Identification of a new prp locus required for propionate catabolism in *Salmonella typhimurium* LT2


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Abstract

A new propionate (prp) locus of *S. typhimurium* was defined by mutation, was located to minute 8 of the chromosome, and was shown to be transcribed in the clockwise direction. A plasmid carrying the wild-type prp+ locus was isolated by complementation, and its initial physical characterization is presented. Transcriptional regulation of prp was studied using MudI1734(lacZ+) operon fusions. Propionate stimulated prp transcription in a merodiploid strain containing prp+ and a prp::MudI1734 fusion, but failed to stimulate transcription of the same fusion in a haploid genetic background. prp transcription was reduced by a factor of 2 in strains deficient in the synthesis of the global regulatory protein FruR; fruR mutants failed to grow on propionate. Propionate blocked growth of prp mutants on medium containing succinate as carbon/energy source.

Keywords: Propionate catabolism; *Salmonella*; Reactive aldehyde intermediates; DNA polymerase I; Glutathione

1. Introduction

Possible pathways for the breakdown of propionate in *Salmonella typhimurium* LT2 have been proposed [1]. However, the biochemistry, genetics, and regulation of these pathways are largely undocumented. In fact, in this bacterium, only one genetic locus has been reported to be somehow involved in propionate catabolism. The prpA locus (prp for propionate) was mapped around minute 98 between argl and pyrB [2]. However, the function(s) of the gene product(s) encoded by this locus remains unknown, and it is unclear whether this locus is directly or indirectly involved in the breakdown of propionate. Recent work in *E. coli* suggested the existence of multiple catabolic pathways for propionate [3,4]. However, as far as we know, no genetic or biochemical analysis of these pathways has been reported.

We have recently shown that growth of *S. typhimurium* LT2 on ethanolamine, 1,2-propanediol, and propionate requires the involvement of glutathione (GSH) [5], and DNA polymerase I (PolI) [6]. The hypothesis proposed to explain these results suggests that GSH may play a role in quenching reactive aldehyde intermediates which may inacti-
vate enzymes, and/or transport or regulatory pro-
teins, while PolI is required to repair DNA modified
by unquenched aldehydes [5,6]. In support of this
idea, exogenously provided acetaldehyde (which in
vivo is endogenously derived from ethanolamine
catabolism) and propionaldehyde (which in vivo is
derived endogenously by the 1,2-propanediol uti-
Hization pathway) were shown to have deleterious
effects on the growth of polA and gshA mutants,
with the polA gshA double mutant displaying ex-
treme sensitivity to these aldehydes [6]. Putative
reactive aldehydes of the propionate catabolic path-
way(s) were not proposed because genetic data sup-
porting the proposed biochemistry of this pathway
are missing in S. typhimurium.

To improve our understanding of propionate
catabolism in this bacterium we pursued the isolation
and genetic analysis of mutants unable to grow with
propionate as carbon/energy source. Some of these
mutants carry lesions at minute 8 region of the
chromosome. This is a new prp locus which is
thought to encode functions needed for propionate
breakdown.

One additional locus also needed for propionate
utilization was identified as fruR, which encodes the
global transcriptional regulatory protein FruR [7].

2. Materials and methods

2.1. Bacteria, culture media, and growth conditions

All bacterial strains used were derivatives of S.
typhimurium LT2. Of these strains the following
were derivatives of TR6583 (metE205 ara-9): 
JE2167, JE2170, JE2437, and JE3056. All TT and
TR strains were obtained from J.R. Roth (University
of Utah). LJ2443 was from M. Saier (University of
California–San Diego). The JE strains described were
constructed during the course of these studies. Cloning experiments utilized E. coli DH5α F' [8]
and the restriction-proficient, modification-proficient
S. typhimurium strain JR501 [9].

Nutrient broth (0.8%, wt/vol) containing 85 mM
NaCl was used as a complex medium. No-carbon E
(NCE) medium supplemented with 1 mM MgSO4
[10], and E medium [11] supplemented with 11 mM
glucose were used as minimal media. The final
centrations of compounds provided in the culture
medium, unless stated otherwise, were as follows:
glucose, 25 mM; propionate, 25 mM; glycerol, 50
mM; succinate, 50 mM; methionine, 0.5 mM; threo-
nine, 0.5 mM; 5-bromo-4-chloro-3-indolyl-β-D-
galactopyranoside (X-gal), 20 mg/liter. Antibiotic
concentrations in rich medium were (mg/liter):
kanamycin, 50; ampicillin, 40; tetracycline, 20; and
chloramphenicol, 20. In minimal medium: kanamyc-
in, 25; ampicillin, 20; tetracycline, 10; chloramphen-
icol, 5.

