Genetic studies of the *phs* locus of *Escherichia coli*, a mutation causing pleiotropic lesions in metabolism and pH homeostasis

G.C. Rowland, P.M. Giffard and I.R. Booth*

Department of Microbiology, Marischal College, University of Aberdeen, Aberdeen AB9 1AS, Scotland

Received 18 May 1984; revised version received 12 June 1984

In *Escherichia coli* a pleiotropic mutation, *phs*, has been reported to affect Na^+-linked metabolic functions and pH homeostasis. The *phs* mutation was previously mapped by its proximity to a *met* marker, presumed to be *metB* at 89 min. We have shown that a second mutation to auxotrophy, *cymX*, which is satisfied by either methionine or cysteine, is closely linked to *phs*. The *cymX* and *phs* lesions map close to *trkB* and *rpsL* at 73.5 min and we postulate that they are alleles of *cysG* and *crp*, respectively. The basis of the pH sensitivity of DZ3 is discussed in the light of this new information.

E. coli *pH regulation* *Melibiose* *Glutamate*

1. INTRODUCTION

It is well established that a wide range of bacteria exhibit a remarkable degree of cytoplasmic pH homeostasis despite variations in external pH [1,2]. However, the molecular mechanisms which contribute to this homeostasis are only vaguely understood [2]. The development of our understanding of many regulatory phenomena in bacteria has been consequent upon genetic studies [3–5]. Thus, the isolation of mutants defective in pH homeostasis has been a major aim of workers in the field [6–8]. Of such mutants one of the most important has been a mutation described as affecting the Na^+/H^+ antiport in *Escherichia coli* (*phs*) which results in a failure to grow at alkaline pH [8–10].

The *phs* mutant was isolated after UV mutagenesis and penicillin enrichment as a strain unable to grow on either melibiose or glutamate [8]. Both of these carbon sources are believed to be taken up predominantly by Na^+-dependent mechanisms. These systems are dependent upon an Na^+ gradient established by the Na^+/H^+ antiport [11,12]. Loss of the Na^+/H^+ antiport would be expected to severely impair transport of these solutes and this was the rationale used to isolate the *phs* mutant [8]. The mutant was subsequently shown to be unable to regulate its cytoplasmic pH at alkaline values of pH. The mutation was mapped in conjugation studies by its relationship to *metB* (89 min [13]) and was suggested to lie at approx. 89.4 min [9].

We were interested in this mutant as part of our investigation of the mechanisms of pH homeostasis in bacteria. We wish to report that the *phs* locus does not map close to *metB* and that the explanation for the earlier data [8] is that *phs* is closely associated with an auxotrophic requirement which is cryptic in a *metB* background. This cryptic lesion affects cysteine biosynthesis and maps close to *trkB* [14].

2. MATERIALS AND METHODS

2.1. *Bacterial strains*

A list of the bacterial strains used is given in table 1.

2.2. *Growth media*

Complex medium (LBK) was used for routine growth of organisms for genetic studies, and con-

* To whom correspondence should be addressed

Published by Elsevier Science Publishers B.V.

Table 1

Escherichia coli strains used

Strain	Genotype	Origin
CS71	Hfr PO2A *metB*1, *gltS*$_o$, *lacY*	[a]
DZ3	CS71 *phs* (*cymX*)	[a]
GR300	DZ3 *phs*$^+$, *zgd*::Tn*10*	[b]
GR301	DZ3 *phs*, *zgd*::Tn*10*, *cymX*	[b]
GR302	MJF1 *phs*, *zgd*::Tn*10*, *cymX*	[b]
JM2137	F$^-$ *araDB*9, Δ(*lac-argF*)$_{u169}$ *rpsL*, *thi*, *ptsF*$^+$, *araE*, Δ(his-gnd)	[c]
MJF1	JM2137 *gltS*$_o$	[d]
Gif106M1	F$^-$, *thrA*1101, *metLM*, *lysC*1001, *ilvA*296, *malA*1, *xyl*7, *ara*13, *mtl*-2, *rpsL*9	[e]
AT2446	Hfr PO1, *metC*69, *thi*-1	[e]
JC158	Hfr PO1, *thi*-1, *serA*6, *relA*1, *spoT*1	[e]
AB1927	Hfr PO1, *metA*28, *argH*1, *purF*1, *xyl*-7, *supE*-44	[e]
TK121	F$^-$, *thi*-1, *rha*-4, *lacZ*82, *gal*33, *trkC*121	[f]
TK110	Tk121 *trkC*$^+$, *trkB*110	[f]
1101	Hfr PO1, *his*-62, *ptsH*1, *relA*1, *bglR*11, *thi*-1, *spoT*1	[e]
ctr-7	Hfr PO45 *ptsI*7, *relA*1, *bglR*10, *thi*-1	[e]
RK4904	F$^-$, *proC*32, *trpE*38, *lysA*23, *metE*70, *argH*1, *zij*-602::Tn*10*, *rpoB*308, *thi*-1, *lacZ*36, *xyl*-5, *mtl*-1, *rpsL*109, *cys*-19, *tsx*-67, λ^-, *supE*44	[e]

