Phosphate Transport in *Halobacterium halobium* Depends on Cellular ATP Levels

MARIO ZORATTI¹* AND JANOS K. LANYI²

Consiglio Nazionale delle Ricerche Unit for the Physiology of Mitochondria and Institute of General Pathology, University of Padova, 35131 Padova, Italy,¹ and Department of Physiology and Biophysics, University of California at Irvine, Irvine, California 92717²

Received 5 January 1987/Accepted 28 July 1987

Freshly harvested *Halobacterium halobium* cells grown in the presence of 0.5 mM P_i took up phosphate with a low apparent K_m . Import depended on intracellular ATP levels; sodium and proton (electro)chemical gradients alone were not competent to drive P_i uptake. Although most of the phosphate accumulated as P_i in the cells, efflux of P_i was difficult to achieve.

Bacteria transport P_i either in symport with protons (30) or by means of a transport apparatus involving periplasmic binding proteins (22, 29).

The two systems may coexist (28, 33, 34) and, importantly, their expression and properties depend on cell growth conditions (28, 34). Other transport mechanisms might exist (2), but they are not well characterized. In contrast to eucaryotic systems, Na^+ involvement has never been observed.

Other nutrients are known to be taken up in symport with Na^+ in halophiles (16, 18), which have adapted to live in extremely high salt concentrations and maintain a large transmembrane electrochemical sodium gradient ($\Delta\bar{\mu}_{Na^+}$), as well as in alkalophiles (15). Ando et al. (2) investigated P_i transport in vesicles from an alkalophilic bacillus; they observed P_i fluxes in response to artificially applied H^+ or K^+ gradients, with no indication of Na^+ involvement.

We have begun a study of P_i transport in *Halobacterium halobium*, the best-known representative of halophilic archaeobacteria. The transport systems of *H. halobium* studied so far for amino acids (20) and Ca^{2+} (6) utilize the Na^+ gradient as the immediate driving force. The primary energy source is, of course, the $\Delta\bar{\mu}_{H^+}$, which can be generated by light or by respiration (5). This bacterium therefore seemed to be a most promising organism for a search for Na^+ -driven P_i transport in procaryotes.

MATERIALS AND METHODS

The abbreviations used are as follows: MOPS, 3-(*N*-morpholino)propanesulfonic acid; TPP, tetraphenylphosphonium; DMO, 5,5-dimethyloxazolidine-2,4-dione; DCCD, *N,N'*-dicyclohexylcarbodiimide; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone; $\Delta\psi$, transmembrane electrical potential gradient; ΔpH , transmembrane pH gradient.

H. halobium S9-3 cells were grown at 40°C and at pH 7 to late exponential growth phase (4 to 5 days). Growth medium was composed of 250 g of NaCl, 20 g of $MgSO_4 \cdot 2H_2O$, 10 g of peptone (Oxoid), 3 g of sodium citrate, 2 g of KCl, 0.2 g of $CaCl_2$, and 0.1 ml of a trace metals solution (27) per liter. The medium initially contained 0.55 mM phosphate (from the

peptone), which declined to some 0.4 mM by harvest time. After the cells were harvested by gentle centrifugation and washing, they were suspended in basal salts (growth medium minus peptone) at pH 7, and the experiments were started within 2 h of isolation. In other cases, the cells were stirred with aeration for 6 to 8 h at 40°C and then used or stored at 4°C for future use (starved cells). The properties of the cells thus stored did not change appreciably for at least a week (14). Protein concentration was determined by the biuret or Lowry method.

Phosphate uptake experiments were generally started by the addition of the cells (to 0.2 to 2.5 mg of protein per ml) to the suspension medium. The suspension was magnetically stirred and could be illuminated with a 150 W slide projector lamp regulated by a Variac rheostat. The light intensity was 0.5 to 1 W/cm². The incubation temperature was 20 to 22°C unless otherwise mentioned. [³²P] P_i flows were monitored either by the Millipore filtration technique, essentially as described previously (21), or by a centrifugation procedure. In the latter case, the pellet was rinsed with nonradioactive incubation medium (P_i free) before lysis with 50 mM P_i . Control experiments showed that the two procedures gave identical results.

