. However, the amount of mitochondrial material in these cells is very large and the mitochondrion usually contacts with its closest neighbors (21). These contacts, as it was shown in our group, are specifically organized (intermitochondrial junctions; references 2–4). The junction zone was found to represent a disk with a diameter of 0.1–1.0 μm. In this zone, membranes and intermembrane spaces are of higher density. Two outer membranes of contacting mitochondria appear to be maximally approached to each other in a manner similar to that observed in tight junctions of the outer cell membrane. Each mitochondrion was shown to be connected with its neighbors by several such junctions.

Assuming that mitochondrial junctions are electrically conductive like gap junctions of the outer animal cell membrane or like microplasmadesmata of cyanobacteria, one

may suggest that power is transmitted along chains of the junction-connected mitochondria. To test such a possibility, we also locally damaged an ethylrhodamine-stained mitochondrion in cardiomyocyte with a narrow laser beam. In certain experiments, local mechanical damage of a single mitochondrion was also used.

The accompanying paper (23) summarizes the data of similar experiments on cyanobacterial trichomes. (For preliminary publications of fibroblast study, see references 5, 9.)

Materials and Methods

Human skin fibroblasts (fetal or postnatal) were grown on coverslips in MEM supplemented with 5% BSA and 5% FCS.

For primary culture of cardiomyocytes, 3–5-d-old rats were used. Hearts were placed in a glass petri dish containing medium 199 with 0.005% trypsin and 0.015% EDTA and were left overnight at 0–2°C. Thereafter dishes were incubated for 1 h at 37°C, trypsin was removed, hearts were rinsed with fresh medium, and heart cells were disaggregated by strong shaking. Cells were grown on coverslips in flasks containing medium 199 supplemented with 10% FBS. Cells were stained in culture medium by use of an aqueous ethylrhodamine (gift of Dr. V. Runov) stock solution (1 mg/ml)
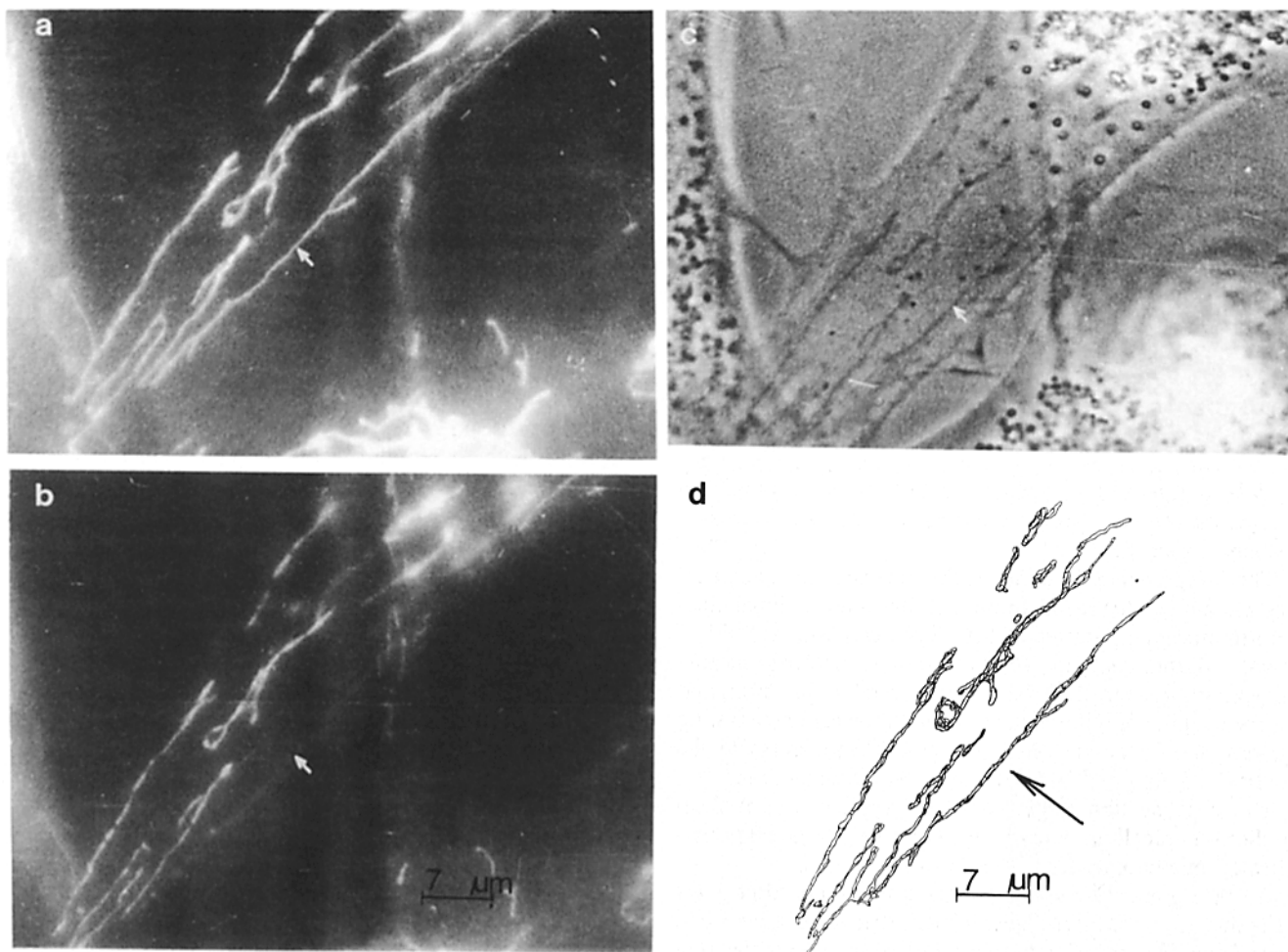

