The eup Genetic Locus of Escherichia coli and Its Role in H+/Solute Symport*

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A number of active transport systems in the bacterium Escherichia coli derive their energy from an electrochemical gradient of protons, the PMF (Δp) which is composed of a membrane potential (Δψ, inside negative) and a pH gradient (ΔpH, inside alkaline) according to the relationship:

$$\Delta p = \Delta \psi - Z \Delta \text{pH}$$

where Z is a coefficient for converting the transmembrane pH gradient to millivolts and equals 59 at 25 °C (4). It has been demonstrated that these transport systems, such as those for lactose and proline in E. coli, function as H+/solute symport systems; i.e. the movement of protons into the cell down their electrochemical potential gradient is coupled to the intracellular accumulation of proline or lactose up their respective chemical potential gradients (5-9). What remains to be elucidated is the molecular mechanism(s) by which the coupling of the PMF to a given transport system is achieved. We have previously reported the isolation and partial characterization of a mutant of E. coli that exhibits a complex phenotype (10). In contrast to the wild type strain of E. coli from which it was derived, this mutant was resistant to neomycin; insensitive to colicin K; unable to grow on nonfermentable carbon sources such as succinate, acetate, malate, or D-lactate; and was defective in the PMF-coupled transport of proline and TMG but normal in the binding protein-dependent transport of glutamine and arginine. These latter two transport systems are not coupled to the PMF but rather derive their energy from ATP or an energy source derived from ATP (11, 12), possibly acetyl phosphate (13). Oxidation of electron transport chain substrates was unimpaired in the mutant as was its ATPase activity. Reversion analysis indicated that all of the traits of this mutant were due to a mutation at a single site. We concluded at the time that the gene product of the eup locus, eup, is defective in certain proton motive force-coupled active transport systems (Plate, C. A. (1978) J. Bacteriol. 125, 467-474). Three independent mutations conferring this phenotype have now been mapped at a locus which lies between rha and pfkB near minute 87 on the E. coli linkage map. We have designated this locus eup for energy-uncoupled phenotype. A mutation in the eup locus is shown to result in diminished transport of proline, alanine, and methyl-D-thiogalactopyranoside while enhancing the transport of tyrosine. The eup mutation does not affect the ability of the strain harboring it to generate a membrane potential or a pH gradient. Rather, it appears to uncouple proton translocation from transport across the membrane and transport across the membrane and transport across the membrane and transport across the membrane (ApH, inside alkaline) according to the relationship:

$$\Delta p = \Delta \psi - Z \Delta \text{pH}$$

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§ The abbreviations used are: PMF, proton motive force; TMG, methyl-D-thiogalactopyranoside; EMS, methanesulfonic acid ethyl ester; TFMPI, triphenylmethylphosphonium ion; ONPG, o-nitrophenyl-D-galactopyranoside.

Experimental Procedures

Bacterial Strains and Media.—The bacterial strains used were all derived from E. coli K12 and are listed in Table I. Isolation of eup mutants after EMS mutagenesis (18) followed a previously described procedure (10). The media used were LB broth (19) or Okzuki medium base (20) supplemented with a carbon source (0.4%), thiamine (0.5 μg/ml), and required amino acids (50 μg/ml). Solid Okzuki minimal and LB media contained 1.5% agar.

Transductions—Bacteriophage P1 transduction was carried out by the method of Lemox (21).

Testing for Colicin K Sensitivity.—Each isolate to be tested was grown overnight at 37 °C in LB broth containing 0.4% glucose. Aliquots from these cultures were spotted onto LB plates containing colicin K, incubated overnight at 37 °C, and scored for growth. The plates containing colicin K were prepared by growing E. coli K235(Col

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**Table I**

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<tr>
<th>Strain</th>
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<td>MNNGμ mutagenesis (10)</td>
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<tr>
<td>M72</td>
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<td>E. Signer</td>
</tr>
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</tr>
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* For genetic nomenclature see Bachmann and Low (14).
μ MNNG, N-methyl-N'-nitro-N-nitrosoguanidine.

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**RESULTS**

**Mapping the eup Locus**—To locate the eup-1 mutation in strain CJ2 (previously designated B51 (10)), interrupted mating experiments were done which placed the mutation 3 to 4 minutes from ilu at approximately minute 87 on the E. coli linkage map (14). This location was confirmed by bacteriophage P1-mediated cotransduction—the mutation cotransduced with metB at a frequency of 21% (45/217) and with glnA at a frequency of 8% (20/258).