In some experiments, the pH or the concentration of the lipophilic ions TPP or triphenylmethylphosphonium, whose redistribution measures changes in $\Delta\psi$ (3), were continuously monitored with a glass electrode or a specific membrane electrode, respectively. ΔpH was assessed on the basis of [¹⁴C]DMO and [¹⁴C]methylamine distributions (3), which were determined in centrifugation experiments. Non-radioactive DMO and methylamine (0.4 mM) were always present in the assays to minimize any binding artifacts.

Free P_i was determined by the molybdate method (19) or, in a few cases, by ³¹P nuclear magnetic resonance determinations on cell extracts. The two techniques agreed closely.

Cellular ATP content was determined by diluting samples of cell suspension into buffered 2 mM EDTA, which resulted in immediate cell lysis. Samples of this solution were then used for the ATP assay by the luciferin-luciferase method (11) in a Packard Pico-Lite luminometer. Rates of oxygen consumption were determined by using a Clark electrode (Yellow Springs, Ohio) in a closed, thermostatted, and stirred chamber.

* Corresponding author.

FIG. 1. Phosphate and serine uptake by variously treated fresh cells. Uptake was monitored by the filtration technique. The suspension medium (see Materials and Methods) also contained 10 mM glycerol, 50 mM Tris-MOPS (pH 7), 20 μ M $[^{32}\text{P}]\text{P}_i$, or 10 μ M $[^3\text{H}]\text{serine}$. (A) Phosphate uptake. Controls received either 10 μ M FCCP and 10 mM P_i (●) or 0.2 mM TPP (▼) at 140 min. In two other incubations, 10 μ M FCCP (□) or 0.2 mM TPP (○) were present from the start. One of the control incubations (▽) was illuminated in the interval indicated. Rates of $[^{32}\text{P}]\text{P}_i$ uptake in the control incubations were 0.076 nmol per mg of protein per min (●) and 0.070 nmol per mg of protein per min (▽). (B) Serine uptake. Either 10 μ M FCCP (□) or 0.2 mM TPP (○) was present from the start in two incubations. Symbols: ▼, cells pretreated with 50 μ M DCCD for 4 h; ●, control cells.

Materials. $[^3\text{H}]\text{serine}$, $[^{14}\text{C}]\text{DMO}$, $[^{14}\text{C}]\text{CH}_3\text{NH}_2$, and carrier-free $[^{32}\text{P}]\text{P}_i$ were obtained from ICN Radiochemicals, Irvine, Calif., or from Amersham Int., Little Chalfont, England. Arginine and serine were obtained from Sigma Chemical Co., St. Louis, Mo., and DCCD was obtained from Fluka AG Chemische Fabrik, Buchs, Switzerland.

RESULTS

The bacteria had characteristics in good general agreement with published data. We measured an average endogenous free-phosphate pool of 78 nmol per mg of protein, corresponding to about 30 mM P_i in if the cell volume was about 2.5 μ l per mg of protein (4, 13, 14, 32), in agreement with previous observations (13, 25). Energy-requiring processes in *H. halobium* may be driven either by light absorption or by substrate oxidation (5). The ATP content of fully energized cells varied somewhat from determination to determination, remaining within the range of values reported by Oesterhelt and co-workers (2.5 to 8 nmol per mg of protein) (11, 12, 25, 26; higher values were reported in references 8, 9, and 13). Freshly isolated cells, oxidizing substrates of the endogenous pool, maintained a $\Delta\psi$ of 180 to 190 mV, as judged from TPP distribution without attempting binding corrections (4, 24, 26). In these cells, the addition of exogenous substrate (glycerol) or illumination produced only a negligible increase in $\Delta\psi$. At a medium pH of 7, ΔpH was found to be very close to zero (4), and again, neither illumination nor glycerol caused anything more than negligible variations after initial transients.