Figure 1. Discharge of the membrane potential in a 40-μm-long mitochondrial filament in a human fibroblast by illumination with a narrow (≤0.5 μm) laser beam. A cell of the primary culture of fibroblasts was stained with ethylrhodamine before (a) and after (b–d) laser treatment. (a and b) Fluorescent microscopy; (c) phase-contrast microscopy; (d) the top view on the model of mitochondria, reconstituted with the use of the serial section technique. Arrow shows the place of the laser illumination.
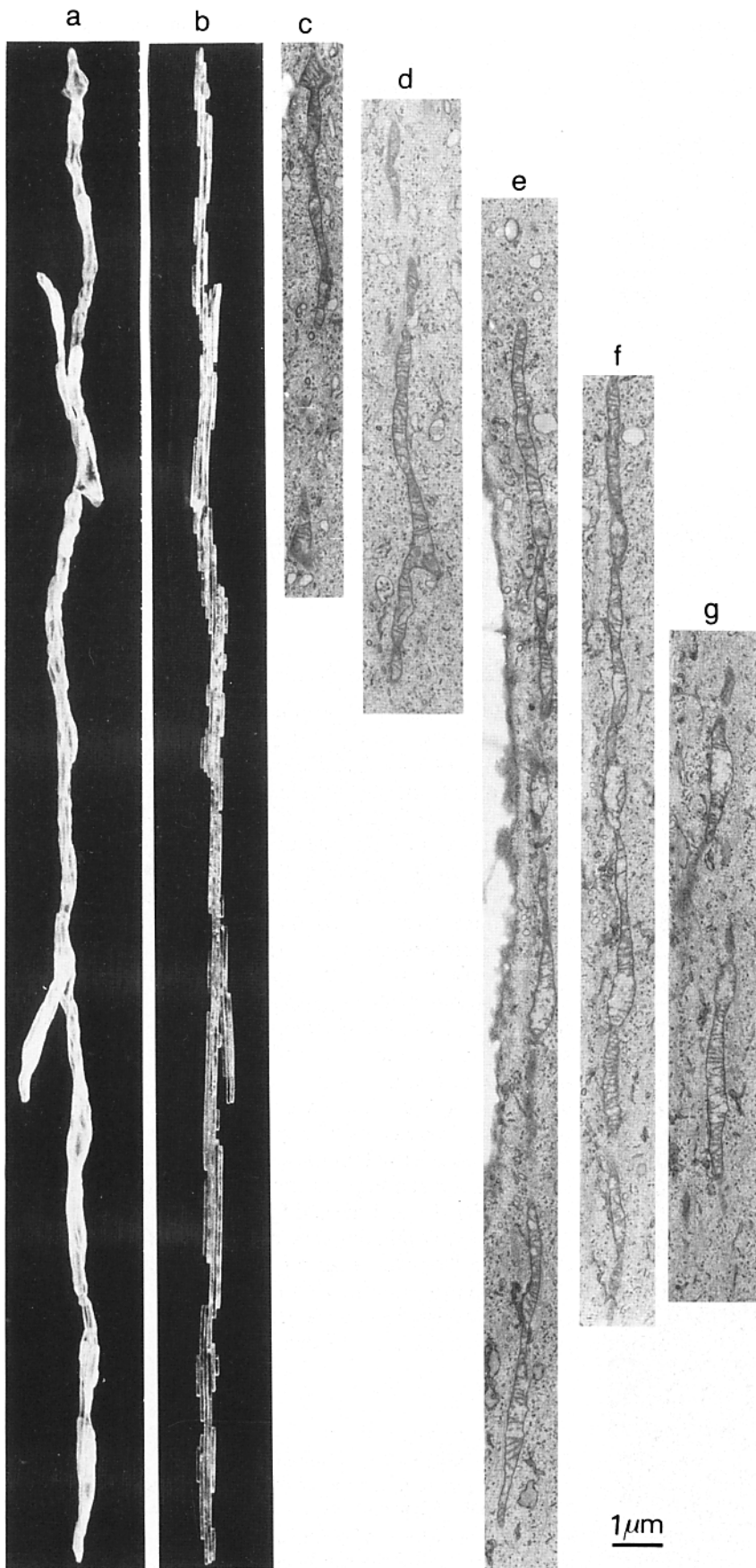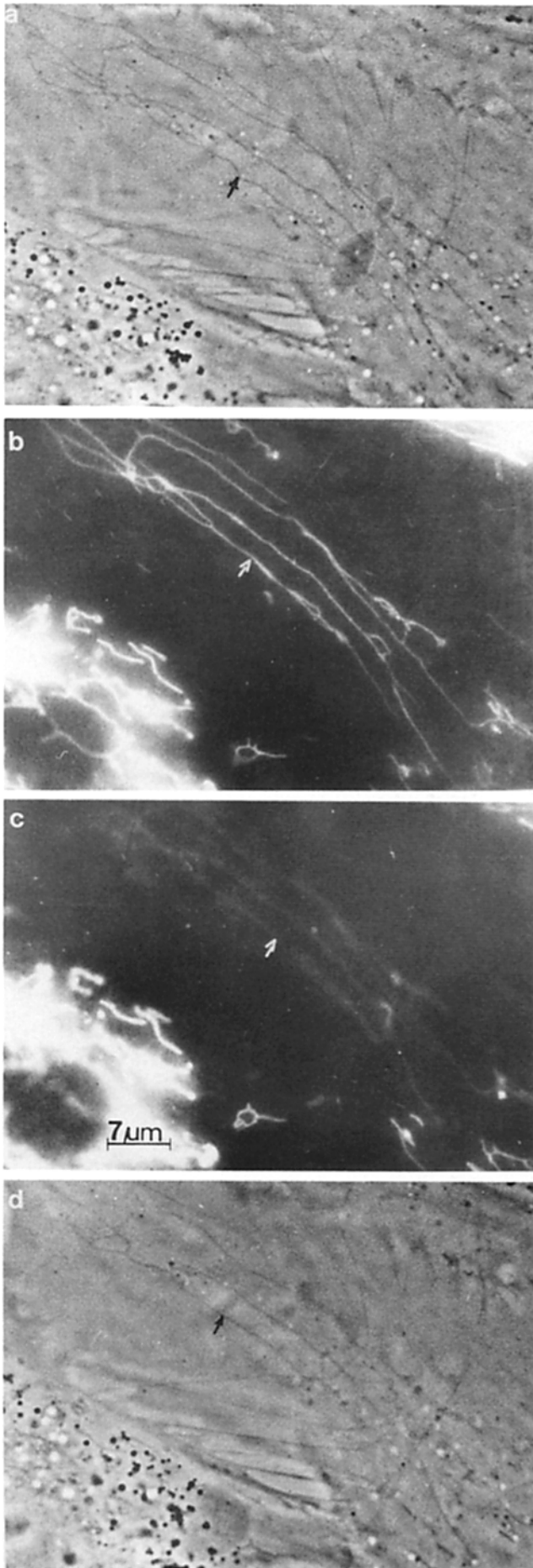
Figure 2. (*a* and *b*) Side and top views on a three-dimensional model of the laser-illuminated filament shown in Fig. 1. (*c–g*) Five electron microscopic sections of the same filament.

1 μm

added into the medium to make the final concentration 10 μg/ml. After staining for 10 min at 37°C, they were washed with the culture medium for 1-3 min and placed for visualizing in the same chamber filled with the same medium. The upper surface of used coverslips was calibrated for searching the cell treated. The microscope used was a Zeiss Universal fitted with a 50-W mercury lamp as the light source for fluorescence with broad band-filtered 420–490-nm light. Observations were made at λ = 500 nm. Photomicrographs were taken using RF-3 film (1,200-1,600 ASA) (TASMA, Kazan) processed to an ASA of 3,200 using D-76 developer. The exposure time of fluorescent images varied from 5 to 10 s.

The microirradiation technique (6) was applied to illuminate mitochondria in the cell. A continuous argon laser (at λ = 488; model ILA-120; Carl Zeiss, Jena, East Germany) with 120 mW of energy output was used. The duration of the light laser irradiation was 40-100 ms. The size of the focused laser spot we evaluated after Berns as $\leqslant 0.5$ μm. (For other details of the device used, see reference 9.)

