Evidence for a Protonmotive Force Related Regulatory System in *Escherichia coli* and Its Effects on Lactose Transport†

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ABSTRACT: Strains of *Escherichia coli* with mutations in the *eup* (energy-uncoupled phenotype) locus do not grow on nonfermentable carbon sources, have reduced growth yields on limiting glucose, are insensitive to colicins A and K, exhibit resistance to aminoglycoside antibiotics, and are defective in protonmotive force coupled active transport. *eup* mutations do not result in lowered protonmotive force. Here we show that deenergization of a *eup*$^+$ strain results in the appearance of a new low K_T, low V_{max} form of the lactose carrier; in a strain deleted of the *eup* locus, deenergization does not evoke the low K_T, low V_{max} form of the lactose carrier. Cells bearing a *eup* point mutation and exhibiting the Eup$^-$ phenotype possess the low K_T, low V_{max} form of the lactose carrier even when energized. In addition to affecting the kinetic parameters of the lactose carrier, the *eup* point mutation also reduces the K_T and V_{max} of the proline carrier. On the basis of these findings, we suggest that the normal *eup* gene product mediates a novel regulation of lactose carrier function following deenergization. The defect in proline and lactose transport caused by *eup* point mutations may stem from an altered *eup* product aberrantly mediating the regulation under energized conditions. Finally, the pleiotropy asociated with *eup* point mutations may be indicative of those protonmotive force driven functions that are subject to *eup* regulation.

Studies carried out independently in three laboratories have resulted in the identification of a genetic locus in *Escherichia coli* which has been variously designated *eup*, *ecfB*, and *ssd* (Plate, 1976; Plate & Suit, 1981; Thorbjarnardottir et al., 1978, Morris & Newman et al., 1981, 1982). Single-site mutations in this locus result in lowered growth yields on limiting glucose, resistance to aminoglycoside antibiotics, reduced sensitivity to certain colicins (A and K) (Plate, 1976; Luria, 1982), inability to grow on nonfermentable carbon sources, and defective protonmotive force (PMF)-coupled active transport. Electron transport and BF_0F_1 ATPase hydrolytic activity are not adversely affected by *eup* mutations (Plate, 1976); neither is the ability to generate and maintain a PMF (Plate & Suit, 1981; Hitchens et al., 1982; Kashket, 1982). This phenotype, along with the finding that a *eup* mutation results in an apparent uncoupling of proton fluxes from thiomethyl β-D-galactopyranoside (TMG) and proline fluxes, led us to postulate that the role of the *eup* gene product is to function in coupling the PMF to the processes driven by it (Plate & Suit, 1981).

Subsequently in a series of elegant studies, Foster et al. (1982) have shown that highly purified lactose carrier protein carries out lactose/proton symport when incorporated into liposomes. Furthermore, lactose accumulation by these proteoliposomes can be driven by an artificially imposed pH gradient (inside alkaline) and/or a membrane potential (inside negative). They concluded from these findings that the coupling of the lactose carrier to the PMF is direct and need not involve the participation of other proteins.

In this report, we present findings that reconcile these disparate conclusions as well as provide new insight into the role of the *eup* locus in bacterial physiology. The availability of a *eup* deletion strain has enabled us to determine unequivocally that a *eup* gene product is not required for PMF-coupled active transport or the other PMF-coupled processes adversely affected by *eup* mutations. Yet kinetic studies comparing the influx of lactose and *o*-nitrophenyl β-D-galactopyranoside (ONPG) into strains carrying a *eup*$^+$ allele vs. a *eup* deletion reveal that a normal *eup* gene product functions in regulating lactose carrier activity under deenergizing conditions. The effect of *eup* mutations on lactose and proline transport and other PMF-coupled functions rendered defective by these mutations appear to stem from an altered *eup* product that mediates an aberrant form of this regulation.

Table I: Bacterial Strains Used

strain	genotype	source
CJ49	*lac*Z(Am) *trp*(Am) *thi*	Plate & Suit (1981)
CJ48	*lac*Z(Am) *trp*(Am) *thi eup*-5	Plate & Suit (1981)
CF877	HfrH *lac*Z *thi zih*-1::Tn10 Δ(*rha-pfk*A)15	D. Fraenkel
CJ57	*lac*Z(Am) *trp*(Am) *thi zih*-1::Tn10 (*rha-pfk*A)15	
CJ49s	As CJ49 but *sup*[a]	this study
CJ48s	As CJ48 but *sup*[a]	this study
CJ57s	As CJ57 but *sup*[b]	this study

[a] Isolated as Lac$^+$ Trp$^+$ colonies. [b] Isolated as described under Experimental Procedures.