Sources of strains: [a] S. Schuldiner; [b] here; [c] M. Jones-Mortimer; [d] transductant of JM2137 with P1 from CS71, to growth on glutamate; [e] CGSC; [f] W. Epstein

tained (per l): tryptone, 10 g; yeast extract, 5 g; KCl, 7 g. Minimal medium S was used for testing auxotrophy and carbon source utilisation and is a wholly K^+-based version of medium A [15]. Antibiotics were added to complex media at the following concentrations: rifampicin, 50 μg/ml; chloramphenicol, 12.5 μg/ml; streptomycin, 100 μg/ml; tetracycline, 25 μg/ml. When tetracycline was added to minimal medium the concentration was reduced to 12.5 μg/ml. Thiamin was added at 1 μg/ml. Carbon sources were present at 0.2% (w/v).

2.3. *Genetic methods*

Transductions using phase P1 *cml clr*100 and conjugation were carried out as in [16]. A Tn10 pool was made in strain TK121 as in [17]. Phage P1 *cml clr100* was lytically propagated on this Tn10 pool and was used to transduce strain DZ3 simultaneously to *phs*$^+$ and tetracycline resistance. As predicted [17] such transductants arose at a frequency approx. 100-fold lower than transductants to either *phs*$^+$ and tetracycline resistance alone. One such transductant, GR300, was shown to have a Tn10 inserted close to *phs* by P1-mediated transduction. With GR300 as donor and DZ3 as recipient the Tn10 was found to be 60 and 42% cotransducible with *phs* when tetracycline resistance and growth on glutamate, respectively, were the selected markers. This Tn10 was used to transfer the *phs* mutation into other strains and facilitated mapping studies.

3. RESULTS

3.1. phs *is not linked to* metB

Earlier conjugation studies established that *phs* lay between the origin of HfrC (PO2A) and a *met* locus presumed to be *metB* [8]. It was provisionally placed at 89.4 min because of its apparent close linkage to this *met* locus and since the parent strain, CS71, carried only *metB*. We sought to map this region using a strain (RK4904) multiply marked in the 89–90 min region and carrying a Tn10 close to *argH* (*zij*::Tn*10* linked 75% to *argH*). Transductants of DZ3 (*metB, phs*) to tetracycline resistance failed to grow on melibiose or glutamate and were thus not *phs*$^+$. However, the transductants acquired *argH* and *rpoB* lesions at the expected frequencies (table 2). No transduc-

Table 2

Mapping the *metB* region

Donor	Recipient	Cysteine present	Selected marker	No. of transductants	Percent non-selected donor marker				
					phs$^+$	*metB*$^+$	*argH*	*rpoB*	Tn10
RK4904	CS71	–	Tn10	150	–	31.3	77.3	81.3	–
	DZ3	–	Tn10	200	0	0	68.5	77.5	–
	DZ3	+	Tn10	50	0	32	74	72	–
	CS71	–	*metB*$^+$	100	–	–	24	11	23
DZ3	RK4904	–	*argH*$^+$	250	–	15.2	–	N.D.	72.4

Transductions were carried out as in [16]

tants to methionine independence were obtained in this cross. Indeed it was routinely observed that DZ3, unlike its parent, CS71, could not be transduced to *metB*$^+$ either directly or indirectly by coinheritance with nearby markers (table 2).

Strain DZ3 was shown to carry the *metB* lesion by transducing RK4904 to arg$^+$ and observing coinheritance of methionine auxotrophy. In such a cross 15% (38/250) of arg$^+$ transductants became methionine requiring consistant with the inheritance of *metB*. These data demonstrate that the *phs* locus does not map adjacent to *metB*, and that DZ3 probably carries a second lesion in methionine biosynthesis, which we term *cymX*.

