ATP-linked Sodium Transport in *Streptococcus faecalis*  

II. ENERGY COUPLING IN EVERTED MEMBRANE VESICLES*  

Donald L. Heefner, Hiroshi Kobayashi, and Franklin M. Harold  

From the Division of Molecular and Cellular Biology, National Jewish Hospital and Research Center, Denver, Colorado 80262 and Department of Biochemistry, Biophysics and Genetics, University of Colorado School of Medicine, Denver, Colorado 80262

Everted membrane vesicles of *Streptococcus faecalis* accumulated \(^{22}\text{Na}^+\) to a nominal concentration gradient of about 100 when incubated with ATP and \(\text{Mg}^{2+}\). The characteristics of \(^{22}\text{Na}^+\) accumulation varied in a complex manner as a function of the pH and of the ionic environment. To illustrate, when the vesicles were prepared in potassium maleate:Hepes:sucrose buffer (0.45 N \(\text{K}^+\)) and incubated in the same buffer at pH 7.4, uptake of \(^{22}\text{Na}^+\) was specifically dependent upon ATP and inhibited by \(\text{N},\text{N}'\)-dicyclohexylcarbodiimide (DCCD) and by proton conductors; when the same vesicles were incubated at pH 8, uptake of \(^{22}\text{Na}^+\) was resistant to the inhibitors. When the vesicles were incubated at pH 7.4 in Tris:maleate:sucrose buffer, \(^{22}\text{Na}^+\) uptake was again unaffected by the inhibitors; moreover, ADP and UTP now supported uptake almost as well as did ATP. The results suggest that there are two modes of \(^{22}\text{Na}^+\) uptake, one dependent upon the proton-motive force (interior positive and acid) and the other upon ATP. Independent support comes from studies on revertants of mutant 7683, known to lack \(\text{Na}^+\) transport in both intact cells and membrane vesicles. Revertants that regained this capacity fell into two classes: in one, \(\text{Na}^+\) movements appear to depend primarily upon the proton-motive force, in the other upon ATP. The present results, as well as those from intact cells (Heefner, D. L., and Harold, F. M. (1980) *J. Biol. Chem.* 255, 11396-11402) can be rationalized by the hypothesis that *S. faecalis* contains two transport systems for sodium: one a primary, ATP-driven sodium pump and the other a secondary porter linked to the proton circulation but activated by ATP. However, we consider it more likely that these are two modes of sodium transport mediated by a single, ATP-linked system.

---

The experiments described in the preceding paper (Heefner and Harold, 1980) led us to conclude that sodium movements in *Streptococcus faecalis* cannot be ascribed to a secondary \(\text{Na}^+/\text{H}^+\) antipporter. We proposed instead that the cells establish a circulation of sodium across the membrane: sodium enters the cells down the electrochemical potential gradient by a route of low affinity, probably a relatively nonspecific "leak." Sodium extrusion is mediated by a distinct transport system that is selective for \(\text{Na}^+\) and requires "ATP" (either ATP itself or a related metabolite), even when the \(\text{Na}^+\) moves outward down a steep concentration gradient. Under certain conditions, "ATP" is a sufficient energy source for sodium extrusion against the concentration gradient; under others a proton-motive force is required as well. In this paper we describe studies on sodium transport with everted membrane vesicles, undertaken to establish the chemical identity of the effector and to clarify its interaction with the proton circulation.

**MATERIALS AND METHODS**

Organisms and Media—*S. faecalis* (faecium) ATCC 9790, and mutant 7683, were grown as described in the companion paper (Heefner and Harold, 1980). Spontaneous revertants were selected by plating 5 x 10\(^7\) cells of 7683 on NaTY medium, pH 8; colonies were picked after 3 days at room temperature and recloned before use. Sodium movements in intact cells were followed as described previously (Heefner and Harold, 1980).

