An ecf Mutation in Escherichia coli Pleiotropically Affecting Energy Coupling in Active Transport but Not Generation or Maintenance of Membrane Potential*

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A temperature-sensitive ecf mutant of Escherichia coli has been isolated and is described in this paper. The mutant is pleiotropically defective in the active transport of amino acids and β-methyl-D-thiogalactoside at 37° or above. A membrane potential of about −86 mV (interior negative), however, is established and maintained under conditions in which active transport is defective (pH 7.0 at 39°). In the wild type a membrane potential of −86 mV under these conditions is sufficient for active transport. This result indicates that the electrochemical gradient, although necessary, is not sufficient to drive the active transport of these substrates in the mutant.

At the nonpermissive temperature in the presence of glucose the initial rate of uptake for all solutes is normal. This uptake is always followed by efflux of the accumulated solute at a rate essentially identical with the proton conductance of the membrane. It has been established that the rapid dissipation of the membrane potential cannot be attributed to an increased membrane permeability to protons. During the last few years several laboratories have applied genetic methods to the problem of energy coupling in the hope of discovering necessary coupling factors other than those of the electron transport chain and the (Ca,Mg)-ATPase. Two such factors may actually exist in Escherichia coli as mutants have been isolated in this organism which are pleiotropically defective in active transport, yet normal in the (Ca,Mg)-ATPase and respiratory activities (6, 7). One of these was designated ECF (for energy coupling factor) and its genetic locus has been located at 64 min on the revised E. coli genetic map (8). Recently Lieberman et al. (8) have shown that the defect of the ecf mutant (MAL300) in transport is due to its inability to efficiently maintain the transmembrane electrical potential. Although the mechanism whereby the membrane potential is dissipated in the mutant is presently unknown it has been established that the rapid dissipation of the membrane potential cannot be attributed to an increased membrane permeability to protons.

This paper describes the isolation and characterization of a transport-defective ecf mutant which is capable of maintaining a chemical component according to the following relationship:

\[ \Delta \mu_H^+ = \Delta \Psi - (2.3 \frac{RT}{F}) \Delta \phi \]

where \( \Delta \Psi \) is the transmembrane electrical potential and \( \Delta \phi \) the chemical difference in proton concentrations across the membrane. The inward movement of solute molecules across the membrane against their concentration gradient is thought to occur via the obligatory coupling to the inward movement of protons (symport) or the outward movement of hydroxide ions (antiport) mediated by solute specific membrane-bound carrier molecules.

Implicit in this theory is the capability of the proton motive force as the direct, immediate driving force for the transport of solutes. Basically, the proton motive force is both necessary and sufficient for transport. Although there is no doubt that the \( \Delta \mu_H^+ \) is of prime importance as the source of energy, it remains to be established whether the coupling of the \( \Delta \mu_H^+ \) to active transport is a direct one as postulated in the chemiosmotic theory or an indirect coupling involving general coupling factors.

It is now generally accepted that energy coupling to bacterial membrane transport can best be explained in terms of the chemiosmotic hypothesis of Mitchell (1–3; for review, see Refs. 4 and 5). According to this hypothesis the direct driving force for active transport is the electrochemical gradient of protons (\( \Delta \mu_H^+ \)), which is composed of an electrical and a chemical component according to the following relationship:

\[ \Delta \mu_H^+ = \Delta \Psi - (2.3 \frac{RT}{F}) \Delta \phi \]

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This paper describes the isolation and characterization of a transport-defective ecf mutant which is capable of maintain-
ing the $\Delta \mu_m$. On the basis of this finding it is concluded that the coupling of $\Delta \mu_m$ to active transport appears to be an indirect process and requires the ECF protein.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Media**

The strains used were all derived from Escherichia coli K12 and are listed in Table I. The media used are nutrient broth and minimal salts E (11) supplemented with 0.5% glucose or 0.5% succinate and 40 $\mu$M thiamine. All plates contained 1.5% agar. The lactose operon was induced by including IPTG (0.5 mM) in the succinate medium.