After making photographs, the coverslip with the cell was fixed with 4% glutaraldehyde in 0.1 mM phosphate buffer (pH 7.4) with the following postfixation of 1% osmium tetroxide, dehydration in ethanol, staining by uranyl acetate, and embedding in Epon 812. In such Epon blocks, we were making a search of irradiated cells by comparing photographs made before fixing with the image in the microscope field. The studied cell was marked. On the base of the marked piece, the pyramid was cut out and then used for making serial thin sections by an ultramicrotome (model No. LKB-4800). Thin sections were examined at 75 kV in a Hitachi HU-12 electron microscope.

Results

Filamentous Mitochondria in Fibroblasts

Combining a laser and a fluorescent microscope, we succeeded in the treatment of a single mitochondrial filament in a human fibroblast cell stained with ethylrhodamine. A very narrow laser beam (the diameter of the light spot commensurable with the thickness of a mitochondrial filament) was used to cause local damage of the filament.

As seen in Fig. 1, a and b, the laser treatment resulted in the disappearance of the ethylrhodamine fluorescence in the entire 40-μm filament. It is essential that (a) other filaments remain fluorescent so that the laser effect is not the result of a nonspecific damage of the cell and (b) the illuminated filament retains its continuity when scrutinized under a phase-contrast (Fig. 1 c) and electron (Fig. 1 d and Fig. 2) microscope. One can see some differences between images of mitochondria before and after the laser shot (compare Fig. 1 a with Fig. 1 $b-d$). We render it to the known phenomena of saltatory movements of organelles in fibroblasts that we actually observed within these cells. The time interval between Fig. 1 a and Fig. 1 b and c is $\sim$30 s, and the interval between Fig. 1 b and the cell fixation corresponding to Fig. 1 d is $\sim$60 s. Within this time interval, there could be some changes in the mitochondria position in the cell.

The effect of laser-induced disappearance of the fluorescence in the single mitochondrial filament can not be explained by the change of focus in the microscope because in

Figure 3. The local laser illumination–induced discharge of the membrane potential in a network formed by mitochondrial filaments. (a and b) Before the laser treatment; (c and d) after the treatment. (a and d) Phase contrast microscopy; (b and c) fluorescent microscopy. Short mitochondria in the right lower corner belong to the same cell whereas rather thick filaments in the left lower corner are localized in the other cell. Arrows show the place of the laser illumination.

Figure 4. Mitochondria in the cardiomyocyte cell culture. The majority of the mitochondrial profiles represents large mitochondria. However, some mitochondrial filaments are also seen (*white arrows*). Mitochondrial junctions are indicated by black arrows.

well-spread cells (human fibroblast is within this number) mitochondria dispose closely at one plane.

In fact, no detectable traces of laser-induced damage were found. Irradiated as well as nonirradiated mitochondria were in orthodox conformational state. They looked like long hollow double-membranous tubules with some cristae (Fig. 2).

After uncoupling of oxidative phosphorylation by the addition of protonophore to fibroblast cells, mitochondria show a transition from an orthodox to a condensed state (data not shown). Since this did not occur in the laser-treated mitochondrion of fibroblast (Fig. 2), two possible assumptions may be made. (*a*) The laser-induced damage increases the

permeability of the fibroblast mitochondrion to ion(s) other than H^+ so that $\Delta\psi$ is converted to ΔpH without any dramatic $\Delta\bar{\mu}H$ decrease. This means that after the laser treatment, the mitochondrion is still energized. (*b*) The damaged mitochondrion recovered before the cell was fixed with glutaraldehyde. Such a recovery was shown in experiments with cardiomyocytes (see below; see Fig. 10).

As one can see in Fig. 2, *c–g*, 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 discharge 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. An example of such an effect is given in Fig. 3. Note that the small group of short mitochondria in the right lower corner of the photograph, which are localized in the same cell but are out of the network, retain fluorescence after the laser irradiation. In the left corner of Fig. 3, mitochondria of another fibroblast cell are seen. It is clear that laser treatment of the adjacent cell is without any effect upon these mitochondria.

Mitochondrial Clusters in Cardiomyocytes

In the culture of myocardial cells of 3–5-d-old rats, the main portion of the mitochondrial material represents large (several μm long) spherical or ellipsoid mitochondria. Moreover there are rather long thin filaments that often are in contact with large mitochondrial bodies (Fig. 4). Mitochondrial junctions of the same structure as in the intact heart can be seen (Figs. 4 and 5).

The laser irradiation of a large roundish mitochondrion was shown to give rise to a fast (within 1–2 s) quenching of not only the irradiated organelle but also a mitochondrial cluster composed of many mitochondria. In the experiment shown in Fig. 6, *a–c*, this cluster includes mitochondrion N1 (which was illuminated by laser), its neighbours (mitochondria N2 and 3), as well as mitochondria N7–N10, N14–N19, N23, and N24. Note that some mitochondria, being very close to the illuminated one (e.g., N4), are fluorescing whereas other mitochondria, localized at a distance as long as 18 μm from it (N14, N15), are nevertheless completely quenched. Electron microscopic analysis revealed that mitochondria, which become quenched after the laser treatment, are connected with the illuminated mitochondrion by intermitochondrial junctions, whereas those retaining fluorescence are not (Fig. 6, *d* and *e*).

As one can see from Fig. 6 *d*, the quenched mitochondria can be easily recognized in an electron micrograph; they look much darker being of higher electron density than those that were fluorescing when studied with a fluorescent microscope. Control experiments showed that the addition of protonophorous uncouplers (1 mM 2,4-p-dinitrophenol or 2 × 10^{-5} M trifluoromethoxycarbonyl cyanide phenylhydrazone)
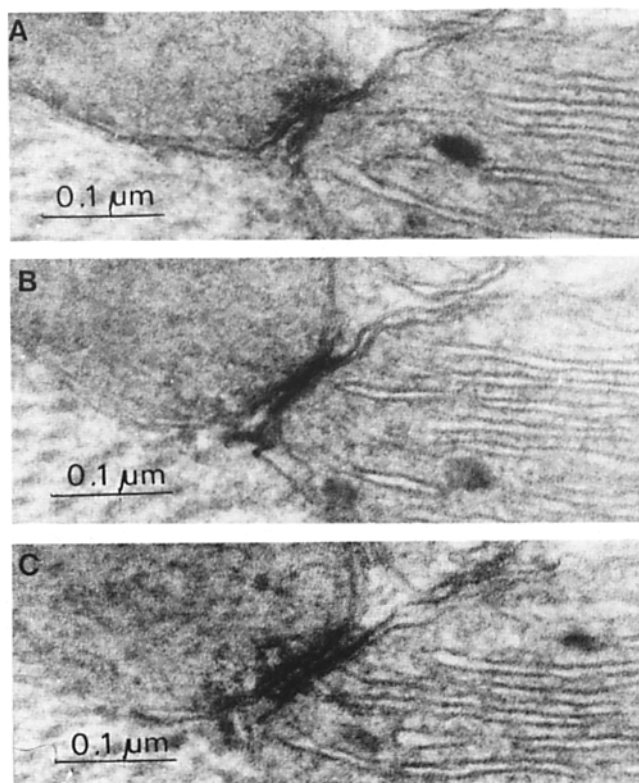

Figure 5. A mitochondrial junction in a cell of cardiomyocyte culture. Series of three sections.

transforms the entire mitochondrial population in the cell from orthodox to this dark state characterized by a very condensed matrix space (not shown in Figs.).