3.2. *Linkage of* cymX *to* phs

To determine the relationship of *phs* to the *cymX* locus, we isolated Tn10 insertions proximal to *phs*. Using P1 phage lytically propagated on a random Tn10 pool of *E. coli* TK121, strain DZ3 was simultaneously transduced to growth on glutamate (*phs*$^+$) and tetracycline resistance. Several Tn10 insertions were isolated in this way and one GR300, was utilised to generate a tetracycline-resistant *phs* mutant (GR301; table 1). This Tn10 was introduced into MJF1 from GR301 thus creating a *phs* derivative of MJF1 (GR302) by transductional linkage. All the transductants which became *phs* (i.e., were unable to grow on L-glutamate or melibiose) simultaneously inherited the *cymX* locus. Of 190 such transductants all required methionine for growth. To date we have not been able to separate these two loci by transduction.

3.3. *The identity of* cymX

Genetic and metabolic studies were undertaken to determine the identity of the lesion in the methionine biosynthetic pathway. Homocysteine and cystathionine which metabolically complement *metC* and *metB* lesions, respectively, replace the methionine requirement of CS71, DZ3 and GR302. A known *metC* lesion (strain AT2446) was not complemented by cystathionine. Homoserine did not replace methionine as a supplement for GR302 and this placed the lesion in either the synthesis of *o*-succinylhomoserine (*metA*) or cystathionine (*metB*). Metabolic steps prior to *metA* could also be ruled out by the observation that neither DZ3 nor GR302 required threonine or lysine [13].

Using GR300 as the donor in transductions the relationship of the Tn10 to *metA* (AB1927), *metB* (CS71) and *metC* (AT2446) loci was examined. In 100 tetracycline-resistant transductants no linkage to these loci was observed. Further, using the *serA* (JC158) marker no linkage of the Tn10 to *metK* was observed to occur (100 transductants scored). Conjugation using GR300 as donor and Gif106M1 as recipient placed the transposon distal to the *malA* locus at 75 min (table 3). In this conjugation it was observed that only the *malA*$^+$ and *xyl*$^+$ recombinants showed any co-inheritance of tetracycline resistance (5 and 0.04%, respectively). The tetracycline-resistant colonies coinherited the other markers at frequencies of 35–61% as expected for proximal markers [16].

Thus, the *cymX* lesion did not appear to map at any of the known methionine loci. The mutation

Table 3
Conjugation of DZ3 with Gif106M1

Selected marker	Map position (min)	No. of conjugants ($\times 10^{-2}$/ml)
$ThrA^+$	0/100	7351
$LysC^+$	91	829
Arg^+	89.5	514
$IlvA^+$	84.5	363
MtL^+	81	196
Xyl^+	80	139
Mal^+	76	66
Tc^R		6

Conjugation to determine the gradient of transfer of markers from DZ3 to Gif106M1 was carried out as in [16] for 90 min and appropriate dilutions were plated on selective media containing streptomycin to select against the donor. $ThrA^+$ was selected in the presence of 2 mM L-methionine and 10 mM L-lysine to repress $metLM^+$ and $lysC^+$. *LysC* was selected in the presence of 2 mM L-methionine and 2 mM L-threonine to repress $metLM^+$ and $thrA^+$ [27]

had the characteristics of a *metA* or *metB* strain but did not map close to either of these loci (tables 2,3). From the biosynthetic pathway [18] it was apparent that two other metabolites were essential to methionine biosynthesis, succinyl-CoA and cysteine. Cysteine was found to replace methionine completely as a growth requirement for GR302. Further, when DZ3 was transduced to tetracycline resistance using RK4904 as donor and screened for methionine independence in the presence of cysteine it was observed that such transductants arose at the frequency expected for *metB* (table 2). Cysteine did not suppress the methionine requirement of DZ3 or any methionine-requiring strain other than GR302. Thus, it appears that the *cymX* lesion actually affects the synthesis of cysteine and its effect on methionine biosynthesis is only indirect. Because of its similarity to previously described *cym* mutants of *Salmonella typhimurium* [19] we call the mutation *cymX*.