**Growth and Preparation of Cells for Transport Assay**

Cells were grown on minimal glucose medium at 25$,^\circ$, harvested in midlog phase (2 to 4 $\times$ 10$^9$ cells/ml), washed twice with 0.1 M potassium phosphate, pH 7.0, containing 1 mM MgCl$_2$, and resuspended in the same plus 100 $\mu$g of chloramphenicol/ml at a density of 3 to 10$^9$ cells/ml. These cells were directly utilized for transport assays.

**Treatment of Cells for TPMP$^+$ Uptake**

To render cells permeable to TPMP$^+$, cells were treated as follows: Immediately after having been collected from the growth medium by centrifugation, they were washed once with 25 mM Tris/HCl, pH 7.3, then washed a second time with 0.1 M potassium phosphate, pH 6.0, and resuspended in the same to about 5 to 10 $\times$ 10$^9$ cells/ml. After a 4-min incubation at 25$,^\circ$, EDTA (K$^+$) was added to a final concentration of 10 mM and the incubation continued for 2 more min with swirling. The cells were then collected by centrifugation, washed twice with 0.1 M potassium phosphate buffer, pH 7.0 or 7.5, containing 1 mM MgCl$_2$, and finally resuspended in the same buffer plus 100 $\mu$g of chloramphenicol/ml at about 3.6 to 4 $\times$ 10$^9$ cells/ml ($\Delta \mu_m = 4.5$). Cells were stored at 0$.^\circ$. All transport assays were done within 1 h after cell preparation, otherwise the cells' transport ability rapidly declines.

**$\Delta p$H Measurement by Flow Dialysis Method**

Flow dialysis was performed according to the method of Ramos et al. (3). The apparatus used was similar to that described by Ramos et al. (3), except that it also had a water circulation jacket around the two chambers for temperature maintenance. Cells suspended in 5 mM potassium phosphate, pH 6.0, containing 100 mM KCl and 1 mM MgCl$_2$ were added to the upper chamber (total volume 0.8 ml), which was flushed with a water-saturated stream of oxygen during the duration of the experiment. The same buffer was pumped through the lower chamber at a rate of 6.0 ml/min using a Pharmacia pump P-30. Before the start of the dialysis, glucose was added to the cells at a final concentration of 2.8 mM, followed 10 s later by 5.5 ($\mu$Ci)dimethyloxazolidine (0.25 mM). Fractions of about 1.7 ml were collected and sampled for radioactivity by liquid scintillation spectrometry.

**Transport Assays**

Unless indicated otherwise the procedure described by Lieberman and Hong (6) was followed. Cells were always preincubated for 3 min at the assay temperature before the assay was performed. Millipore filters (0.45-$\mu$m pore size) were used for amino acid or sugar uptake assays and cellulose acetate filters (0.45-$\mu$m pore size) for TPMP$^+$ uptake. The buffer used to wash cells was always the same buffer in which the cells were resuspended for the transport experiments.

The specific activities and final concentrations of the radioactive substrates used were as follows: L-[U-14C]Alanine (159 Ci/mmol), 12.6 $\mu$M; L-[U-14C]Choline (236 Ci/mmol), 8.3 $\mu$M; L-[U-$\alpha$-$\beta$-$\gamma$-14C]lysine (312 Ci/mmol), 5.6 $\mu$M; L-[U-$\alpha$-$\beta$-$\gamma$-14C]serine (156 Ci/mmol), 12.9 $\mu$M; L-[U-$\alpha$-$\beta$-$\gamma$-14C]tyrosine (180 Ci/mmol), 37 $\mu$M; $\alpha$-methyl-$\alpha$-$\beta$-$\gamma$-[14C]glucopyranoside (198 Ci/mmol), 37 $\mu$M; 3-[14C]-thigalactoside (TMDG) (14.4 Ci/mmol), 100 $\mu$M; [3H]triphenylmethylphosphonium bromide (TPMP$^+$Br$^-$) (80 Ci/mmol), 0.4 mM.