Fig. 7 demonstrates that there are thin filamentous mitochondria between two groups of large, quenched mitochondria due to illumination of one of them. Electron microscopy showed that (*a*) all the quenched mitochondria in the cluster, spherical and filamentous, are in the condensed state, and (*b*) there are junctions between mitochondrial clusters and filaments (not shown in the Fig.).

The simplest explanation of these data is the following. In a cardiomyocyte, there are several "octopus-like" mitochondrial clusters formed by many mitochondria joined by mitochondrial junctions. These junctions are of high electric conductance so that the cluster is de-energized when at least one of the cluster-composing mitochondria becomes leaky.

Sometimes, it was seen that two mitochondrial clusters were localized inside the cell at two different levels. In Fig. 8, a cardiomyocyte is shown in which there is a rather large portion of the mitochondrial material in filamentous form. Illumination of a thick filament resulted in the quenching of many filaments in various parts of the cell. Some of these filaments were at the distance of 35 μm from the illuminated one. At the same time, other filaments retaining their fluorescence seemed to be arranged very close to the locus of illumination. Careful analysis showed that, at least in some places, the quenched cluster was localized above the intact one (see position of the branch of an intact mitochondrial filament, indicated by the *thin arrow* in the *inset* of Fig. 8). The size of the quenched cluster was ∼50 μm.
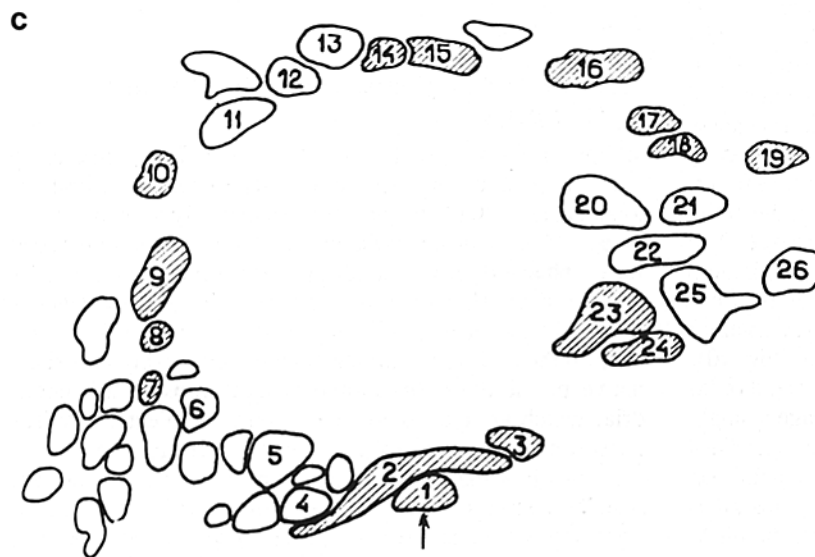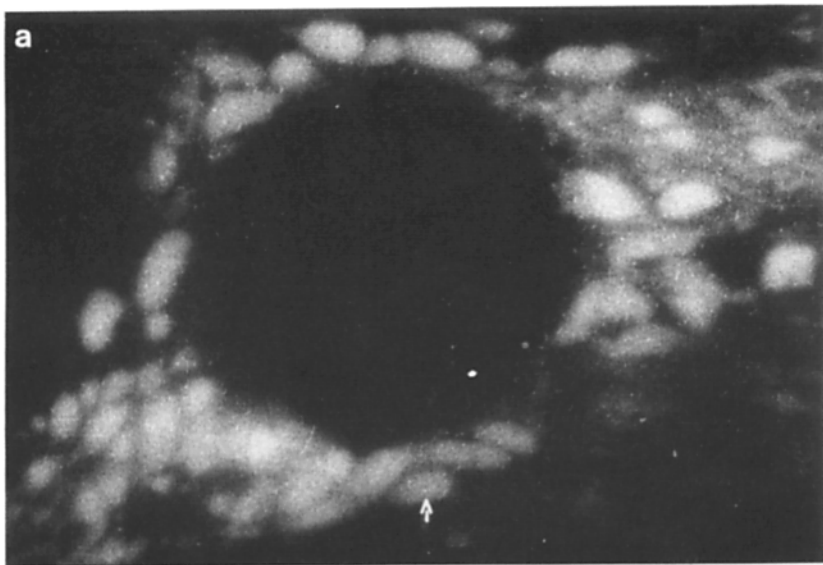
Figure 6. De-energizing of a cluster of mitochondria in a ethylrhodamine-stained cardiomyocyte, induced by the laser illumination of a single mitochondrion. (*a*) Before the laser treatment; (*b, d* and *e*) after the treatment. (*a* and *b*) Fluorescent microscopy; (*c*) a scheme indicating which mitochondria are quenched after laser illumination of mitochondrion N1; (*d* and *e*) electron microscopy. Arrows in *a–d* and arrows in *e* show the illuminated mitochondrion and mitochondrial junctions, respectively.
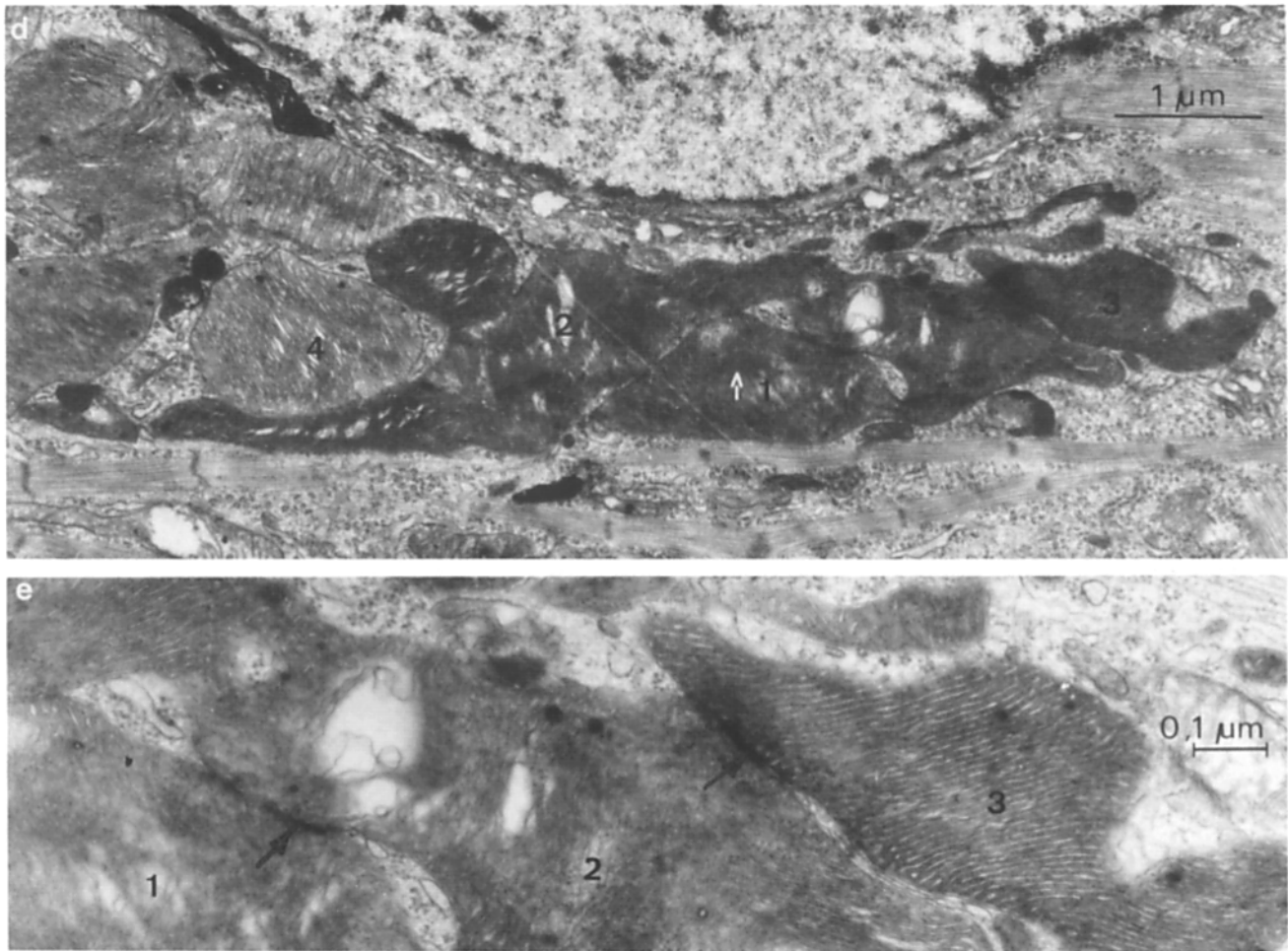
In certain cases, we succeeded in observation of the reversal of the fluorescence quenching. In Fig. 9, *a* and *b*, illumination of a large mitochondrion in the left part of the cell (*arrow 1*) gave rise to the quenching of fluorescence in these mitochondria as well as in several small mitochondria disposed in the direction to the nucleus (large black area). Moreover one more large mitochondrion was quenched in the right part of the cell (*arrow 2*). The photograph shown in Fig. 9 *c* was made 3 min after the laser illumination. It is seen that both mitochondria 1 and 2 fluoresced again although not so strong as before the treatment. On the other hand, fluorescence of adjacent mitochondria decreased. The latter effect may be due to redistribution of ethylrhodamine between intact mitochondria and damaged and then repaired mitochondria. Reparation was accompanied by the transition of mitochondria from condensed to orthodox state (Fig. 10).