**Membrane Vesicles**—The procedure was modified from that described by Kobayashi et al. (1978). Cells were grown overnight in 1 liter of NaTY medium (7683 was grown on KTY), washed twice with 200 ml of 2 mM MgSO\(_4\), and suspended in 40 ml of 0.5 mM glycyglycine buffer (pH 7.3) containing 2 mM MgSO\(_4\), 10 mM mercaptoethanol, and 20 mg of lysozyme. The suspension was incubated at 37°C for 60 min and the protoplasts collected by centrifugation (30,000 x g, 10 min at 4°C). They were then suspended in 10 ml of ice-cold buffer containing K\(^+\) maleate:K\(^+\) Hepes:sucrose:MgSO\(_4\):mercaptoethanol (0.2 mM:0.05 mM:0.25 mM:10 mM; pH 7.4) and 0.1 mg of DNase. The suspension was homogenized by hand in a Potter-Elvehjem homogenizer while being kept in ice. The homogenate was passed through a chilled French pressure cell at 30,000 p.s.i. and then centrifuged at 4°C (twice for 20 min at 27,000 x g) to remove cell debris. The vesicles were then collected (90 min at 48,000 x g) and resuspended in 0.5 ml of ice-cold buffer (K\(^+\) maleate:K\(^+\) Hepes:sucrose:MgSO\(_4\); pH 7.4, as above); fresh vesicles were used for all the experiments. In some early experiments vesicles were prepared in buffer containing Tris:maleate:Tris/Hepes:MgSO\(_4\); (0.05 mM:0.25 mM:0.25 mM) but in general, the procedure described above gave the most active preparations. Characterization of the preparation, which consists of a mixture of right-side-out and everted vesicles, was reported by Kobayashi et al. (1978).

\(^{22}\text{Na}^+\) Uptake—Typically, vesicles suspended in K\(^+\) maleate:K\(^+\) Hepes:sucrose:MgSO\(_4\); were diluted into more of the same buffer, supplemented with \(^{22}\text{NaCl\,(10}\,\text{mM}\) and ATP (Mg\(^{2+}\) salt; 4.7 mM). Inhibitors and ionophores were added as required; the assay mixtures were stirred mechanically at room temperature and uptake was initiated by addition of the vesicles (0.6 to 1.1 mg/ml; final volume 1.5 ml). For sampling, 0.2-ml aliquots were diluted with 1.8 ml of buffer containing 50 mg of poly(l-lysine), mixed for 20 s, and filtered through Millipore filters, pore size 0.65 \(\mu\)m (Lancaster and Hinkle, 1978). The filters were washed once with 3 ml of buffer, dried, and counted in a gas-flow counter.

**Other Procedures**—ATPase activity was measured in the standard  

1 The abbreviations used are: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TCS, tetrachloroethylene; CCCP, carbonylcyanide m-chlorophenylhydrazone; DCCD, \(\text{N},\text{N}'\)-dicyclohexylcarbodiimide.
buffer as described by Harold et al. (1969). Quinacrine fluorescence was monitored as described by Kobayashi et al. (1978). Protein was determined by the Lowry procedure. Reagents were purchased from standard sources.

RESULTS

Accumulation of $^{22}\text{Na}^+$ by Everted Membrane Vesicles—

Fig. 1 documents the basic observations. French press vesicles, prepared and incubated in Tris/maleate buffer, pH 7.4, with MgATP and 10 mM $^{22}\text{Na}^+$, accumulated $^{22}\text{Na}^+$ to a nominal concentration gradient of about 100 (only about half the vesicles are everted, so the true concentration gradient is probably larger). Addition of the proton conductors TCS and CCCP elicited rapid efflux of the $^{22}\text{Na}^+$; DCCD also induced efflux but rather more slowly, as would be expected. Other experiments (not shown) indicated that preincubation of the vesicles with the inhibitors prevented $\text{Na}^+$ accumulation. ATP could be replaced by GTP, which is also a substrate for the proton-translocating ATPase, but not by CTP, UTP, ADP, AMP, or PP. These results are consistent with the interpretation that $\text{Na}^+$ accumulates by antiport for protons, with the proton-motive force (interior positive and acid) providing the driving force; they stand in sharp contrast to the conclusions drawn from intact cells in the preceding paper (Heefner and Harold, 1980).

If $^{22}\text{Na}^+$ accumulation were indeed mediated by a secondary $\text{Na}^+/\text{H}^+$ antiporter, one would expect an artificial pH gradient (interior acid) to substitute for ATP. Accordingly, vesicles were prepared in Tris/maleate buffer of pH 5.5 and diluted into buffer at pH 8.0. No $^{22}\text{Na}^+$ accumulation was seen, except when the sodium-ionophore monensin was also added (data not shown). Other experiments, in which we tried to impose both a pH gradient and a membrane potential (interior positive), were equally unsuccessful. Although hardly strong evidence, these negative results are consistent with indications from intact cells that the sodium transport system itself requires ATP.

