A SINGLE LOCUS IN *ESCHERICHIA COLI* GOVERNS GROWTH IN ALKALINE pH AND ON CARBON SOURCES WHOSE TRANSPORT IS SODIUM DEPENDENT

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1. Introduction

Respiring *Escherichia coli* cells maintain their intracellular pH almost constant at 7.7 through the external pH range 6.0–8.5 [1,2]. ΔpH generated at the cytoplasmic membrane by the proton pumps [3] markedly changes, decreasing from 1.8 at the lower pH to −0.4 at the basic pH, with the inversion point at 7.7. The marked decrease in ΔpH is partially compensated by a parallel increase of $\Delta\psi$ [4–6]. Thus, the cytoplasmic membrane, while maintaining a high electrochemical gradient of protons ($\Delta\tilde{\mu}_{H^+}$ = 160 mV), can also regulate the internal pH.

The decrease in ΔpH with increasing external pH may be partly attributed to the function of pH-dependent cation/proton antiporters [1,7]. Such a pH dependence has been shown [4,8] in the case of the sodium/proton antiport in *E. coli* [9].

Sodium/proton antiport is also most probably required for maintaining sodium gradient [7] across the cell membrane. Therefore a mutant defective in this antiport is expected to be both sensitive to alkaline pH as well as unable to grow on carbon sources which are accumulated by sodium-dependent transport systems. The isolation of such a mutant is described here.

Abbreviations: FCCP, carbonyl cyanide p-trifluoromethoxyphenyl hydrazone; ΔpH = pH in − pH out; $\Delta\psi$, membrane potential; $\Delta\tilde{\mu}_{H^+}$, the electrochemical proton gradient

2. Materials and methods

2.1. *Bacteria and growth media*

The *E. coli* K-12 strains used are described in table 1.

Cells were grown on minimal medium A [11] lacking citrate, supplemented with L-methionine (50 μg/ml) and containing one of the following

Table 1
Characteristics of *E. coli* K-12 strains used in this work

Strain	Mating type	Genotype	Source
CS7	Hfr	*gltC metB*	Y. S. Halpern [10]
CS71	Hfr	*gltC metB lacY1*	Transduction AB2874 → CS7
CS72	Hfr	*gltC lacY1*	Transduction CS101B Met[+] → CS71
DZ3	Hfr	*gltC metB lacY1 phs*	This study
DZ31	Hfr	*gltC metB lacY1 nalA phs*	Transduction L-1821 → DZ3
CS101B Met[+]	Hfr	*gabC*	Y. S. Halpern [16]
AB2874	F[−]	*thil iluC7 argE3 his4 proA4 aroF363 mtl1 xyl5 galK2 lacY1 tfr3 tfx358 sup44 nalB18*	Y. S. Halpern [22]
L-1821		*metB gabC 'nut' nalA*	Y. S. Halpern [16]

carbon sources; 0.5% glycerol; 1% dipotassium succinate; 0.5% sodium-L-glutamate; 10 mM D-melibiose plus 10 mM NaCl. Solid media were prepared by addition of 1.5% Difco agar.

L-Broth, used for conjugation and transduction experiments, contained KCl instead of NaCl.

2.2. *Isolation of mutants*

Mutants unable to grow on melibiose and glutamate as carbon source were induced in *E. coli* CS71 by UV irradiation. Mutants were isolated after two cycles of penicillin enrichment [12] in melibiose and sodium L-glutamate medium. Selection was performed by replica plating. All the steps were performed at 30°C, because of the temperature sensitivity of the melibiose operon [13].

2.3. *Transduction*

Plkc lysates of the desired donor bacteria were prepared by the method in [14] and transduction was done as in [15].

2.4. *Mating experiments*

Mating experiments were performed essentially as in [16] except that glycerol was used instead of succinate.

2.5. *pH controlled growth curves*

Growth at different pH was carried out in a BioFlo Model C30 chemostat (New Brunswick Scientific), as batch culture. pH was controlled utilising a Modcon (Kiryat Motzkin, Israel) pH titrator. The medium $MgSO_4$ was reduced to 0.005%.

