

# Coupling Membranes As Energy-transmitting Cables.

## II. Cyanobacterial Trichomes

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**Abstract.** Power transmission along trichomes of filamentous cyanobacteria *Phormidium uncinatum* has been studied with the use of ethylrhodamine fluorescence as a probe for the transmembrane electric potential difference ( $\Delta\Psi$ ). It is found that agents preventing the light-induced  $\Delta\Psi$  formation (photosynthetic redox chain inhibitor dibromothymoquinone) or dissipating  $\Delta\Psi$  (uncoupler tetrachlorotrifluoromethylbenzimidazole) strongly decrease the fluorescence of the ethylrhodamine-stained trichomes.  $K^+-H^+$  antiporter nigericin converting  $\Delta pH$  to  $\Delta\Psi$  increases the fluorescence. These relationships are in agreement with the assumption that ethylrhodamine electrophoretically accumulates inside the cyanobacterial cells.

Illumination of a single cell in the *P. uncinatum* trichome gives rise to quenching of the fluorescence in this cell and usually in one or two neighbor cells, whereas the rest of trichome remains fluorescing. A

small light spot (5% of the trichome length) causes an increase in the ethylrhodamine fluorescence not only in the illuminated but also in the nonilluminated parts of the trichome up to the laser-treated cell or its neighbor(s). It is concluded ethylrhodamine can be used to monitor the power transmission which was previously demonstrated by microelectrode studies of the cyanobacterial trichomes.

In certain trichomes, several "dark" cells appear during the storage of the trichomes without energy sources. Illumination for several minutes results in dark cells becoming fluorescing. Thus some cells or cell clusters can be reversibly excluded from the lateral  $\Delta\Psi$ -transmitting system of the trichome, the rest being still electrically connected. This means that filamentous cyanobacteria possess mechanisms to transmit power along the trichome and to switch off this transmission.

**I**N the accompanying paper (1), an hypothesis on the power transmission along coupling membranes has been reported in a study on eucaryotic cells. It was found that local laser-induced damage in an extended mitochondrial system, such as a mitochondrial filament, a mitochondrial network (*Reticulum mitochondriale*), or chain of contacting mitochondria (*Streptio mitochondriale*), gives rise to irradiation of the  $\Delta\Psi$  dissipation over the entire system which may be as long as 50  $\mu m$ . Discharge of  $\Delta\Psi$  was monitored fluorimetrically using fluorescent penetrating cation ethylrhodamine which is electrophoretically accumulated by mitochondria. In this study we applied the same approach to a multicellular procaryote, filamentous cyanobacteria *Phormidium uncinatum*.

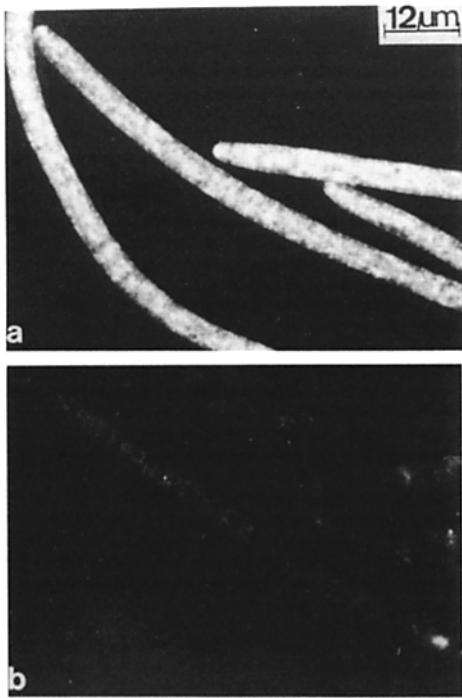
*P. uncinatum* filament (trichome) is a sequence of many (up to several hundred) cells that can be connected with microplasmadesmata; i.e., very thin and short tubules crossing intercellular gaps (4). The inner (cytoplasmic) membrane of the cyanobacterial cell falls to the category of coupling membranes containing  $\Delta\bar{\mu}H$  generators and consumers. It was *P. uncinatum* that proved to be the first system where long-distance  $\Delta\bar{\mu}H$  transmission was directly shown. For

this purpose, we used a focused light beam to energize a small part of the trichome and extracellular microelectrodes to monitor  $\Delta\Psi$  transmission from one trichome end to the other. In this way, cable properties of the trichome were demonstrated (3, 6, 8). Unfortunately, microelectrodes cannot be effectively applied for extended mitochondrial systems. This is why it seemed desirable to compare ethylrhodamine responses in mitochondria and cyanobacteria.