In the last series of experiments, we applied, instead of laser illumination, quite another technique to damage a single mitochondrion in cardiomyocyte. A glass microcapillary (∼0.2 μm in diameter) was stuck into a large mitochondrion inside the cell stained with ethylrhodamine. Such a mechanical damage of the mitochondrial membrane was found to cause the fluorescence loss in this and some other mitochon-

dria, the rest of the mitochondrial population remaining fluorescent (Fig. 11).

Discussion

Cable Properties of Mitochondria in Fibroblast and Cardiomyocyte

Results of the study on fibroblasts were, in fact, predicted by the concept assuming that filamentous mitochondria and mitochondrial reticulum represent electrically united (cable) systems. This concept explains why the laser beam–induced local damage of a very small part of the mitochondrial filament or network gives rise to the quenching of ethylrhodamine fluorescence in the entire filament (network).

As to the culture of cardiomyocytes, there were two alternative possibilities. (*a*) Roundish and ellipsoid mitochondria, which are typical for these cells, are electrically isolated from each other so that long-distance transmission of the mitochondrial electric potential does not occur. (*b*) Specific contacts formed by these mitochondria (intermitochondrial junctions) are of high electrical conductance and, hence, all the junction-connected mitochondria can be elec-
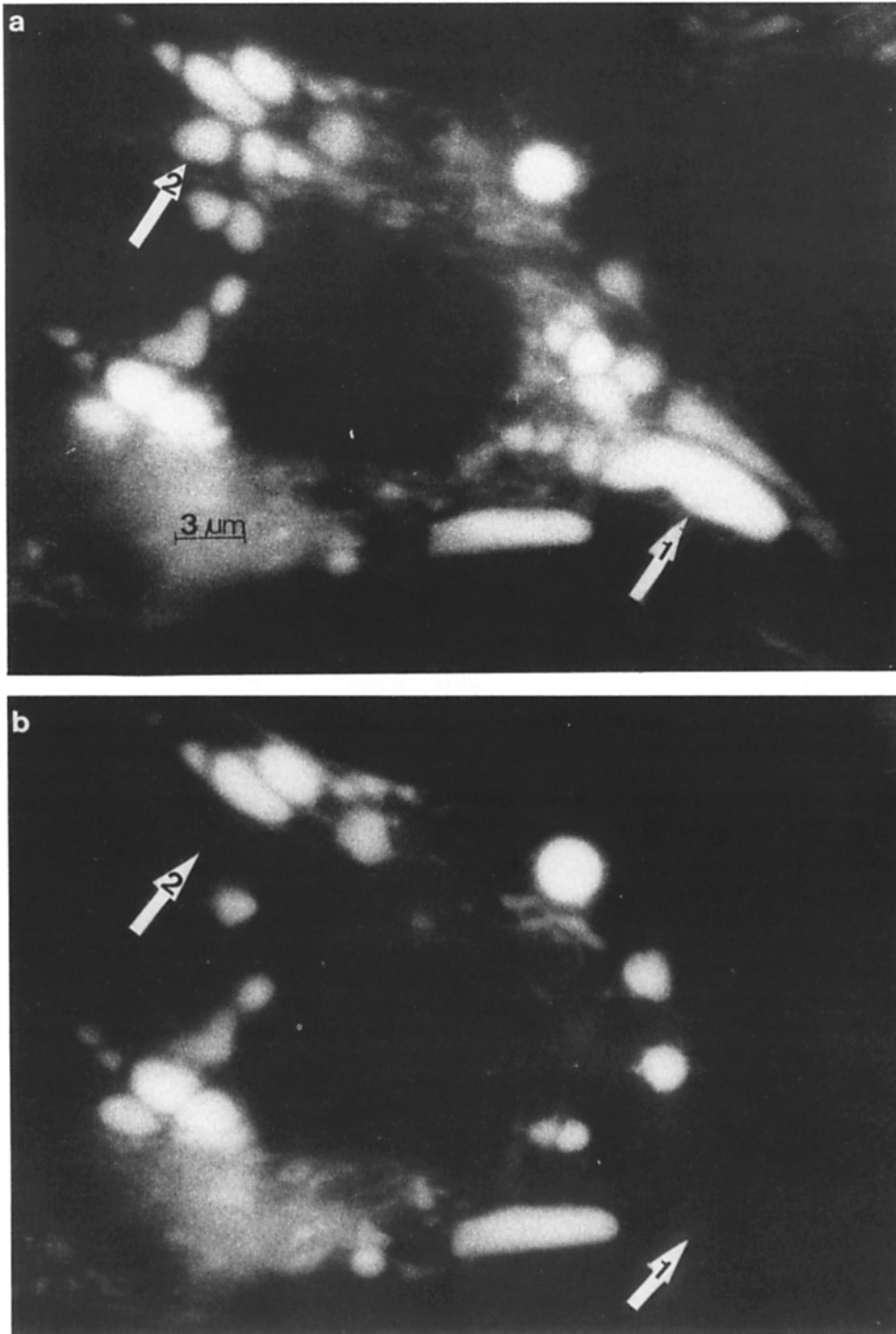
Figure 7. Two groups of large ellipsoid mitochondria in a cluster are connected with thin mitochondrial filaments. (*a*) Before the laser treatment; (*b* and *c*) after the treatment. (*a* and *b*) Fluorescent microscopy; (*c*) phase-contrast microscopy; (*d*) a drawing showing profiles of the cluster-forming, junction-connected mitochondria (according to the electron microscopic three-dimensional reconstruction). Arrows 1 and 2 indicate the illuminated mitochondrion and the distal group of quenched mitochondria, respectively.