2.6. *Sodium efflux assays*

Starved cells were prepared by overnight incubation at 37°C of a mid-log culture in 0.1 M potassium phosphate buffer (pH 7.2) containing 0.01% $MgSO_4$ and 50 μg/ml methionine. Cells were harvested and resuspended in 0.1 M phosphate buffer (pH 7.2) at 2 mg cell protein/ml. ^{22}Na was added to 10 mM final conc. (2.5×10^5 cpm/μmol) and the cells were incubated on ice for 2 h. Efflux was assayed as in [8].

2.7. *Protein determination*

Protein was determined by the method in [17].

2.8. *Chemicals and isotopes*

$^{22}NaCl$ (carrier free) was from New England Nuclear; FCCP from DuPont.

3. Results and discussion

3.1. *Isolation of mutants unable to grow on melibiose and glutamate as carbon sources*

The rationale for the isolation of a mutant, defective in the sodium/proton antiport and sensitive to alkaline pH, was based on the assumption, that this antiport is responsible both for controlling the internal pH as well as for maintaining the cell sodium gradient, needed for the transport of several growth substrates. Accumulation of melibiose [18] and glutamate [19] in *E. coli* has been shown to be driven by an artificially imposed sodium gradient in the absence of any other energy source. Inability to grow on both carbon sources is most likely due to a defect in a common function indispensable for the utilisation of these substrates. Such a common step may be the sodium/proton antiport.

Mutants defective in growth on both melibiose and glutamate were obtained by UV mutagenesis and enrichment by penicillin in the presence of both substrates (table 2). When glutamate was used as a carbon source for growth at pH 7 and 30°C, only one of the six mutants isolated showed measurable but very slow growth: 7.8 h doubling time as compared to 4.4 h of the wild-type. The doubling time of the mutants tested on melibiose was between 2.5–4.5-times longer than that of the wild-type (1.8 h) and they also showed a lag of 5–10 h.

With glutamate as carbon source, colonial growth of the mutants on agar solidified minimal medium, was not detected for 80 h and with melibiose as car-

Table 2
Doubling times of *E. coli* CS71 and mutants on several carbon sources at pH 7.0

Strain	Carbon source		
	Glycerol	L-Glutamic acid	D-melibiose
CS71	1.4	4.4 (0)	1.8 (0)
DZ3	1.4	∞	8.2 (5)
DZ43	1.8	7.8 (6)	4.6 (10)
DZ70	2.2	∞	6.6 (0)
DZ92	1.6	∞	6.2 (10)
DZ99	1.9	∞	6.0 (6)
DZ223	1.6	∞	4.8 (10)

The various strains were grown on glycerol minimal medium, washed once with cold 0.1 M phosphate buffer (pH 7.0), starved for 2 h at 30°C and used for inoculation. Doubling times and the lags (in parenthesis) are given in hours

bon source, small colonies were detected only after 72 h. Although other interpretations are possible, the very slow growth on both substrates is expected for a mutant normal in metabolic pathways and possessing carriers, but impaired in the active transport capacity for these substrates. Indeed growth on succinate (not shown) as well as on glycerol (table 2) was identical to that of the wild-type. Strain DZ3 was chosen for further thorough investigation.

The strain DZ3 (*metB gltC*), to which *nalA* resistance was transduced (DZ31), was conjugated with a derivative of the wild-type strain, which is an HfrC, to which the wild type *metB* allele was introduced by transduction (CS72). In these strains *metB* maps exactly at half the distance between the *mel* and *glt* loci and *mel* is expected to penetrate first [19]. Recombinants capable of growth on melibiose tested in the presence of methionine, appeared before Met^+ recombinants (growing on glycerol without methionine). All the Met^+ recombinants were capable of growth on glutamate suggesting that a single locus (*phs*) is responsible for growth on both carbon sources. Although not all the recombinants capable of growth on both melibiose and glutamate were Met^+, all the Met^+ recombinants were capable of growth on both substrates. Hence a strong linkage is suggested between *metB* locus and the *phs* locus which enters first this Hfr strain. It should be noted that neither the *mel* gene [20] nor the *glt* gene [10] are linked to *metB* gene. Further genetic characterisation of the *phs* locus will be published elsewhere, (in preparation). A preliminary analysis of the product of the *phs* locus on SDS–polyacrylamide gels indicates that a polypeptide with app. mol. wt 24 000 is missing in strain DZ3.