**Synthesis of [3H]Triphenylmethylphosphonium Bromide**

The synthesis procedure was developed by D. Lipsich and M. A. Lieberman and is described by Lieberman (12). Basically, the synthesis was done in two steps. The first was the formation of [3H]TPMP$^-$ through the condensation of triphenylphosphine (P$_3$H$_3$) and [3H]methyl iodide (NEN). The second step was to exchange the I$^-$ for a Br$^-$ via passage of the [3H]TPMP$^+$ through an ion exchange column. The product was then recrystallized from a chloroform/benzene mixture.

**ApH Measurement by Flow Dialysis Method**

Step 1. CH$_3$I (80 mCi/mmol, 1.78 mg/cm$^3$, 45 mg), in benzene, was added to a 15% molar excess of P$_3$H$_3$ dissolved in benzene (1.0 ml final volume). The solution was stirred for 2 h at room temperature during which a precipitate (TPMP$^+$P$_3$) formed. The reaction flask was then stored overnight at 4$.^\circ$.

Step 2–The [3H]TPMP$^+$ was collected by filtration on a sintered glass funnel and the crystals recovered via solvation in absolute ethanol. The [3H]TPMP$^+$, in ethanol, was then placed on a Resyn 291 (Fisher) anion exchange column (1.5 x 14 cm) which had been pre-equilibrated with 500 ml of saturated sodium bromide (excess NaBr was removed by washing with approximately 1.0 liter of distilled water). The product was eluted from the column with 300 ml of distilled H$_2$O, and the H$_2$O removed by rotary evaporation. The CHCl$_3$-soluble material was recovered, the volume reduced, and the crystallization of [3H]TPMP$^+$Br$^-$ induced by the dropwise addition of benzene. A second crystallization was performed, the material dried, and the melting point taken. The melting point of TPMP$^+$Br$^-$ is about 190$,^\circ$, TPMP$^+$Br$^-$ 231–235$.^\circ$. For the material synthesized the melting point was 230–235$.^\circ$.

The synthesis was also done with nonradioactive CH$_3$I.

The following example analysis was obtained for the material from that synthesis:

C$_6$H$_5$BrP

Calculated: C 63.88, H 5.08, Br 22.37, I 0, P 8.67

Found: C 63.72, H 5.11, Br 22.39, I < 0.01, P < 0.34

**Isolation of ecf Mutants**

Since the ecf gene is closely linked with the metC gene, metC mutants were readily isolated by localized mutagenesis (13) using the metC mutant JSH211 as a recipient for transduction with the mutagenized phage. Strain JSH211 was grown overnight on nutrient broth at 25$,^\circ$, and CaCl$_2$ and MgCl$_2$ were added to the culture to a final concentration of 5 mM and 10 mM, respectively. Hydroxylation-mutagenized P1 phage lysate prepared according to the method of Cunningham-Rundles and Maas (14) was then added. After 15 min at 37$,^\circ$, cells were pelleted and resuspended in the same volume of minimal salts medium. Aliquots (0.2 ml) were spread onto minimal glucose plate lacking methionine and the plates were incubated at 25$.^\circ$. Only Met$^+$ colonies will grow on these plates, and about 20 to 40 $\times$ 10$^3$ small colonies appeared. The colonies were then transferred to a 43$^\circ$ incubator for 5 h, after which they were transferred back to 25$^\circ$ to allow further growth for 24 h. The resulting small colonies (which are presumably those unable to grow at 43$^\circ$) were then picked and spotted onto succinate and glucose plates. Succinate plates were incubated at 43$^\circ$ and glucose plates at 25$^\circ$, both for 2 days. A total of 790 small colonies were picked among 15,000 total Met$^+$ transductants, and 30 mutants were found to be unable to grow on succinate at 43$^\circ$. One of these is characterized in this paper.

