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Isolation and Genetic Characterization of *Escherichia coli* Mutants Defective in Propionate Metabolism

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Escherichia coli mutants defective in propionate metabolism (Prp^-) were isolated after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Prp^- mutants demonstrate a phenotypic inability to grow on odd-chain-length fatty acids. The new genetic locus for the Prp^- phenotype maps at approximately 98 min on the *E. coli* chromosome.

In *Escherichia coli*, propionate generally arises as the terminal three-carbon fragment in the oxidation of odd-chain-length fatty acids (16, 17). Proprionate metabolism in *E. coli* has been studied in some detail, but the mechanism of metabolism is still unclear. The mechanism of propionate metabolism seems complex and involves at least two pathways: (i) α-oxidation to pyruvate (considered the major route of metabolism) and (ii) metabolism via the hydroxyglutarate pathway (7, 13, 16-19).

In 1971, Salanitro and Wegener (13) isolated an *E. coli* mutant that was capable of growing on even-chain-length fatty acids, but not on odd-chain-length fatty acids. These investigators showed that their mutant, N_3V^-, could not oxidize propionate. However, no further genetic or biochemical studies were performed to determine the nature of the propionate defect in their mutant.

In the course of studying mutants defective in fatty acid degradation (*fad*), we have isolated *E. coli* mutants that fail to grow on odd-chain-length fatty acids due to a defect in propionate metabolism. The genotype of mutants defective in propionate metabolism will be referred to as *prp*. We report on the isolation of Prp^- mutants and the mapping of the *prp* locus. This mutation (*prp*) represents a new genetic locus in an unpopulated region of the *E. coli* linkage map.

The bacterial strains employed are listed in Table 1. Mutants defective in the utilization of propionate were isolated from an *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-mutagenized culture of LS5218 (*atoC fadR*) which was ampicillin enriched in medium containing nonanoate as the sole source of carbon and energy and selected on minimal agar plates containing acetate as the sole carbon and energy source. Approximately 250 colonies were picked and replica plated onto minimal media containing various fatty acids as sole carbon sources. Five colonies were able to grow on even-chain-length fatty acids, but not on short (C_3 and C_5) and medium (C_7, C_9 and C_{11}) odd-chain-length fatty acids as sole carbon sources. The growth properties of the parent strain, LS5218 (prp^+), and the mutant strain, LS5221 (*prp*), are shown in Table 2. Although the Prp^- strain LS5221 was capable of growing slowly on longer odd-chain-length fatty acids (Table 2), it appeared that the expression of the Prp^- phenotype (inability to grow on odd-chain-length fatty acids) was primarily a function of the ratio of propionate to acetate produced from the oxidation of a given odd-chain-length fatty acid. When this ratio was high, as in the case of oxidation of shorter odd-chain-length fatty acid (especially C_3 and C_5), culture growth did not occur. A decrease in this ratio, as in the case of oxidation of longer odd-chain-length fatty acids, lowered the accumulation of propionate and some limited culture growth occurred. Thus, the phenotypic inability of the Prp^- mutant to grow on odd-chain-length fatty acids appears to be dependent on the ability of the organism to metabolize endogenously generated propionate. Alternatively, it is possible that the accumulation of endogenously generated propionate, or a further metabolite of propionate, is toxic to the cells. We have observed that *prp* strains do not grow well on a mixture of even-chain-length fatty acids and propionate or other odd-chain-length fatty acids (data not shown).

The new genetic locus *prp* has been mapped in strain LS5221. Figure 1 shows the origins and direction of chromosome transfer of six Hfr strains (KL14, KL16, KL983, LK99, HfrH, and HfrC) used to determine the approximate location of the *prp* locus by the method of Low (8). Phenocopy cultures of LS5225 were obtained by starvation of logarithmic-phase cells in medium E for 5 h at 37°C (9). prp^+ Streptomycin-resistant (Str^r) recombinants were selected on minimal media containing either propionate or non-