Everted vesicles incubated with ATP and Mg$^2+$ generate the expected proton-motive force (interior acid and positive) as shown by the quenching of quinacrine fluorescence. Beck and Rosen (1979) showed that, in Escherichia coli vesicles, the addition of sodium partly reversed the quenching and attributed this to alkalization of the vesicles by Na$^+/\text{H}^+$ antiport. Similar results were obtained with our vesicles: sodium sulfate, but not potassium sulfate, elicited partial reversal of fluorescence quenching, albeit only at concentrations far higher than those used in transport studies (10 to 50 mM; data not shown).

Effect of Ionic Environment and pH on $^{22}\text{Na}^+$ Uptake by Vesicles—The ionic environment in the preceding experiments was very different from that found in intact cells, which often contain as much as 0.5 M K$^+$. Fig. 2A illustrates the accumulation of $^{22}\text{Na}^+$ by vesicles prepared and incubated in 0.2 M potassium maleate, at pH 7.4. The fact that the presence of 0.4 M K$^+$ did not inhibit the uptake of $^{22}\text{Na}^+$ (present at 10 mM) testifies to the selectivity of the transport system, but we could not obtain a satisfactory $K_a$. Once again, DCCD and TCS inhibited $^{22}\text{Na}^+$ accumulation, but less drastically than in the experiment of Fig. 1. Finally, when the experiment was repeated at pH 8.2 accumulation of $^{22}\text{Na}^+$ was much reduced, but entirely resistant to DCCD and to TCS (or to TCS plus valinomycin) (Fig. 2B).

It may be argued that under the conditions of Fig. 2B (0.4 M K$^+$ and pH 8.2), DCCD and the ionophores simply fail to block the proton-motive force, and that the observations are therefore of no significance. We believe this to be unlikely, for three reasons. First, we showed in the preceding paper that DCCD and TCS plus valinomycin are effective inhibitors of intact cells under these conditions. Second, DCCD was found to block both the hydrolytic activity of the ATPase, and quinacrine quenching, at pH 7.4 with 0.4 M K$^+$ present. At pH 8.2 both activities were too low to be measured directly, but vesicles treated with DCCD at pH 8.2 were subsequently found to be blocked at pH 7.4. Finally, we shall later describe experiments done with vesicles from revertants of mutant 7683, which are very sensitive to DCCD and to ionophores under these conditions. We therefore believe that, as in the case of intact cells (Heefner and Harold, 1980), $^{22}\text{Na}^+$ uptake under these conditions requires ATP but not the proton-motive force.

The effects of ionic conditions on the characteristics of $^{22}\text{Na}^+$ accumulation are even more strikingly illustrated by experiments in which vesicles prepared in 0.2 M K$^+$/maleate were then incubated in Tris/maleate buffer with $^{22}\text{Na}^+$; under these conditions, there should be gradients of both K$^+$ and maleate from the vesicle lumen outward. As shown in Fig. 3, there was a small basal uptake of $^{22}\text{Na}^+$ by the vesicles. Addition of Mg$^2+$ATP elicited excellent accumulation of $^{22}\text{Na}^+$, which was entirely resistant to inhibition by DCCD or

![Fig. 1. Accumulation of $^{22}\text{Na}^+$ by everted membrane vesicles, and its efflux in response to ionophores. Vesicles were prepared in Tris/maleate/sucrose buffer (0.05 M, 0.2 M, pH 7.4) and resuspended in the same buffer at 1.3 mg of protein/ml. $^{22}\text{NaCl}$ (10 mM) was added at 0 min, ionophores at 4 min (arrow). Symbols: (C) no additions; (O) MgATP, 5 mM, only; (A) MgATP plus 10 mM TCS; (A) MgATP plus 0.2 mM DCCD; (B) MgATP plus 10 mM CCCP; (B) MgATP plus 10 mM K$^+$ maleate and 2 mM valinomycin.