Fresh, energized cells took up labeled phosphate at a low rate (Fig. 1A). The uptake was characterized by a K_m of 5 to 10 μ M, comparable to the values found in other bacterial systems (1, 2, 28, 34) and by a V_{max} of only 0.08 to 0.1 nmol per mg of protein per min at 20 to 22°C (data not shown). Interestingly, higher rates, up to 0.35 nmol per mg of protein per min, were sometimes observed with starved cells. Most

(up to 85%) of the phosphate taken up by cells could be recovered by the molybdate complexation-extraction procedure (data not shown). Thus, the phosphate entering the cells persisted largely as P_i in the endogenous pool. ^{31}P nuclear magnetic resonance experiments failed to reveal significant amounts of perchloric acid-soluble P-containing compounds other than P_i in cell extracts after protracted (up to 7 h) glycerol-sustained uptake. In particular, no polyphosphates were detected. Cyclic 2,3-diphosphoglycerate has been found to accumulate in another archaeobacterium, *Methanobacterium thermoautotrophicum* (31). The ^{31}P nuclear magnetic resonance spectra of our cell extracts showed a broad, weak signal at the position expected for this compound, but its positive identification was not attempted.

$[^{32}\text{P}]\text{P}_i$ uptake by fresh cells was not influenced by illumination (Fig. 1A), and it continued at the same rate for at least 7 h, without noticeably leveling off (data not shown). P_i uptake was inhibited by protonophores and lipophilic ions, either added during the uptake or present from the beginning (Fig. 1A). Both agents caused collapse of $\Delta\psi$ to unmeasurable levels and a strong decrease of cellular ATP content (Table 1) (11). The addition of TPP to respiring or illuminated bacteria also caused a massive loss of protons from the cells (data not shown) (4), with consequent formation of a ΔpH of 2 to 3 U, alkaline inside. Efflux of $[^{32}\text{P}]\text{P}_i$ was difficult to achieve: the addition of excess unlabeled phosphate, excess protonophore, or both, or of TPP stopped the uptake of label, but P_i efflux from the cells was very slow (Fig. 1A; data not shown).

Protonophores, TPP, and DCCD reduced, but did not block, serine uptake (Fig. 1B). In *H. halobium*, serine transport is believed to be driven by both the electrical and the chemical components of the $\Delta\mu_{\text{Na}^+}$ maintained by the primary pumps and the Na/H antiporter (17, 20). The $\Delta\mu_{\text{Na}^+}$ of fresh cells is large and decays over a period of days because of the permeability characteristics of the membrane (16). The serine uptake in the presence of uncouplers or TPP

FIG. 2. Effect of DCCD treatment on respiration, cellular ATP content, and P_i uptake. Freshly harvested cells were incubated at room temperature for 4 h with various concentrations of DCCD. (A) Cells were then incubated aerobically with 10 mM glycerol for 10 min, and ATP was measured (○) or a sample was mixed with medium in the oxygraph to follow the rate of respiration (●). (B) Phosphate uptake was monitored by the filtration method. The incubation medium used is described in the legend to Fig. 1A. Symbols: Δ , control; $\square$, 15 μ M DCCD; $\blacksquare$, 50 μ M DCCD. The rates of $[^{32}P]P_i$ uptake were 0.072 nmol per mg of protein per min for control cells and 0.010 nmol per mg of protein per min for cells treated with 15 μ M DCCD.

(Fig. 1B) showed that a sodium chemical activity gradient existed and was competent to drive amino acid transport under those conditions. While the reasons for the subsequent loss of label (Fig. 1B) are not clear, the experiments shown in Fig. 1A and B, taken together, indicate that a sodium gradient is not by itself sufficient for P_i uptake and

FIG. 3. Arginine-induced restoration of P_i uptake by DCCD-treated cells. Phosphate uptake by control (●) and DCCD-treated (0.1 mM, 4 h) (○, Δ) cells in the same suspension medium as for the experiment shown in Fig. 1A. The medium of one of the incubations (○) also contained 5 mM KCN, which other experiments showed to have no effect on arginine-supported P_i uptake. Centrifugation technique. When indicated, 5 mM arginine was added to one suspension (○). The rates of $[^{32}P]P_i$ uptake were 0.084 nmol per mg of protein per min for control cells and 0.051 nmol per mg of protein per min for cells treated with DCCD and arginine.

point to a substantial difference in the transport mechanism of amino acids and phosphate in *H. halobium*.