3.4. *Mapping of* cymX

The genetic loci for cysteine biosynthesis consist of one cluster of 5 genes (*cysC, D, H, I, J*) and 6 genes (*cysA, B, E, G, K, Z*) that are widely separated [13,14,18]. The *cymX* mutation and the nearby Tn10 were found to be highly cotransducible with the *rpsL* and *trkB* loci at 73.5 min (table 4). The *trkB* locus is close to *cysG* [13]. The Tn10 was approx. 63% cotransducible with *trkB* (strain TK110) and 71% cotransducible with *rpsL* (strain JM2137). We have thus designated the Tn10 zgd corresponding to a map position of 73.5 min (table 1). In transductions of TK110 to tetracycline resistance using GR301 as donor all classes of transductants arose at high frequency (table 4).

Table 4
Transductional linkage of *trkB, rpsL, cymX* and the Tn10 insertion

Donor	Recipient	Selected marker	No. of transductants	Non-selected marker			Percentage contraduction
				rpsL	*trkB*	*cymX*	
GR300 (Tn10)	JM2137 (*rpsL*)	Tn10	211	D	–	–	71
				R	–	–	29
GR300 (Tn10)	TK110 (*trkB*)	Tn10	100	–	D	–	63
				–	R	–	37
GR301 (Tn10, *cymX*)	TK110 (*trkB*)	Tn10	98	–	D	D	37
				–	D	R	27.5
				–	R	D	26.5
				–	R	R	9

Transductions were carried out as in [16]. The *trkB* locus was scored by the ability of $trkB^+$ cells to grow when replica plated onto minimal agar plates lacking K^+. For such plates the agar was washed twice with distilled water prior to sterilisation

Thus, it is probable that the transposon lies between *trkB* and (*phs–cymX*). The *phs* locus cannot be scored in TK110 since its phenotypic expression requires mutations in *gltSo* and *lacY* [8]. However, on the basis of these data *phs* maps close to *trkB* and *rpsL* at 73.5 min.

The *cymX* mutation could be satisfied by thiosulphate (20 mM) but not sulphite (1 mM) or sulphate (1 mM). This is consistent with a lesion in *cysG* which specifies a component of sulphite reductase [20].

In control transductions no linkage of either *phs* or the nearby Tn10 to the other *cys* loci was observed using the following nearby markers in coinheritance studies: the *cys* cluster, *srl; cysB, trpE; cysA, K* and *Z, ptsI* and *ptsH* (not shown).

The *cymX* and *phs* lesions do appear to be independent, though tightly linked, mutations since cysteine does not suppress the growth defect on either glutamate or melibiose. Further, revertants of GR302 to independence of cysteine retain the *phs* phenotype. It is not known whether the revertants are at the *cymX* locus or are suppressor mutations [21].

4. DISCUSSION

Our data clearly demonstrate that the *phs* mutation does not map close to *metB*. The reason for the incorrect positioning of this gene is that there is a closely associated metabolic lesion which is cryptic in the *metB* strain in which the *phs* mutation was isolated. This lesion we have demonstrated affects cysteine biosynthesis and thence methionine synthesis. The lesion is suppressible metabolically either by homocysteine, cystathionine, methionine or thiosulphate. It thus resembles *cym* mutations of *cysG* which have been studied in *S. typhimurium* [19]. We therefore suggest that *cymX* is probably an allele of *cysG*.

The close proximity of (*phs, cymX*) to *trkB* and *rpsL* places the *phs* mutation close to the regulatory locus *crp*, which specifies the catabolite repressor protein. Mutations in *crp* have been described in both *E. coli* and *S. typhimurium* which limit the range of 'poor' carbon sources which may be utilised for growth [22,23]. The original *phs* mutation was isolated as the result of the failure of cells to grow on two poor carbon sources which is a standard procedure for isolation of *crp* mutants [16]. We therefore suggest that the failure of DZ3 to grow on glutamate and melibiose is due to a *crp* allele which affects the induction of the genes of the *mel* and *glt* systems, but not those required for other poor carbon sources.

In this model pH sensitivity would arise due either to general effects of the *crp* mutation or to the *cym* lesion. Cysteine, through glutathione, may be an important component of the regulation of K^+ transport [24]. Specifically, cells unable to synthesise glutathionine are K^+ leaky [24]. Potassium cycling has been proposed to play a major role in pH regulation in *E. coli* [2,25,26]. It may be that the pH sensitivity of DZ3 results from a shortfall in cysteine biosynthesis acting on K^+ transport.

