ELECTROGENIC PROTON TRANSPORT IN THE PLASMA MEMBRANE OF *NEUROSPORA*

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A variety of experiments involving direct measurement of membrane potentials with microelectrodes have led to the notion that the generalized "chemiosmotic" hypothesis (4) can account for the major transport processes occurring in the plasma membrane of *Neurospora*; and that fungal plasma membranes, despite their apparent lack of redox elements, are useful models for many transport events which take place in energy-conserving membranes.

The diagram in Fig. 1 represents the current working hypothesis for transport by *Neurospora*. The measured resting membrane potential lies in the range -160 to -240 mV (cell interior negative) and is sustained largely by ejection of protons coupled to the splitting of ATP (8).[1] The membrane potential then serves as the main energy distributor for transport, and—together with a small inward chemical gradient for protons—drives the uptake of a variety of different substances. Direct evidence exists that glucose and its analogues are handled in this manner (9), and indirect evidence that amino acids, phosphate, and potassium are as well.[2] From the measured membrane resistance (ca. $10{,}000\Omega \cdot \text{cm}^2$ under standard conditions; ref. 7) and potential, the ion flux driven by the proton pump can be estimated at $200\ \text{pmol/cm}^2 \cdot \text{s}$. If the density of transport sites on the membrane were roughly the same as that of F_1-ATPase sites on the mitochondrial inner membrane ($3{,}000/\mu\text{m}^2$, ref. 1), the flux would yield a turnover rate of 200/site · s, for a two-charge process.

Major evidence in support of Fig. 1 comes from a survey of the speed and extent of depolarizations caused by certain ions, metabolic inhibitors, and antibiotics. Hydrogen ions, almost alone among the common inorganic cations and anions, have a steep depolarizing effect: 30–40 mV for each unit decrease of pH_o, at least in the range pH 5 to pH 3 (6). Cyanide, at concentrations just sufficient to block cytochrome oxidase,

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[1] Ion flux and electrical measurements suggest that the ATPase of the *Neurospora* plasma membrane resembles the bacterial membrane ATPase more closely than the mitochondrial ATPase. It is insensitive to oligomycin and rutamycin, but sensitive to Dio 9, chlorhexidine, and *N,N'*-dicyclohexylcarbodiimide. C. L. Slayman and C. W. Slayman. Unpublished experiments.

[2] C. W. Slayman, H. Lowendorf, and C. L. Slayman. Unpublished experiments.

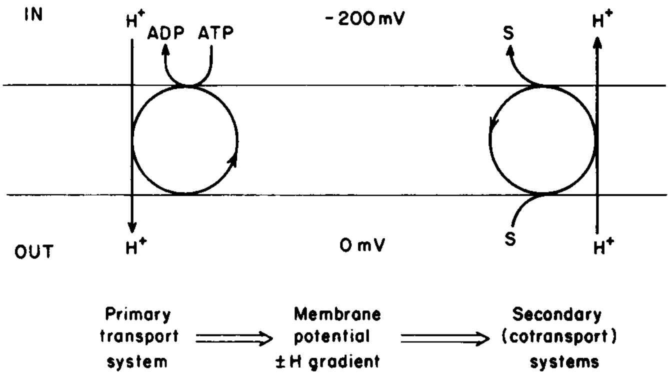

FIGURE 1 A chemiosmotic model for transport in the plasma membrane of *Neurospora*.

depolarizes about 75%, with an exponential time constant of 5.7 s, closely tracking the intracellular ATP concentration (8). Most other depolarizing agents work more rapidly: tyrocidin and nystatin, which presumably create large holes or pores in the membrane, nearly abolish the membrane potential within 2–4 s (3); 5 mM EDTA depolarizes by 60% with a time constant of 3–5 s, apparently by extracting Mg^{++}, not Ca^{++}, from the pump;[2] 50–100 mM NH$_4^+$, perhaps acting as a lipid-soluble ion, depolarizes 75% with a time constant of ca. 0.5 s (footnote 2); and glucose, at concentrations which saturate the energy-dependent glucose transport system in carbon-starved *Neurospora*, depolarizes by 60% with a time constant of 0.5 s (9). Proton efflux occurs during the ammonium depolarization, and stoichiometric proton and glucose influx occur during glucose depolarization.