Cultures for β-galactosidase assays were routinely
grown in 5 ml of medium in a 125-ml Klett flask and
vigorously shaken at 400 rpm to ensure aeration.
Approximately 10⁶ cfu were inoculated into 5 ml of
fresh medium (i.e., approx. 2 × 10⁹ cfu/ml), and the
cultures were grown to mid-exponential phase (60–
80 Klett Units, or approx. 2 X 10⁷ cfu/ml). Cell
growth was monitored with a Klett-Summerson col-
orimeter equipped with a red filter. Cultures for
growth curves were grown in test tubes as described
above. Absorbance was monitored with a Spectronic

2.2. Genetic techniques

2.2.1. Transductions

Genetic crosses involving phage P22 HT105 int-
201 [12,13] were performed as previously described
[10,14].

2.2.2. Generation of pools of insertion mutations

A pool of mutant strains carrying a transposition-
defective derivative of Tn10, TnIOd(Tc) [15], was ob-
tained as described elsewhere [16]. The method of
Hughes and Roth [17] was used to generate a pool of
independent MudI1734(lacZΔA, Km') [18] (hereafter
referred to as MudI).

2.2.3. Isolation of TN10dTc and MudI insertions

that render the cell unable to use propionate as
carbon/energy source

Strain TR6583 (metE205 ara-9 prp +) was trans-
duced to tetracycline resistance with a P22 phage
lysate grown on a pool of approx. 80,000 inde-
dependent Tn10dTc mutants. Tc⁰ transductants unable to
grow on minimal medium supplemented with propi-
onate and methionine were freed of phage, and analyzed further.

A similar approach was used to isolate \textit{prp}::MudJ strains. In this case TR6583 was transduced to Km resistance using a P22 phage lysate grown on a pool of approx. 50,000 strains each carrying one MudJ insertion.

The MudJ or Tn10d(Tc) element was considered to be responsible for the Prp phenotype when \( \geq 1000 \) Km' (Tc') strains also displayed a Prp phenotype.

### 2.2.4. Mapping

The general location of a Tn10d(Tc) element in the chromosome was identified using P22 phage lysates obtained by induction of Mud-P22 prophages in the mapping strain kit of Benson and Goldman [19,20], as donors, and the Tn10d(Tc)-containing strains as recipients. Bochner's medium [21,22] was used to increase the frequency of isolation of Tc-sensitive transductants. The chromosomal location of the \textit{prp-121::Tn10d(Tc)} element was further established by co-transduction to nearby markers [23].

The Mud-P22 mapping strain kit was also used to determine the location of MudJ insertions, except that growth on minimal NCE-propionate medium instead of Bochner medium was used to establish the general chromosomal location of the insertion.

### 2.2.5. Direction of \textit{prp} transcription

The direction of \textit{prp} transcription was determined genetically by the method of Hughes and Roth [24]. Mutation \textit{prp-114::MudJ} was converted to a Mud1-8 (hereafter referred to as MudA) by the method described elsewhere [18]. This was done to increase the homology, thus the frequency of recombination between two MudA elements. Briefly, we employed a P22 lysate grown on the \textit{prp-114::MudA} (JE2437) strain to co-infected strain TR6583 (\textit{prp}'). The second donor was P22 phage grown on a strain carrying a \textit{thr::MudA} insertion of known orientation (strain TT8370 (\textit{thr-458::MudA}) or TT8371 (\textit{thr-469::MudA})). Ampicillin-resistant (Ap') transductants selected on rich medium containing ampicillin were patched on a nutrient agar-Ap (NA-Ap) plate, grown for about 6 h, and replica printed to NCE-propionate medium lacking threonine, E-glucose medium with and without threonine, and NA-Ap medium. Prp+ Thr+ Ap' transductants can only arise when the two MudA elements recombine prior to insertion into the chromosome, and yield a hybrid that upon recombination with the chromosome generates a duplication with the MudA insertion at the join point.

### 2.2.6. Construction of a merodiploid strain needed to study \textit{prp} transcription

Strain TT13632, which contained a Tn10-held duplication of the \textit{S. typhimurium} LT2 chromosome from 7 to 23 minutes (DUP1033[\textit{proC-pyrC}]), was transduced to Km resistance with phage grown on strain JE2170 (\textit{prp-114::MudJ}). Km' transductants were selected on NA medium containing both Tc and Km. Tc was always present in the medium to ensure maintenance of the duplication. The resulting strain, JE3110 (DUP1033[\textit{proC-pyrC}]\textit{Tn10\*prp-114::MudJ}) was transduced to Cm resistance with phage grown on strain JE3644 containing \textit{fruR426::MudP(Cm)} insertion to yield strain JE3651 (DUP1033[\textit{proC-pyrC}]\textit{prp-114::MudJ fruR426::MudP}).