Note added in proof

Further genetic studies have shown that the *phs* mutation is a single pleiotropic lesion affecting transport and utilisation of glutamate, melibiose, sulphate and arabinose. It maps between *trKA* and *rpsL* close to *spc*. Our speculation that *phs-cymX* is a double mutation affecting *crp* and *cysG* is incorrect. It seems probable that *phs* is a selective defect in either transcription *(rpoA)* or translation/membrane protein insertion.

ACKNOWLEDGEMENTS

The authors wish to thank Dr S. Schuldiner, Dr M. Jones-Mortimer, Dr W. Epstein and Dr B. Bachmann for *E. coli* strains. We wish to acknowledge financial support from SERC. We wish to thank the following for helpful comments: Dr C.F. Higgins, Dr P. Postma, Dr H. Shuman, Dr W. Epstein and Dr E.P. Bakker.

REFERENCES

[1] Padan, E., Zilberstein, D. and Rottenberg, H. (1981) Biochim. Biophys. Acta 650, 151–166.
[2] Booth, I.R. and Kroll, R.G. (1983) Biochem. Soc. Trans. 11, 70–72.
[3] Postma, P.W. and Roseman, S. (1976) Biochim. Biophys. Acta 457, 213–257.
[4] Kornberg, H.L., Watts, P.H. and Brown, K. (1980) FEBS Lett. 117, K28–K36.
[5] Garcia, F., Bancroft, S., Rhee, S.G. and Kustu, S. (1977) Proc. Natl. Acad. Sci. USA 74, 1662–1666.

[6] Plack, R.H. and Rosen, B.P. (1980) J. Biol. Chem. 255, 3824–3825.
[7] Schuldiner, S. and Padan, E. (1981) in: Membranes and Transport (Martonosi, A. ed.) vol.2, pp.65–74.
[8] Zilberstein, D., Padan, E. and Schuldiner, S. (1980) FEBS Lett. 116, 177–180.
[9] Zilberstein, D., Agmon, V., Schuldiner, S. and Padan, E. (1982) J. Biol. Chem. 257, 3687–3691.
[10] Zilberstein, D., Agmon, V., Schuldiner, S. and Padan, E. (1984) J. Bacteriol., in press.
[11] MacDonald, R.E., Lanyi, J.K. and Greene, R.V. (1977) Proc. Natl. Acad. Sci. USA 74, 3167–3170.
[12] Tsuchiya, T., Raven, J. and Wilson, T.H. (1977) Biochem. Biophys. Res. Commun. 76, 26–31.
[13] Bachmann, B.J. (1983) Microbiol. Rev. 47, 180–230.
[14] Boronat, A., Britton, P., Jones-Mortimer, M.C., Kornberg, H.L., Lee, L.G., Murfitt, D. and Parra, F. (1984) J. Gen. Microbiol. 130, 673–685.
[15] Ahmed, S. and Booth, I.R. (1983) J. Gen. Microbiol. 129, 2521–2529.
[16] Miller, J.H. (1972) in: Experiments in Molecular Genetics, Cold Spring Harbor Laboratory.
[17] Kleckner, N., Roth, J. and Botstein, D. (1977) J. Mol. Biol. 116, 125–159.
[18] Umbarger, H.E. (1978) Annu. Rev. Biochem. 47, 533–606.
[19] Qureshi, M.A., Smith, D.A. and Kingsman, A.J. (1975) J. Gen. Microbiol. 89, 353–370.
[20] Mizobuchi, K., Demerec, M. and Gillespie, D.H. (1962) Genetics 47, 1617–1627.
[21] Kingsman, A.J., Smith, D.A. and Hulanicka, M.D. (1978) Genetics 89, 419–437.
[22] Alper, M.D. and Ames, B.N. (1978) J. Bacteriol. 133, 149–157.
[23] Daniel, J., Joseph, E., Ullman, A. and Danchin, A. (1981) FEMS Lett. 10, 389–393.
[24] Meury, J. and Kepes, A. (1982) EMBO J. 1, 339–343.
[25] Brey, R.N., Beck, J.C. and Rosen, B.P. (1978) Biochem. Biophys. Res. Commun. 83, 1588–1594.
[26] Kroll, R.G. and Booth, I.R. (1981) Biochem. J. 198, 691–698.
[27] Theze, J., Margarita, D., Cohen, G.N., Borne, F. and Patte, J.C. (1974) J. Bacteriol. 117, 133–143.