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Propionate Catabolism in *Salmonella typhimurium* LT2: Two Divergently Transcribed Units Comprise the *prp* Locus at 8.5 Centisomes, *prpR* Encodes a Member of the Sigma-54 Family of Activators, and the *prpBCDE* Genes Constitute an Operon

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We present the initial genetic and biochemical characterization of the propionate (*prp*) locus at 8.5 centisomes of the *Salmonella typhimurium* LT2 chromosome (T. A. Hammelman et al., FEMS Microbiol. Lett. 137: 233–239, 1996). In this paper, we report the nucleotide sequences of two divergently transcribed transcriptional units. One unit is comprised of the *prpR* gene (1,626 bp) encoding a member of the sigma-54 family of transcriptional activators; the second unit contains an operon of four genes designated *prpB* (888 bp), *prpC* (1,170 bp), *prpD* (1,452 bp), and *prpE* (1,923 bp). The heme biosynthetic gene *hemB* was shown by DNA sequencing to be located immediately downstream of the *prpBCDE* operon; *hemB* is divergently transcribed from *prpBCDE* and is separated from *prpE* by a 66-bp gap. In addition, we demonstrate the involvement of PrpB, PrpC, and PrpD in propionate catabolism by complementation analysis of mutants using plasmids carrying a single *prp* gene under the control of the arabinose-responsive P_{BAD} promoter. Expression of *prpB* to high levels was deleterious to the growth of a prp^+ strain on minimal medium supplemented with propionate as a carbon and energy source. We also report the cloning and overexpression of *prpB*, *prpC*, *prpD*, and *prpE* in the T7 system. PrpB, PrpC, PrpD, and PrpE had molecular masses of ca. 32, ca. 44, ca. 53, and ca. 70 kDa, respectively. PrpB showed homology to carboxyphosphonoenolpyruvate phosphonomutase of *Streptomyces hygroscopicus* and to its homolog in the carnation *Dianthus caryophyllus*; PrpC was homologous to both archaeal and bacterial citrate synthases; PrpD showed homology to yeast and *Bacillus subtilis* proteins of unknown function; PrpE showed homology to acetyl coenzyme A synthetases. We identified a sigma-54 (RpoN)-dependent promoter with a consensus RpoN binding site upstream of the initiating methionine codon of *prpB*, the promoter-proximal gene of the *prp* operon. Consistent with this finding, an *rpoN* prp^+ mutant failed to use propionate as carbon and energy source. Finally, we report the location of MudI1734 elements inserted in *prpC* or *prpD* and of a Tn*10*Δ*16*Δ*17* element in *prpB* and provide genetic evidence supporting the conclusion that the *prpBCDE* genes constitute an operon.

The catabolism of propionate has been studied in both procaryotes and eucaryotes, which employ different strategies for the breakdown of this short-chain fatty acid. Propionyl coenzyme A (propionyl-CoA) appears to be the intermediate shared by propionate catabolic pathways (Fig. 1). Pathways identified in bacteria are the malonic semialdehyde-CoA pathway (39, 40, 42), the acrylate pathway (8), and the cobalamin-dependent methylmalonyl-CoA mutase pathway (7, 19). In the yeast *Saccharomyces cerevisiae* and filamentous fungi, propionate is catabolized via the 2-methylcitric pathway (21, 25), while in humans, plants, and insects, the methylmalonyl-CoA pathway has been documented (11, 28, 43).

The catabolism of propionate has been studied to a limited extent in *Salmonella typhimurium*. To our knowledge, only the *prpA* locus (95.5 centisomes [Cs]) has been reported to be required for the breakdown of propionate in this bacterium (9, 29). The gene products encoded by this locus are unknown, and their roles in propionate catabolism are unclear.

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Recently, we identified genetic loci at 8.5, 27.3, 62, and 86.5 Cs whose functions are needed for propionate catabolism in *S. typhimurium*. The gene at 27.3 Cs was shown to be *cobB*, which encodes an alternative phosphoribosyltransferase involved in cobalamin biosynthesis (35, 37, 38); the gene at 62 Cs was documented to be *gshA*, the first gene involved in the synthesis of glutathione (27); the gene at 86.5 Cs was identified as *polA*, which encodes DNA polymerase I (26); and the locus at 8.5 Cs was previously unknown and was referred to as *prp* (12).

This study focuses on the initial characterization of the *prp* locus at 8.5 Cs (12). We have characterized four genes organized into two divergently transcribed units. *prpR* encodes the putative transcriptional activator of the *prpBCDE* operon; the *prpBCDE* genes appear to encode proteins with enzymatic activity.

MATERIALS AND METHODS

Strains, media, and growth conditions. All strains used in this work were derivatives of *S. typhimurium* LT2; their genotypes and the genotypes of plasmids used or constructed during this work are listed in Table 1. The E medium of Vogel and Bonner (41) and no-carbon E (NCE) medium were used as minimal media. E medium contained citrate (10 mM) as a chelator of Mg(II) ions (1 mM). Routinely, E medium was supplemented with glucose (11 mM). NCE medium did not contain citrate, and Mg(II) ions were added to the medium after autoclaving once the medium reached room temperature to avoid formation of

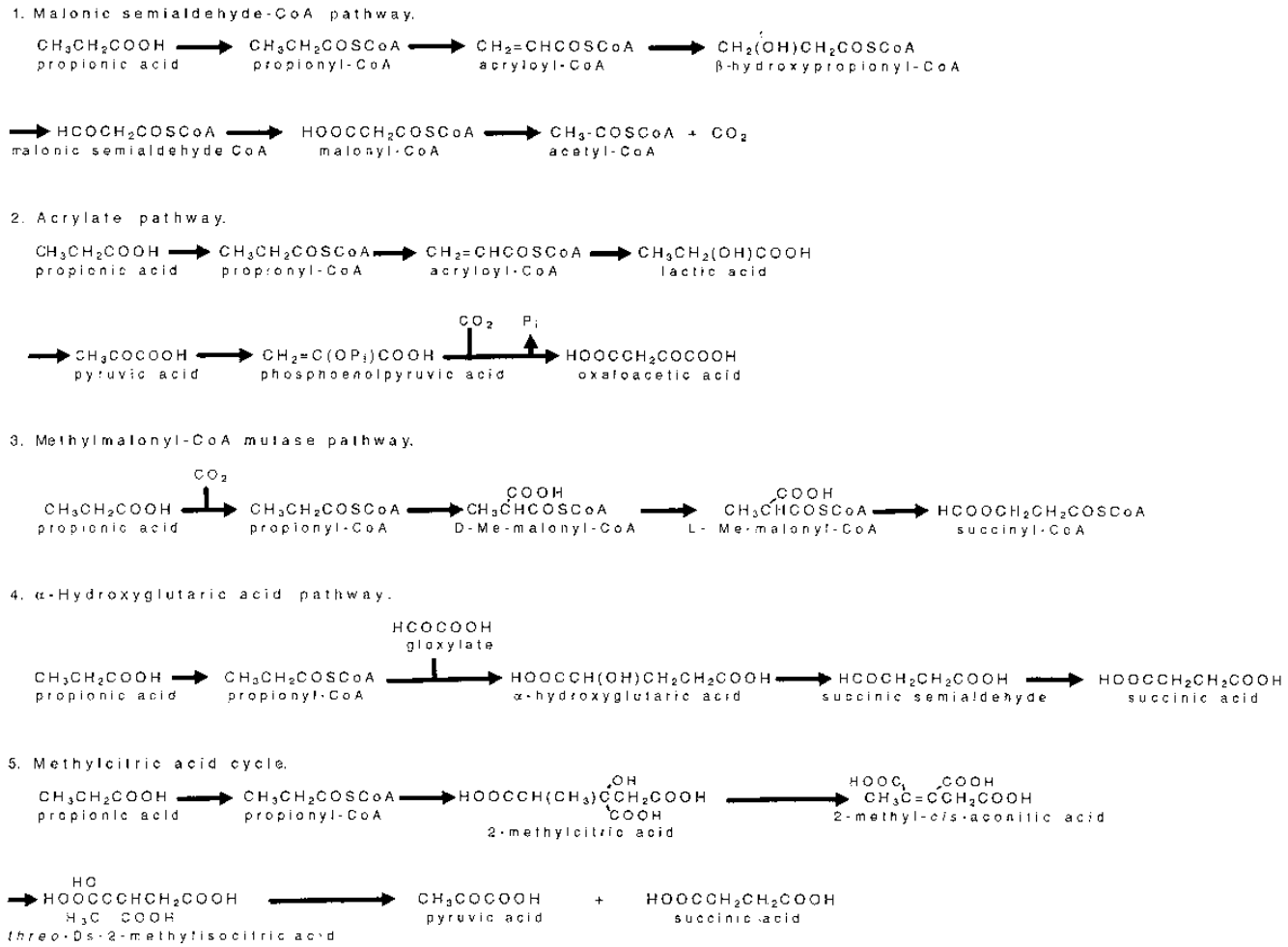


FIG. 1. Pathways of propionate catabolism in procaryotes. The conversion of propionate to propionyl-CoA is thought to be a shared step in all five pathways (42). None of these pathways have been demonstrated in *S. typhimurium*.

insoluble magnesium phosphate. Nutrient broth and Luria-Bertani broth were used as rich media (5). When added to NCE medium, propionate was at 30 mM, succinate was at 50 mM, methionine was at 0.5 mM, EGTA was at 10 mM, D-(+)-arabinose was at 500 μM (or as indicated), and $MgSO_4$ was at 1 mM. Antibiotics were used at concentrations previously reported (26). Crosses selecting for kanamycin-resistant or chloramphenicol-resistant transductants were preincubated for 1 h at 37°C without shaking in fresh medium lacking kanamycin or chloramphenicol.