In this paper it will be reported that (a) ethylrhodamine and local laser-induced damage techniques can be used to demonstrate long-distance power transmission along the cyanobacterial trichome and (b) such a transmission can be specifically switched off to isolate leaky cells from the rest of the trichome. (For preliminary communication, see reference 7.)

### Materials and Methods

The filamentous cyanobacteria *P. uncinatum* were grown according to Glagolev et al. (5). One day before the experiment, trichomes of cyanobacteria were transferred from the medium to a 22-mm<sup>2</sup> coverslip moistened with 0.8 ml medium D (see reference 5). After this, the slips were stored



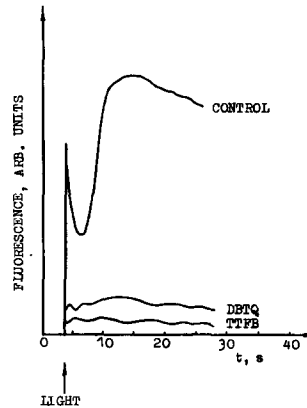
**Figure 1.** *Phormidium uncinatum* trichomes stained with ethylrhodamine without (a) and with (b) protonophorous uncoupler TTFB. *P. uncinatum* trichomes were incubated in medium D containing 2.5  $\mu\text{M}$  ethylrhodamine with or without  $1 \times 10^{-4}$  M TTFB. Aerobic conditions exist.

in a petri dish until microscopic examination. Aqueous stock solution of ethylrhodamine was diluted with medium D to 2.5  $\mu\text{M}$  concentration. Trichomes were incubated in the ethylrhodamine-supplemented medium D for 1 h. Then fluorescence of trichomes was observed under a fluorescent Univar microscope using a 100-W tungsten lamp as a light source and a blue Univar filter N49 so that fluorescence was excited with 400–490 nm light and observed at 500–550 nm. To monitor kinetics in a single cell, a microfluorimeter technique was used. The diameter of the area illuminated by the light was 0.5  $\mu\text{m}$ . The fluorescence was measured with a Univar microscope equipped with an FMEL-1A system. The laser illumination that was used to cause selective damage to a single cell in a trichome was carried out exactly as in the study of mitochondria described in the accompanying paper (1).

## Results

The goal of the first series of the experiments was to show that ethylrhodamine can be used as a probe for the light-supported  $\Delta\Psi$  generation in *P. uncinatum*. It was shown that agents inhibiting the *P. uncinatum* photoredox chain (hence the  $\Delta\Psi$  formation; dibromothymoquinone, DBTQ) or discharging  $\Delta\Psi$  (protonophorous uncoupler tetrachlorotrifluoro-methylbenzimidazole, TTFB) strongly suppress the light-induced increase in the ethylrhodamine fluorescence in *P. uncinatum* trichomes. These effects can be seen in Figs. 1 and 2. On the other hand,  $\text{K}^+-\text{H}^+$  antiporter nigericin converting  $\Delta\text{pH}$  to  $\Delta\Psi$  was found to increase the fluorescence of trichomes (not shown in the figure.).