trically united. It was the latter possibility that was confirmed in the experiments. The laser- or microcapillary-induced damages of a single mitochondrion were found to result in the de-energizing of many mitochondria attached to the damaged one with junctions. Thus, it may be concluded that the cable effect is inherent not only in long filamentous mitochondria but also in chains of junction-connected roundish mitochondria. In fact, this study revealed existence of

functionally linked mitochondrial clusters in the cardiomyocyte cell.

Clusters Formed by Chains of Mitochondria (Streptio mitochondriale)

A mitochondrial cluster described in this study represents branched chains of junction-connected mitochondria. Such

490

c

3 μm

d

3 μm
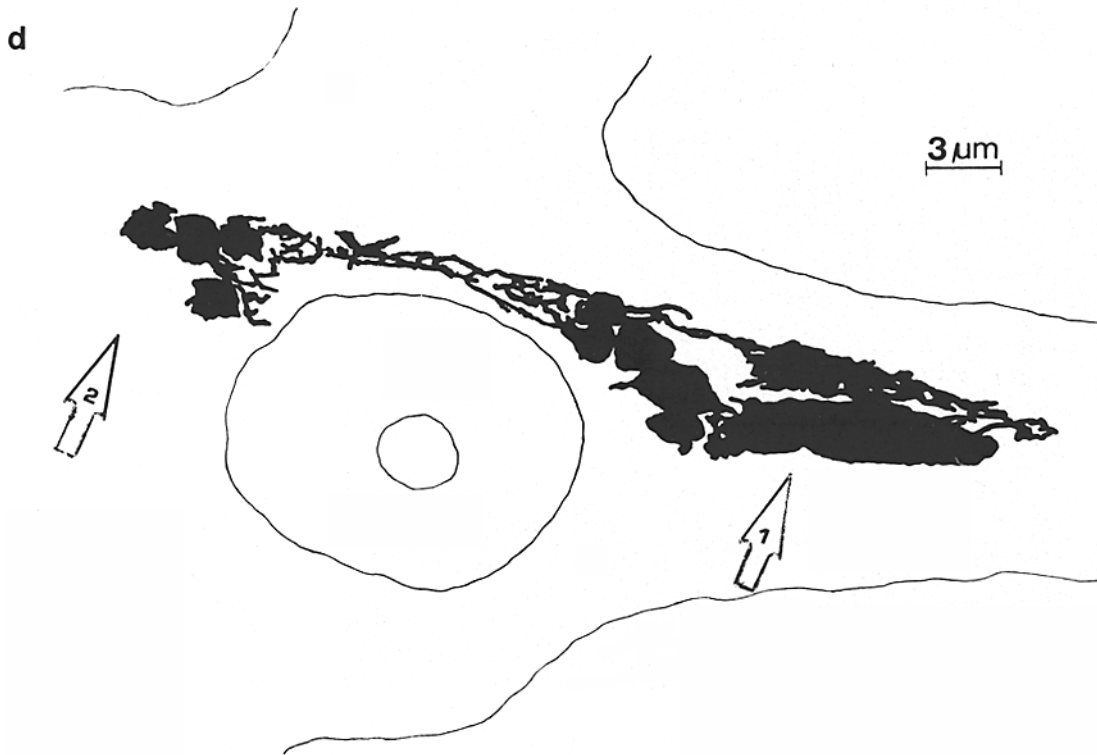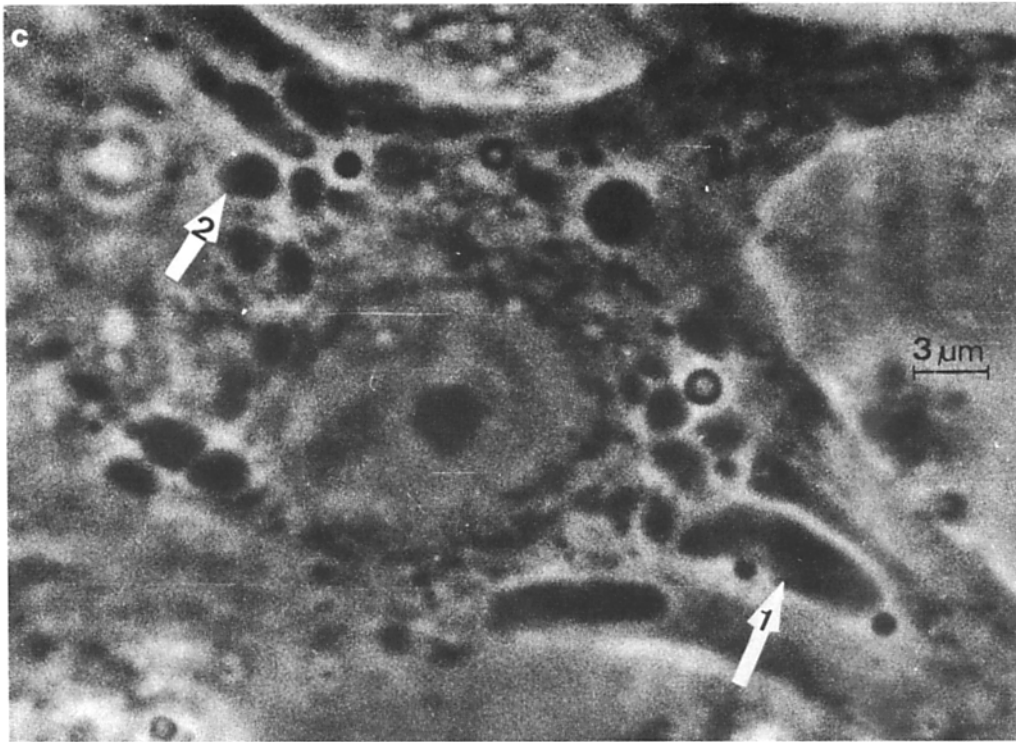
a type of mitochondrial organization may be defined as *Streptio mitochondriale*. It can be found not only in a culture of cardiomyocytes (Figs. 1 and 2) but also in intact heart (personal data) as well as in diaphragm and skeletal muscles (17).