Experimental Procedures

Bacterial Strains and Media. The bacterial strains used are listed in Table I. To measure lactose carrier mediated ONPG hydrolysis, it was necessary to overcome the *lac*Z(Am) defect in strains CJ49, CJ48, and CJ57. Therefore, the derivative CJ49s, CJ48s, and CJ57s strains were isolated, each carrying a nonsense suppressor. The CJ49s and CJ48s derivatives were isolated spontaneously on minimal media lacking tryptophan with lactose as the sole carbon source. A sup-

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pressor-containing derivative of strain CJ57[Δ(*rha-pfk*A)15] was isolated as a blue colony on minimal plates containing gluconate as the carbon source and no tryptophan and supplemented with isopropyl β-D-galactopyranoside (IPTG; 1 mM) and 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (40 μg/mL) (Miller, 1972). The media used were LB broth (Luria et al., 1960) or Ozeki medium base (OM) (Nagel de Zwaig & Luria, 1967) supplemented with a carbon source (0.4%), thiamin (0.5 μg/mL), and required amino acids (50 μg/mL). Induction of the *lac* operon was achieved by adding IPTG (1 mM). Solid OM and LB media contained 1.5% agar.

Transduction. Bacteriophage P1 transduction was carried out by the method of Lennox (1955).

Lactose and Proline Transport. Cultures were harvested by centrifugation, the cells were washed once with 50 mM potassium phosphate buffer (pH 6.6) containing 10 mM $MgSO_4$, and the washed cells were resuspended in this same buffer to yield cell suspensions containing 0.5–1.0 mg of cell protein/mL. Glycerol (0.4%) was added, and the cells were maintained on ice. Aliquots (100 μL) of cell suspension were preincubated at 27 °C for 30 s with shaking. [^{14}C]Lactose (1–4.6 Ci/mol; final concentration ranging from 0.27 to 40 μM) was then added, and 15 s later the cells were collected on a nitrocellulose filter (Millipore) and washed with 4 mL of 0.1 M LiCl. The filters were counted in a Packard liquid scintillation counter at 83% efficiency.

Hydrolysis of ONPG. Cells were washed twice and resuspended in OM containing chloramphenicol (100 μg/mL). For energized conditions, glycerol (0.4%) was added; for deenergized conditions, sodium azide (30 mM) was added. ONPG hydrolysis was continuously monitored at 420 nm with a Gilford recording spectrophotometer. Under the experimental conditions used the molar extinction coefficient was found to be 2330. The total β-galactosidase level was determined by using toluene-treated cells.

Protein Determinations. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Materials. [D-*glucose*-1-^{14}C]Lactose was obtained from Amersham/Searle and L-[U-^{14}C]proline from New England Nuclear. Tetracycline hydrochloride, amikacin, IPTG, and ONPG were obtained from Sigma Chemical Co. All other chemicals were reagent grade.

RESULTS

Properties of a eup Deletion Strain. The primary objective of this study was to establish the consequences of the absence of a *eup* gene product on PMF-driven active transport. Toward this end, we were aided by the availability of *E. coli* strain DF877 which is deleted of the *rha-pfk*A region of the chromosome (Daldal & Fraenkel, 1981). Previously we presented data consistent with the *eup* locus being located near minute 88 between the markers *rha* and *pfk*A (Plate & Suit, 1981; Bachmann, 1983). Thus, a strain having a deletion extending from *rha* through *pfk*A should be a *eup* deletion as well, and such a strain should manifest the effects of the total absence of *eup* gene product on such a function as active transport. An additional advantage is that strain DF877 carries the transposon Tn10 (tetracycline resistance) in close linkage to the site of the *rha-pfk*A deletion (44% cotransduction frequency, counterclockwise) (Daldal & Fraenkel, 1981). This allowed us to transfer the *rha-pfk*A deletion into the strain background we have used for most of our studies, minimizing the possibility that genetic differences between strains at chromosomal locations other than the *eup* locus have an effect on the manifestation of the *eup* null phenotype.