DCCD inhibits *H. halobium* ATP synthase fairly specifically (24, 25), and it can be used to investigate ATP-dependent processes. Treatment with DCCD decreased the rate of respiration and ATP content of cells (Fig. 2A). Low concentrations of DCCD were sufficient to cause an increase of the $\Delta\psi$, which then remained constant between 50 and 200 μ M DCCD (data not shown) (24, 25). Δ pH increased with DCCD concentration at least up to 200 μ M (data not shown). These effects are as expected for an inhibitor of the ATP synthase without unwanted side effects. Our observations are in agreement with previous results with illuminated cells (24, 25).

Inhibition of P_i uptake by DCCD was marked even at low DCCD levels (Fig. 2B). A comparison with the effect of DCCD on serine uptake (Fig. 1B) again shows that a $\Delta\mu_{Na^+}$ existed in the presence of the inhibitor and could drive amino acid but not phosphate uptake. Inhibition could be largely relieved by the addition of arginine (Fig. 3), which mediates substrate-level phosphorylation in *H. halobium* (12, 13) with a consequent increase in cellular ATP levels (Table 1). The experiment also showed that DCCD did not inhibit P_i transport by reacting with the carrier itself.

In several experiments, short time lags in phosphate uptake were evident. The lag developed progressively in time after cell harvest. Figure 4 shows the behavior of starved cells with respect to phosphate uptake. No phosphate was taken up either in the dark or under illumination; uptake began only after a variable lag, sometimes as long as 45 to 50 min, upon the addition of glycerol. Interestingly, glycerol caused an immediate return of the depressed ATP levels of starved cells to the values found in fresh cells

FIG. 4. Phosphate uptake by starved cells and by cyanide-poisoned fresh cells. Filtration technique. The incubation medium (see Materials and Methods) contained 50 mM Tris-MOPS (pH 6) and 50 μ M [32 P]P_i. Starved cells (●, ○, △): light was turned on, when indicated, to illuminate one suspension (△), while the control (○) always remained in the dark and did not receive glycerol. Glycerol (20 mM) and 7.5 μ M 3,5-di-*tert*-butyl-4-hydroxybenzylidenemalonitrile plus 9 mM P_i were added when indicated, to the third incubation (●). The rate of [32 P]P_i uptake after the glycerol addition (●) was 0.18 nmol per mg of protein per min. Fresh cells (□): the same incubation medium was used, plus 5 mM NaCN. The suspension was illuminated, and DCCD (0.4 mM) was added as indicated. The rate of [32 P]P_i uptake during the first illumination was 0.078 nmol per mg of protein per min.

(Table 1), as well as an immediate increase in $\Delta\psi$ and proton ejection (data not shown). These effects were obtained also by illumination (Table 1; data not shown) (9, 25) without, however, any P_i uptake. On the other hand, illumination completely restored phosphate uptake (Fig. 4), as well as $\Delta\psi$ (data not shown) and ATP levels (Table 1), in fresh cells inhibited with cyanide, which resemble starved cells in having lower ATP (Table 1) and $\Delta\psi$ than untreated fresh cells.