Extension of the mitochondrial cluster in cardiomyocyte may be estimated using ethylrhodamine staining and local laser damage techniques. It was found that the laser-induced quenching of the ethylrhodamine fluorescence in the entire cluster is accompanied by a characteristic change in the electron microscope picture. The affected cluster can be easily
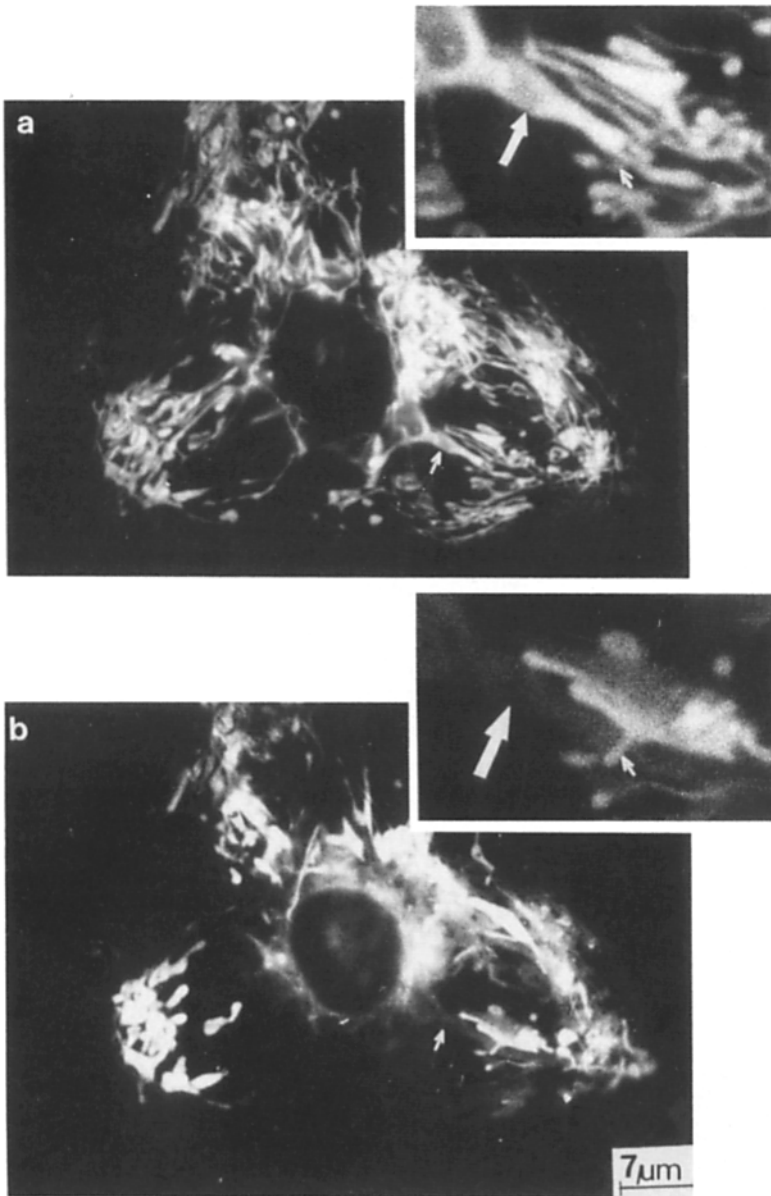
Figure 8. Mitochondrial clusters localized at different levels inside a cardiomyocyte before (*a*) and after (*b*) the laser illumination. Fluorescent microscopy is used. In photographs at lower magnification, arrows indicate site of the laser illumination. In inset, laser illumination is shown by large arrows whereas the small ones indicate a branch of the lower mitochondrial cluster that remained fluorescing when the upper one was quenched.
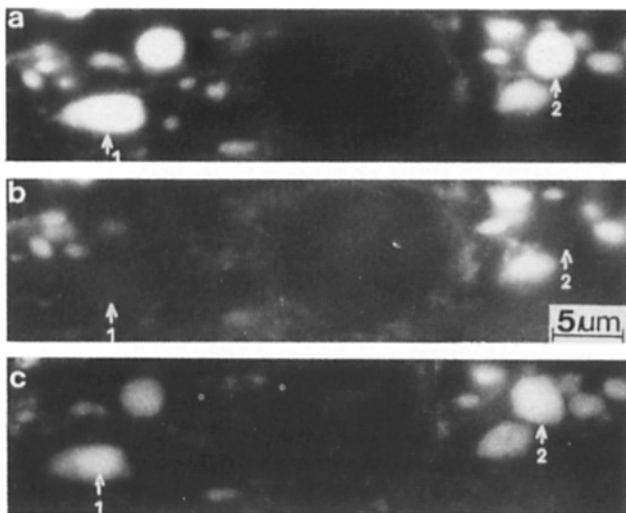

