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Lateral Transport of Energy Along the Coupling Membranes of Cyanobacteria

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Abstract

The light-induced electric potential changes brought about local illumination of trichomes of cyanobacteria *Phormidium uncinatum* have been studied by means of extracellular electrodes. Responses of several electrodes located at various distances from the illuminated area of the trichome were monitored simultaneously. They turned out to be similar in shape: a rapid increase to the maximum value was followed by a slow decay toward a nonzero residual level. The results offer strong evidence in favor of power transmission along the trichome. The computerized experimental data lend support to the notion of a unified system of coupling membranes acting as a passive cable for electrical propagation, the cable parameters being $\tau C = 440$ sec cm^{-2} and $\lambda = 0.07$ cm.

Key Words: Cynaobacteria; electrical potential; cable theory; power transmission.

Introduction

The energy accumulated in coupling membranes in the form of a transmembrane electrochemical potential difference ($\Delta\mu_{H^+}$) due to the action of proton pumps is transportable along these membranes. This means that an electrical potential difference ($\Delta\psi$) generated by a proton pump in a certain region of the membrane propagates along the membrane, to be utilized in some other region. The concept of a coupling membrane acting as an electrical cable for energy transport in the cell was formulated in 1969–1971 (Skulachev, 1969, 1971) and applied recently to studying the role of giant mitochondria in muscle (Mitchell, 1976; Skulachev, 1977) and yeast (Davidson and Jarland, 1974). In fact, any coupling membrane contains many individual generators and consumers of $\Delta\mu_{H^+}$. All of them can be unified in a common energy system due to the transportability of $\Delta\mu_{H^+}$.

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Theoretically, transport of $\Delta\mu_{H^+}$ in the form of Δ pH by means of proton diffusion in the bulk phase is also possible. Moreover, this transport can take place if a part of the functional proton current does not enter the bulk aqueous phases but is carried along specialized H^+ pathways at the surface of the membrane. The cyanobacteria *Ph. uncinatum* investigated in this work consists of individual cells lined in several millimeter long trichomes. A trichome is composed of cylindrical cells about 6 μm in diameter and about 2μm long, enclosed in a common multilayered cell wall and slime. Electron micrographs of thin sections of filamentous cyanobacteria have suggested the presence of "intercellular connections" or "microplasmodesmata" between adjacent cells (Pankratz and Bowen, 1963; Wildon and Mercer, 1963). Freeze-fracture micrographs (Giddings and Stachelin, 1978) confirm the existence of 200–300 microplasmodesmata with an outside diameter less than 200 Å between the vegetative cells of cyanobacterium *Anabaena cylindrica.*

Hader (1978*a*) was the first to observe a light-induced electric potential difference between the two ends of a trichome. The results were obtained both by intracellular and extracellular electrodes. In his experiments, the whole trichome was illuminated uniformly. Hader (1978*b*) suggested that the changes of electric potential difference between the ends of a trichome serve as a signal that transmits information between the photoreceptor and the motor apparatus of the cells. The aim of the present work is an experimental investigation of the light-induced electric potential changes in cyanobacteria *Phormidium uncinatum* and a theoretical study of the energy transport mechanisms along coupling membranes.

Materials and Methods

Phormidium uncinatum was cultivated according to Glagoleva *et al.* (1980). Trichomes were grown in Petri dishes for 24 hr on the surface of 2% agar under cool light illumination at 25°C. The mineral salts medium contained 0.05 M KNO_3, 0.57 mM K_2HPO_4, 0.14 $MgSO_4$, 1.5×10^{-5} M $FeCl_3$ and 1.5×10^{-5} M ammonium citrate.

For measurements of photoelectric responses, the extracellular recording technique employed by Hodgkin and Rushton (1946) for neurophysiological investigation was used. Recently this technique was applied by Hader (1978*a*, *b*) and by Chailachian *et al.* (1980) in experiments with filamentous cyanobacteria. A bunch of 20 to 30 trichomes in parallel orientation was placed in the groove ($1.2 \times 10^5 \times 100 \times 100$ μm) of a chamber (Fig. 1) described by Chailachian *et al.* (1980). Each of the four points of the bunch, which divided it into three equal parts, was connected to a hollow in which a silver electrode was immersed. The groove and the hollows were filled with