DISCUSSION

The experiments presented show that P_i uptake by whole, freshly isolated *H. halobium* cells grown in about 0.5 mM P_i depends on cellular ATP levels. Uptake was inhibited by uncouplers or by the lipophilic cation TPP, both of which severely depressed cellular ATP levels. Inhibition occurred despite the presence of a sizeable $\Delta\bar{\mu}_{\text{Na}^+}$, which is competent to drive amino acid uptake, as well as, in the case of TPP, the presence of a large ΔpH (Fig. 1). In the experiments involving inhibition of P_i uptake by DCCD and its reestablishment by arginine addition (Fig. 2 and 3), DCCD-treated cells had higher $\Delta\psi$ and ΔpH than untreated cells did and a comparable $\Delta\bar{\mu}_{\text{Na}^+}$. Yet phosphate uptake was strongly inhibited, presumably as a consequence of the lowered intracellular ATP levels. When intracellular ATP was increased by arginine fermentation, uptake was restored.

At low cellular ATP levels, the cell membrane appears to possess a low permeability to phosphate, which is taken up or released only very slowly (Fig. 1A). Even under the most favorable conditions, efflux of [32 P]P_i was slower than might have been expected on the basis of both the observed rates of uptake by energized cells and the size of the inner P_i pool. This result agrees with the observations of Melhorn et al.

(23) and Helgersen et al. (13) regarding the impermeability of vesicles to phosphate and the constancy of the cellular P_i pool. At normal cellular ATP levels, [32 P]P_i flows appeared to take place mostly toward the cell interior, as judged from the lack of [32 P]P_i efflux after the addition of excess unlabeled phosphate. This irreversibility of the uptake is

TABLE 1. Effects of various treatments on the ATP content of *H. halobium* cells

Cell type	Addition(s)	% ATP content ^a (n)
Fresh	None	99 $\pm$ 5 (5)
	Light	102 $\pm$ 8 (5)
	Glycerol (10 mM)	100 ^b
	FCCP (10 μ M)	27 $\pm$ 9 (2)
	TPP (0.2 mM)	47 $\pm$ 11 (3)
	NaCN (5 mM)	59 $\pm$ 15 (4)
	NaCN (5 mM), light	96 $\pm$ 4 (4)
	DCCD ^c (0.1 mM)	20 $\pm$ 3 (5)
Starved	DCCD ^c (0.1 mM), 5 mM arginine	86 $\pm$ 9 (3)
	None	47 $\pm$ 5 (5)
	Light ^d	98 $\pm$ 7 (3)
	Glycerol ^d (10 mM)	100 ^{b,e}

^a The bacteria were incubated in basal salts at 20 to 22°C for 10 to 30 min. The data are expressed as percentages of glycerol-supplemented control cells (measured for each preparation). The ATP content of controls for both fresh and starved cells was 2.5 to 5 nmol per mg of protein. Figures given are averages of *n* measurements on different cell preparations $\pm$ the standard (or average, for *n* = 2) deviation.

^b By definition. Control.

^c The cells were pretreated with 0.1 mM DCCD for 4 h.

^d The increase caused by illumination or glycerol addition took place within 1 min.

^e The ATP content of starved, glycerol-supplemented cells was measured as 107 $\pm$ 4% (*n* = 2) of that of the same cells (plus glycerol) immediately after harvest.

unexpected for simple, unregulated mobile-carrier or channel-based transport mechanisms.

These results might be interpreted as pointing to the existence of a transport ATPase which would hydrolyze ATP to transfer P_i across the cellular membrane. However, this conclusion is unwarranted: ATP might act to regulate P_i transport rather than to energize it. An example of regulation of transport by ATP, via binding to the carrier, has recently been described (7). Regulation might also be achieved via less direct mechanisms. Bacteria in many cases take up substrates precisely at the rate at which they are utilized for cell growth. The mechanism of this control has not been described, to our knowledge, but it does not necessarily involve repression of protein synthesis (22). Clearly, this type of regulation would result in the apparent requirement of ATP for transport, because ATP is necessary for metabolism and cell growth. P_i uptake can be thus regulated: such is apparently the case for *Escherichia coli* grown in P_i -rich media (22, 30). That this type of regulation might be operating in our study is suggested by the low V_{max} of P_i uptake by fresh cells, which is at least an order of magnitude lower than values reported for other bacterial P_i transport systems (1, 2, 28, 34). The fact that the maximal rate of uptake was higher in starved than in fresh cells also conforms to a recognized pattern (22).