![Fig. 2. $^{22}\text{Na}^+$ accumulation by membrane vesicles in K$^+$ maleate buffer. Vesicles were prepared in K$^+$ maleate: Hepes-sucrose buffer, pH 7.4, as described under "Materials and Methods." Uptake was assayed by adding vesicles (to 5 mg/ml) to aliquots of the same buffer, either at pH 7.4 (A) or at pH 8.2 (B) supplemented with 10 μM $^{22}\text{NaCl}$ and the following additions: (C) no ATP; (O) ATP, 4.7 mM; (O) ATP plus 30 mM TCS; (O) ATP plus 0.5 mM DCCD (DCCD added at -10 min).]
ATP-linked Sodium Transport in Bacteria

Revertants of Mutants

Class R-1—Small colonies appeared with a frequency of 7/10° cells plated on NaTY medium, pH 8, at room temperature. These cells had recovered the ability to expel Na⁺ against a concentration gradient when supplied with glucose (Fig. 5A). However, they had not recovered the wild type phenotype, for "Na⁺" extrusion was inhibited by TCS plus valinomycin over the entire range from pH 6 to pH 8 (Fig. 5A; extrusion was also blocked by DCCD, not shown). Everted membrane vesicles, prepared and incubated in K⁺ maleate buffer at pH 7.4, accumulated "Na⁺" in presence of MgATP; accumulation was totally abolished by TCS plus valinomycin and by DCCD both at pH 7.4 (Fig. 5B) and at pH 8 (data not shown). Apparently, then, in both cells and vesicles of R-1, sodium transport depends on the proton-motive force. Experiments with intact cells analogous to those illustrated in Figs. 2 and

**Fig. 3.** "Na⁺" accumulation by vesicles prepared in K⁺ maleate but assayed in Tris/maleate. Vesicles were prepared in K⁺ maleate:Hepes:sucrose buffer as described in Methods. Uptake was assayed by adding vesicles (to 1 mg/ml) to aliquots of Tris/maleate: sucrose:MgSO₄ buffer (0.05 M:0.75 M: 5 mM, pH 7.4) supplemented with 10 µM "NaCl and the following additions: (⃝) no ATP (with or without DCCD); (bone) ATP only; (△) ATP plus 0.5 mM DCCD (added at 10 min); (A) ATP plus 0.03 mM TCS and 5 µM valinomycin.

by ionophores. Appropriate controls (not illustrated) showed that DCCD blocked both hydrolysis of ATP and generation of the proton-motive force (as judged by quinacrine quenching); once again, "Na⁺" accumulation depends on ATP but not on the proton-motive force.

There is another curious feature to these latter experiments. When vesicles were incubated in either Tris/maleate or potassium maleate buffer under the conditions of Figs. 1 and 2, ATP and GTP were the only nucleotides that supported "Na⁺" accumulation (Fig. 4A). However, in the case of K⁺-loaded vesicles suspended in Tris/maleate buffer, UTP and even ADP would serve as well (Fig. 4B). The following were inactive under all conditions: CTP, AMP, α,β-methylene adenosine 5'-triphosphate (AMP-(CH₂)₂PP), β,γ-methyleneadenosine 5'-triphosphate (AMP-P(CH₂)₂P), and β,γ-imidoadenosine 5'-triphosphate (adenylyl imidodiphosphate). It is not likely that either ADP or UTP takes the place of ATP as a generator of the proton-motive force: (i) neither was contaminated with ATP, as judged by paper chromatography; (ii) neither supported "Na⁺" uptake in the absence of ATP; (iii) neither supported quinacrine quenching; (iv) both were hydrolyzed very slowly by our vesicles; (v) finally, "Na⁺" uptake supported by ADP and UTP was again resistant to DCCD and to TCS plus valinomycin (data not shown).

We can, thus, discern two modes of "Na⁺" accumulation by vesicles. One mode, exemplified by Figs. 1 and 2A, requires the proton-motive force; since in this system the proton-motive force is itself generated by ATP hydrolysis we cannot demonstrate a direct involvement of ATP in sodium transport per se. In the second mode, illustrated by Figs. 2B, 3, and 4, "Na⁺" uptake is not inhibited by DCCD or by TCS and is judged to be independent of the proton-motive force. Whatever the mechanism may be, it clearly requires an appropriate nucleotide. Do these two modes reflect the presence of two distinct transport systems? Vesicles of mutant 7683 failed to accumulate "Na⁺" under any of these conditions. In the hope of dissecting out distinct transport systems we examined a series of revertants of this mutant that had recovered the capacity to grow on the usual Na⁺-rich medium NaTY.