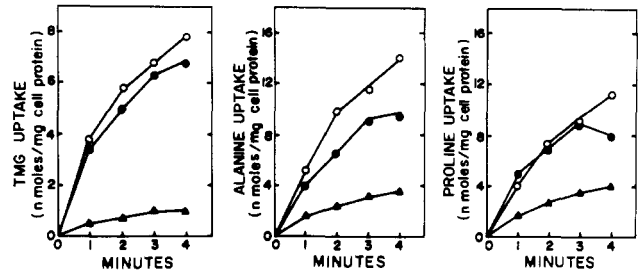

FIGURE 1: Active transport by strains CJ49, CJ48, and CJ57. Cultures of strain CJ49 (●), CJ48 (▲), and CJ57 (○) were grown in Ozeki medium base with gluconate as the carbon source. Active-transport assays were carried out as previously described (Plate & Suit, 1981). The specific activities and initial concentrations of the transport substrates were as follows: [*methyl*-^{14}C]TMG (10 μCi/μmol), 10 μM; L-[U-^{14}C]alanine (10.6 μCi/μmol), 9 μM; L-[U-^{14}C]proline (10 μCi/μmol), 10μM.

Accordingly, P1 lysates were prepared on strain DF877 and were used to transduce strain CJ49(*eup*$^+$) to tetracycline resistance (Tet^r). The Tet^r transductants were then scored for Δ(*rha-pfk*A)15 (no growth on rhamnose or mannitol as the carbon source) and for the Eup$^-$ phenotype (no growth on succinate and resistance to amikacin). All of the Tet^r transductants exhibited a Eup$^+$ phenotype, irrespective of having acquired Δ(*rha-pfk*A)15. While this result implies that the *eup* null phenotype is Eup$^+$, two additional possibilities need to be considered. One is that a *eup* suppressor mutation exists in strain DF877 that cotransduces with Δ(*rha-pfk*)15 and obscures the *eup* null phenotype. A second possibility is that the perceived location of the *eup* locus is wrong and it lies outside of the region covered by Δ(*rha-pfk*A)15.

To determine if a *eup* suppressor mutation was involved, P1 lysates prepared on strain DF877 were used to transduce strain CJ48(*eup*-5) to tetracycline resistance and the Tet^r transductants were scored for Δ(*rha-pfk*A)15 and the Eup$^-$ phenotype. From a total of 51 Tet^r transductants, 20 (39%) had acquired Δ(*rha-pfk*A)15, and all exhibited a Eup$^+$ phenotype. The remaining 31 transductants (61%) did not acquire the deletion and retained the Eup$^-$ phenotype of the recipient CJ48(*eup*-5) strain. These findings argue against the existence of a *eup* suppressor mutation cotransducing with Δ(*rha-pfk*A)15.

To establish that the *eup* locus lies in the region covered by Δ(*rha-pfk*A)15, a P1 lysate was prepared on strain CJ48(*eup*-5) and was used to transduce strain CJ57[Δ(*rha-pfk*A)15] to *pfk*A$^+$ selecting for growth on mannitol. The *pfk*A$^+$ transductants were scored for the Eup$^-$ phenotype and the ability to grow on rhamnose. All (269/269) *pfk*A$^+$ transductants were found to be *eup* and *rha*$^+$. These data are fully compatible with the *eup* locus mapping between *rha* and *pfk*A as previously reported (Plate & Suit, 1981).

Thus, deleting the *eup* locus results in a Eup$^+$ phenotype or, more correctly in a quasi-Eup$^+$ phenotype. When compared with strain CJ49(*eup*$^+$), the deletion strain CJ57[Δ(*rha-pfk*A)15] exhibited an increased sensitivity to amikacin and grew more slowly on succinate. Of particular significance was the finding that, in contrast to the *eup*-5 mutation, deleting the *rha-eup-pfk*A region of the chromosome did not adversely affect the transport of proline, alanine, and TMG (Figure 1). This result clearly rules out any mechanistic role for a *eup* gene product in solute/proton cotransport and raises the possibility that the Eup$^-$ phenotype is due to the presence of an altered *eup* product encoded by the mutant *eup*-5 allele. Evidence compatibile with this hypothesis was obtained from a series of kinetic studies of ONPG and lactose influx into strains bearing the *eup*$^+$ and *eup*-5 alleles and the *eup* deletion strain.