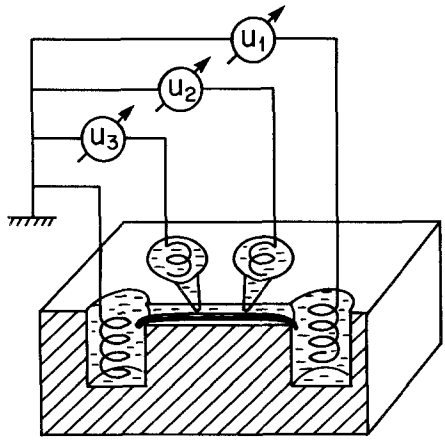

Fig. 1. The experimental chamber.

distilled water to achieve maximal increase in the extracellular resistance. In the present experiments, the resistance in the groove was about 50 MΩ. The electric responses were registered by means of a cathamplifier with an input resistance of 10^3 MΩ and then displayed on a dual-trace Disa oscilloscope. The time resolution of the electric measurements was less than 1 msec. After the bunch of trichomes was placed in the groove, a small part of it near its end was illuminated by a focused light beam; either an incandescent electric lamp or a helium–neon laser served as a light source. The electric potentials of the electrodes where registered simultaneously relative to a reference electrode fixed near the nonilluminated end of the bunch.

Results and Discussion

Experiments with Cyanobacterial Trichomes

A characteristic electric response of *Ph.uncinatum* during local illumination is presented in Fig. 2a. When the trichome region adjacent to the electrode was illuminated, the electric potential reached a maximal value within several seconds (t_{max}); then the potential slowly decayed with a characteristic time of about 30 sec and approached some value near zero. After a prolonged illumination, switching off the light induced an overshoot of potential. When the light was switched off soon after the maximal potential was reached, there was no overshoot. When the laser beam was used, the potential showed a biphasic response after switching on the light (Fig. 2b).

When the potentials of several electrodes were registered simultaneously

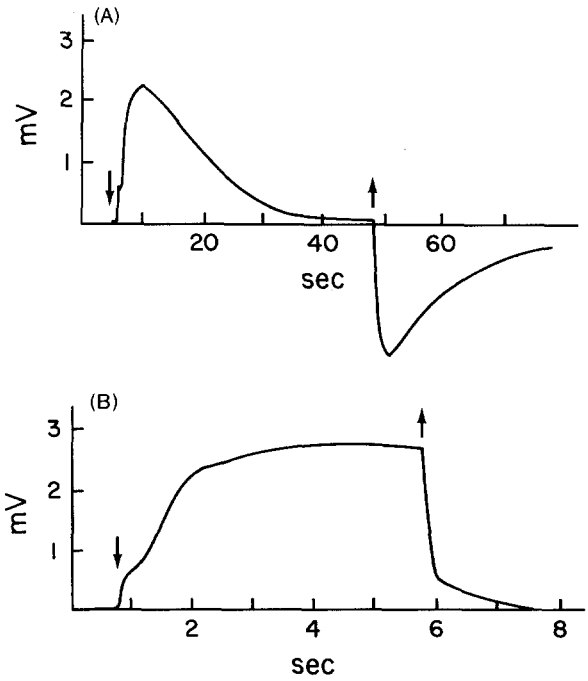

Fig. 2. (A) Characteristic electric responses of *Phormidium uncinatum* to local illumination. (B) The biphasic character of the electric potential increase. See text for explanation.

(Fig. 3), the plots followed the same pattern. The greater the distance between the illuminated area and the registering electrode, the lower was the maximum potential value and higher the t_{max}. As evidenced by the potential versus time plots, the electric potential difference was generated across the membrane, the cell interior being negative. The amplitude of the potential varied with the number of trichomes in the bunch from 2 to 3 mV for 20 trichomes and up to 20 mV for thick bunches.