Genetic techniques. (i) Transductions. All transductional crosses were performed as described elsewhere (6). To minimize lysogen formation, a lysate of bacteriophage P22 mutant HT105/1 *int201* was used (30, 31).

(ii) Localized mutagenesis and isolation of *prp* point mutants. The procedure of Hong and Ames (15) as described by Davis et al. (5) was used to introduce point mutations into the *prp* locus, using hydroxylamine as the mutagen. A P22 lysate (4×10^{11} PFU/ml) grown on strain JE3813 [*prp*$^+$ *zai-6386*::Tn*10d*(Tc)] was prepared as described elsewhere (5); the Tn*10d*(Tc) element present in strain JE3813 was 90% cotransducible with *prpD109*::MudJ. Hydroxylamine-mutagenized P22 lysate grown on JE3813 was used as the donor to transduce TR6583 (*prp*$^+$) to tetracycline resistance. To identify propionate mutants, tetracycline-resistant transductants were replica printed to NCE minimal plates containing propionate or succinate, EGTA, methionine, Mg(II), and tetracycline.

Recombinant DNA techniques. (i) DNA isolation and manipulations. Chromosomal DNA used to determine the DNA sequence flanking the *prpB121*::Tn*10d*(Tc) and *prpD109*::MudP elements was isolated from P22 lysates obtained after induction of the *prpD109*::MudP element, a Mud-P22 prophage inserted within *prp* (4, 44). The procedure used for the isolation of DNA from P22 lysates has been described elsewhere (26). To determine nucleotide sequences other than the ones flanking *prpB121*::Tn*10d*(Tc) and *prpD109*::MudP, plasmid DNA was isolated by using a QIAprep Spin Plasmid Miniprep kit from Qiagen Inc. (Chatsworth, Calif.). Restriction enzymes were purchased from New England Biolabs (Beverly, Mass.) and Promega (Madison, Wis.). DNA fragments were isolated from agarose gels by using a QIAquick gel extraction kit from Qiagen.

(ii) DNA sequencing. The nucleotide sequences of the DNA flanking the *prpB121*::Tn*10d*(Tc) and *prpD109*::MudP elements were determined with a SequiTherm Cycle Sequencing kit (Epicenter Technologies, Madison, Wis.), using [^{35}S]ATP, as instructed by the manufacturer. For this purpose, the oligomer 5′-TCCATTGCTGTTGACAAAGG-3′ was used as the primer. The oligomer bound to IS*10*-L of the *prpB121*::Tn*10d*(Tc) element, and the oligomer 5′-ATC CCGAATAATCCAATGTCC-3′ annealed to the MuL end of *prpD109*::MudP.

A Sequenase kit (version 2.0; Amersham Life Science, Arlington Heights, Ill.) was used to sequence *prpB* and portions of *prpC* and *prpR*. Primers used in this work were synthesized by Genosys (The Woodlands, Tex.) and National Biosciences, Inc. (Plymouth, Minn.). The rest of the *prpC*, *prpD*, *prpR*, *prpE*, and *hemB* DNA sequence was determined by using ABI PRISM dye terminator cycle sequencing at the UW-Madison Biotechnology Center; primers were synthesized at the UW Biotechnology Center. Plasmid pPRP1 was used as the DNA template to sequence *prpRCD*. Plasmid pPRP29 was used as template to sequence *prpE* and part of *hemB*. The coding and noncoding DNA strands of each gene were each sequenced at least twice.

(iii) Sequence data analysis. Sequence data were compared with sequences deposited in GenBank, using the BLAST algorithm developed by the National Center for Biotechnology Information and the National Library of Medicine (1). The alignments presented were obtained with the ClustalW 1.6 multiple sequence alignment program through the BCM Search Launcher of the Human Genome Center, Baylor College of Medicine, Houston, Tex.

(iv) PCR amplifications. All PCR amplifications of DNA encoded on plasmids were performed as described elsewhere (2). PCR amplification using overnight cell cultures was performed as previously described (16, 22). Deep Vent DNA polymerase from New England Biolabs was used in all PCR amplifications. PCR

TABLE 1. Strains and plasmids used in this study[a]

Strain or plasmid	Genotype	Reference or source
Strains		
S. typhimurium		
SK284	*ntrA209*::Tn*10 hisF645*	S. Kustu
JR501	*hsdSA29 hsdSB121 hsdL6 metA22 metE551 trpC2 ilv-452 rpsL120 xyl-404 galE719 H1-b H2-en,n,x* [cured of *Fels2*(−)] *fla-66 nml*	36
TR6583[b]	*metE205 ara-9*	K. Sanderson via J. Roth
Derivatives of TR6583		
JE3056	*prpB121*::Tn*10d*(Tc)	
JE2167	*prpD109*::MudJ	
JE2170	*prpC114*::MudJ	
JE3719	*prpD109*::MudP	
JE3760	*prpD109*::MudP *prpB121*::Tn*10d*(Tc)	
JE3813	*zai-6386*::Tn*10d*(Tc)	
JE3907	*zai-6386*::Tn*10d*(Tc) *prpC167*	
JE3946	*zai-6386*::Tn*10d*(Tc) *prpB195*	
JE3956	*zai-6386*::Tn*10d*(Tc) *prpD205*	
JE4015	pPRP15 *prpB*$^+$ *cat*$^+$ (Cm^r)	
JE4016	pPRP21 [P_{BAD} *prpB*$^+$ *bla*$^+$ (Ap^r)]	
E. coli		
K38	HfrC (λ)	34
DH5α/F′	F′/*endA1 hsdR17* (r_K^- m_K^+) *supE44 thi-1 recA1 gyrA* (Nal^r) *relA1* Δ(*lacZYA-argF*) U169 deoR [ϕ80d*lac*Δ(*lacZ*)*M15*]	New England Biolabs
Plasmids		
PBAD30	P_{BAD} expression vector; *bla*$^+$ (Ap^r)	10
pGP1-2	Plasmid carrying the T7 *rpo*$^+$ RNA polymerase, Km^r	34
pT7-6	Overexpression vector; *bla*$^+$ (Ap^r)	34
pT7-7	Overexpression vector; *bla*$^+$ (Ap^r)	34
pPRP1	*prpBCD*$^+$ *bla*$^+$ (Ap^r) *cat*$^+$ (Cm^r)	12
pPRP3	*prpBCD*$^+$ derived from pPRP1; Km^r	
pPRP8	*prpBC*$^+$ derived from pPRP3; Km^r	
pPRP12	*prpBCD*$^+$ derived from pPRP3; Km^r	
pPRP14	*prpBC*$^+$ derived from pPRP8; *bla*$^+$ (Ap^r) Km^r	
pPRP15	*prpB*$^+$ derived from pPRP14; *cat*$^+$ (Cm^r)	
pPRP19	*prpB*$^+$ in pT7-7; *bla*$^+$ (Ap^r)	
pPRP21	*prpB*$^+$ in pBAD30; *bla*$^+$ (Ap^r)	
pPRP22	*prpC*$^+$ in pT7-7; *bla*$^+$ (Ap^r)	
pPRP24	*prpD*$^+$ in pT7-7; *bla*$^+$ (Ap^r)	
pPRP29	*prpBCD*$^+$ *bla*$^+$ (Ap^r); *cat*$^+$ (Cm^r)	
pPRP34	*prpC*$^+$ in pSU21; *cat*$^+$ (Cm^r)	
pPRP35	*prpC*$^+$ in pBAD30; *bla*$^+$ (Ap^r)	
pPRP36	*prpD*$^+$ in pBAD30; *bla*$^+$ (Ap^r)	
pPRP37	*prpE*$^+$ in pSU20; *cat*$^+$ (Cm^r)	
pPRP38	*prpE*$^+$ in pT7-6; *bla*$^+$ (Ap^r)	
pPRP12-5.4	*prpBCD*$^+$ derived from pPRP12; Km^r	
pSU19	Cloning vector; *cat*$^+$ (Cm^r)	18
pSU20	Cloning vector; *cat*$^+$ (Cm^r)	18
pSU21	Cloning vector; *cat*$^+$ (Cm^r)	18
pSU38	Cloning vector; Km^r	3
pSU39	Cloning vector; Km^r	3

[a] All *S. typhimurium* strains are derivatives of the LT2 strain. For transfer of plasmids between *E. coli* and *S. typhimurium*, the plasmids were first passed through their respective modification-proficient, restriction-deficient strains, i.e., DH5α/F′ for *E. coli* and JR501 for *S. typhimurium*.
[b] Formerly SA2979.

amplifications were carried out in a Temp-Tronic Thermocycler (Thermolyne, Dubuque, Iowa).