Additional eup mutants were isolated and one of these, strain CJ35(eup-4) was used to construct strain CJ39. Strain CJ39 served as the donor in a series of P1-mediated cotransduction experiments, the results of which are summarized in Fig. 1 and Table I and establish the location of the eup locus relative to known markers on the E. coli chromosome.

Mapping data obtained for strain CJ44(eup-5) are presented in Table III. Bacteriophage P1 was grown on CJ44 and used to transduce strain OSA30 to metB+. The metB+ transductants were then analyzed with respect to rha, eup, and glpK as unselected markers. The data place the mutation in strain CJ44 between rha and glpK, and the cotransduction frequency between the mutation and metB (17%) is consistent with the mutation having occurred in the eup locus.

One point needs to be made regarding the scoring of eup mutations. In many of our mapping experiments, eup mutations were scored as conferring a succinate-negative phenotype (Suc-) on a recipient strain. When the eup-4 and eup-5 mutations were transduced into strain OSA30, the Suc- phenotype was found to be suppressed. Transductants were obtained that were insensitive to colicin K, resistant to low levels of neomycin, and defective in proline transport. Moreover, the Suc- phenotype could be recovered when the eup-4 and eup-5 mutations were transduced from the OSA30 strain background into strain CJ29. Thus when OSA30 was used in crosses, eup mutations were scored as conferring either colicin K insensitivity or neomycin resistance to this recipient strain.

**Active Transport in Strain CJ44(eup-5)**—The ability of strain CJ44(eup-5) to accumulate seven different solutes was compared with its isogenic eup+ derivative strain CJ45. As previously reported for another eup mutant (10), the binding protein dependent uptake of glutamine and arginine was the same in both strains, as was the phosphoenolpyruvate-phosphotransferase-mediated uptake of α-methylglucoside (data not shown). The uptake of proline, alanine, tyrosine,
and TMG are all mediated by PMF-coupled transport systems (12, 25), and the uptake of these solutes by strains CJ45(eup +) and CJ44(eup-5) is shown in Fig. 2. Proline, alanine, and TMG uptake were all depressed in strain CJ44(eup-5) relative to the levels of uptake achieved for each of these solutes by CJ45(eup +). The uptake of tyrosine, on the other hand, was enhanced nearly 2-fold in strain CJ44(eup-5) as compared to CJ45(eup +). Tyrosine uptake in both strains was nearly completely inhibited by the presence of either unlabeled tryptophan or phenylalanine, indicating that the tyrosine uptake seen was being mediated by the general aromatic amino acid transport system (26, 27) (data not shown).

The data presented in Fig. 2 were obtained using glucose-grown cells. Glycerol grown cells yielded similar results for proline, alanine, and tyrosine uptake. TMG uptake was enhanced in the glycerol-grown cells relative to the glucose-grown cells, reaching a steady state level of 7.4 nmol/mg of cell protein, respectively. Thus lactose carrier function does not affect the levels of uptake achieved for each of these solutes by strains CJ45 and CJ44.

To ascertain whether the eup-5 mutation was affecting lactose carrier function, nonsense suppressor-bearing derivatives of strains CJ45(eup +) and CJ44(eup-5) were constructed and carrier-mediated ONPG hydrolysis was measured. The rate of carrier-mediated ONPG hydrolysis obtained for CJ45(eup +) was 0.91 A420 units/min/mg of cell protein, while CJ44(eup-5) yielded the lower rate of 0.38 A420 units/min/mg of cell protein. The levels of β-galactosidase present in both strains were comparable, as toluenized extracts of the eup + and eup-5 derivatives yielded ONPG hydrolysis rates of 9.4 A420 units/min/mg of cell protein and 7.4 A420 units/min/mg of cell protein, respectively. Thus lactose carrier function does not serve as a convenient measure of the ability of the vesicles to establish a pH gradient (inside acid) as a result of electron transport or proton translocating ATPase activity (23, 32, 33). Everted membrane vesicles of strain E. coli served as a convenient measure of the ability of the vesicles to establish a pH gradient (inside acid) as a result of electron transport or proton translocating ATPase activity (23, 32, 33). Everted membrane vesicles of strain E. coli served as a convenient measure of the ability of the vesicles to establish a pH gradient (inside acid) as a result of electron transport or proton translocating ATPase activity (23, 32, 33). Everted membrane vesicles of strain E. coli served as a convenient measure of the ability of the vesicles to establish a pH gradient (inside acid) as a result of electron transport or proton translocating ATPase activity (23, 32, 33). Everted membrane vesicles of strain E. coli served as a convenient measure of the ability of the vesicles to establish a pH gradient (inside acid).
Fig. 3. Uptake of TPMP\(^+\) and proline by Tris-EDTA-treated cells of CJ45(eup\(^+\)) and CJ44(eup-5). Cultures were grown in minimal medium with glucose as carbon source and treated with Tris-EDTA as described under “Experimental Procedures.” TPMP\(^+\) and proline uptake by the Tris-EDTA-treated cells was measured as described under “Experimental Procedures.” The specific activity and initial concentrations of the radiolabeled solutes were: A, [methyl-\(^3\)H]TPMP\(^+\) (3.59 Ci/mmol), 5 μM; and B, L-[\(^1\)\(^4\)C]proline (10.6 μCi/μmol), 8.5 μM.