Calculation of the $\Delta\psi$ Transmission Parameters

The appearance of an electric potential on the electrode placed in the unilluminated area of the trichomes (Fig. 3) prompts one to adopt the idea of energy transport along the trichome. It seems reasonable to consider the mechanism of $\Delta\psi$ transport along trichomes as a current of electricity along a cable. An electric sheme of this cable is shown in Fig. 4. Initially, the whole trichome is in the darkness; $\Delta\mu_{H^+}$ generators do not work. This means that

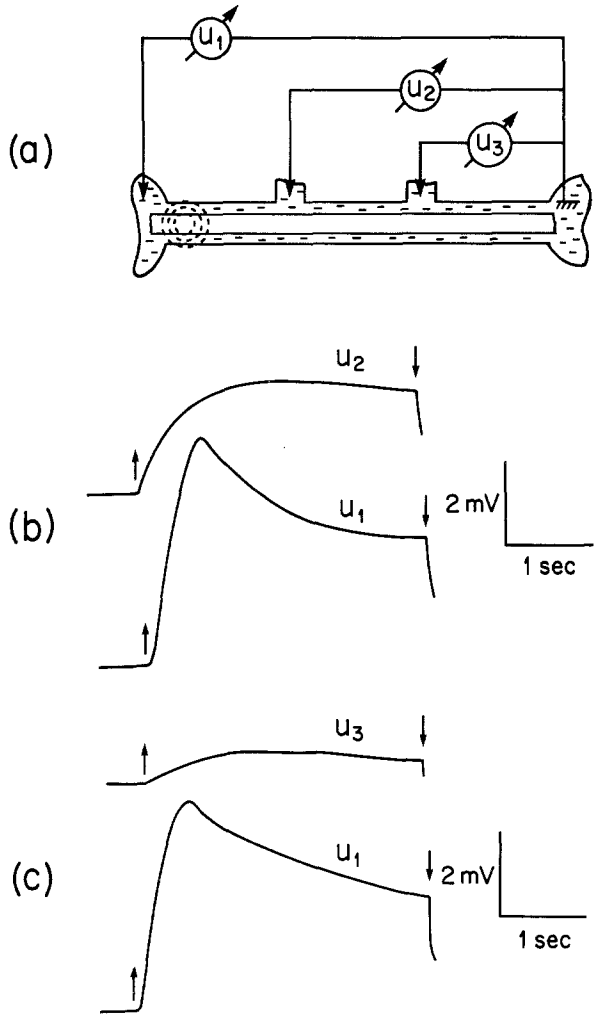

Fig. 3. A light-spot-induced electric potential difference, measured simultaneously by four extracellular electrodes situated along the bunch of trichomes of *Phormidium uncinatum*. The distances between electrode 4 and electrodes 1, 2, and 3 were 1100, 750, and 400 μm, respectively. The 100-μm-long end part of trichomes near electrode 1 was illuminated with a light beam. (a) A scheme of the groove with four electrodes; (b) synchronous measurements of electric potential differences between electrodes 2 and 4 (U_2) and 1 and 4 (U_1); (c) The same between electrodes 3 and 4 (U_3) and 1 and 4 (U_1).

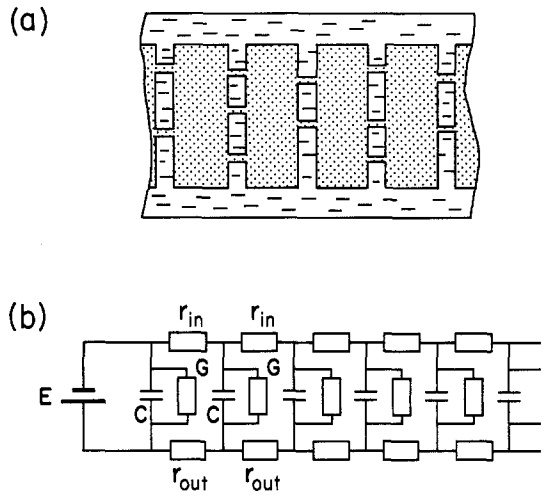

Fig. 4. The cable structure of *Phormidium uncinatum*. (a) A scheme of trichome; (b) the equivalent electrical scheme. E is the emf corresponding to the illuminated area of the trichome; r_{in}, G, C, and r_{out} are the resistance of the intracellular substance and connections between cells, the conductivity of the insulation (coupling membrane), the capacitance of the membrane, and the resistance of the groove outside the trichomes, respectively, per unit length.