Table II: Kinetic Parameters of ONPG Influx into Strains CJ49s, CJ48s, and CJ57s under Energized and Deenergized Conditions

strain	additions	K_T (mM)	V_{max} [nmol of ONPG hydrolyzed min^{-1} (mg of cell protein)$^{-1}$]	β-galactosidase level (μmol min^{-1} mg^{-1})
CJ49s	glycerol	0.84	640	2.05
	azide	0.09[a]	64[a]	1.79
		0.83	216	
CJ48s	glycerol	0.09[a]	50[a]	1.26
		1.10	265	
	azide	0.61	75	2.15
CJ57s	glycerol	1.00	505	1.47
	azide	1.30	188	2.12

[a] Eadie–Hofstee plots were biphasic and yielded two apparent K_T and V_{max} values (cf. Figure 2).

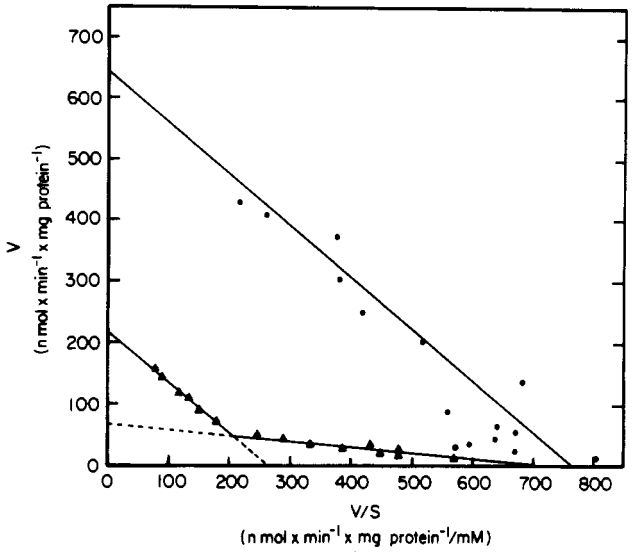

FIGURE 2: Eadie–Hofstee plot of the rate of ONPG influx into energized (+glycerol) (●) and deenergized (+azide) (▲) CJ49s(*eup*$^+$) cells as a function of external ONPG concentration. Maximum velocities (V_{max}) were derived from the y intercepts of the plots, and apparent K_T's were estimated from the slopes of each function.

Kinetics of ONPG Influx. The kinetics of ONPG influx into strain CJ49s(*eup*$^+$) under energized (+glycerol) and deenergized (+30 mM sodium azide) conditions were determined, and the results obtained are given in Figure 2 and Table II. Under energized conditions, the kinetics of ONPG influx were monophasic, yielding an apparent K_T of 0.84 mM and a V_{max} of 640 nmol of ONPG hydrolyzed min^{-1} (mg of cell protein)$^{-1}$. Treating the cells with 30 mM sodium azide, an azide concentration that completely inhibits the active transport of lactose (data not shown), resulted in biphasic kinetics with the appearance of a new low K_T, low V_{max} form of the lactose carrier not evident in the energized cells. That this azide-induced low K_T, and V_{max} ONPG influx was mediated by the lactose carrier, and did not represent ONPG entry by some other route, was indicated by the fact that it was completely inhibited by 5 mM thiodigalactoside, a specific, high-affinity substrate of the lactose carrier (data not shown). Azide treatment also reduced the V_{max} of the high K_T component (0.84 mM) by 66%.

The kinetics of ONPG influx into energized and azide-treated cells of the *eup* deletion strain CJ57s[Δ(*rha-pfk*A)15] are presented in Figure 3. In azide-treated cells of this strain, no low K_T, low V_{max} form of the lactose carrier was evident, the effect of azide being to lower the V_{max} of ONPG influx by 63%.

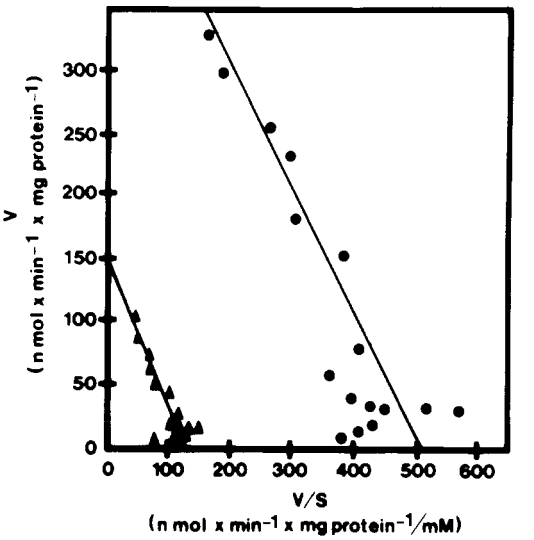

FIGURE 3: Eadie–Hofstee plot of the rate of ONPG influx into energized (+glycerol) (●) and deenergized (+azide) (▲) cells of strain CJ57s[Δ(*rha-pfk*A)15] as a function of external ONPG concentration.