Table I: Bacterial strains used

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>JSH211 F⁺ thi strA metC-69 metJ</td>
<td>From JSH210 (8) by resistance to ethionine according to the method of Shu and Greene (9)</td>
</tr>
<tr>
<td>JSH270 F⁺ thi strA metC-27*</td>
<td>This work, from JSH211 by localized mutagenesis</td>
</tr>
<tr>
<td>JSH212 F⁺ thi strA metC</td>
<td>Met⁺ transductant of JSH211</td>
</tr>
<tr>
<td>MAL300 F⁺ thi strA ecf-4* metC⁺</td>
<td>Ref. 8</td>
</tr>
<tr>
<td>MAL321 F⁺ thi strA ecf-9* metC⁺ ilv</td>
<td>Ref. 10</td>
</tr>
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Protein Determinations
Protein was determined by the method of Lowry et al. (15) using bovine serum albumin as the standard.

Materials
Except for [3H]TPMP+Br- all labeled compounds were obtained from New England Nuclear. CCCP was obtained from Sigma, cellulose acetate filters (EHWF0500) from Millipore.

RESULTS
Genetic and Growth Properties—The transport-defective mutant JSH270 is presumed to be an ecf mutant as its growth phenotype, reversion pattern, and mutational site are similar to those exhibited by the well characterized ecf mutant MAL300 (6, 8). JSH270 grows normally on glucose, succinate, fumarate, malate, d-lactate, or acetate as the sole carbon source at 25° but is unable to grow on any of these compounds or nutrient broth at 37° or above. The viability of JSH270 decreases following prolonged periods (>2 h) of incubation at the nonpermissive temperature. After overnight incubation at 42° on nutrient broth plates or minimal glucose or succinate agar plates supplemented with methionine, survivors (revertants) appear at a frequency at 10-2. These revertants are of two classes; Class I which does not require methionine for growth and Class II which are methionine auxotrophs (metC). The ecf−metC mutation is about 90% co-transducible with the metC gene.

Defective Transport—Fig. 1 shows the pleiotropic temperature-sensitive transport defect of JSH270. These assays were performed at 25° and 43° with washed cells in the absence of any exogenous energy source. The normal α-methylglucoside uptake at 43° by the mutant suggests that the transport defect is not due to a nonspecific membrane lesion.

The addition of glucose (1 mM) to the mutant cells at the nonpermissive temperature (37° and above) brings about a large stimulation in the uptake of solutes (proline, lysine). The extent of the stimulation by glucose is dependent upon the temperature (Fig. 2). Regardless of the temperature used the glucose stimulation is always immediately followed by a rapid efflux of transported solutes. As there was no significant difference in the amount of proline transiently accumulated when either 0.4 mM or 4 mM glucose was used, as shown in Fig. 3, it can be concluded that the onset of the efflux process is not due to a lack of cellular energy.

It is possible, however, that the onset of the efflux process could be due to either a build-up of glucose metabolites (which would be inhibitory to the retention of solute against a concentration gradient) or a disappearance of glucose metabolites (which would be necessary for the transport process to occur). In order to distinguish between these two possibilities cells were first preincubated for 8 min at 39° in the presence or absence of glucose (1 mM) and then assayed for their ability to transport proline. If no glucose was added at this point no uptake of proline was observed (Fig. 4). If, however, glucose (1 mM) was added after the 8-min incubation, accumulation of proline could be measured and the two possibilities distinguished. As shown in Fig. 4, preincubation with glucose reduced stimulation by glucose of the rate and extent of proline uptake. This result indicates that glucose uptake and utilization in the mutant under nonpermissive conditions prior to the transport assay can lead to an inhibition of proline uptake. The simplest explanation for this phenomenon is that a production of a glucose metabolite (or metabolites) is inhibiting the energy-coupling process in JSH270, and this inhibition is most likely responsible for the efflux of accumulated solutes.

Both Class I and Class II revertants of JSH270 regain at nonpermissive temperature the ability to grow on glucose, acetate, or other Krebs cycle intermediates as sole carbon sources as well as the ability to accumulate solutes (data not shown). This finding shows that the inability to retain accumulated solutes is due to the ecf mutation.