If ATP played only a regulatory role, concentrative phosphate uptake would have to be driven by another energy source, presumably a transmembrane ion (electro)chemical gradient. Harold and Spitz (10) have presented such a model for P_i transport in *Streptococcus faecalis*. P_i uptake in that organism was proposed to take place in exchange for OH^- , under the control of cellular ATP levels. Thus, the intriguing possibility still remains that in *H. halobium* phosphate transport might be driven by the sodium gradient, under the control of cellular ATP.

ACKNOWLEDGMENTS

We thank G. F. Azzone for his interest, R. Jacobs and C. Paradisi for nuclear magnetic resonance measurements, B. Schobert for the use of her TPP electrode, and F. Engqvist for technical support.

M.Z. was the grateful recipient of a Fulbright Travel Grant and of the Assunta Baccarini-Melandri fellowship administered by the Italian Group for Bioenergetics. The work was financed partly by Public Health Service grant GM 29498 from the National Institutes of Health.

LITERATURE CITED

- Alfasi, H., D. Friedberg, and I. Friedberg. 1979. Phosphate transport in arsenate-resistant mutants of *Micrococcus lysodeikticus*. J. Bacteriol. 137:69-72.
- Ando, A., S. Irie, L. M. Masuda, T. Matsushita, T. Fujii, M. Yabuki, and I. Kusaka. 1983. H^+ or K^+ -dependent transport systems of phosphate in alkalophilic bacillus. Biochim. Biophys. Acta 734:290-294.
- Azzone, G. F., D. Pietrobon, and M. Zoratti. 1984. Determination of the proton electrochemical gradient across biological membranes. Curr. Top. Bioenerg. 13:1-77.
- Bakker, E. P., H. Rottenberg, and S. R. Caplan. 1976. An estimation of the light-driven proton pump as sources of energy for active glutamate transport in *Halobacterium halobium*. Biochim. Biophys. Acta 440:557-572.
- Belliveau, J. W., and J. K. Lanyi. 1977. Analogies between respiration and a light-driven proton pump as sources of energy for active glutamate transport in *Halobacterium halobium*. Arch. Biochem. Biophys. 178:308-314.
- Belliveau, J. W., and J. K. Lanyi. 1978. Calcium transport in *Halobacterium halobium*. Arch. Biochem. Biophys. 186:98-105.
- Carruthers, A. 1986. ATP regulation of the human red cell sugar transporter. J. Biol. Chem. 261:11028-11037.
- Danon, A., and S. R. Caplan. 1976. Stimulation of ATP synthesis in *Halobacterium halobium* R1 by light-induced or artificially created proton electrochemical potential gradients across the cell membrane. Biochim. Biophys. Acta 423:133-140.
- Danon, A., and W. Stoeckenius. 1974. Photophosphorylation in *Halobacterium halobium*. Proc. Natl. Acad. Sci. USA 71:1234-1238.
- Harold, F. M., and E. Spitz. 1975. Accumulation of arsenate, phosphate, and aspartate by *Streptococcus faecalis*. J. Bacteriol. 122:266-277.
- Hartmann, R., and D. Oesterhelt. 1977. Bacteriorhodopsin-mediated photophosphorylation in *Halobacterium halobium*. Eur. J. Biochem. 77:325-335.
- Hartmann, R., H. D. Sickinger, and D. Oesterhelt. 1980. Anaerobic growth of halobacteria. Proc. Natl. Acad. Sci. USA 77:3821-3825.
- Helgerson, S. L., C. Requadt, and W. Stoeckenius. 1983. *Halobacterium halobium* photophosphorylation: illumination-dependent increase in the adenylate energy charge and phosphorylation potential. Biochemistry 22:5746-5753.
- Helgerson, S. L., and W. Stoeckenius. 1985. Transient proton inflows during illumination of *Halobacterium halobium* cells. Arch. Biochem. Biophys. 241:616-627.