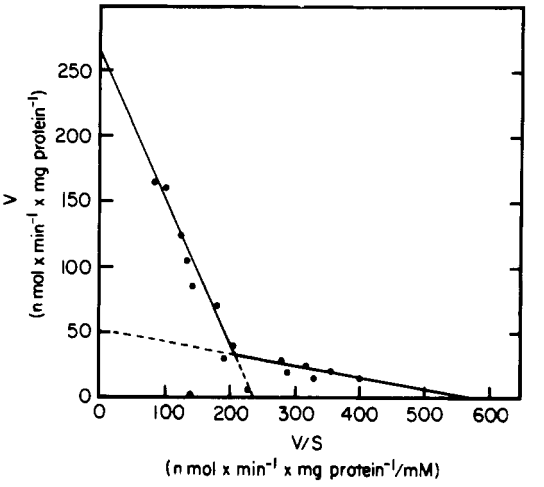

FIGURE 4: Eadie–Hofstee plot of the rate of ONPG influx into energized (+glycerol) cells of strain CJ48s(*eup*-5) as a function of external ONPG concentration.

With the Eup$^-$ strain CJ48s(*eup*-5), the kinetics of ONPG influx under energized conditions were biphasic (Figure 4, Table II) and yielded kinetic constants similar to those obtained with strain CJ49s(*eup*$^+$) treated with azide. Treating strain CJ48s(*eup*-5) with azide resulted in monophasic kinetics for ONPG influx which yielded an apparent K_T of 0.61 mM and a V_{max} of 75 nmol of ONPG hydrolyzed min^{-1} (mg of cell protein)$^{-1}$.

Two points emerge from these results. First, azide treatment of CJ49s(*eup*$^+$) results in the emergence of a new low K_T, low V_{max} form of the lactose carrier, a form not previously reported. Second, the absence of this low K_T, low V_{max} form of the lactose carrier in the azide-treated *eup* deletion strain, together with the finding that the *eup*-5 mutation results in the presence of the low K_T, low V_{max} form of the lactose carrier even under energized conditions, suggests that a *eup* gene product is associated with this kinetic transition.

Kinetics of Lactose Influx. Previous studies reporting the kinetics of lactose influx into *E. coli cells* have employed the *lacZ* strain ML308-225. Under energized conditions, monophasic kinetics have been reported, yielding an apparent K_T of approximately 0.6 mM and a V_{max} of 300 nmol min^{-1} (mg of cell protein)$^{-1}$ (Wrinkle & Wilson, 1966; Ghazi & Shecter, 1981). Deenergization of intact cells or membrane vesicles

Table III: Kinetic Parameters of Lactose Influx into Strains CJ49, CJ48, and CJ57

strain	K_T (mM)	V_{max} [nmol min^{-1} (mg of protein)$^{-1}$]
CJ49	0.02[a]	8.2[a]
	3.50	333
CJ48	0.60	40
CJ57	2.80	200

[a] The double-reciprocal plot was biphasic and yielded two apparent K_T and V_{max} values (cf. Figure 5).

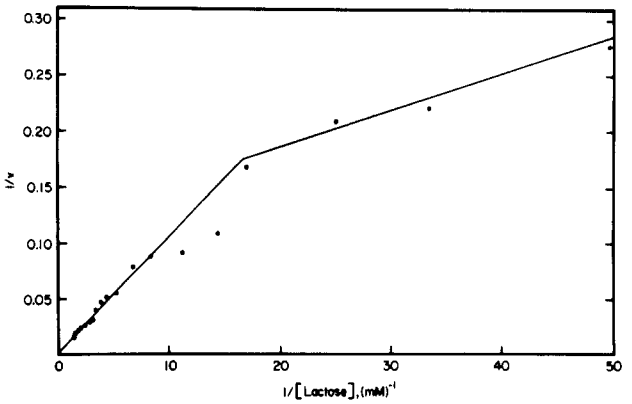

FIGURE 5: Double-reciprocal plot of lactose influx into cells of strain CJ49(*eup*$^+$). Each point represents the average of three determinations.

derived from this strain results in a marked increase in the apparent K_T for lactose (15–20 mM) with little if any effect on the V_{max} (Ghazi & Shecter, 1981; Robertson et al., 1980; Wright et al., 1981). Partial deenergization results in biphasic kinetics, one component exhibiting the apparent K_T of active transport (0.2–0.6 mM) and one the apparent K_T of facilitated diffusion (15–20 mM) (Ghazi & Shecter, 1981; Robertson et al., 1980).