(v) Localization of the *prp-114*::MudJ element. The location of the *prp-114*::MudJ element was determined by PCR amplification of sequences flanking one end of the element. For this purpose, we used the *prpB* primer 5′-GAGGAGA TGGTAGACCGAAT-3′ and the MuL primer described above, which bound to the MudJ element (44). Primer 5′-CGATCCTGCAAAACAACA-3′ was used to determine the exact location of the *prp-114*::MudJ element by DNA sequencing of the amplified DNA fragment.

Construction of a plasmid carrying only *prpB*$^+$. Plasmid pPRP1 (*prp*$^+$) (12) (Fig. 2) was used to construct a plasmid carrying only the *prpB*$^+$ gene. Related plasmids were constructed as follows.

(i) Plasmid pPRP3. To facilitate subcloning of the *prpBCD*$^+$ operon, the entire *prpBCD*$^+$ region was moved from pPRP1 into the cloning vector pSU39 (3), resulting in plasmid pPRP3 (Fig. 2). This was achieved in two steps. First, pPRP1 was digested with *Nhe*I and cloned into *Xba*I-digested pSU38 (3). The resulting plasmid was digested with *Sal*I to remove pBR328 and religated to yield pPRP3.

(ii) Plasmid pPRP8. Plasmid pPRP3 was digested with *Cla*I to remove a 4.8-kb fragment containing *prp*$^+$, which was cloned into *Cla*I-digested pSU20 (18) to yield pPRP8 (Fig. 2). This plasmid carried *prpBC*$^+$, truncated *prpD*, and *prpR*.

(iii) Plasmid pPRP15 (*prpB*$^+$ single-gene plasmid). Plasmid pPRP8 was digested with *Bsp*HI and ligated together with pGEM-5zf(+) to yield pPRP14. A 1-kb *Sph*I-*Stu*I fragment containing *prpB*$^+$ was removed from pPRP14 and ligated into pSU19 to yield pPRP15.

Construction of the smallest plasmid that complemented a strain carrying insertion *prpB121*::Tn*10d*(Tc). Plasmid pPRP3, which complemented a mutant carrying the *prpB121*::Tn*10d*(Tc) element, was used to construct smaller complementing plasmids.

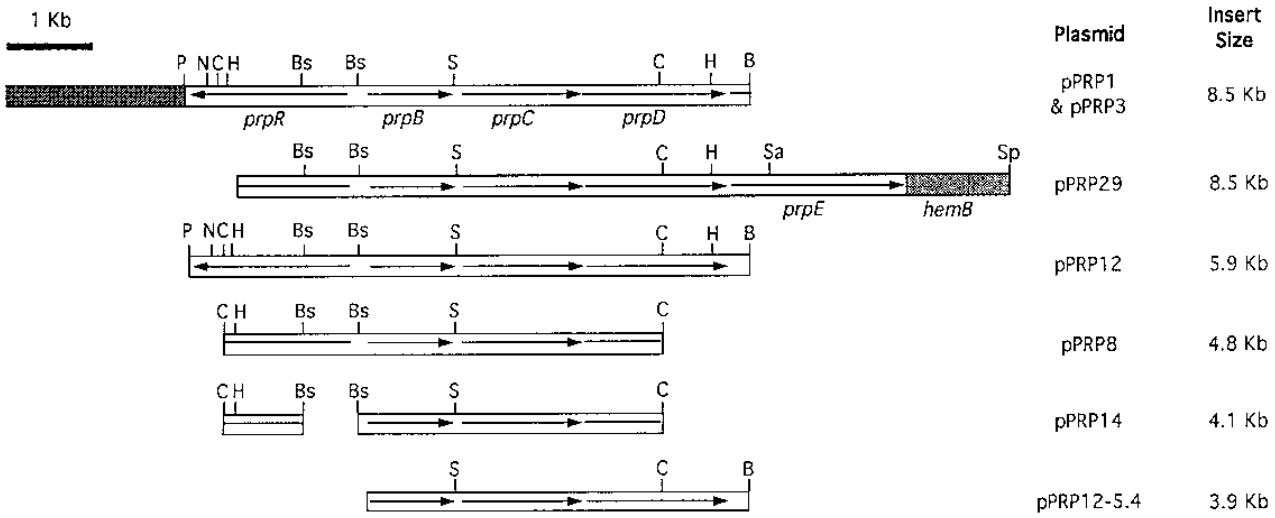


FIG. 2. Construction of plasmids carrying more than one gene. The shaded region indicates DNA not essential for propionate catabolism. The white regions show locations of the *prpR*, *prpB*, *prpC*, *prpD*, and *prpE* genes. Restriction enzyme sites: B, *Bam*HI; C, *Cla*I; Bs, *Bsp*HI; H, *Hin*dIII; N, *Nru*I; P, *Pst*I; S, *Stu*I; Sa, *Sal*I; Sp, *Sph*I.

(i) Plasmid pPRP12. Plasmid pPRP3 was digested with *Bam*HI and *Pst*I and cloned into *Bam*HI- and *Pst*I-digested pSU39 to yield pPRP12 (Fig. 2). This plasmid carried $prpR^+$ and $prpBCD^+$.

(ii) Plasmid pPRP12-5.4. Plasmid pPRP12 was digested with *Sph*I and *Nru*I for preparation of exonuclease III digestions. Exonuclease digestions were performed with Epicentre's Discrete Delete kit. To determine the extent of the deletion, the DNA was sequenced by using the −40 multiple cloning site primer, 5′-GGTTTTCCCAGTCACGAC-3′. This plasmid carried $prpBCD^+$ but lacked *prpR* and the *prpB* RpoN promoter.

Construction of derivatives of plasmid pBAD30 carrying *prpB*, *prpC*, or *prpD*. Plasmid pBAD30 (10) was kindly provided by Jon Beckwith (Harvard University).

(i) Plasmid pPRP21 (P_{BAD} *prpB*$^+$). The *prpB* gene was cloned into plasmid pBAD30 by PCR using pPRP15 as a template. PCR amplification was performed with primer 5′-TGAATAACAAGGTACCAACATGAGG-3′ (which introduced a *Kpn*I site immediately upstream of the *prpB* ribosome binding site) for one end and the −40 multiple-cloning-site primer (see above) for the other end. The amplified fragment (900 bp) was digested with *Kpn*I and *Xba*I and then cloned into pBAD30 digested with the same enzymes. To remove possible errors generated during amplification, a 788-bp *Sfi*I-*Xba*I piece of the 3′ end of *prpB* was cut from plasmid $P_{BAD}prpB^+$ and replaced with one from pPRP15. The remaining 100 bp on the 5′ end of *prpB* was sequenced by using primer 5′-CCGCTA CGCCGCCGCCAGAAAG-3′. The resulting plasmid was designated pPRP21.

(ii) Plasmid pPRP35 (P_{BAD} *prpC*$^+$). A plasmid carrying only a wild-type copy of *prpC* under the control of the P_{BAD} promoter was constructed. To facilitate the cloning of *prpC* into pBAD30, a 2.3-kb *Stu*I-*Cla*I fragment from plasmid pPRP12-5.4 was cloned into pSU21 digested with *Hin*cII and *Cla*I. The resulting plasmid was designated pPRP34. Plasmid pPRP34 was digested with *Kpn*I and *Hin*dIII to remove the 2.3-kb $prpC^+$ fragment. This piece was cloned into pBAD30 cut with the same enzymes. The resulting plasmid was designated pPRP35.