- Krulwich, T. A. 1986. Bioenergetics of alkalophilic bacteria. J. Membr. Biol. 89:113-125.
- Lanyi, J. K. 1978. Light energy conversion in *Halobacterium halobium*. Microbiol. Rev. 42:682-706.
- Lanyi, J. K. 1978. Coupling of aspartate and serine transport to the transmembrane electrochemical gradient for Na^+ in *Halobacterium halobium*. Translocation stoichiometries and apparent cooperativity. Biochemistry 17:3011-3018.
- Lanyi, J. K. 1979. The role of Na^+ in transport processes of bacterial membranes. Biochim. Biophys. Acta 559:377-397.
- LeBel, D., G. G. Poirier, and A. R. Beaudoin. 1978. A convenient method for the ATPase assay. Anal. Biochem. 85:86-89.
- MacDonald, R. E., R. V. Greene, and J. K. Lanyi. 1977. Light-activated amino acid transport systems in *Halobacterium halobium* envelope vesicles: role of chemical and electrical gradients. Biochemistry 16:3227-3235.
- MacDonald, R. E., and J. K. Lanyi. 1975. Light-induced leucine transport in *Halobacterium halobium* envelope vesicles: a chemiosmotic system. Biochemistry 14:2882-2889.
- Medvecsky, N., and H. Rosenberg. 1970. The phosphate-binding protein of *Escherichia coli*. Biochim. Biophys. Acta 211:158-168.
- Melhorn, R. J., B. Schobert, L. Packer, and J. K. Lanyi. 1985. ESR studies of light-dependent volume changes in cell envelope vesicles from *Halobacterium halobium*. Biochim. Biophys. Acta 809:66-73.
- Michel, H., and D. Oesterhelt. 1976. Light-induced changes of ΔpH and $\Delta \psi$ in *Halobacterium halobium*. FEBS Lett. 65:175-178.
- Michel, H., and D. Oesterhelt. 1980. Electrochemical proton gradient across the cell membrane of *Halobacterium halobium*: effect of DCCD, relation to intracellular ATP, ADP and P_i concentration, and influence of the K^+ gradient. Biochemistry 19:4607-4614.
- Michel, H., and D. Oesterhelt. 1980. Electrochemical proton gradient across the cell membrane of *Halobacterium halobium*: comparison of the light-induced increase with the increase of intracellular ATP under steady-state illumination. Biochemistry 19:4615-4619.
- Oesterhelt, D., and W. Stoeckenius. 1974. Isolation of the cell membrane of *Halobacterium halobium* and its fractionation into red and purple membrane. Methods Enzymol. 31:667-678.
- Rosenberg, H., R. G. Gerdes, and K. Chegwidden. 1977. Two systems for the uptake of phosphate in *Escherichia coli*. J. Bacteriol. 131:505-511.
- Rosenberg, H., R. G. Gerdes, and F. M. Harold. 1979. Energy coupling to the transport of P_i in *E. coli* K12. Biochem. J. 178:133-137.

30. Russell, L. M., and H. Rosenberg. 1980. The nature of the link between K^+ transport and P_i transport in *E. coli*. *Biochem. J.* **188**:715–723.
31. Seely, R. J., and D. E. Fahrney. 1983. A novel diphospho-P,P'-diester from *Methanobacterium thermoautotrophicum*. *J. Biol. Chem.* **258**:10835–10838.
32. Wagner, G., R. Hartmann, and D. Oesterhelt. 1978. Bacteriorhodopsin-mediated photophosphorylation in *Halobacterium halobium*. *Eur. J. Biochem.* **89**:169–179.
33. Willsky, G. R., R. L. Bennett, and M. H. Malamy. 1973. Inorganic phosphate transport in *Escherichia coli*: involvement of two genes which play a role in alkaline phosphatase regulation. *J. Bacteriol.* **113**:529–539.
34. Willsky, G. R., and M. H. Malamy. 1980. Effect of arsenate on inorganic phosphate transport in *Escherichia coli*. *J. Bacteriol.* **144**:366–374.