When we determined the kinetics of lactose influx into strain CJ49(*eup*$^+$), the results obtained were different from those previously reported for strain ML308-225. A double-reciprocal plot of the initial rate of lactose uptake by energized CJ49-(*eup*$^+$) cells as a function of external lactose concentration was biphasic, with apparent K_T values of approximately 0.02 and 3.5 MM and respective V_{max} values of 8.2 and 333 nmol min^{-1} (mg of cell protein)$^{-1}$ (Figure 5, Table III). Both phases of the double-reciprocal plot were dependent upon *lac* operon induction, as uninduced cells yielded nonsaturation kinetics indicative of passive diffusion (data not shown).

Two points can be made regarding the data in Figure 5. First, linear regression analysis of the data indicates that the lines of best fit are the two slopes shown. Second, to ensure that the biphasic kinetics obtained with strain CJ49(*eup*$^+$) were not an artifact of the assay procedure used, the kinetic experiments were repeated with *E. coli* strain ML308-225. When the same procedures and the same concentration range indicated in Figure 5 were used, the double-reciprocal plot obtained with ML308-225 was monophasic and yielded an apparent K_T of 0.6 mM and a V_{max} of 333 nmol min^{-1} (mg of cell protein)$^{-1}$. These values are very close to those previously reported by Wrinkler and Wilson (1966) for this *E. coli* strain.

The double-reciprocal plot for strain CJ57[Δ(*rha-pfk*A)15] was monophasic and yielded an apparent K_T of 2.8 mM and a V_{max} of 200 nmol min^{-1} (mg of cell protein)$^{-1}$, values similar to the high K_T, high V_{max} component of lactose influx in CJ49(*eup*$^+$). In the case of strain CJ48(*eup*-5), the double-reciprocal plot was also monophasic and yielded an apparent

Table IV: Kinetic Parameters of Proline Influx into Strains CJ49, CJ48, and CJ57

strain	K_T (μM)	V_{max} [nmol min^{-1} (mg of protein)$^{-1}$]
CJ49	2.6	6.3
CJ48	1.4	1.5
CJ57	2.9	5.0

K_T of 0.6 mM and a V_{max} of 40 nmol min^{-1} (mg of cell protein)$^{-1}$.

Although the kinetics of lactose influx into strain CJ49-(*eup*$^+$) differ from those obtained by us and others for ML308-225, they are consistent with what was found regarding the kinetics of ONPG influx under deenergized conditions in the *eup*$^+$ background. In the absence of β-galactosidase, lactose influx occurs against its chemical potential gradient in symport with protons and results in partial dissipation of the PMF (Schuldiner & Kaback, 1975; Therisod et al., 1982; Ahmed & Booth, 1983). We suggest that this lactose-induced deenergization, together with a gene product that is absent in the (*rha-pfk*A) deletion strain, results in the low K_T, low V_{max} component of lactose influx seen in Figure 5 and that this is analogous to the situation that prevails in azide-treated CJ49s(*eup*$^+$) cells in which a low K_T, low V_{max} component of ONPG influx is also evident.

Effect of the eup-5 Mutation on Proline Influx. Previously we have shown that *eup* mutations result in reduced steady-state levels of proline accumulation (Plate, 1976; Plate & Suit, 1981). A kinetic analysis of proline influx into strains CJ49(*eup*$^+$), CJ48(*eup*-5), and CJ57[Δ(*rha-pfk*A)15] under energized conditions revealed that the *eup*-5 mutation affected the kinetic parameters of the proline carrier in a manner similar to its effects on the lactose carrier (Table IV). Strain CJ48(*eup*-5) exhibited a 2-fold reduction in the apparent K_T for proline and 5-fold reduction in the V_{max} of proline influx as compared to strains CJ49(*eup*$^+$) and CJ57[Δ(*rha-pfk*A)15]. This is consistent with the notion that the proline carrier, like the lactose carrier, is subject to aberrant regulation mediated by an altered *eup* product. We have attempted to determine if the normal *eup* product regulates proline carrier activity in deenergized cells, but these experiments are difficult to do, and this question remains unresolved at present.