Fig. 2 demonstrates kinetics of the ethylrhodamine fluorescence changes in a single cell of the *P. uncinatum* trichomes after switching on the light. In the absence of in-



**Figure 2.** Kinetics of the light-induced ethylrhodamine fluorescence of a single cell in the *P. uncinatum* trichome. Anaerobic conditions exist. Incubated mixture, medium D supplemented with 20 mM glucose and glucose oxidase (0.01 mg/ml), catalase 0.2 mg/ml. Additions,  $2 \times 10^{-5}$  M TTFB and  $1 \times 10^{-5}$  M DBTQ. Fluorescence is in arbitrary units; time is in seconds.

hibitors, a three-phase response could be revealed. First, very rapid fluorescence increase occurred. Its kinetics proved faster than time resolution of the monitoring system. Then partial decrease in the fluorescence was observed which was completed within 2 s. Finally the third phase, i.e., the fluorescence increase, developed. It took  $\sim 5$  s. A special study showed that the transient fluorescence decrease requires extracellular  $\text{Ca}^{2+}$ . It is probably a result of the phototaxis signal and may be accounted for by  $\text{Ca}^{2+}$  influx. As shown in Fig. 2, DBTQ and TTFB very strongly inhibit the light-induced fluorescent response.

In Figs. 3 and 4, effect of the laser damage of a trichome-composing cell is demonstrated. A cell was illuminated with very narrow ( $\sim 0.5$   $\mu\text{m}$  in diameter) laser beam, using the same technique as in the accompanying paper (1). As one may see in Fig. 3, a and b, the laser treatment gave rise to a strong fluorescence quenching in the treated cell and in one of its neighbors, whereas other cells showed the same or slightly lower fluorescence. In other experiments, the number of quenched cells usually varies from one to five. Recovery of fluorescence in the quenched cells occurred in a few minutes (Fig. 3 c). Phase-contrast microscopy did not reveal any visible changes in the trichome structure (Fig. 3 d and e).

In Fig. 3, total illumination of the trichome by the actinic light was used both to energize photosynthetic  $\Delta\bar{\mu}\text{H}$  generators in cyanobacterial membranes and to excite the ethylrhodamine fluorescence, whereas the membrane damage was caused by the laser light. Fig. 4 shows results of another experiment, also with laser treatment, in which total illumination (a and c) or, alternatively a small beam of the actinic light illuminating only  $\sim 5\%$  of the trichome length (b) were employed to energize the trichome. Due to cable properties of the trichome, partial illumination should cause transmission of the produced  $\Delta\Psi$  along the trichome (3, 6, 8). It is obvious from Fig. 4 b, that the laser treatment of a cell in the middle part of trichome interrupts  $\Delta\Psi$  transmission along the trichome since the light spot can induce ethylrhodamine response in the trichome part before, not after, the laser-damaged cell. In the experiment of Fig. 4 b, trichome was illuminated with a light spot for 20 s, then the total illumination was switched on and a photograph was immediately taken. Under such conditions energizing of the light spot-illuminated part of the trichome (up to the damaged cell) already reached its maximal level (see above;