(iii) Plasmid pPRP36 (P_{BAD} *prpD*$^+$). Plasmid pPRP29 was used as a source of *prpD* due to restriction sites which facilitated cloning. This plasmid was isolated similarly to pPRP1 as described elsewhere (12). Plasmid pPRP29 was cut with *Pvu*I, blunt ended with DNA polymerase I Klenow fragment, and cut with *Sal*I to generate a 1.8-kb fragment containing $prpD^+$. This fragment was cloned into pBAD30 cut with *Sma*I-*Sal*I. The resulting plasmid was designated pPRP36.

Construction of pT7-7 overexpression plasmids for *prpB*, *prpC*, and *prpD*. (i) Plasmid pPRP19 (P_{T7} *prpB*$^+$). The *prpB* gene was cloned into plasmid pT7-7 to increase the amount of mRNA and protein synthesized. For this purpose, an *Nde*I site was constructed at the methionine start codon, using the mutagenic primer 5′-GAGGACGACATATGTCTTTACA-3′. This primer and pSU vector multiple-cloning-site primer 5′-GGTTTTCCCAGTCACGAC-3′ were used to amplify a 900-bp DNA fragment containing $prpB^+$ from plasmid pPRP15. The DNA fragment was digested with *Nde*I and *Bam*HI and cloned into plasmid pT7-7 digested with the same enzymes. The resulting plasmid, designated pPRP19, was used to overexpress *prpB*.

(ii) Plasmid pPRP22 (P_{T7} *prpC*$^+$). To clone $prpC^+$ into plasmid T7-7, an *Nde*I site was constructed at the methionine start codon by using the mutagenic primer 5′-GACGAGGACCATATGACAGACACG-3′. This primer and the pSU cloning site primer described above were used to amplify a 2.2-kb DNA fragment from plasmid pPRP8. The fragment was digested with *Nde*I and *Bam*HI and cloned into pT7-7 digested with the same enzymes. The resulting plasmid, designated pPRP22, was used to overexpress *prpC*.

(iii) Plasmid pPRP24 (P_{T7} *prpD*$^+$). To clone $prpD^+$ into plasmid T7-7, an *Nde*I site was constructed at the methionine start codon by using the mutagenic primer 5′-GGAAACGTACCATATGTCTACCC-3′. This primer and the pSU cloning site primer described above were used to amplify a 1.6-kb fragment containing $prpD^+$ from plasmid pPRP12. The DNA fragment was digested with *Nde*I and *Bam*HI and cloned into pT7-7 digested with the same enzymes. The resulting plasmid, designated pPRP24, was used to overexpress *prpD*.

Construction of pT7-6 overexpression plasmid for *prpE*. (i) Plasmid pPRP37. Plasmid pPRP29 was digested with *Hin*dIII and *Sph*I to remove a 3-kb fragment containing $prpE^+$, which was cloned into pSU20 cut with the same enzymes. The resulting plasmid was named pPRP37.

(ii) Plasmid pPRP38. Plasmid pPRP37 was digested with *Bam*HI and *Hin*dIII to remove the 3-kb $prpE^+$ fragment, which was then cloned into plasmid pT7-6 cut with the same enzymes. This plasmid carried $prpE^+$ under the control of a bacteriophage T7 promoter and was used to overexpress *prpE*.

Overexpression of *prpB*, *prpC*, *prpD*, and *prpE*. Plasmids pPRP19, pPRP22, pPRP24, and pPRP38 were transformed into *E. coli* K38 carrying plasmid pGP1-2. The latter contains the gene for T7 RNA polymerase, and expression of the protein was sensitive to temperature (34). The resulting *E. coli* strains were maintained at 30°C in Luria-Bertani broth containing ampicillin and kanamycin. Overexpression protocols have been described elsewhere (23).

Complementation studies. Propionate point mutants generated as described above were transformed with pPRP21 (P_{BAD} $prpB^+$), pPRP35 (P_{BAD} $prpC^+$), or pPRP36 (P_{BAD} $prpD^+$) to identify mutations in *prpB*, *prpC*, or *prpD*, respectively. Ampicillin-resistant transformants were replica printed to NCE minimal medium supplemented with Mg(II), propionate, methionine, ampicillin, and arabinose (500 μM). Control experiments used plasmids pBAD30 (negative control) and pPRP12-5.4 ($prpBCD^+$, positive control).

Other procedures. Cell extracts were obtained by sonication at 50% duty cycle for 5 min, using a Fisher model 550 Dismembrator (Fisher Scientific, Itasca, Ill.). Cell debris was removed by high-speed centrifugation at 43,146 × *g* for 1 h at 4°C. The Laemmli system for denaturing polyacrylamide gel electrophoresis was used to resolve proteins in cell extracts (17). Proteins were visualized by staining with Coomassie brilliant blue (Sigma, St. Louis, Mo.) as described elsewhere (33).

Nucleotide sequence accession numbers. The GenBank accession number for the sequence shown in Fig. 3 is U51879.

RESULTS AND DISCUSSION

Two divergently transcribed units comprise the *prp* locus at 8.5 Cs. Figure 3 shows the nucleotide sequence of an 8,371-bp fragment of the 8.5-Cs region of the *S. typhimurium* chromosome. This fragment contained five open reading frames hereafter referred to as *prpR*, *prpB*, *prpC*, *prpD*, and *prpE*. These genes were organized into two divergently transcribed units. Computer analysis of the sequence data yielded the following

information about the region between the two loci and about the *prp* genes.

(i) *prpR*. *prpR* was 1,626 bp long and constituted by itself one of the two transcriptional units comprising the *prp* locus at 8.5 Cs. The DNA sequence predicted a protein of 541 amino acids with a mass of 60.4 kDa and a pI of 9.03. The PrpR protein had striking homology to 50 members of the sigma-54 (RpoN) family of transcriptional activators. A comparison of the amino acid sequence of PrpR to those of the *Escherichia coli* AtoC protein and the *S. typhimurium* NtrC protein is shown in Fig. 4.

(ii) The promoter region of the *prpBCDE* operon. *prpR* and the *prpBCDE* operon were separated by 264 nucleotides. This region contained the site $TGGCATN_5TTGCT$ upstream of *prpB*, the promoter-proximal gene of the *prpBCDE* operon. This sequence was in excellent agreement with the consensus RpoN binding site $TGGCACN_5TTGC(A/T)$ (20), strongly suggesting the involvement of RpoN in the transcription of the *prp* operon. Consistent with the presence of an RpoN binding site, *rpoN* $prpR^+$ $prpBCDE^+$ mutant strain SK284 failed to grow on medium containing propionate as a carbon and energy source and histidine as a supplement. Putative ribosome binding sites for *prpR* and *prpB* were also identified, as was a putative promoter for *prpR* (Fig. 3).

(iii) The *prpBCDE* operon. The promoter-proximal gene *prpB* gene was 888 bp long; the sequence predicted a protein of 295 residues with a mass of 31.9 kDa and a pI of 5.8. *prpB* was separated from *prpC* by a 122-bp gap. *prpC* was 1,170 bp long; the sequence predicted a protein of 389 amino acids with a mass of 43.3 kDa and a pI of 6.17. *prpD* was separated from *prpC* by a 39-bp gap and was 1,452 bp long; the sequence predicted a protein of 483 residues with a mass of 53.9 kDa and a pI of 5.74. *prpE* was separated from *prpD* by a 40-bp gap; the sequence predicted a protein of 640 residues with a molecular mass of 70.8 kDa and a pI of 6.14.

Computer analysis of *prpB*, *-C*, *-D*, and *-E* and of the sequences between them failed to identify good internal promoters, suggesting that transcription of these genes is driven by the RpoN promoter located upstream of *prpB*.

Location of insertion elements within the *prpBCDE* operon. Figure 3 shows the locations of insertion elements *prp-121*::Tn*10d*(Tc), *prp-114*::MudJ, and *prp-109*::MudJ within the *prp BCD* operon. *prp-121*::Tn*10d*(Tc) was located within *prpB*; *prp-114*::MudJ was located within *prpC*; and *prp-109*::MudJ was located approximately in the middle of *prpD*. Hereafter, these insertions are referred to as *prpB121*::Tn*10d*(Tc), *prpC114*::MudJ, and *prpD109*::MudJ. Insertions within *prpE* have not been sequenced.

Genetic evidence that the *prpBCDE* genes constitute an operon. To investigate the possibility that *prpBCDE* constituted an operon, complementation studies were performed. Plasmid pPRP12-5.4 (which carried a 3.9-kb insert containing *prpB*, *prpC*, and *prpD* [Fig. 2]) restored growth of strains JE3056 [*prpB121*::Tn*10d*(Tc)], JE2170 (*prpC114*::MudJ), and JE2167 (*prpD109*::MudJ). Removal of DNA from either end of the insert caused a loss of complementation. Because insertion elements are notorious for their polar effects on the expression of downstream genes if they are part of the same transcriptional unit, these results suggested *prpBCDE* constituted an operon.