Discussion

To date, the existence of *eup* mutants has proven enigmatic. On the one hand, this class of mutants exhibits pleiotropic deficiencies in PMF-coupled functions while apparently maintaining the PMF, leading to suggestions that a product of the *eup* locus might function by coupling the PMF to those cellular functions driven by it (Plate & Suit, 1981). On the other hand, evidence has been presented that such energy-transducing proteins as the lactose carrier of *E. coli* (Foster et al., 1982) and the proton-translocating ATPase (Futai & Kanazawa, 1983) themselves respond to the PMF, thus obviating any need for coupling proteins. The objectives of the present study were to resolve these seemingly disparate findings and to elicit clues as to the role of the *eup* locus and its product in *E. coli* physiology.

Here we have presented genetic data confirming that the *eup* locus indeed lies between *rha* and *pfk*A as previously reported (Plate & Suit, 1981). In addition, we have shown that a strain deleted of the *rha-pfk*A region of the *E. coli* chromosome exhibits a quasi-Eup$^+$ phenotype and normal PMF-driven transport. These findings clearly preclude any obligatory role for a *eup* gene product in such PMF-driven functions as proton/solute cotransport or oxidative phospho-

rylation. Therefore, it would appear that the Eup$^-$ phenotype originates not from the functional loss of a normal *eup* gene product but rather results from the presence of a mutationally altered *eup* product.

Having made this distinction, two questions need to be addressed. What, if any, role does a *eup* product play in energy transduction, and how do *eup* mutations disrupt such energy-coupled functions as active transport? With regard to the latter question, it has been suggested that *eup* mutations could exert their effects by causing a reduction in the PMF (Booth et al., 1984; Ferguson, 1985). This seems unlikely since it has been shown that *eup* mutations do not result in a reduced membrane potential, in increased proton permeability, or in defective proton translocation (Plate & Suit, 1981); Hitchens et al., 1982; Kashket, 1982). Although it is possible that small differences were not detected in these studies, it seems reasonable to seek alternative explanations for the Eup$^-$ phenotype.

The results obtained from the kinetic studies of ONPG and lactose influx afford additional insight into the possible functioning of a *eup* gene product in *E. coli* physiology. In azide-treated CJ49s(*eup*$^+$) cells, we have found a hitherto undetected low K_T, low V_{max} component of ONPG influx that is mediated by the lactose carrier. This low K_T, low V_{max} form of the lactose carrier is not evident in energized cells of this strain nor is it found in azide-treated cells of the deletion strain CJ57s[Δ(*rha-pfk*A)15]. It would appear that membrane deenergization, a consequence of azide treatment, in concert with the functioning of a gene product that is absent in the *rha-pfk*A deletion strain results in a portion of the lactose carriers assuming this low K_T, low V_{max} kinetic form. The low K_T, low V_{max} form of the lactose carrier was also detected in the kinetic studies of lactose influx into strain CJ49(*eup*$^+$), presumably appearing in response to the partial membrane depolarization known to occur as a result of lactose/proton symport into cells lacking β-galactosidase (Schuldiner & Kaback, 1975; Therisod et al., 1982; Ahmed & Booth, 1983).

The (*rha-pfk*A) deletion removes approximately 0.5 min of the *E. coli* chromosome (Bachmann, 1983) and, assuming an average gene size of 1.1 kilobase (kb) (Ingraham et al., 1983), could delete as many as 20 genes, one of which is the *eup* locus. That it is the *eup* gene product that functions in converting a portion of the lactose carriers into the low K_T, low V_{max} form is suggested by the finding that this form of the lactose carrier is detectable in energized cells that carry the *eup*-5 mutation. It is conceivable, therefore, that the *eup*-5 mutation results in an altered *eup* product whose functioning reduces the K_T and V_{max} of the lactose carrier but, unlike the normal *eup* product, the altered *eup*-5 product functions aberrantly under energy-replete conditions.