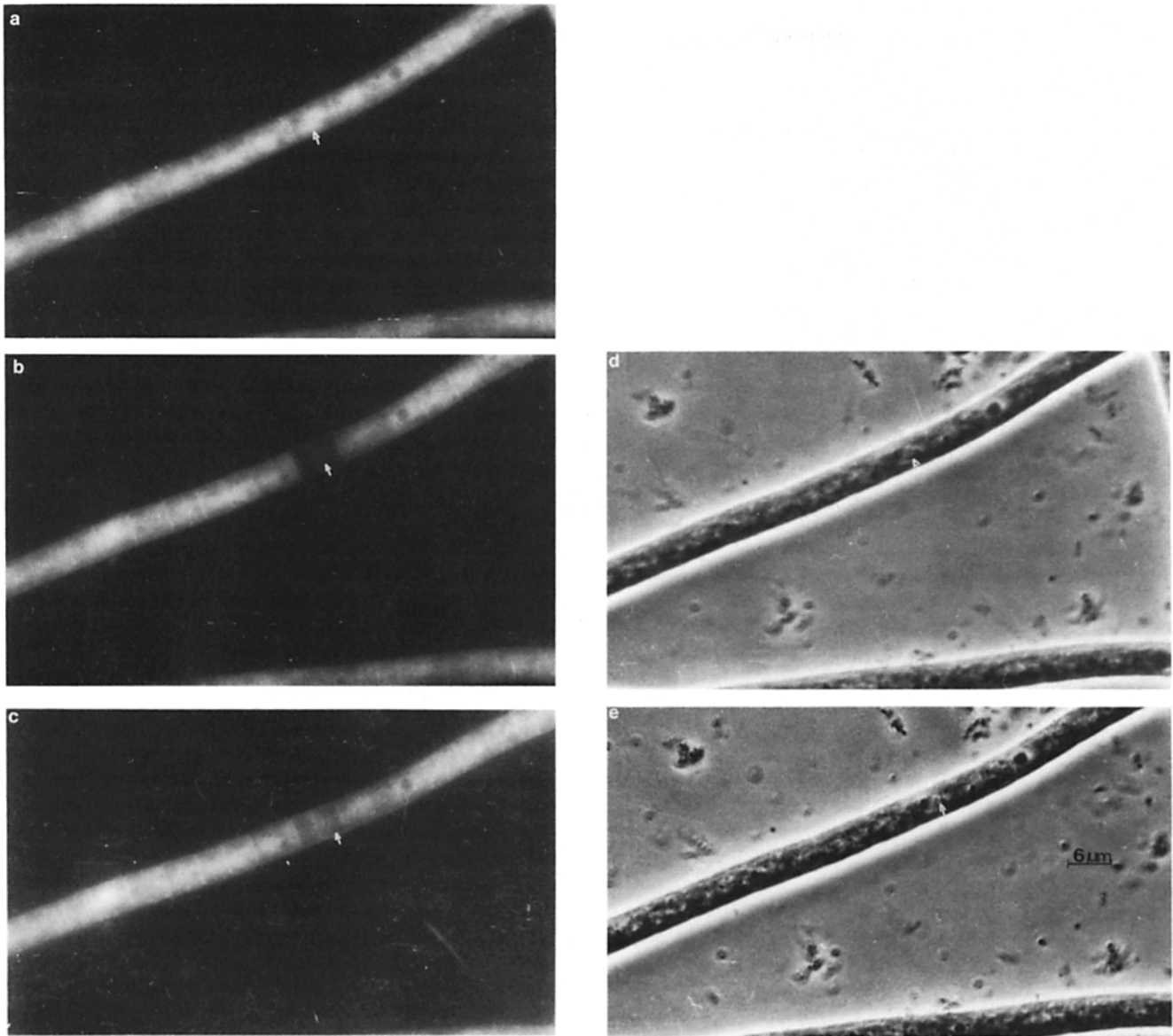


Figure 3. Effect of the local laser illumination (arrow) upon a trichome of *P. uncinatum*. (a–c) Fluorescent microscopy; (d and e) phase contrast microscopy. (a and d) Before the laser treatment; (b) 2 min after the laser illumination of a cell in the middle part of the trichome; (c and e) 15 min after the laser illumination.

Fig. 2). If the trichome was photographed for >15 s after switching on the total illumination, no difference between two parts of the trichome could be observed (Fig. 4 c). This means that not only the proximal but also the distal part of the studied trichome were competent in the ethylrhodamine response.