Consistent with these results, plasmid pPRP21 (P_{BAD} $prpB^+$) failed to restore growth of strain JE3056, which carries the *prpB121*::Tn*10d*(Tc) insertion. In contrast, this plasmid complemented the Prp phenotype *prpB* point mutants (see below). Taken together, these results were interpreted to mean that the *prpB121*::Tn*10d*(Tc) element had polar effects on the expression of *prpC*, *prpD*, and *prpE*.

Homology of PrpB, -C, -D, and -E to other proteins. (i) *PrpB*. The amino acid sequence of PrpB showed homology to the carboxyphosphonoenolpyruvate phosphonomutase (CPEP mutase) of *Streptomyces hygroscopicus* (14, 24). The CPEP mutase and PrpB proteins were the same length and showed 35% end-to-end identity (Fig. 5). Both proteins showed extensive identity to a CPEP mutase homolog of *Dianthus caryophyllus* (a carnation) and to the product of the *Bacillus subtilis* gene *yqiQ* (GenBank accession D84432), whose function is unknown in this bacterium. No CPEP mutase activity has been demonstrated for either the *D. caryophyllus* or the *B. subtilis* protein. PrpB exhibited less but significant identity to phosphonoenolpyruvate phosphonomutases of *S. hygroscopicus* (GenBank accession number P29247) (13, 32) and to isocitrate lyases of many organisms (data not shown).

The homology of PrpB to CPEP mutase of *S. hygroscopicus* is most intriguing given the fact that CPEP mutase catalyzes the synthesis of a phosphonate (C-P) bond (13, 23). A phosphonate intermediate has not been proposed in any of the known pathways for propionate breakdown (7, 38), and we feel it unlikely that the propionate catabolic pathway in *S. typhimurium* involves a phosphonate compound. It is possible that the reactions catalyzed by PrpB and CPEP mutase are similar from a mechanistic standpoint, but the substrates and products of the reactions are different. The homology between these two enzymes with vastly different roles (one catabolic and one anabolic) is relevant from an evolutionary point of view.

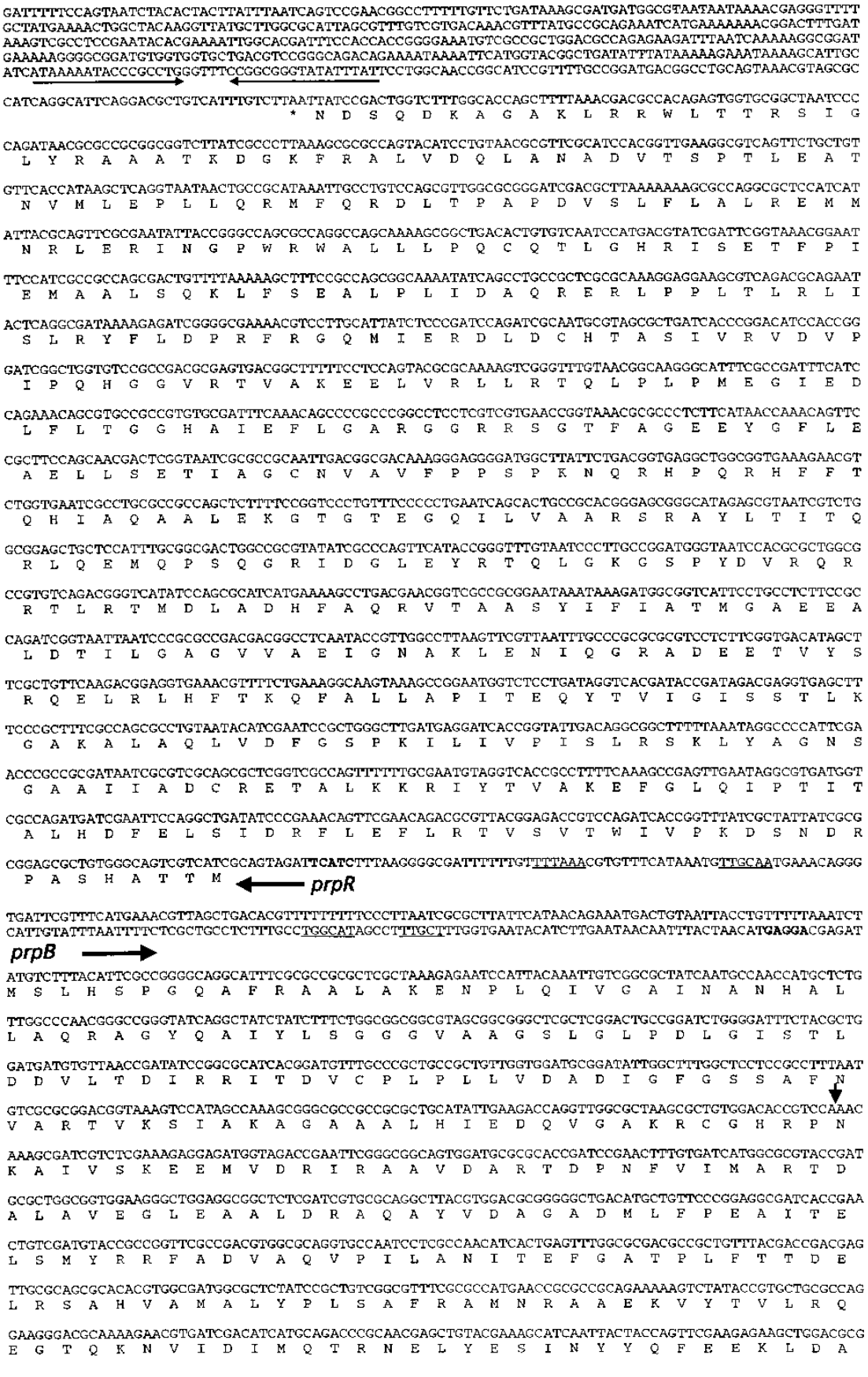
(ii) PrpC. Computer analysis of PrpC showed homology to citrate synthases (EC 4.1.3.7) from archaea and bacteria (e.g., *Pyrococcus furiosus* and *B. subtilis*). The most striking homology was to the archaeal enzyme from *P. furiosus* (GenBank accession number S81109) (Fig. 6). Bacterial citrate synthases from *B. subtilis* (GenBank accession number P39120) and *Mycobacterium smegmatis* (SWISS-PROT accession number P26491) were also homologous to PrpC.

(iii) PrpD. PrpD was homologous to a number of proteins of unknown functions, the most striking of which are YqiP in *B. subtilis* and the hypothetical yeast protein YP9723.02 (PIR accession number S52815) (Fig. 7). The involvement of PrpD in propionate catabolism in *S. typhimurium* is suggestive of the role of these proteins in these organisms.

(iv) PrpE. PrpE was homologous to several acetyl-CoA synthetases from bacteria and archaea (e.g., *Pseudomonas aeruginosa* [SWISS-PROT accession number P36333], *E. coli*, *Synechocystis* sp., *Alcaligenes eutrophus*, and *Methanothrix soehngenii*) and fungi (e.g., *Penicillium chrysogenum*) (Fig. 8).

***prpB*, *-C*, and *-D* encode proteins of a size predicted by their DNA sequences.** Figure 9 shows the results of the overexpression of *prpB*, *prpC*, and *prpD* in *E. coli* under the control of the T7 promoter and ribosome binding site in plasmid pT7-7. PrpB, PrpC, and PrpD were overexpressed to levels significantly above background. PrpB, PrpC, and PrpD had molecu-

FIG. 3. DNA and amino acid sequences for *prpR* and the *prpBCDE* operon. The arrows indicate the locations of the following insertion elements: *prpB121*::Tn*10d*(Tc), *prpC114*::MudJ, and *prpD109*::MudJ. Putative ribosome binding sites are identified in boldface. The underscored sequence $TGGCATN_5TTGCT$ upstream of *prpB* is in excellent agreement with the consensus RpoN binding site $TGGCACN_5TTGC(A/T)$. The underlined region downstream of *prpB*, *prpR*, and *prpE* indicates a predicted hairpin loop. The underlined sequences upstream of *prpR* identify the putative promoter for this gene. Asterisks identify termination codons. The DNA and amino acid sequences shown here are available under GenBank accession number U51879.