### 2.2.7. Transformations

All transformations were performed by electroporation as described previously [25].

### 2.3. \(\beta\)-galactosidase enzyme activity assays

\(\beta\)-galactosidase enzyme activity was assayed as reported [26].

### 3. Results and discussion

#### 3.1. Mutants with a \textit{Prp} phenotype

Twelve mutants unable to use propionate as the sole source of carbon and energy were isolated. Of these, 11 carried MudJ insertions, 1 carried a Tn10d(Tc) element. Eleven of the 12 MudJ insertions isolated were co-transducible with the Tn10d(Tc) element. Since most of these mutations appeared to be clustered, they were assumed to affect the same locus, and thus they were referred to as \textit{prp} mutants. One MudJ insertion was not co-transducible with the Tn10d(Tc) element, and was later mapped to the \textit{fruR} locus (see below).
Insertion prp-109::MudJ (strain JE2167) was 65% co-transducible with prp-121::Tn10d(Tc) (strain JE3056) suggesting a considerable distance between these markers, and thus raising the possibility that this region may contain more than one gene.

3.2. Chromosomal location of the new prp locus

The prp-121::Tn10d(Tc) element was initially placed within the minute 8 region of the chromosome as described above. Two-factor crosses showed 7% co-transduction between insertion prp-121::Tn10d(Tc) (strain JE3056, donor) and proC963::MudP (strain TT15230, recipient); no linkage to proAB was observed.

During the preparation of this paper, the new edition of the S. typhimurium genetic map was published [27]. In this new edition we noticed a reference to a prpB locus around the minute 8 region reported as unpublished results by Tittensor and co-workers. It is likely that we have identified the same genetic locus. However, since we believe there is more than one gene within it, we refrain from referring to it as prpB.

3.3. Direction of transcription of prp

The direction of transcription of prp was determined genetically as described above. Co-infection of TR6583 with phage lysates grown on TT8370 (thr-458::MudA) and JE2437 (prp-114::MudA) yielded only 6 Prp+ Thr+ Ap+ transductants amongst 291 Ap+ transductants tested, a frequency of 2%, which is within the range of spontaneous duplications of random regions of the chromosome [28]. In contrast, when phage on strain TT8371 (thr-469::MudA) was used to co-infect TR6583 with phage grown on strain JE2437 (prp-114::MudA), 39 Prp+ Thr+ Ap+ transductants were identified amongst 257 Ap+ transductants tested, a frequency of 15%. The appearance of Prp+ Thr+ Ap+ transductants required a recombination event between MudA elements inserted into genes with the same direction of transcription. On the basis of these data, the direction of prp transcription was determined to be clockwise. Similar conclusions were obtained when the experiment was performed using leu::MudA insertions (strains TT9625 (leuC1184::MudA) or TT9623 (leuC1184::MudA)). Only the co-infection involving phage grown on TT9625 (clockwise, MudA element) yielded prototrophic transductants capable of growing on propionate as carbon/energy source (data not shown).

3.4. Initial physical analysis of the prp locus

Plasmid pPRP1 (prp+) was isolated from a sized (approx. 8–9 kb fragment) clone bank of S. typhimurium LT2 chromosomal DNA digested with Sau3A and cloned into the BamHI site of the tetA gene of vector pBR328 (Ap+ Cm+4.9 kb). This plasmid was introduced into all of our prp mutants with lesions in the minute 8 region. When introduced into strain JE3177 (E. coli DH5αF'/pPRP1 prp+), the size of the prp fragment (heavy black segment) was calculated to be approx. 8 kb. The number of EcoRI sites within the prp fragment was greater than the ones shown in the figure; only those shown were clearly discernible. The numbers correspond to the distance (kb) of the restriction sites clockwise from position 0.

TT9623 (leuC1184::MudA)). Only the co-infection involving phage grown on TT9625 (clockwise, MudA element) yielded prototrophic transductants capable of growing on propionate as carbon/energy source (data not shown).