TABLE 1. *Bacterial strains employed*

Strain	Sex	Relevant genotype[a]	Source
LS3010	F+	*fadR1*	R. W. Simons et al. (14)
LS5218	F+	*fadR atoC*	This work
LS5219	F+	*prp* derivative of LS5218	This work
LS5220	F+	*prp* derivative of LS5218	This work
LS5221	F+	*prp* derivative of LS5218	This work
LS5222	F+	*prp* derivative of LS5218	This work
LS5223	F+	*prp* derivative of LS5218	This work
LS5225	F+	*prp* Str^r derivative of LS5221	This work
LS5432	F+	*prp* derivative of LS5218	This work
AB4141	F−	*valS7 metC56 thi-1*	B. B. Magee (3) strain via CGSC[b]
JBl	Hfr	*metB1 uxuA1*	J. M. Robert-Baudouy and R. C. Portalier strain (12) via CGSC
PCl	F−	*leuB6 thyA47 dnaC1 strA153*	P. Carl (4) strain via CGSC
AB313	Hfr	*thr-1 leuB6 thi-1*	A. L. Taylor (15) strain via CGSC
JC1552	F−	*argG6 metR1 his-1 leu-6 trp-31 str-104*	A. J. Clark (5) strain via CGSC
HfrH	Hfr	Prototrophic	W. Hayes (2) strain via CGSC
HfrC	Hfr	Prototrophic	K. B. Low (8) strain via CGSC
KL14	Hfr	Prototrophic	K. B. Low (8) strain via CGSC
KL16	Hfr	Prototrophic	K. B. Low (8) strain via CGSC
KL98	Hfr	Prototrophic	K. B. Low (8) strain via CGSC
KL99	Hfr	Prototrophic	K. B. Low (8) strain
LS6035	F−	*dnaC1 thyA47 leuB6 fadR*::Tn*5*	This work
LS6036	F−	*dnaC1 thyA47 leuB6 fadR*::Tn*5 prp*	This work
LS6037	F+	*zji*::Tn*10*[c] *prp*	This work
LS6038	F−	*metB1 uxuA fadR*::Tn*5*	This work

[a] Only relevant and auxotrophic markers are listed; see references for complete genotype.

[b] CGSC, Coli Genetic Stock Center, Yale University, New Haven, Conn.

[c] Transposon insertions are designated as previously described (6). When a Tn insertion is not within a known gene, it is given a three-letter symbol starting with *z*, and the second and third letters indicate the approximate location in minutes (e.g., *zaj* corresponds to 9 min, and *zji* corresponds to 98 min).

anoate as the sole carbon source. These recombinants were formed only in matings (30 min) with Hfr strains H and C, implying that the *prp* gene was located between 96 and 13 min on the genetic map (1). *prp*$^+$ *str*$^+$ recombinants were formed after 5 min of mating LS5225 with strain HfrH and after 15 to 20 min of mating LS5225 with strain HfrC. The latter results suggested that the *prp* gene is closer to 96 min on the genetic map (1).

A cross between strains HfrH (*dnaC*$^+$ *prp*$^+$ *leu*$^+$ Str^r) and LS6036 (F$^-$ *dnaC leu prp* Tet^r Str^r) revealed that 90% of the *prp*$^+$ Str^r recombinants were *dnaC*$^+$, and that 23% of the *leu*$^+$ Str^r recombinants were *prp*$^+$. These results suggested that the *prp* locus was close to the *dnaC* locus, which maps at 99 min on the *E. coli* linkage map.

The *prp* locus was more precisely mapped by determining cotransduction frequencies of four different alleles of this gene with *valS*, *uxuA*, *dnaC*, and *thr*, which were previously localized between 96 and 100 min on the *E. coli* chromosome (Fig. 1). The different *prp* mutants were weakly cotransducible (about 3.5%) with *dnaC* and not at all with the distal markers *valS* and *thr*. The frequency of cotransduction of the different *prp* mutations with *uxuA* was approximately 44%, suggesting a gene order of *uxuA-prp-dnaC*. We could not precisely order the *prp*

TABLE 2. *Growth behavior of LS5218 and LS5221 on various fatty acids*[a]

Chain length of fatty acid (carbon source)[b]	Extent of growth (no. of doublings)	
	LS5218	LS5221
C_2	4	4[c]
C_3	4	NG
C_4	4	4
C_5	4	NG
C_6	4	4
C_7	4	NG
C_8	4	4
C_9	4	NG
C_{10}	4	4
C_{11}	4	NG
C_{12}	4	4
C_{13}	4	1
C_{14}	4	4
C_{15}	4	1
C_{16}	4	4
C_{17}	4	1
$C_{18:1}$	4	4

[a] Cultures of LS5218 and LS5221 were pregrown to exponential phase in M9-Brij (10) containing acetate (40 mM) and propionate (10 mM). A sufficient inoculum from these cultures was added to M9-Brij containing the indicated fatty acid to produce a turbidity of 10 to 15 Klett-Summerson units. Cell growth was monitored at 540 nm in a Klett-Summerson Colorimeter.