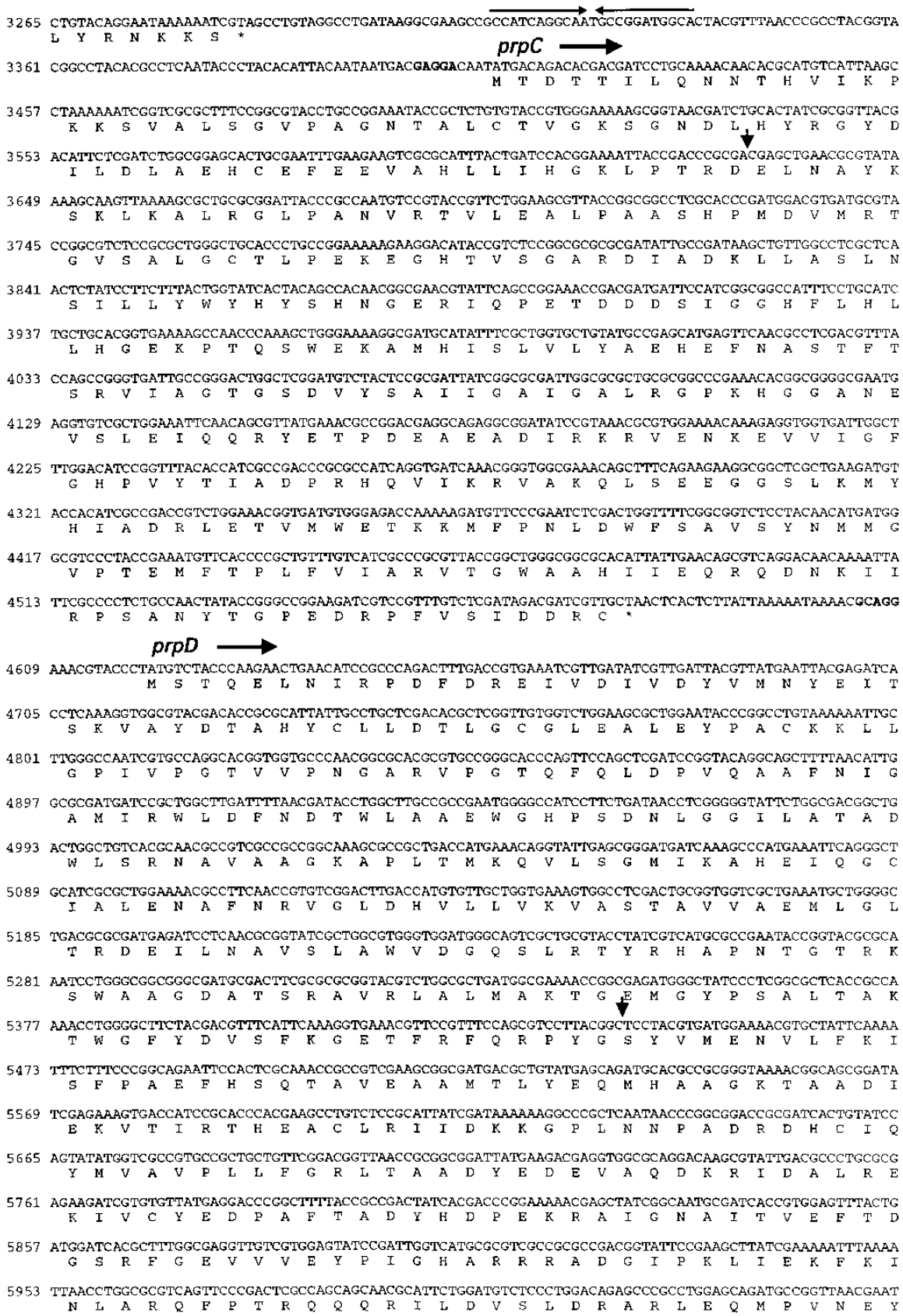


FIG. 3—*Continued.*

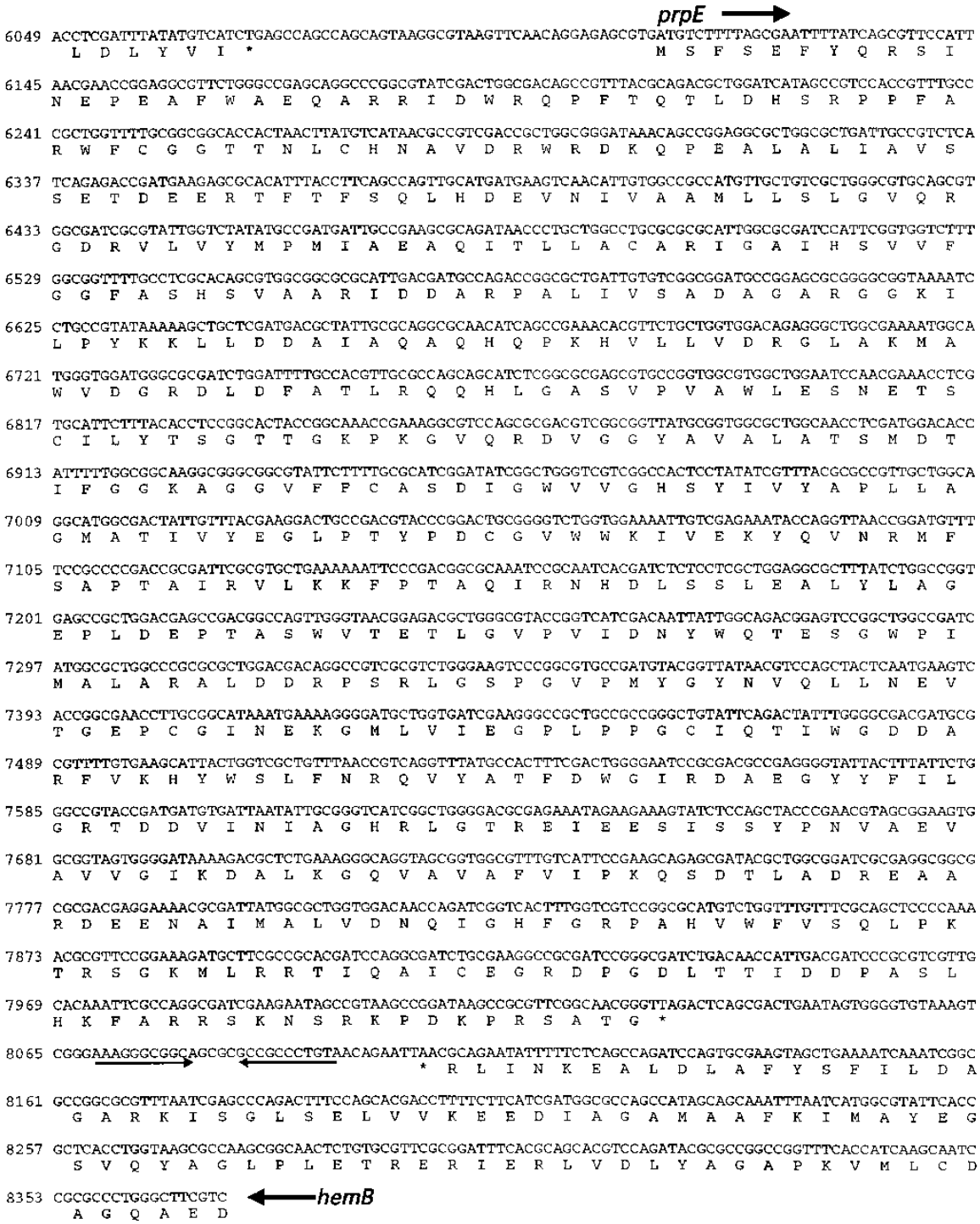


FIG. 3—*Continued.*

lar masses of ca. 32, ca. 44, and ca. 53 kDa, respectively. The results demonstrated that *prpB*, *-C*, and *-D* direct the synthesis of proteins with masses predicted by the DNA sequences of the genes.

The solubility of the overexpressed proteins was addressed by determining the amount of Prp protein remaining in the supernatant after centrifugation of cell extracts. Abundant amounts of PrpB, PrpC, and PrpD remained soluble, with PrpD showing the largest loss to inclusion bodies.