We are not certain why the low K_T, low V_{max} form of the lactose carrier has not been detected in previous studies of ONPG and lactose transport. To our knowledge, this represents the first report of kinetic studies of ONPG and lactose influx into whole *E. coli* K-12 cells, previous work having employed either intact cells of *E. coli* ML strains (Winkler & Wilson, 1966; Ghazi & Schecter, 1981) or cytoplasmic membrane vesicles prepared from *E. coli* ML or K-12 strains (Robertson et al., 1980; Wright et al., 1981). If a *eup* product is required for the low K_T, low V_{max} form of the lactose carrier to be manifest, two reasons why it was not detected in previous studies can be advanced. The ML strains of *E. coli* are genetically distinct from *K*-12 strains and might not possess a functional *eup* locus. This would not be immediately evident since the *eup* null phenotype is quasi-Eup$^+$. Furthermore, it is difficult to determine this genetically since ML strains of *E. coli* are not amendable to genetic manipulation. In addition, the *eup* product may be a cytoplasmic or peripheral membrane protein that is lost in the course of membrane vesicle preparation. The eventual isolation of the *eup* locus and identification of its product, and elucidation of the cellular location of this product, should resolve this question.

Placing the findings presented here in context with what is currently known regarding the properties of the lactose carrier, we propose the following scheme. Robertson et al. (1980) and Wright et al. (1981) have shown that membrane deenergization results in a conformation change of the lactose carrier, as indicated by a 100-fold increase in the apparent K_T for lactose (16-fold increase in the case of ONPG) with little if any effect on the V_{max}. The apparent K_T values of the deenergized lactose carrier for ONPG and lactose are in the range of 15–20 mM. We have not attempted measurements in this concentration range but presume that this same increase in the K_T of the lactose carrier occurs upon deenergization of the *eup* deletion strain and that this accounts for the azide-induced decrease in the V_{max} for ONPG hydrolysis (Table II). That is, with azide-treated CJ57s cells, 67% of the lactose carriers have an apparent K_T of 15 mM or higher and are not detected in our kinetic measurements. We postulate that it is these deenergized lactose carriers that are recruited by a *eup* gene product in a *eup*$^+$ strain and through some as yet undefined mechanism are converted into the low K_T, low V_{max} lactose carriers seen in azide-treated *eup*$^+$ cells. In the case of cells bearing the *eup*-5 mutation, we propose that an altered *eup* product aberrantly functions to convert energized lactose carriers into the low K_T, low V_{max} form, resulting in the defective lactose transport associated with *eup* mutations.

In a broader perspective, we view the role of the *eup* system as being regulatory in nature with one of its functions being to modify the kinetic properties of the lactose carrier in response to deenergization. Whether deenergization of *eup*$^+$ cells evokes a reduction in the K_T and/or V_{max} in any PMF-coupled transport carrier proteins besides the lactose carrier is not yet known. The fact that the *eup*-5 mutation results in a lowering of the K_T and V_{max} of proline transport, which resembles its effects on lactose transport, raises the possibility that other PMF-coupled carriers are subject to *eup* regulation. Considering that in their natural habitat bacteria undoubtedly encounter feast to famine extremes in the availability of exogenous nutrients, and that under famine conditions it may be difficult for them to maintain an adequate PMF, such a response would be physiologically beneficial. Under conditions of a declining PMF, the triggering of a mechanism that simultaneously reduces carrier activity and increases affinity for substrate would both reduce the drain on any existing PMF and also increase the cell's ability to capture dwindling carbon and energy sources.

As pointed out earlier, *eup* mutations, in addition to affecting PMF-coupled transport, also result in increased resistance to aminoglycoside antibiotics, cause insensitivity to colicins A and K, prevent growth on nonfermentable carbon sources, and reduce growth yields on limiting glucose. These latter two properties are characteristic of ATPase-defective *unc* mutants (Gibson, 1983) and suggest that *eup* mutants may be defective in oxidative phosphorylation, though this has yet to be directly shown. The PMF is required for each of these functions (Futai & Kanazawa, 1983; Damper & Epstein, 1981; Bryan & Kwan, 1983; Jetter & Jetter, 1975). If, as seems apparent for lactose transport, mutation to Eup$^-$ in effect removes the deenergization requirement for the *eup* product

to act, it is likely that *eup* regulation transcends transport function and applies generally to PMF-coupled processes. If so, a normal *eup* product may serve a homeostatic function under all conditions by balancing the activity of various PMF-driven processes in accordance with the cell's ability to maintain an optimal PMF for growth. While many details regarding *eup* function remain to be elucidated, this system holds the promise of providing new insight into the manner in which energy-transducing processes are regulated.

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Registry No. Lactose, 63-42-3; L-proline, 147-85-3.

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