**Fig. 4.** Efficacy of various nucleotides in supporting "Na⁺" uptake by membrane vesicles. A, vesicles were prepared in K⁺ maleate:Hepes:sucrose buffer, pH 7.4, as usual. Uptake was assayed by adding vesicles (to 1.1 mg/ml) to aliquots of the same buffer, pH 7.4, supplemented with 10 µM "NaCl and additions as listed below. B, the same vesicles added to buffer containing 50 mM Tris/maleate, 0.75 M sucrose, and 5 mM MgSO₄, pH 7.4, plus 10 µM "NaCl. Additions: (⃝) none; (bone) ATP, 4.7 mM; (△) CTP, 4.7 mM; (A) ADP, 4.7 mM (ADP points were recalculated from a separate experiment).

**Fig. 5.** Sodium transport by revertant R-1. A, intact cells. Potassium-loaded cells were suspended in 0.45 N K⁺ buffer (maleate:Hepes), pH 8.0, and supplemented with 20 µM "NaCl. After 1 h, glucose was added. (⃝) no glucose, TCS, 20 µM, and 4 µM valinomycin present throughout; (bone) no ionophores, plus glucose; (△) TCS and valinomycin, plus glucose. B, membrane vesicles. Membrane vesicles were prepared in K⁺ maleate:Hepes:sucrose buffer as described under "Materials and Methods," and assayed in the same buffer, pH 7.4, with 10 µM "NaCl and the following additions: (⃝) no ATP; (bone) ATP, 4.7 mM; (△) ATP plus 0.4 mM DCCD; (A) ATP plus 20 µM TCS and 4 µM valinomycin.

Sodium Transport by Cells and Membrane Vesicles from Revertants of Mutants 7683—Spontaneous revertants of 7683 isolated as described under "Materials and Methods" fell into two classes by colony size. Examination of six independent isolates confirmed the existence of two phenotypes.
ATP-linked Sodium Transport in Bacteria

We now must try to define the role of ATP in sodium transport and at this point we encounter serious ambiguity. The early stages of this research were guided by the working hypothesis (proposed also by Skulachev, 1978) that sodium is transported by a reversible, secondary Na"/H" antiporter, electroneutral or more likely electrogenic, that can move Na" against a concentration gradient at the expense of the proton-motive force but becomes "mobile" only in presence of ATP (one might, for example, envisage phosphorylation of the carrier protein, or its allosteric activation by ATP). This hypothesis seemed to be supported by the discovery that Na" accumulation by vesicles is strongly inhibited by proton conductors and by DCCD, a covalent inhibitor of the proton-translocating ATPase (Figs. 1 and 2A). However, it fails utterly to account for the findings, presented in the preceding paper, that, under certain circumstances (excess K" and alkaline cytoplasmic pH), intact cells can expel Na" against a concentration gradient of 100-fold in the absence of any measurable proton-motive force. Indeed, even with vesicles one can find conditions, again involving high levels of K" and an alkaline pH, under which Na" accumulation requires only ATP and is independent of the proton-motive force (Figs. 2 and 3). Even the nucleotide specificity is altered under some conditions (Fig. 4). The alternative hypothesis, that sodium extrusion is energized exclusively by ATP without any direct involvement of the proton-motive force, fares no better. Both in intact cells and in vesicles, ATP serves as sufficient energy donor only under stringent conditions of pH and K" concentration; other media (Figs. 1 and 2A; also Fig. 6 of the preceding paper) call for a proton-motive force. It appears, then, that an ATP-driven Na" pump would have to be restricted to rather special ionic environments; in most situations, sodium transport requires the collaboration of ATP and the proton circulation.

The two alternative modes of Na" extrusion may differ, not only with respect to the energy donor but also in theircounterion. It is clear from the work of Harold and Papineau (1972) that sodium extrusion does not carry positive charge outward. Sodium must move by exchange for a counterion which has not been rigorously identified but is probably H". Could Na" exchange directly for K"? We believe that at low concentrations of K" (5 mM or less) the answer is no. Direct exchange offers no explanation for the inhibition of cation movements by proton conductors or DCCD, and is inconsistent with the observation that Na" can exchange for a variety of ions (including H", Ca"+, and organic bases); conversely, cells can take up K" in the absence of any added Na" (Bakker and Harold, 1980). We therefore continue to maintain the simplest hypothesis, that Na" is expelled by exchange for protons which, in turn, are extruded by the proton pump (Fig. 7). But the case may be otherwise at high K" concentrations (50 mM and above): it may well be that under these conditions we see ATP-driven exchange of Na" for K".