Figure 9. Reversibility of the laser-induced quenching of fluorescence of a mitochondrial cluster. (*a*) Before the laser treatment; (*b*) immediately after the treatment; (*c*) 3 min after the treatment. Arrows 1 and 2 indicate, respectively, illuminated mitochondrion and a distal mitochondrion responding synchronously with the illuminated one. Fluorescent microscopy is used.
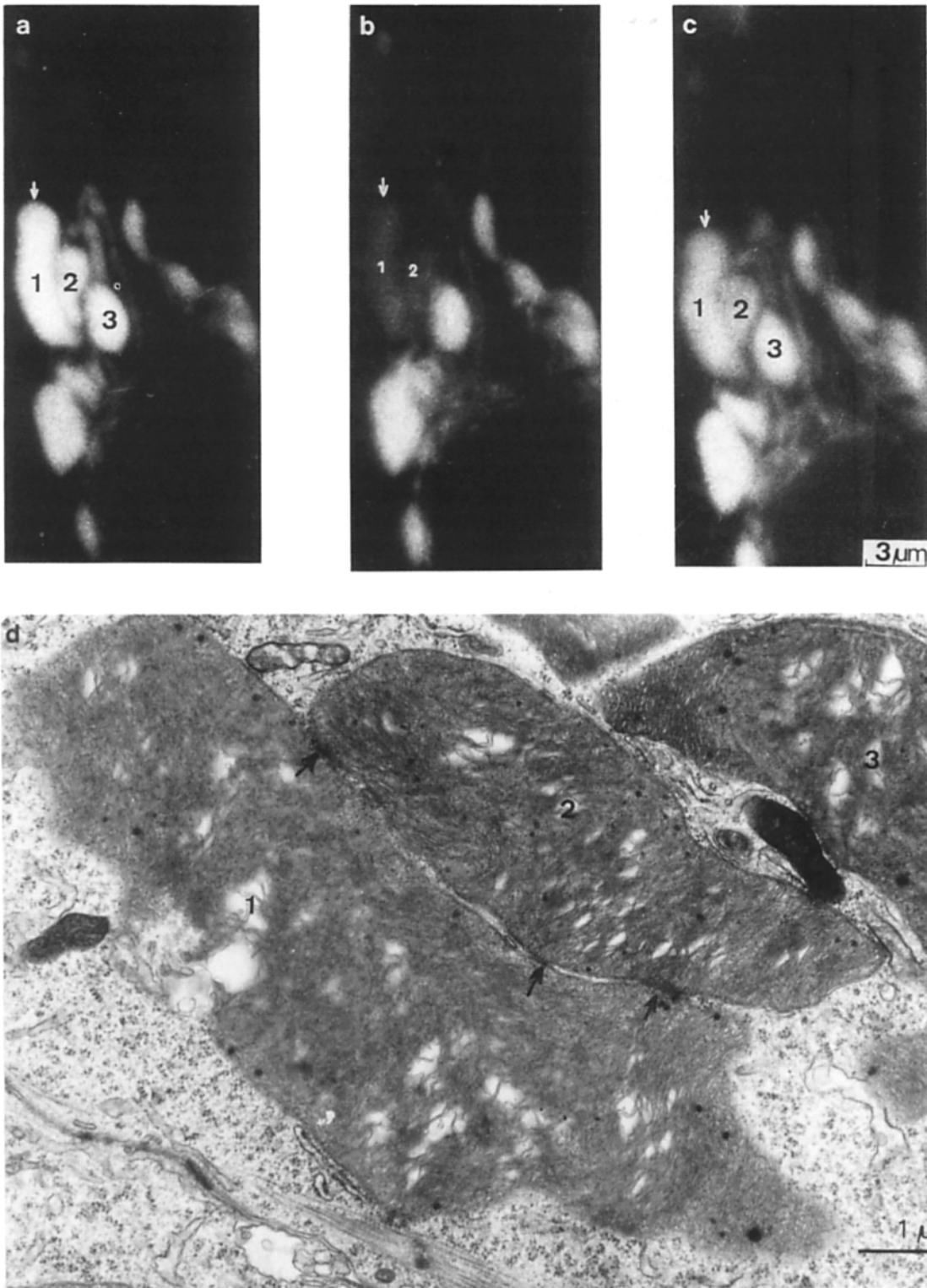
Figure 10. Re-energizing of a laser-treated mitochondrion is accompanied by condensed-to-orthodox transition. (*a*) Before the laser treatment; (*b*) immediately after the treatment. (*c* and *d*) 3 min after the treatment. (*a–c*) Fluorescent microscopy; (*d*) electron microscopy. *White arrow*, irradiated mitochondrion. *Black arrows*, intermitochondrial junctions.

recognized since, after the laser treatment of a single mitochondrion, all the cluster-composing organelles change from an orthodox to a condensed state. Apparently both effects, the fluorescence decrease and morphological change, are due to a laser-induced de-energizing that results in the downhill ethylrhodamine efflux and in shrinkage of mitochondria, respectively. Under the same conditions, addition of protonophorous uncouplers was shown to induce appear-
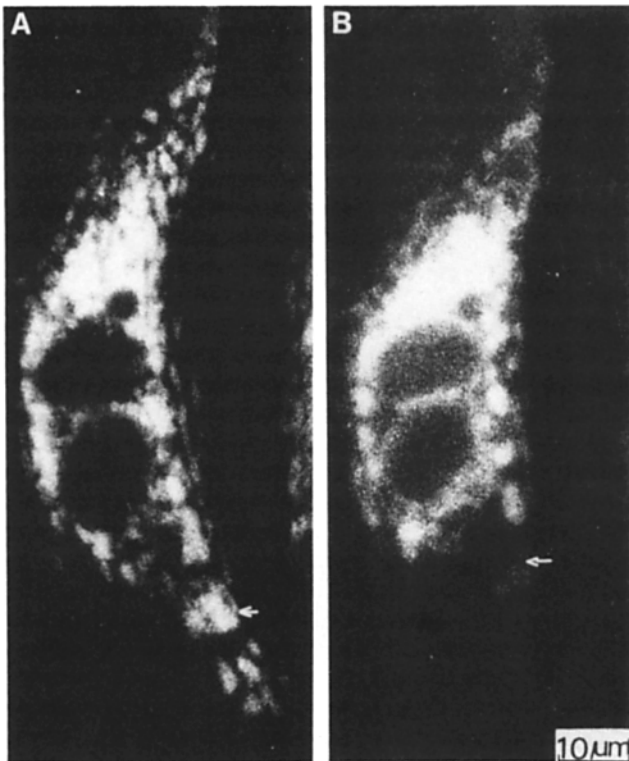
Figure 11. Quenching of a mitochondrial cluster caused by damage of a mitochondrion with a microcapillary (*arrow*). Fluorescent microscopy used. Before (*A*) and after (*B*) damage.

ance of the condensed state. In this case, however, the entire mitochondrial population proved to be involved so that clusterization could not be revealed.

What is the mechanism of the laser-induced de-energizing of mitochondrial cluster? It is hardly a mechanical effect (percussion action) of the laser flash. In some experiments we slowly increased the laser energy input from zero to the level that resulted in the fluorescence loss. Also in this case, cluster quenching of mitochondria was observed. 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 to damage a mitochondrion inside the cell. A large ellipsoid mitochondrion in a cardiomyocyte was punctured with a glass microcapillary. This resulted in an effect similar to that in laser experiments; i.e., fast (within 1–2 s) quenching of a mitochondrial cluster while other mitochondria continue to fluoresce (see Fig. 11).

Only these properties must be inherent in mitochondrial structures if they were involved in the long-distance power transmission inside the cell (27). Since the energy is accumulated by the mitochondria as a transmembrane difference in H^+ potentials, one may conclude that it is the hydrogen ion that is transported along extended mitochondrial systems. This implies, in particular, that junctions between mitochondria-forming clusters in cardiomyocytes are H^+ permeable, resembling intercellular junctions (microplasmadesmata) of cyanobacteria.

As shown in the accompanying paper (23), power trans-

mission via cyanobacterial junctions can be interrupted when one of the trichome-composing cells is damaged by the laser illumination. It is not excluded that such an effect takes place in mitochondrial junctions in cardiomyocytes. If it were the case, it would be reasonable to consider mitochondrial clusters as a result of partial decomposition of a united system of mitochondria in cardiomyocyte culture cells. 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" in the cardiomyocyte. 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 preexisting clusterization. At present, it is difficult to discriminate between these two possibilities.

Possible Functions of Extended Mitochondrial Systems

The present study was undertaken to verify the hypothesis about a possible role of extended mitochondrial structures as intracellular power-transmitting protonic cables. The data obtained can be accounted for by the H^+ transport along extended mitochondrial profiles.

At least for the mitochondrial filaments, it seems obvious that the above consideration may also be applied to components other than the hydrogen ion. In fact, the filament might be the route for any substance that is concentrated in a mitochondrial compartment. 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 a 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.

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. Among them free fatty acids and their carnitine and CoA esters should be mentioned (for example see reference 29).

The lateral transport of molecular oxygen poses one more intriguing problem. There are several effects favorable for the use of the inner mitochondrial membrane as a route for the intracellular oxygen transport (1, 2, 10, 13, 15, 16, 24, 27, 28, 32).

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