Fig. 4. Energy-dependent quenching of quinacrine fluorescence by everted membrane vesicles prepared from strains CJ45(eup\(^+\)) (A) and CJ44(eup-5) (B). Incubation mixtures contained 10 mM Tris-HCl (pH 8), 5 mM MgCl\(_2\), 0.14 mM KCl, 0.2 μM quinacrine dihydrochloride, and 0.24 mg of membrane protein. D-Lactate (D-LAC), KCN, and ATP were added to final concentrations of 5, 2, and 0.5 mM, respectively.

CJ45(eup\(^+\)) and CJ44(eup-5) quenched quinacrine fluorescence to the same extent when incubated either with D-lactate as an oxidizable substrate or upon addition of ATP to vesicles inhibited in electron transport by 2 mM potassium cyanide (Fig. 4).

Thus the effects of the eup-5 mutation in strain CJ44 on PMF-coupled transport do not appear to be secondary to effects of the mutation on the formation of a membrane potential and/or pH gradient.

Apparent Lack of H\(^+/\)Solute Symport in CJ44(eup-5)—The fact that CJ44(eup-5) appeared normal in its ability to generate a PMF raised the possibility that the eup-5 mutation was affecting the coupling of either the membrane potential or the pH gradient to the affected transport systems. Thus the mutation appears to be affecting H\(^+/\)solute symport is suggested by the following findings.

Using cells of CJ44(eup-5) and CJ45(eup\(^+\)) that had been depleted of endogenous energy reserves, we found that an artificial pH gradient (outside acid) could drive TMG accumulation in strain CJ45(eup\(^+\)) but was ineffective in driving TMG accumulation in strain CJ44(eup-5) (Fig. 5). In the case of strain CJ45(eup\(^+\)), addition of HCl sufficient to reduce the external pH from 8 to 6 resulted in an increase in the intracellular TMG concentration to a level 14 times that of the extracellular concentration. With strain CJ44(eup-5), on the other hand, this same reduction in pH did not result in an increase in the intracellular TMG concentration above that present extracellularly. On the other hand, the lactose transport system was functional in the energy-starved CJ44(eup-5) cells, as indicated by their ability to take up TMG in the presence of D-lactate as energy source, albeit to a lesser extent than the CJ45(eup\(^+\)) cells (Fig. 6). The fact that CJ44(eup-5) cells were able to accumulate TMG against a concentration gradient in the presence of D-lactate but not when an artificial pH gradient was imposed was presumably due to the generation of a membrane potential as a result of D-lactate oxidation. This would suggest that in the mutant TMG uptake was responding only to the membrane potential and not to the ΔpH component of the PMF.

Fig. 5. TMG accumulation in response to an artificial pH gradient by energy-depleted cells of CJ45(eup\(^+\)) and CJ44(eup-5). Glycerol-grown cultures were starved of endogenous energy reserves as described under “Experimental Procedures.” Incubation mixtures at 27 °C contained 3 ml of cells (0.24 mg of cell protein) in 0.2 M potassium phosphate buffer (pH 8), chloramphenicol (100 μg/ml), and [methyl-\(^1\)\(^4\)C]TMG (10 μCi/μmol) at a final concentration of 50 μM. Aliquots were removed at the indicated times to determine the basal level of TMG accumulation. Then sufficient 2 mM HCl was added to reduce the pH from 8 to 5, additional aliquots were removed, and TMG uptake was determined. ——, external concentration of TMG.