[b] Fatty acids of various chain lengths were suspended in 10% Brij 58, neutralized with KOH, sterilized, and added as the sole source of carbon at the following concentrations: C_3 to C_6, 10 mM; C_7 to C_{13}, 5 mM; and C_{14} to C_{18}, 2.5 mM.

[c] NG, No growth within 48 h.

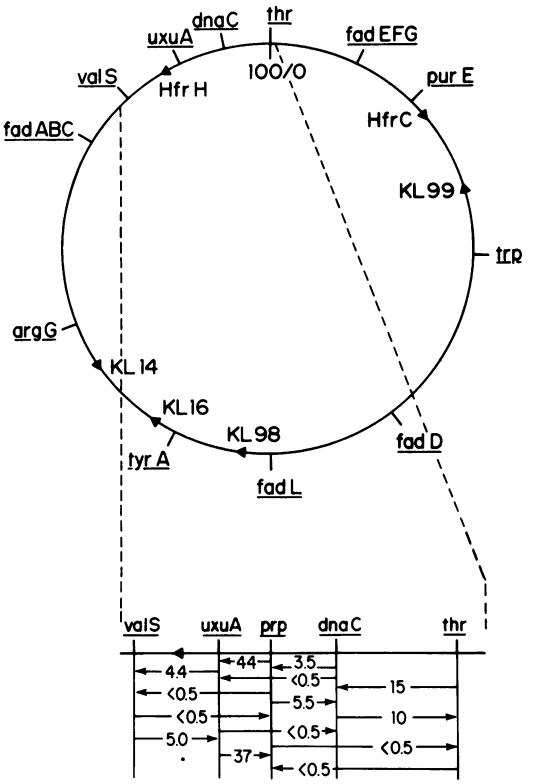

FIG. 1. *Location of the prp gene on the genetic map of E. coli. Circular map adapted from Bachmann and Low (1). The origin and direction of chromosome transfer are taken from Low (8). The values at the bottom are P1 cotransduction frequencies, with the arrowheads indicating the selected markers. The enlarged portion is not drawn to scale. Transduction and phage stock preparations were carried out as described by Nunn et al. (10, 11). Conjugation experiments were as described by Miller (9). The genotypes of strains used in these studies are listed in Table 1.*

locus with respect to *uxuA* and *dnaC* due to an inability to demonstrate cotransduction between *uxuA* and *dnaC*. These results indicated that the *uxuA* and *dnaC* markers were too far apart to be used in a three-factor cross. The absence of other genetic markers with selectable phenotypes in the 97- to 99-min region also prevented precise mapping of the *prp* gene. The 97- to 98-min region on the *E. coli* chromosome remains sparsely populated, containing loci with phenotypes that are not easily selected. Mapping of the *prp* gene in this region provides a marker with a readily selectable or identifiable phenotype in cotransduction studies with new markers mapping in this region.

We have been unsuccessful in our attempts to identify the *prp* defect in our mutants. The fact that spontaneous Prp^+ revertants obtained by selection for growth of strains LS5221, LS5219, LS5223, and LS5220 on propionate can grow on odd-chain-length fatty acids indicates that these mutants are indeed defective in propionate metabolism. Two pathways of propionate metabolism in *E. coli* have been postulated: (i) α-oxidation to pyruvate and (ii) metabolism via the hydroxyglutarate pathway. The metabolism of propionate via α-oxidation to propionate requires functional propionyl coenzyme A (CoA) synthetase, propionyl CoA dehydrogenase, acrylyl CoA hydratase, and lactate dehydrogenase activities. Our results showed that the Prp^- strain, LS5221, incorporated [1-^{14}C]propionate into phospholipids at rates comparable to those for the Prp^+ strain (data not shown), indicating that the *prp* defect is not in propionyl CoA synthetase activity. Secondly, our mutants appeared to have functional propionyl CoA dehydrogenase activity. Our studies also indicated that the hydroxyglutarate synthetase and succinate semialdehyde activities (required for metabolism of propionate via hydroxyglutarate) of our Prp^- mutants were comparable to the activity found in the Prp^+ strains (data not shown). The accurate identification of the *prp* defect in our mutant may help resolve the question of which pathway is utilized in propionate metabolism in *E. coli*.

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