3.5. Insertions outside the prp locus which result in a Prp phenotype

One of the 12 mutants displaying a Prp phenotype was shown to carry a MudJ insertion at minute 3 of...
the linkage map. This MudJ mutation was 86% co-transducible by P22 with leu, and was identified as an allele of fruR based on two lines of evidence. First, the MudJ insertion was > 99% linked to the bona fide fruR51::TnlO mutation (strain LJ2443). Second, the strain carrying this MudJ insertion (strain JE3128) displayed all the growth phenotypes reported for fruR mutants [29]. Therefore, the MudJ insertion in question was referred to as fruR426::MudJ (strain JE3128).

The simplest explanation for the Prp phenotype of the fruR mutant (JE3128) was that the observed drop in prp expression in this strain (Table 1) was sufficient to prevent propionate catabolism even after prolonged incubation. Since the effect of a lack of FruR may be indirect, the physical analysis of the promoter region of the prp locus is needed to ascertain whether FruR directly regulates prp transcription.

3.6. Regulation of prp transcription

The data presented in Table 1 show that the operon fusion prp-114::MudJ strain (JE2170) was not expressed in a haploid genetic background in response to propionate in the medium. These results could be due to polar effects of the MudJ element on a positive regulator needed for the expression of the prp operon, or to polar effects on the expression of functions needed for the synthesis of a metabolite of propionate which is the effector recognized as a signal of propionate in the environment.

To document the need for wild-type prp+ functions, we introduced insertion prp-114::MudJ into a strain carrying a duplication of the region containing the prp locus (strain TT13632). The resulting strain (JE3110) (prp+ prp-114::MudJ) was grown to mid-exponential phase in the presence or absence of propionate in the medium, and β-galactosidase activity levels were determined. Expression of prp in JE3110 increased 39-fold in the presence of propionate over the levels measured in the absence of propionate.

The data in Table 1 also show that a functional FruR regulatory protein was needed for full expression of prp. Lack of the global regulatory FruR protein resulted in a reproducible 2-fold decrease in prp expression in the merodiploid strain JE3651 (prp+ prp-114::MudJ fruR426::MudP), both in the presence and absence of propionate in the medium. It was surprising that the prp+ fruR strain JE3128 was unable to use propionate as carbon/energy source, in spite of the substantial prp transcription (153 β-galactosidase units) observed in the merodiploid strain JE3651.

3.7. Effect of propionate on the ability of prp mutants to utilize alternative carbon sources

Fig. 2 demonstrates the negative effect of propionate on growth of a prp mutant (strain JE2170) on alternative carbon/energy sources. The utilization of glucose, glycerol, and succinate were affected to different degrees by the presence of propionate. In the case of glucose (Fig. 2, closed, open squares), the rate of growth and the final cell density of the culture were largely unaffected whether or not propionate was present in the culture medium, and a slight delay in the onset of growth was noted when propionate was present in the medium. When glycerol was

Table 1
Effect of the lack of FruR function on prp expression in haploid and merodiploid strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>β-Galactosidase (U/μg)</th>
<th>NCE glycerol with added</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JE2170</td>
<td>prp-114::MudJ</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>JE3110</td>
<td>prp+ * Tn10* prp-114::MudJ</td>
<td>9</td>
<td>351</td>
</tr>
<tr>
<td>JE3651</td>
<td>prp+ * Tn10* prp-114::MudJ</td>
<td>5</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>fruR426::MudP</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*β-Galactosidase enzyme activity was assayed in cultures grown to approx. 2 x 10⁸ cfu/ml (mid-exponential phase). Reported values are the average of 5 separate experiments; standard deviation was ± 10.
provided as the source of carbon/energy, propionate had a very small effect on the rate and the lag (Fig. 2; closed, open circles), and the culture eventually reached the same cell density as the control culture.

The most dramatic effect of propionate was noted when succinate was the sole source of carbon and energy (Fig. 2; open, solid triangles). The culture growing with succinate in the absence of propionate reached full density within 28 h of incubation. However, when propionate was included in the medium, the culture failed to grow even after prolonged incubation (50 h). This drastic effect of propionate on succinate catabolism in a \textit{prp} mutant was observed even when the concentration of propionate in the medium was reduced 25-fold to 1 mM (data not shown). It is unclear whether this negative effect of propionate is due to a build up of a toxic metabolite or a regulatory signal.

This negative effect of propionate appeared to be specific since inclusion in the medium of fatty acids (final concentration of 50 mM) with carbon skeleton length ranging from formate (C-1), acetate (C-2), butyrate (C-4), caproate (C-5), valerate (C-6), and octanoate (C-8), did not have a negative effect on the ability of strain JE2170 (\textit{prp-114::MudJ}) to utilize succinate as carbon/energy source (data not shown).

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**References**


activator and repressor functions which is homologous to the periplasmic ribose-binding protein. Res. Microbiol. 142, 951–963.