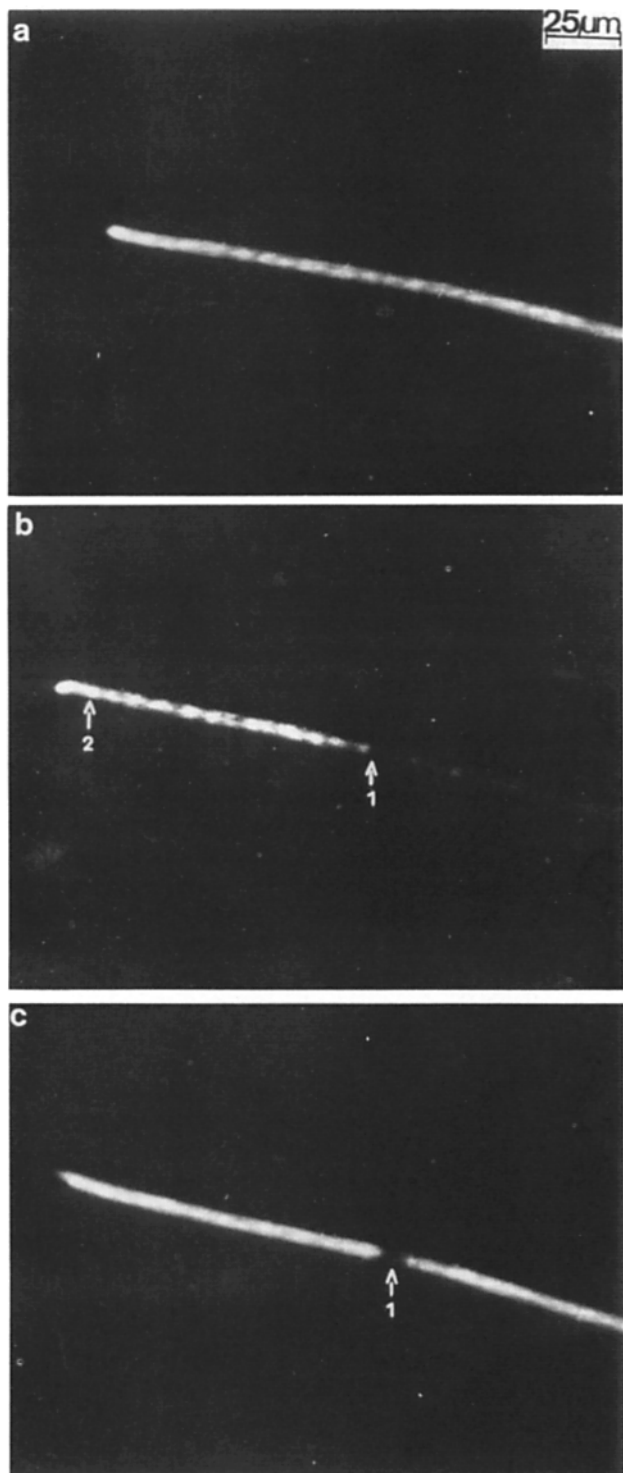
As a rule, all cells of the trichome before the laser treatment showed a similar intensity of fluorescence. However, sometimes we succeeded in finding a trichome that contained dark cell(s) displaying a much lower intensity of fluorescence than other cells in the same filament. Some of the dark cells became fluorescent, being illuminated for several seconds (Fig. 5, a and b). Experiments on the dark cell-containing trichomes showed that the light spot failed to induce an

ethylrhodamine fluorescence in that part of the trichome that was separated from the light spot by dark cell(s).

In certain trichomes, the light spot failed to energize an entire trichome even if it did not contain the dark cells. An example of this kind is given in Fig. 5, c and d.

### Discussion

The above data confirmed our previous observations about cable properties inherent in cyanobacterial trichomes. In particular, it is found that the ethylrhodamine-reported energizing of a small part of trichome by the light spot can spread along the trichome (see Figs. 4 and 5, c and d). In this respect, a cell in the trichome resembles a heart muscle



**Figure 4.**  $\Delta\Psi$  transmission along the laser-treated *P. uncinatum* trichome. Fluorescent microscopy was used (a) before the laser treatment; (b and c) after the laser treatment of a cell in the middle part of the trichome (arrow 1). (a and c) Total illumination by actinic light initiating both photosynthetic electron transfer and ethylrhodamine fluorescence. (b) Trichome was illuminated with a spot of actinic light covering  $\sim 5\%$  of the trichome length (arrow 2). Then the total illumination started to excite the ethylrhodamine fluorescence and the photograph was taken (exposure was 4 s).

mitochondrion connected to its neighbors with mitochondrial junctions (1). The advantages of the cyanobacterial system are that here we can (a) induce not only local de-energizing by laser illumination but also local energizing by a small spot of the light-actuating photosynthetic  $\Delta\bar{\mu}H$  generators; and (b) follow  $\Delta\Psi$  transmission not only by ethylrhodamine probe but also directly by extracellular microelectrodes.