Fig. 6. Effect of D-lactate on TMG uptake by energy-starved cells of CJ45(eup\(^+\)) (A) and CJ44(eup-5) (B). Glycerol-grown cultures were starved of endogenous energy reserves as described under “Experimental Procedures.” Incubation mixtures at 27 °C contained 1.0 ml of cells (0.08 mg of cell protein) in 0.2 M potassium phosphate buffer (pH 8) and chloramphenicol (100 μg/ml). At zero time, [methyl-\(^1\)\(^4\)C]TMG (10 μCi/μmol) was added to a final concentration of 50 μM and aliquots were removed at the indicated times to determine TMG uptake. When added, D-lactate was present at a final concentration of 20 mM. ——, external concentration of TMG.
Another indication that $H^+$/solute symport was being affected by the eup-5 mutation came from an examination of the effect of added TMG on the membrane potential of cells of strains CJ45(eup+)* and CJ44(eup-5). It has been shown using membrane vesicles prepared from E. coli cells that addition of lactose results in a partial depolarization of the membrane, due to proton entry in symport with lactose (30). As can be seen from the data presented in Fig. 7, addition of 1 mM TMG to Tris-EDTA-treated CJ45(eup+) cells also resulted in a depolarization of the membrane, as indicated by the transient efflux of TMG+ from the cells following TMG addition. Addition of 1 mM TMG to the CJ44(eup-5) cells did not elicit any significant efflux of TMG+ from the cells. This could reflect poor functioning of the lactose transport system in the mutant or it could mean that TMG uptake by the CJ44(eup-5) cells is electroneutral, i.e. occurs without proton cotransport. We favor the notion that the eup mutation results in an uncoupling of proton fluxes from TMG fluxes, since 1) significant lactose transport function is retained by the mutant, as measured both by TMG uptake and carrier-mediated ONPG hydrolysis; 2) a substantial TMG gradient was imposed; and 3) this would be consistent with an artificial pH gradient being unable to drive TMG accumulation in strain CJ44(eup-5).

A problem with both of these experiments is that the cells used were physiologically abnormal, in one case having been put through the starvation regime while in the other case having their permeability properties altered as a result of the Tris-EDTA treatment. In an effort to verify the above findings with physiologically normal cells, we examined the effects of adding unlabeled TMG to cells that had reached steady state in radiolabeled proline accumulation at pH 7.5. Since the driving force for proline accumulation is the membrane potential at this external pH (31), the influx of protons in cotransport with TMG might partially collapse this potential, resulting in proline efflux. The presence of lactose has been shown to partially inhibit proline uptake by E. coli membrane vesicles (30). Addition of 1 mM TMG to cells of strain CJ45(eup+) did result in an enhanced rate of proline exit compared to those cells not receiving TMG (Fig. 8). However, addition of 1 mM TMG to preloaded cells of strain CJ44(eup-5) did not enhance proline exit as compared to cells not receiving TMG.

The reciprocal experiment was also carried out, i.e. adding unlabeled proline to cells preloaded with radiolabeled TMG, and similar findings were obtained (Fig. 9). Addition of 1 mM proline to cells of CJ45(eup+) resulted in TMG exit and the establishment of a lower steady state level of TMG accumulation. Addition of proline to CJ44(eup-5) cells had no effect on the steady state level of TMG accumulation. The results obtained with the untreated cells are thus consistent with the conclusion that the eup-5 mutation in strain CJ44 results in a dissociation of proton and solute cotransport for two active transport systems that are coupled to the PMF, namely the proline and lactose transport systems.

**DISCUSSION**

Single site mutations that yield E. coli strains defective in PMF-coupled active transport, resistant to neomycin, insensitive to colicin K, and unable to grow on nonfermentable carbon sources have been shown to reside in a genetic locus near minute 57 on the E. coli linkage map (14) that we have designated eup. The eup locus maps in the same region as the ecfB locus described by Thorbjarnardottir et al. (34). While mutations in the ecfB locus also result in resistance to low levels of neomycin and poor growth on succinate, the question of whether the eup and ecfB loci are allelic remains open until complementation studies are performed.
While the relationship of the various phenotypic manifestations of eup mutations remains to be established, the present study does provide insight into a possible role for the eup gene product in chemiosmotically coupled transport systems. Efforts to elucidate the molecular mechanisms involved in chemiosmotic coupling to active transport systems have in large measure been focused on the lactose transport system of E. coli. It is now well established that this transport system mediates a coupled flux of lactose (or lactose analogs) and protons with a 1:1 stoichiometry (5,9,35). Lactose influx into energy-depleted E. coli cells is accompanied by an alkalization of the external medium (9,36), while lactose efflux from either energy-depleted intact cells (37) or membrane vesicles (8) gives rise to a membrane potential (inside negative). Lactose efflux in the presence of the proton specific ionophore carbonylcyanide-m-chlorophenylhydrazone does not give rise to a membrane potential, implicating protons as the charged species that exit with lactose (8,37). What has not been established, and is central to an understanding of how this as well as other PMF-coupled transport systems function, is how these proton movements are being mediated. It is in this capacity that we attribute a role for the eup gene product.