3.2. *An impaired ability to extrude sodium*

Being a common locus determining the utilisation of two substrates which are accumulated by sodium-dependent transport systems, it is likely that the *phs* locus is related to the mechanism responsible for maintaining a sodium gradient across the cell membrane. This suggestion has been tested in fig.1. Starved cells were equilibrated with $^{22}Na^+$ at 10 mM. At the onset of the experiment glycerol was added and the amount of radioactivity retained in the cells was determined at various time points. In the wild-type strain a rapid efflux of $^{22}Na^+$ was observed with initial rate of 25 nmol . mg protein^{-1} . min^{-1} reaching a steady state after ~2 min. In the mutant DZ3, ⩾3.5-

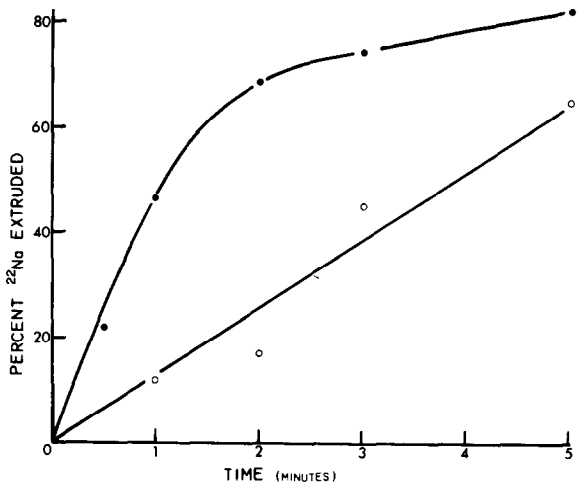

Fig.1. Energy-dependent sodium efflux in *E. coli CS71* and *DZ3*. Sodium efflux assay was done as in section 2. The assay was in triplicate at 25°C: (•–•) CS71; (○–○) DZ3. No efflux was observed either in the absence of glycerol or in the presence of 25 μM FCCP.

times slower rate of efflux was dectected and accordingly the steady state level was reached after 6–8 min (not shown).

3.3. *Sensitivity of growth to alkaline pH*

Fig.2 summarises the growth pattern of the wild-type and strain DZ3 as a function of the external pH. With both, glycerol and glucose as carbon sources the doubling time of the wild-type was constant at pH 7.2–8 (1.2 and 1.1 h, respectively) and up to pH 8.5 only a slight increase in the doubling time was detected.

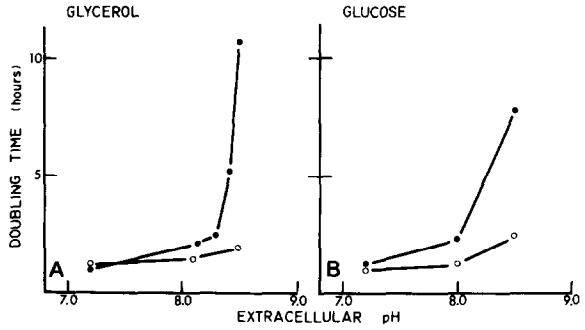

Fig.2. Growth of *E. coli CS71* and *DZ3* at different pH values. Cells were grown under pH-controlled conditions. The carbon source was either 0.5% glycerol (A) or 0.5% glucose (B): (○–○) CS71; (•–•) DZ3.

The mutant DZ3 exhibited similar doubling time to that of the wild type up to pH 8.0. However, above this pH a marked progressive inhibition of the growth on either substrate was observed. Thus, while the doubling time of strain DZ3 at pH 8.1 was only 1.2–2-fold longer than that of the wild type, at pH 8.5 it was 4–6-fold longer.

We conclude therefore that the *phs* locus is responsible for maintaining the sodium gradient across the cell membrane needed for the ultilisation of both melibiose and glutamate and its wild-type allele is also required for growth at alkaline pH. Indeed the recombinant described above that regained the ability to grow on melibiose and glutamate as carbon sources also regained the ability to grow at the alkaline pH.

Work with a non-alkalophilic mutant of *Bacillus alcalophilus* [21] supports the conclusion that the mechanism responsible for maintaining the sodium gradient across the cell membrane is required for growth at alkaline pH.

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