***prpB*, *prpC*, and *prpD* functions are required for propionate catabolism.** To document the involvement of the PrpB, PrpC, and PrpD proteins in propionate breakdown, we sought to demonstrate complementation of function by single-gene plasmids carrying *prpB*, *prpC*, or *prpD*. To this end, we isolated 54 *prp* missense mutants. Fifty-two of these mutants were complemented with plasmid pPRP12-5.4 (*prpBCD*$^+$). The remaining two mutants were not analyzed further. To avoid possible deleterious effects due to high gene dosages (see below), plas-

```
AtoC    --------------- --------------- --------------- --------------- --------------- -----MTAINRILIV   10
NtrC    --------------- --------------- --------------- --------------- --------------- ------MQRGIVWVV    9
PrpR    MTTAHSAPRDNSDKP VIWTVSVTRLFELFR DISLEFDHLATITPI QLGFEKAVTYIRKKL ATERCDAIIAAGSNG AYLKSRLSIPVILIK   90

                 *  *                    *                     *                        *
AtoC    DDEDNVRRMLSTAFA LQG-FETHCANNGRT ALHLFADIHPDVVLM DIRMPEMDGIKALKE MRSH--ETRTPVILM TAYAEVETAVEALRC   97
NtrC    DDDSSIRWVLERALA GAG-LTCTTFENGNE VLAALASKTPDVLLS DIRMPGMDGLALLKQ IKQR--HPMLPVIIM TAHSDLDAAVSAYQQ   96
PrpR    PSGFDVLQALAKAGK LTSSIGIVTYQETIP ALLAFQKTFHLRLEQ RSYVTEEDARGQINE LKANGIEAVVGAGLI TDLAEEAGMTAIFIY  180

         *        *           *                          *      * *            *   ***  ** ****** * * *
AtoC    GAFDYVIKPFDLDEL NLIVQRALQLQSMKK EIRHLHQALSTSWQW GHILTNSPAMMDICK DTAKIALSQASVLIS GESGTGKELIARAIH  187
NtrC    GAFDYLPKPFDIDEA VALVERAISHYQEQQ QPRNIEVNGPTTDMI G----EAPAMQDLFR IIGRLSRSSISVLIN GESGTGKELVAHALH  182
PrpR    SAATVRQAFHDALDM TRLTRRQRVDYP--- SGKGLQTRYELGDIR G----QSPQMEQLRQ TITLYARSRAAVLIQ GETGTGKELAAQAIH  263

                         **   *  *    *  * **** * *****      * * ** *   *** ***** ***   *  *** *      *
AtoC    YN--------SRRAK GPFIKVNCAALPESL LESELFGHEKGAFTG AQ-TLRQGLFERANE GTLLLDEIGEMPLVL QAKLLRILQEREFER  268
NtrC    RH--------SPRAK APFIALNMAAIPKDL IESELFGHEKGAFTG AN-TIRQGRFEQADG GTLFLDEIGDMPLDV QTRLLRVLADGQFYR  263
PrpR    QTFFHRQPHRQNKPS PPFVAVNCGAITESL LEAELFGYEEGAFTG SRRGGRAGLFEIAHG GTLFLDEIGEMPLPL QTRLLRVLEEKAVTR  353

         **     ** * *  **   *      * * ***** **      * **** *  **  **   **                         *
AtoC    IGGHQTIKVDIRIIA ATNRDLQAMVKEGTF REDLFYRLNVIHLIL PPLRDRREDISLLAN HFLQKFSSENQR--- -DIIDIDPMAMSLLT  354
NtrC    VGGYAPVKVDVRIIA ATHQNLERRVQEGKF REDLFHRLNVIRIHL PPLRERREDIPRLAR HFLQVAARELGV--- -EAKLLHPETETALT  349
PrpR    VGGHQPIPVDVRVIS ATHCDLDREIMQGRF RPDLFYRLSILRLTL PPLRERQADILPLAE SFLKQSLAAMEIPFT ESIRHGLTQCQPLLL  443

           **** * * *                     *                   *                     *               *
AtoC    AWSWPGNIRELSNVI ERAVVMNSGPIIFSE DLPPQIRQPVCNAGE VKTAP---------- ------VGERNLKEE IK-RVEKRIIMEVLE  427
NtrC    RLAWPGNVRQLENTC RWLTVMAAGQEVLIQ DLPGELFEASTPDSP SHLPPDSWATLLAQW ADRALRSGHQNLLSE AQPELERTLLTTALR  439
PrpR    AWRWPGNIRELRNMM ERLALFLS-----VD PAPTLDRQFMR---- -QLLP---------- ------ELMVNTAEL TPSTVDANALQDVLA  507

           *     *  **   *  *   *
AtoC    QQEGNRTRTALMLGI SRRALMYKLQEYGID PADV-  461
NtrC    HTQGHKQEAARLLGW GRNTLTRKLKELGME -----  469
PrpR    RFKGDKTAAARYLGI SRTTLWRRLKAGAKD QSDNZ  542
```

FIG. 4. Homology of PrpR to representative members of the sigma-54 family of transcriptional activators. AtoC, acetoacetate metabolism regulatory protein from *E. coli* (SWISS-PROT accession number Q06065); NtrC, nitrogen regulatory protein from *S. typhimurium* (EMBL accession number X85104); PrpR, propionate metabolism regulatory protein from *S. typhimurium* (GenBank accession number U51879). Asterisks indicate amino acids conserved in all three proteins.

mids pPRP21 (P_{BAD} *prpB*$^+$), pPRP35 (P_{BAD} *prpC*$^+$), and pPRP36 (P_{BAD} *prpD*$^+$) were used in complementation tests performed on minimal medium containing propionate (30 mM) and D-(+)-arabinose at 500 μM. The above-mentioned plasmids were introduced into 20 *prp* mutants, and their Prp phenotypes were assessed. Plasmid pPRP21 (P_{BAD} *prpB*$^+$) failed to restore growth of any of these mutants on propionate; plasmid pPRP35 (P_{BAD} *prpC*$^+$) restored growth of six mutants; plasmid pPRP36 (P_{BAD} *prpD*$^+$) restored growth of eight mutants. To identify a missense mutation in *prpB*, the 32 remaining *prp* mutants were transformed with pPRP21 (P_{BAD} *prpB*$^+$), and the growth of four mutants was restored on propionate. Figure 10B illustrates the effectiveness of plasmid pPRP21 (P_{BAD} *prpB*$^+$) in restoring growth of a representative *prpB* point mutant (JE3946) on propionate. Similar complementation was observed with *prpC* and *prpD* mutants carrying plasmids pPRP35 and pPRP36, respectively.

These data support the conclusion that PrpB, PrpC, and

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                                                    *  *      *    *    *    *  *  ******
S. hygr --------------- -----MAVTKAR--T FRELMNAPEILVVPS AYDALSAKVIQQAGF PAVHMTGSGTSASML GLPDLGFTSVSEQAT    68
D. cary MAPPNGTTNGETEVA TQGSYTAVSTGRKTT MHRLIEEHGSVLMPG VQDALSAAVVEKTGF HAAFVSGYSVSAAML GLPDFGLLTTTEVVE    90
S. typh --------------- ----MSLHSPGQ--A FRAALAKENPLQIVG AINANHALLAQRAGY QAIYLSGGGVAAGSL GLPDLGISTLDDVLT    69

            *            * * * *     *  *                ****  * ***   *     **    * * *  *  *  *   **
S. hygr NAKNIVLAVD-VPVI MDADAGYGN-AMSVW RATREFERVGIVGYH LEDQVNPKRCGHLEG KRLISTEEMTGKIEA AVEAREDEDFTIIAR   156
D. cary ATRRITAAAPNLCVV VDGDTGGGG-PLNVQ RFIRELISAGAKGVF LEDQVWPKKCGHMRG KAVVPAEEHALKIAA AREAIGDSDFFLVAR   179
S. typh DIRRITDVCP-LPLL VDADIGFGSSAFNVA RTVKSIAKAGAAALH IEDQVGAKRCGHRPN KAIVSKEEMVDRIRA AVDARTDPNFVIMAR   158

        ***    **     *       ****  * * *                     **  * * **   *  *            *    *
S. hygr TDARESFGLDEAIRR SREYVAAGADCIFLE AMLDVEEMKRVRDEI DAPLLANMVEGGKTP WLTTKELESIGYNLA IYPLSGWMAAASVLR   246
D. cary TDARAPHGLEEGIRR ANLYKEAGADATFVE APANVDELKEVSAKT KGLRIANMIEGGKTP LHTPEEFKEMGFHLI AHSLTAVYATARALV   269
S. typh TDALAVEGLEAALDR AQAYVDAGADMLFPE AITELSMYRRFADVA QVPILANITEFGATP LFTTDELRSAHVAMA LYPLSAFRAMNRAAE   248