All of the above phenomena had been qualitatively predicted, for mitochondrial and/or bacterial membranes, on the basis of flux measurements and indirect arguments. However, two properties of the *Neurospora* membrane have recently emerged which could not have been predicted without the direct electrical measurements. Both are attributes of the proton extrusion system. First, under conditions of fractional metabolic downshift, such as can be maintained by cyanide treatment of the cytochrome mutant *poky f* (10), the membrane potential undergoes damped oscillations; these begin with a depolarization of 30–100 mV, repolarize slightly beyond the control voltage, then damp out for 2–4 cycles, and end with the potential only slightly below the control value. Since intracellular ATP does not show such oscillations, the possibility exists that some kind of feedback control mechanism superimposes itself on the proton pump (2).

Second, the proton pump seems to behave like an ideal current source, driving a fixed current outward through the plasma membrane at all voltages in the range −300 to −50 mV. The experimental results underlying this conclusion are shown in Fig. 2. The mutant *poky f* was chosen for this experiment; and a hypha was impaled with three

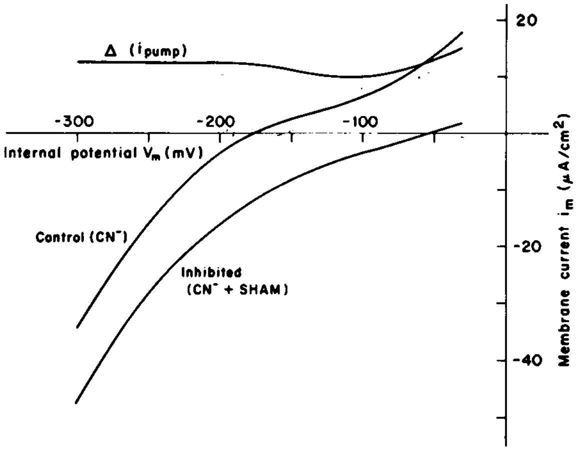

FIGURE 2 Estimation of the current-voltage curve for the electrogenic proton pump in the plasma membrane of *Neurospora*.

microelectrodes: one to pass a polarizing current, and two—separated by about 130 μm—to record the transmembrane voltage. From the measured currents and a mathematical manipulation taking account of the cable properties of the *Neurospora* hypha,[3] actual membrane current densities (ordinate scale) were calculated at 10-mV intervals. This maneuver was carried out in the presence of cyanide and then in the presence of cyanide plus salicylhydroxamic acid (SHAM). (SHAM inhibits a compensatory, cyanide-insensitive respiratory path in *poky f* without any direct effect on the plasma membrane; cyanide alone adds a significant conductance to the membrane; together the two inhibitors abolish ATP synthesis in *poky f*, and strongly depolarize the membrane.) The difference between the resultant membrane I-V curves represents the I-V curve for the proton pump (Δ, i_{pump}). The fact that it is essentially horizontal means that the pump is insensitive to the membrane potential, and that its internal resistance is very large. With the customary sort of cyclic carrier for transport systems, this result could arise if the chemical reactions at the membrane boundaries, rather than the voltage-sensitive steps of membrane transit, were rate-limiting.

One additional result can also be seen which may be significant to mechanisms of energy conservation. The I-V curve for the *Neurospora* membrane, with or without the proton pump, is quite nonlinear. Clearly, changes in membrane potential need not be proportional to changes in membrane current. It is therefore inappropriate to infer, for example, that ADP control of mitochondrial respiration does not operate via the membrane potential (5), simply because uncoupling agents and ADP have disproportionate effects on respiration (a measure of current) and computed membrane potential, in state 4 mitochondria.

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