It was found that the local energizing of a laser-treated trichome energizes only a part of the trichome, right up to its damaged site. This means that the local laser treatment prevents power transmission from the nontreated cells to the treated one. Hence, filamentous cyanobacteria can, if necessary, switch off the power transmission along the trichome but since usually one or two cells closest to the treated one are also quenched, one may suggest that the trichome-forming cells can be organized in clusters. Inside the cluster, the cells are connected with junctions that are always operative. On the other hand, intercellular junctions between two clusters can be reversibly closed (cf. mitochondrial clusters in cardiomyocytes [1]). An alternative explanation may be that the closest neighbors of the damaged cell have simply no time to actuate a chain of events resulting in switching off the intercellular contacts.

As experiments showed, electric isolation of cell(s) from the rest of the trichome is not a laser-induced artifact. Sometimes, it is also observed in intact trichomes. We mean dark cells showing low ethylrhodamine fluorescence whereas all other cells in the same trichome are strongly fluorescing. Apparent isolation of dark cells is temporary and, within a period of time, they can transform to fluorescing cells (Fig. 5, a and b). Disruption of the functional connection between two cells in the trichome occurs, in certain cases, without the de-energizing of one of them. Such a case was shown in Fig. 5 c.

Here the light spot energized only a half of the trichome. Under total illumination, all the cells in the site where power transmission was interrupted, look energized (Fig. 5 d).

Thus, cyanobacterial trichomes possess the possibility to have lateral power transmission and reversible switching off of this transmission. The latter effect resembles regulation of permeability of gap junctions between animal cells (2).

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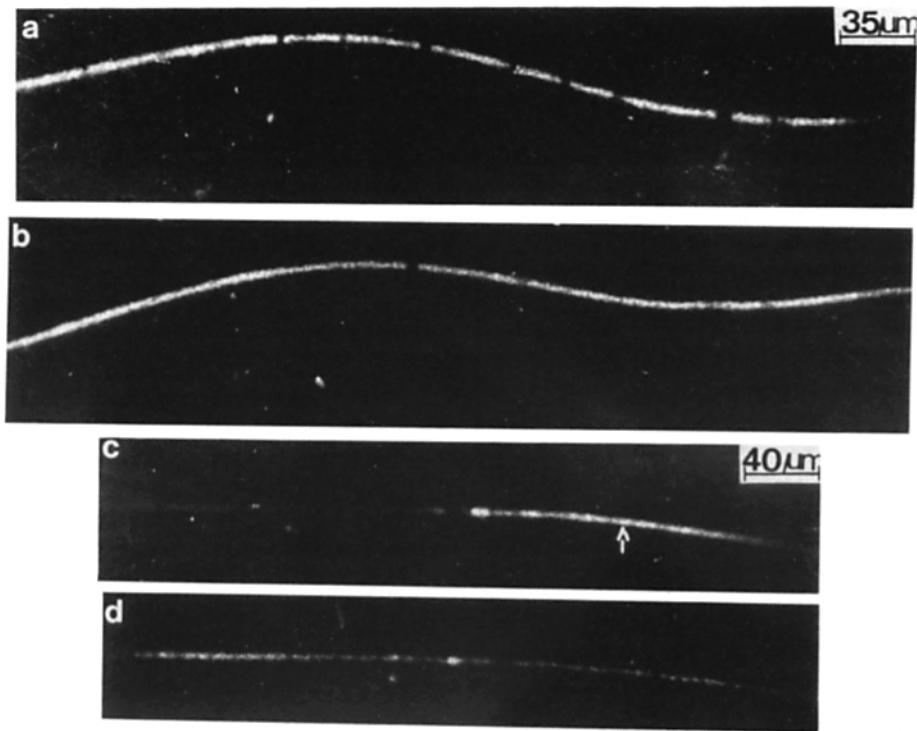


Figure 5. Examples of switching off intercellular power transmission in intact trichomes. (a, b, and d) Total illumination only; (c) illumination with a light spot (arrow) with subsequent total illumination. Photographs were taken 0–4 (a and c) and 18 (b and d) s after the total illumination started.

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