ΔΨ Is Maintained—ΔΨ was indirectly determined by measuring the uptake of the lipophilic cation TPMP+ in cells made permeable to TPMP+ as described under "Experimental Procedures." Fig. 5 shows the uptake of TPMP+, proline, and lysine by glucose-grown mutant (JSH270) and wild type
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FIG. 3 (top). Effect of glucose concentration on the stimulation of proline uptake by JSH270 cells at 39°. Uptake was carried out with glucose-grown (25°) cells in the presence of the following glucose concentrations: 0 mM (○); 0.4 mM (●); 1 mM (▲); 4 mM (△); Glucose was added to the cells 15 s before proline.

FIG. 4 (bottom). Effect of prior glucose metabolism on proline uptake by JSH270 cells at 39°. Glucose-grown cells (25°) were first incubated in the presence or absence of glucose (1 mM) for 8 min at 39° followed by [14C]proline or [14C]proline plus glucose (1 mM) at t = 8, and uptake of proline occurred with or without glucose at t = 0 but no glucose at t = 8 (○); without glucose at t = 0 but glucose added at t = 8 (●); glucose added at t = 0 and t = 8 (▲).

FIG. 5. Uptake of TPMP⁺, proline, and lysine by wild type (JSH212) and mutant JSH270 cells at 25° and 39°. Uptake was done with Tris/EDTA-treated, glucose-grown (25°) cells in the presence of 1 mM glucose which was added to the cells 15 s before radioactive substrate. For the calculation of the ratio [TPMP⁺]intr/[TPMP⁺]extr, the cell volume used was 3.0 µl for 1 ml of cell suspension which had an A₆₆₀ of 4.5 or 1 mg of cell protein/ml. TPMP⁺, ●; proline, ○; lysine, ▲.

FIG. 6. Uptake of TPMP⁺ and TMG by JSH212 and JSH270 cells at 39°. Uptake assays were done with Tris/EDTA-treated, succinate-grown (25°) cells in the presence of 1 mM glucose: A, JSH212, B, JSH270. TPMP⁺, ●; TMG, ○.
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Fig. 7. Effect of temperature shift (25° to 30°) on the accumulation of solutes. Tris/EDTA-treated, glucose-grown (25°) cells were used for the proline efflux experiment and Tris/EDTA-treated, succinate-grown (25°) cells which had been induced for the lac operon were used for TMG experiment. Cells were first allowed to accumulate substrate at 25° in the presence of 1 mM glucose for 7 min after which the temperature was shifted to 30°. The arrows indicate the time at which the temperature shift was made.

Fig. 8. Effect of CCCP on TPMP+ accumulation in JSH7270 cells. Tris/EDTA-treated, glucose-grown (25°) cells were used for this experiment.

medium. Calculations based on the method of Ramos and Kaback (17) yield an internal pH of 7.0 for the mutant and 7.1 for the wild type. On the basis of this data it is therefore concluded that the interior of the mutant cells remains alkaline relative to the external medium at the nonpermissive temperature.

It should be pointed out that the conditions used for the pH measurements differ considerably from those used for the ΔΨ measurements; the buffer used for the ΔpH studies is 5 mM potassium phosphate, pH 6, while that used for ΔΨ study is 100 mM potassium phosphate, pH 7.0. In addition, cells are approximately 13 times more concentrated in the ΔpH measurements than in the ΔΨ measurements. Although the data will not be shown here, cells resuspended in 5 mM potassium phosphate, pH 6.0, containing 100 mM KCl and 1 mM MgCl2 at 1 mg of cell protein/ml exhibit the same transport and ΔΨ properties as previously presented.

lac Carrier Is Not Altered—To demonstrate that the transport defect observed with JSH7270 is not due to an alteration of membrane carrier molecules the ability of the lac carrier protein (the M protein) to carry out the carrier-mediated diffusion of orthnitrophenyl-β-D-galactoside (ONPG) was ex-

Fig. 9. Comparison of amino acid efflux induced by CCCP and the ecf mutation. Tris/EDTA-treated, glucose-grown (25°) JSH212 cells were allowed to accumulate proline or lysine at 30° in the presence of 1 mM glucose for 7 min. CCCP (10 μM) was added and at various intervals thereafter the retention of amino acid by the cells determined. The data for the mutation induced efflux in JSH270 is that previously presented in Fig. 4. JSH212, ○; JSH270, ●.