The findings of the present study suggest two possible functions for the eup gene product in PMF-coupled transport. On the one hand, a solute-specific PMF-coupled carrier (e.g. the lactose or proline carrier) may mediate the movements of both solute and protons and the eup gene product functions in the association and/or dissociation of the proton from the carrier. On the other hand, the solute-specific carrier may function only in solute translocation and the eup gene product functions as a proton symporter. While we presently cannot distinguish between these possibilities, both suggest that a functional PMF-coupled transport system in E. coli is an oligomeric structure comprised of at least two distinct polypeptide species. One of these polypeptides is specific for a given transport system and, in the case of the proline or lactose transport systems in E. coli, would be coded for by the proT or lacY genes, respectively. The second polypeptide species would be the product of the eup locus and would serve in a general capacity for various PMF-coupled transport systems. Either of these functions would account for the pleiotropic transport defect exhibited by the eup mutants described in this and a previous study (10).

A second class of mutants might also be predicted from this model. These would be mutants in which a specific solute carrier polypeptide would be altered such that it could no longer functionally interact with the eup gene product and the cotransport of protons with this specific solute would be abolished. Mutants of E. coli having this phenotype have been studied by Wong et al. (39) and Wilson and Kuschi (39). These investigators have isolated mutants specifically defective in the energy-dependent accumulation of lactose. The mutations in these strains have been mapped within the lacY gene and, while not affecting the ability of the carrier to translocate lactose, do result in an abolition of proton cotransport (40). The lacY mutations in these strains could be affecting a proton-binding site on the lactose carrier itself or they could be altering the interaction between the lactose carrier and the eup gene product. The availability of a method to solubilize and reconstitute the lactose transport system of E. coli in artificial liposomes (41) provides one means for differentiating between those possibilities and should enable us to define more precisely the role of the eup gene product in active transport in the near future.

The model that we are proposing for the role of the eup gene product in PMF-coupled transport in E. coli is analogous to one which has been proposed by Guffanti et al. (42) for Na+-dependent transport in Bacillus alcalophilus. These investigators have reported that a mutantional loss of Na+/H+ antiport activity in this organism leads to a pleiotropic defect in Na+-dependent substrate transport. It has been suggested that these transport systems share a common Na+-translocating subunit.

The product of another genetic locus in E. coli, the ecfA locus near minute 65, has also been implicated in coupling the PMF to active transport systems (43-45). One temperature conditional ecfA mutant has been isolated which, at the restrictive temperature, exhibits a pleiotropic active transport defect while generating and maintaining a normal membrane potential (44). There are no available data which indicate how the products of the ecfA and eup loci might interact in active transport, and excessive speculation seems unwarranted. However, one can conceive of a model in which the eup gene product mediates the coupling of the ΔpH component of the PMF to a given transport system while the ecfA gene product couples the Δψ component to that transport system. Resolution of this question should also be facilitated by reconstitution studies.

While tyrosine uptake is enhanced in strain CJ44(eup-5) relative to strain CJ45(eup+) is not known. The reason may be related to previous findings which indicate that the general aromatic amino acid transport system of E. coli appears to be considerably more resistant to anoxia or cyanide than the transport systems for proline, glycine, or alanine (46,47). This may reflect preferential coupling of the Δψ or ΔpH components of the PMF to different transport systems. Inhibition of respiration by cyanide results in a reduction of the ΔpH component of the PMF (48) while having relatively little effect on the Δψ component, as measured by TPMP+ uptake. Under these conditions, Δψ is presumably maintained by the proton translocating ATPase at the expense of glycolytically derived ATP. In fact, inhibition of phenylalanine uptake by cyanide closely parallels a decline in intracellular ATP levels while the decline in glycine uptake parallels the much more rapid inhibition of oxygen uptake (47). The availability of the eup mutants should facilitate the determination of whether or not such preferential coupling is indeed a reality.

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