every capacitor C_1 representing a single cell membrane is uncharged. After illumination of several cells at the edge of the trichome, the situation drastically changes. Due to the work of the $\Delta\mu_{H^+}$ generators in the membranes of exposed cells, the electric current passes through the resistance of the interior of the cell and the resistance between the two adjacent cells. The conductivity G is introduced in the sheme to account for the imperfect insulating properties of coupling membranes. The resistance r_{in} in the sheme represents the total resistance of the inner cellular substance and that of the intracellular contacts per unit of length; r_{out} is the distributed resistance of the medium outside the trichomes. The sum of the inner (r_{in}) and outer (r_{out}) resistances will be called r:

$$r = r_{in} + r_{out}$$

The potential distribution $U(x,t)$ along the cable is described by the equation

$$\frac{\delta^2 U(x,t)}{\delta x^2} - \tau C \frac{\delta U(x,t)}{\delta t} - \tau G\, U(x,t) = 0 \qquad (1)$$

C being the capacitance of the cable per unit of length. As a first

approximation, we shall consider a semi-infinite cable which has an ideal insulation ($G = 0$):

$$\frac{\delta^2 U(x,t)}{\delta x^2} - \tau C \frac{\delta U(x,t)}{\delta t} = 0 \tag{2}$$

The initial and boundary conditions in this case are as follows:

$$\begin{aligned} U(x,t) &= 0 \qquad \text{for } t = 0,\ x > 0 \\ U(x,t) &= U_0 \qquad x = 0 \end{aligned} \tag{3}$$

The solution of the problem (2), (3) can be written in the form

$$U = U_0 \left[1 - \phi \left(\frac{x}{2} \sqrt{\frac{\tau C}{t}} \right) \right] \tag{4}$$

F being

$$\phi(z) = \frac{2}{\sqrt{\pi}} \int_0^z e^{-z^2} dz \tag{5}$$

It is reasonable to rewrite Eq. (4) in terms of ΔU, i.e., the electric potential difference between two fixed points of the cable:

$$\Delta U = \Delta U_0 \left[\phi \left(\frac{x_i}{2} \sqrt{\frac{2C}{t}} \right) - \phi \left(\frac{x_r}{2} \sqrt{\frac{2C}{t}} \right) \right] \tag{6}$$

where X_r is the coordinate of the reference electrode and X_i is the coordinate of the recording electrode ($i = 1, 2, 3$). Potential plots calculated by means of Eq. (6) are shown in Fig. 5a. By equating the first derivative of Eq. (6) to zero, one obtains t_{max}, when the potential is at its maximal value:

$$t_{max} = \tau C \frac{x_i^2 - x_r^2}{4 \ln (x_i/x_r)} \tag{7}$$

With the conductivity G of the cable insulation taken into account, the differential equation for the same initial conditions (3) will be

$$U_1(x,t) = U(x,t) + \frac{G}{C} \int_0^t U(x,\tau) \cdot e^{-(G/C)\tau} d\tau \tag{8}$$

and

$$\Delta U_1(x,t) = U_1(x_i,t) - U_1(x_r,t) \tag{9}$$

The curves corresponsing to Eq. (9) are shown in Fig. 5b. Analysis of Eq. (6) and (9) shows that introduction of the conductivity G results in a residual electric potential difference.

The residual potential difference corresponds to a state of the cable when

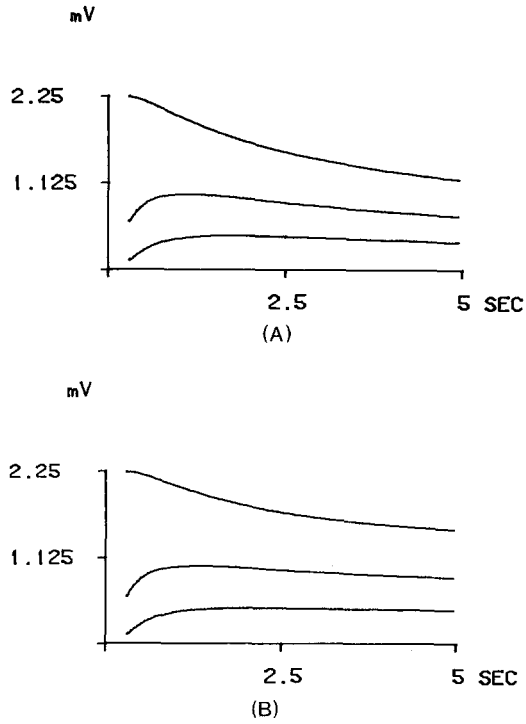

Fig. 5. Computer simulation of a power transport process along cyanobacterial trichome confirming the experimental data shown in Fig. 3. A cable model with parameters $rC = 440$ sec cm^{-2}, $G = 0\ \Omega\ \text{cm}^{-1}$ (A) and $rC = 440$ sec cm^{-2}, $G = 4 \times 10^{-10}\ \Omega\ \text{cm}^{-1}$ (B).