Fig. 10. ΔpH measurement as determined by flow dialysis using DMO. The procedure and conditions used are described under "Experimental Procedures." Tris/EDTA-treated, glucose-grown (25°) cells were used for these experiments. The arrows indicate the point when CCCP (25 μM) was added to the upper chamber in the experiment indicated by ○. The experiment with JSH270 (A) was done with DMO at 0.3 mM (8.3 mCi/mmol). The experiment with JSH212 (B) was done with DMO at 0.25 mM (10 mCi/mmol). No CCCP added, ●; CCCP added, ○.
amined. The data obtained under conditions in which glucose was absent are summarized in Table II. Clearly, the carrier-mediated diffusion of ONPG is normal in the mutant JSH270 at 39° as compared to the wild type JSH312. In view of the fact that glucose was present in all the experiments in which efflux of accumulated solutes were observed, the effect of glucose metabolism on the carrier-mediated diffusion of ONPG was also examined with the mutant. The conditions used were similar to those for transport assays. Cells were first incubated with 1 mM glucose for 8 min at 39° and then assayed for their ability to hydrolyze ONPG in the presence or absence of sodium azide. Similar rates were obtained under these conditions as that obtained in absence of glucose (Table II). These results indicate that the M protein is not altered at the nonpermissive temperature with or without prior glucose metabolism.

Comparison with Other ecf Mutants—The mutant JSH270 displays a transport defect fundamentally different from the other well characterized ecf mutant MAL300 (8). The defect of the latter mutant is primarily due to its inability to efficiently maintain the membrane potential rather than its inability to couple energy to transport, as is the case with JSH270. Thus, at the nonpermissive temperature (43°) the mutant MAL300 can accumulate TPMP⁺ and amino acids only transiently if exogenously supplied energy is limiting. For the purposes of comparison the uptake of TPMP⁺, proline, and lysine by MAL300 was also carried out under similar conditions used for JSH270, and the results are shown in Fig. 11A. Another ecf mutant MAL321, which was isolated on the basis of resistance to colicin K (10), behaves similarly as MAL300 (Fig. 11B). Apparently MAL321 is also unable to retain solutes because of an inability of the cell to efficiently maintain the membrane potential.

**TABLE II**

Rates of ONPG hydrolysis

ONPG hydrolysis was performed according to the method of Rosen (18) utilizing a Zeiss spectrophotometer equipped with a constant temperature block. Cells were induced for the lac operon by growth in minimal medium plus succinate and 0.5 mM isopropyl-β-d-thiogalactoside. Assays were performed in 1.0 ml of 0.1 mM potassium phosphate, pH 7.0, containing 1 mM MgCl₂, 10 μM of NaN₃, and 1.5 x 10⁷ cells/ml. The final concentration of ONPG used was 2 nM.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>JSH212</th>
<th>JSH270</th>
</tr>
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<tbody>
<tr>
<td>25°</td>
<td>62</td>
<td>45</td>
</tr>
<tr>
<td>39°</td>
<td>282</td>
<td>319</td>
</tr>
<tr>
<td>39° + glucose*</td>
<td>266</td>
<td></td>
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</table>

*Performed with cells preincubated with 1 mM glucose for 10 min at 39°.

**Fig. 11.** Uptake of TPMP⁺, lysine, and proline by MAL300 and MAL321. Uptake was done with Tris/EDTA-treated, glucose-grown (35°) cells in the presence of 1 mM glucose. A, MAL300 (pH 7.6, 37°); B, MAL321 (pH 7.6, 39°). TPMP⁺, ○; lysine, △; proline, ▲.

**DISCUSSION**

Previous reports from this laboratory have suggested that the ecf and metC gene products may be required for the maintenance of the membrane potential (8). Surprisingly, the inability of the ecf mutant MAL300 to maintain the membrane potential cannot be attributed to the formation of a "proton hole" or to a nonspecific leakiness of the membrane. This finding has led to the view that the defect observed in this mutant may be due to other ion movements across the membrane and represents a novel lesion in energy transduction.