In principle, all the data now at hand can be rationalized by the proposal that S. faecalis contains two sodium transport systems: one, a primary ATP-driven pump that exchanges Na" for H" or K" and requires an alkaline cytoplasmic pH; the other, a secondary Na"/H" antiporter that is energized by the proton circulation but "mobile" only in the presence of ATP. In an effort to test this hypothesis we turned to revertants of mutant 7683, which is unable to extrude sodium under all our conditions; by the above hypothesis this must be a double mutant that lacks both transport systems. We did, indeed, obtain two kinds of revertants. Class R-1, growing as small colonies on medium NaTY, exhibits the characteristics expected of a secondary Na"/H" antiporter (in presence of ATP only); neither cells nor vesicles were able to transport...
properties, and sufficiently closely related that both were lost.

S. faecalis has two pathways for sodium extrusion, one primary driven by ATP and the other a secondary Na'/H' antiporter.

Vesicles, however, were peculiar: they accumulated Na' only in the presence of both ATP and DCCD; uptake was resistant to uncouplers (Fig. 6). We do not understand the effect of DCCD, but one should keep in mind that the accumulation of Na' by the vesicles is the resultant of two processes, an inward pump and an outward leak. The latter, if electrophoretic (as proposed in the preceding paper) will be reduced by blocking the ATPase that generates a membrane potential, interior positive. This may possibly account for the finding that DCCD unmask ATP-driven sodium transport while blocking the secondary Na'/H' antiporter.

Evidently, there is considerable merit in the hypothesis that S. faecalis has two pathways for sodium extrusion, one primary driven by ATP and the other a secondary Na'/H' antiporter mobile only in presence of ATP; at least, one can resolve genetically the two carriers that the hypothesis predicts. But we find it difficult to accept the existence of two independent transport systems, endowed with such peculiar properties, and sufficiently closely related that both were lost in mutant 7683. Rather, we suspect that we are looking at two modes of sodium transport, one driven by ATP and the other by the proton-motive force, both mediated by a single transport system of modular structure (Fig. 7). On this basis it is possible that sodium transport in response to the proton-motive force, a striking phenomenon in membrane vesicles (Figs. 1 and 2A), may be an artifact arising from disruption of an ATP-driven Na'/H' exchange system during vesicle preparation. In fact, one wonders whether the widespread practice of studying metabolite transport solely in membrane vesicles may not blind us to complexities that would be revealed by concurrent work with intact cells.

An alternative speculation deserves mention, namely, that some transport systems can draw on both the electrochemical potential of protons and on ATP, either alternatively or simultaneously. By analogy, with the popular macroscopic transport carrier, we might term such hypothetical constructs "mopeds." It would be premature to consider possible mechanisms, although a recent article by Mitchell (1979) suggests points of departure. We may note, however, that candidates for "moped coupling" are not uncommon. Examples are the K' transport system of S. faecalis (Bakker and Harold, 1980); the analogous TrkA system of E. coli (Rhoads and Epstein, 1977; Epstein and Laimins, 1979); perhaps even the shock-sensitive transport systems of E. coli that feature periplasmic binding proteins and depend upon both "ATP" and the proton-motive force in a manner that has remained quite obscure (Plate, 1979, Honig et al., 1979; Ferenci et al., 1977).

By comparison with the simple scheme that sufficed to account for the original data (Harold and Papineau, 1972), bacterial cation exchange now appears to be quite a complex process. As a minimum we must invoke a vectorial ATP-linked system for sodium extrusion; a separate and relatively nonspecific leak pathway; and a potassium transport system that requires both "ATP" and the proton-motive force (Bakker and Harold, 1980). Whatever their mechanisms may prove to be, these are not elementary porters.

REFERENCES

Harold, F. M., Baarda, J. R., Baron, C., and Abrams, A. (1969) J. Biol. Chem. 244, 2261-2268
Plate, C. (1979) J. Bacteriol 137, 221-228