all of the capacitors are charged. Therefore the potential distribution is discribed by the equation

$$\frac{\partial^2 U}{\partial x^2} - rGU = 0 \tag{10}$$

Its solution is

$$U = U_0 \exp(-x/\lambda) \tag{11}$$

λ being the constant of the length of the cable, i.e., the distance in which the potential decays by a factor of e:

$$\lambda = (\tau G)^{-\lambda/2} \tag{12}$$

It is now clear that the higher the conductivity G, the greater the residual level of the potential difference. If the finite cable length l is accounted for, the

solution of Eq. (1) will be

$$U_2(x,t) = U_0\left[1 - \phi\left(\frac{x}{2}\sqrt{\frac{2C}{t}}\right) - 1 + \phi\left(\frac{x+2l}{2}\sqrt{\frac{2C}{t}}\right)\right.$$
$$\left. + 1 - \phi\left(\frac{x+4l}{2}\sqrt{\frac{2C}{t}}\right] - 1 + \ldots \right. \quad (13)$$

The additional terms that appear in Eq. (13) are corrections for the waves of the potential reflected at the boundary $x = 1$. Obviously, the presence of the reflected waves will not be noticeable immediately, but only after some time. The effect of the finite cable length on the residual potential difference is opposite to that of non-zero conductivity G.

To estimate the cable parameter τC, one can use Eq. (7):

$$\tau C = \frac{4t_{\max} \ln (x_i/x_c)}{x_i^2 - x_c^2} \quad (14)$$

The experimentally determined value of $t_{\max}$ on a dark electrode is due to two reasons, i.e., (a) the maximal potential on the illuminated area of the electrode is reached during a finite time of about 1 sec rather than instantaneously, and (b) an additional time for the energy transport is necessary as described above [Eq. (7)]. The further the electrode is from the exposed area, the larger is the contribution due to the second effect. Introducing the correction accounting for these effects into (13), one obtains an estimate of 400 to 500 sec · cm^{-2}. Introduction of a finite length of a trichome, $I = 1$ mm, in model equation (12) does not contribute significantly to the cable parameter τC. A best-fit computer determination of the model parameters based on the experimental data (Fig. 3) leads to the following value of the main cable parameter:

$$\tau C = 440 \text{ sec} \cdot \text{cm}^{-2} \quad (15)$$

Another experimental characteristic of the electric potential changes is the residual potential difference V. As follows from formula (10), Eq. (16) allows one to calculate the average constant of the cable length, λ, on the basis of the experimentaly determined values of V_i for a given x_i

$$\lambda = \frac{x_i - x_j}{\ln(V_j/_i)} \quad (16)$$

The experimental values for V_i lead to

$$\lambda = 0.05 \text{ cm} \quad (17)$$

Although we have obtained the constant of the length, it is impossible to calculate the conductivity G of the coupling membranes of cyanobacteria based on Eq. (12) because the value of τ is unknown. Let us assume an average value of the specific capacitance C_{sp} of the coupling membrane based on the data for artificial lipid bilayers (Jain, 1972) and chromatophore membranes (Packham *et al.*, 1978). Hence, using the known geometry of cells of cyanobacteria, it is possible to calculate the value of capacitance per unit of length:

$$\begin{aligned} C_{sp} &= 10^{-6}\ \text{F cm}^{-2} \\ C &= 5 \times 10^{-g}\ \text{F cm}^{-1} \end{aligned} \tag{18}$$

Comparing (15) with (18), we obtain

$$\tau = 8.8 \times 10^{10}\ \Omega\ \text{cm}^{-1} \tag{19}$$

and from (19), (12), (17), we obtain

$$G = 4 \times 10^{-10}\ \Omega^{-1}\ \text{cm}^{-1} \tag{20}$$

The value of G_{sp} corresponding to G, i.e., the conductivity per unit area of a membrane, is

$$G_{sp} = 5 \times 10^{-8}\ \Omega^{-1}\ \text{cm}^{-2}$$

It is noteworthy that the contribution of τ_{out} to the total value of τ,

$$\tau = \tau_{in} + \tau_{out}$$

is not more than 1% because the resistance of the groove with a length of 1 mm is 50 MΩ, which corresponds to $\tau_{out} = 5 \times 10^{8}\ \Omega\ \text{cm}^{-1}$. Consequently,

$$\tau_{in} = 8.8 \times 10^{10}\ \Omega\ \text{cm}^{-1} \tag{21}$$

The corresponding value of the resistance to the electric current between the adjacent cells of cyanobacteria calculated from (21) is consistent with the data for some other objects (Loewenstein and Kanno, 1964).