The results in this communication indicate that the electrochemical gradient of protons cannot, by itself, effect energy coupling without the participation of the ecf and metC gene products. This is inferred from the findings described here that an ecf mutation can be isolated which affects only active transport and not the ability to maintain the transmembrane potential. This finding implicates the ecf and metC gene products as essential elements for energy coupling, and therefore introduces new elements into this coupling process which must be taken into consideration by any model for active transport in *Escherichia coli*. Two possible models are discussed below.

Model I is a modification of the original chemiosmotic hypothesis which allows the coupling factor ECF to be accommodated in it and which still retains the chemiosmotic coupling concept. In the original chemiosmotic hypothesis (1), the transport solute carrier was assumed to serve as both a solute carrier and a proton symporter. In the present model, the ECF protein is postulated to be the proton symporter itself, which is distinct from the carrier. Both the ECF and carrier proteins are required to constitute a functional transport system. In addition, the ECF protein in this model is postulated as a common component to all transport systems which are driven directly by the proton motive force. The role of the ECF protein in energy coupling is to sense and respond to the proton motive force as a proton symporter, but not to transform the proton motive force into other energized forms as an energy transformer.

Model II is identical with Model I in terms of constitution. But the direct driving force for the energy-coupling to active transport in this model is postulated to be conformational energy rather than the electrochemical gradient of protons. In this model the ECF factor is postulated as an energy-transducing factor whose function is to transform the proton motive force into conformational energy which is then directly utilized to effect coupling. The role of the proton motive force in the energy coupling to active transport in this model is therefore an indirect one. This model is in principle similar to
the one put forth by Ji (19) for the mechanism of oxidative phosphorylation in which a hydrophobic protein, distinct from the (Ca,Mg)-ATPase, is hypothesized to act as a coupling factor. It must be pointed out here that the two models presented above are the simplest although not the only possibilities.

According to either one of these two models of energy coupling one should be able to isolate ecf mutants which are uncoupled due to either a faulty interaction between the ECF protein and the carrier (type I) or an inability to sense and respond to the electrochemical gradient of protons (type II). In addition, according to model II, but not model I, one should be able to isolate ecf mutants which are uncoupled due to either a dissipation of the gradient (type III) or an inability to transduce that gradient into conformational energy (type IV). JSH270 is an ecf mutant which may be uncoupled due to either a faulty interaction or to an insensitivity to the proton motive force or an inability in energy transduction described above (mutant type I, II, or IV). MAL300 and MAL321 are ecf mutants which are uncoupled due to their inability to maintain the membrane potential (mutant type III). Thus the conformational coupling theory as presented above can accommodate the data most easily.

It is not known why solutes efflux from cells when the cells have the ability to initially accumulate them under the nonpermissive conditions (Fig. 2). Nevertheless, the results presented in Fig. 4 suggest that the efflux can be attributed to an inhibition of the energy-coupling process by as yet unidentified glucose metabolites. If this is the case then the ecf mutation of the mutant JSH270 has to alter the ECF protein in a manner such that its functioning in energy coupling per se becomes sensitive to these metabolites. According to this view the coupling process would have to be completely inhibited approximately 1 min after the addition of glucose and remain so thereafter for at least 15 min. There is an indication, however, that the degree of the inhibition may gradually decrease with time since the addition of a second dose of glucose 8 min after the initial dose can bring about a second stimulation of proline uptake, albeit smaller in extent than the first (compare Figs. 3 and 4). It is not yet known whether the energy-coupling process is normally subject to inhibition or regulation by metabolites.

Acknowledgments—I thank Dr. Lieberman for making the [3H]TPMP+Br- used in this work and D. Liposh for developing the procedure that made the synthesis of this compound possible.

REFERENCES
An ecf mutation in Escherichia coli pleiotropically affecting energy coupling in active transport but not generation or maintenance of membrane potential.

J S Hong