The overshoot in the off effect after prolonged illumination can be ascribed to a conductivity that is localized in the potential-generating area and remains after switching off the light. Hence, the discharge of the capacitors through this large conductance results in a process which is the reverse of that described by Eqs. (6) and (9). Apparently, the light-induced conductance increase was due to photooxidation of membrane constituent(s), since it was found that the overshoot was absent if anaerobic conditions were used.

Our results (Fig. 3) may be considered evidence in favor of the lateral power transmission hypothesis. In fact, the decrease of the potential as the

distance from the illuminated area increases is in good agreement with the results predicted by the passive cable model. Moreover, the absence of electrode depolarization and the slow decay of the potential on the dark electrode lend further support to the hypothesis. Certainly, a transported $\Delta\psi$ may serve for some signal functions, e.g., to switch on different molecular mechanism, to open or close the ion channels, etc. However, measurements of the motility of cyanobacteria indicate that it is power transmission that is responsible for locomotion (gliding) of trichomes under partial illumination. It was found that illumination of 5% of the trichome length supported motility under conditions where the light was the only available energy source. The experiments with the rings of slime supported the assumption that the energy for the movement is consumed even in the dark part of a trichome (Glagoleva *et al.*, 1980).

One may ask: how is it possible to predict the potential distribution in the inner parts of the trichome by measuring the external currents and potentials? As a matter of fact, the currents in the inner and outer parts of the chain are equal in terms of Kirchhoff's law. Consequently, the potential difference between two given points in the inner part of the chain is proportional to that in the outer part, the factor of proportionality being r_{in}/r_{out}. This enables one to estimate the cable parameter τC on the basis of the invariant experimentally measured value of t_{max}, which is independent of the potential amplitude. This also means that the obtained parameters do not depend on the number of trichomes in the bunch.

It is noteworthy that simple diffusion cannot provide the necessary rate of power transport. It can be seen from the experimental results of this work that energy is transported along the cyanobacteria trichomes over distances of the order of 1 mm during not more than 5 sec. It is possible to estimate the diffusion coefficient necessary to provide the observed speeds of transport by means of the Einstein–Smoluchowski equation (Einstein, 1954):

$$D = \frac{\overline{x}^2}{2\tau}$$

$\overline{x}^2$ being the mean square displacement and τ the characteristic time. Assuming

$$\tau = 5 \text{ sec} \quad \text{and} \quad \overline{x} = 1 \text{ mm}$$

one obtains $D = 10^{-3}$ cm^2 sec^{-1}. It is evident that neither ATP nor other ions and molecules (e.g., $D_{K^+} = 2 \times 10^{-5}$ cm^2 sec^{-1}) could provide such rates. Although all of the diffusion coefficients used were determined for aqueous dilute solutions, they would not be much different in physiological conditions.

The proton diffusion coefficient in aqueous solutions is known to be $D_{H^+} = 9.3 \times 10^{-5}$ sec^{-1} (Antropov, 1975). Thus, normal three-dimensional diffusion of any substance cannot provide the necessary rate of power transport.

As stated above, the usual three-dimensional diffusion cannot provide the necessary energy transport rate. The effect of a reduction in dimensionality in biological diffusion processes has been considered in detail by Adam and Delbrück (1968). It follows from their analysis that if some one- or two-dimensional pathways for proton transport exist, it is quite possible for condition (23) to be satisfied. The convection of the intracellular substantance maintained by wavelike movement of the cell wall structures could also provide the necessary rate of transport. This movement is due to the action of the helical fibrils that coat a trichome under the outer membrane.

The above two mechanisms of the energy transport along the coupling membranes demand significantly different conditions for their realization. A trichome can act as a passive cable [Eqs. (15), (17), and (20)] if conductive channels exist between adjacent cells. The necessary condition for the proton current is more complicated. It would require a continuous system of hydrogen-bonded chains.

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