             *   **                *  *         *
S. hygr KLFTELREAGTTQKF WDDMGLKMSFAELFE VFEYSKISELEARFV RDQDZ--   296
D. cary NIMKILKEKGTTR-- -DDLDQMATFSEFNE LISLESWYEMESKFK NFTPKAT   318
S. typh KVYTVLRQEGTQK-- -NVIDIMQTRNELYE SINYYQFEEKLDALY RNKKSZ-   296
```

FIG. 5. Homology of PrpB to CPEP phosphonomutase. Abbreviations: *S. hygr*, *S. hygroscopicus* CPEP mutase (accession number X67953); *D. cary*, *D. caryophyllus* CPEP mutase homolog (accession number S35145); *S. typh*, *S. typhimurium* PrpB (accession number U51879). Asterisks indicate amino acids conserved in all three proteins.

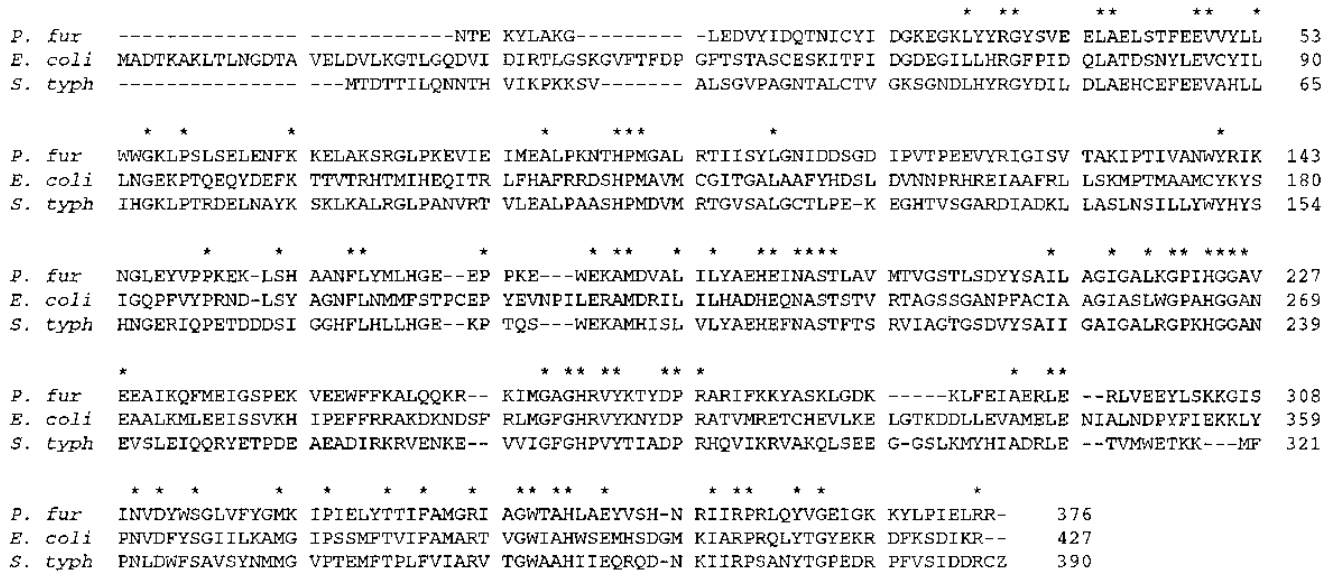

FIG. 6. Homology of PrpC to *P. furiosus* (*P. fur*) citrate synthase (S81109), citrate synthase from *E. coli* (GenBank accession number P00891), and PrpC (propionate catabolism protein) from *S. typhimurium* (*S. typh*) (GenBank accession number U51879). Asterisks indicate amino acids conserved in all three proteins.

PrpD are all required for the catabolism of propionate in *S. typhimurium*.

Is *prpE* required for propionate catabolism? Is it part of the *prp* operon? At present it is unclear whether *prpE* is involved in propionate catabolism. Thus far, all mutations in the 8.5-Cs region that resulted in a Prp^- phenotype were complemented by the plasmid carrying wild-type copies of *prpR* and *prpBCD*. If *prpE* is involved in propionate catabolism, it is not clear why we have not isolated mutations in it. One possible explanation is that there is a function in the cell that can substitute for PrpE. Such redundancies are not uncommon, especially if one considers the results from computer analyses of the *prpE* sequence which showed homology to acetyl-CoA synthetases from bacteria, archaea, and fungi. It is not unreasonable to suggest that PrpE may be the propionyl-CoA synthetase enzyme and that other acyl-CoA synthetases could compensate

FIG. 7. Homology of PrpD to proteins of unknown functions in *S. cerevisiae* and *B. subtilis*. Abbreviations: *S. cere*, *S. cerevisiae* hypothetical protein YP9723.02 (PIR accession number S52815); *B. subt*, *B. subtilis* YqiP protein (DDBJ locus BACJH642, accession number D84432); *S. typh*, *S. typhimurium* PrpD protein (GenBank accession number U51879). Asterisks indicate amino acids conserved in all three proteins.

```
                                                    *  **  *  **                  *
E. coli  --------------- -MSQIHKHTIPANIA DR-----CLINPQQY EAMYQQSINVPDTFW GEQG-KILDWIKPYQ KVKNTSFAPGNVSIK   68
P. chry  MSDGPIQPPKPAVVH EAHEVDTFHVPKAPH DKHPSGTHIKDIEEY KKLYEESIKSPDTFW ARMARELLTFDKDFE TTHHGSFENG--DNA   88
S. typh  --------------- ---------------- ------------MSF SEFYQRSINEPEAFW AEQA-RRIDWRQPFT QTLDHSRPPF---AR   44

         *   *  *   *  * *         * *                 *    *   *  *   *    ** *  * **   **    *** ****
E. coli  WYEDGTLNLAANCLD RHLQENGDRTAIIWE GDDASQSKHISYKEL HRDVCRFANTLLELG IKKGDVVAIYMPMVP EAAVAMLACARIGAV  158
P. chry  WFVEGRLNASFNCVD RHALKNPDKVAIIYE ADEPNEGRKITYGEL MREVSRVAWTLKERG VKKGDTVGIYLPMIP EAVIAFLACSRIGAV  178
S. typh  WFCGGTTNLCHNAVD RWRDKQPEALALIAV SSETDEERTFTFSQL HDEVNIVAAMLLSLG VQRGDRVLVYMPMIA EAQITLLACARIGAI  134

         *** * **        *  *          *  * * *     **  *  *           *     *      *  ** *
E. coli  HSVIFGGFSPEAVAG RIIDSNSRLVITSDE GVRAGRSIPLKKNVD DALKNPNVTSVEHVV VLKRTGGKIDWQEGR DLWWHDLVEQASDQH  248
P. chry  HSVVFAGFSSDSLRD RVLDASSKVIITSDE GKRGGKIIGTKKIVD EAMK--QCPDVHTVL VYKRTGAEVPWTAGR DIWWHEEVEKYPNYL  266
S. typh  HSVVFGGFASHSVAA RIDDARPALIVSADA GARGGKILPYKKLLD DAIAQ-AQHQPKHVL LVDRGLAKMAWVDGR DLDFATLRQQHLGAS  223

                    * **** *******      **    *        *         *  *  **  ** *  * **   *  *   *  *  *
E. coli  QAEEMN-AEDPLFIL YTSGSTGKPKGVLHT TGGYLVYAALTFKYV FDYHPGDIYWCTADV GWVTGHSYLLYGPLA CGATTLMFEGVPNWP  337
P. chry  APESVS-SEDPLFLL YTSGSTGKPKGVMHT TAGYLLGAAMTGKYV FDIHDDDRYFCGGDV GWITGHTYVVYAPLL LGCATVVFESTPAYP  355
S. typh  VPVAWLESNETSCIL YTSGTTGKPKGVQRD VGGYAVALATSMDTI FGGKAGGVFFCASDI GWVVGHSYIVYAPLL AGMATIVYEGLPTYP  313

                  *  *      **** * *        *     * *   *  ***       *       *       *   **** *   *  *
E. coli  TPARMAQVVDKHQVN ILYTAPTAIRALMAE GDKAIEGTDRSSLRI LGSVGEPINPEAWEW YWKKIGNEKCPVVDT WWQTETGGFMITPLP  427
P. chry  NFSRYWDVIDKHDVT QFYVAPTALRLLKRA GDEHIHHKMHS-LRI LGSVGEPIAAEVWKW YFECVGKEEAHICDT YWQTETGSHVITPLG  444
S. typh  DCGVWWKIVEKYQVN RMFSAPTAIRVLKKF PTAQIRNHDLSSLEA LYLAGEPLDEPTASW VTETLG---VPVIDN YWQTESG-WPIMALA  399

                 **    *  *          *     *    * *      *     *  *   *    *         *   *   **  ***
E. coli  GATELKA---GSATR PFFGVQPALVDNE-G NPLEG-ATEGSLVIT DSWP-GQARTLFGDH ERFEQTYFSTF-KNM YFSGDGARRDEDGYY  510
P. chry  GITPTKP---GSASL PFFGIEPAIIDPVSG EEIVGNDVEGVLAFK QPWP-SMARTVWGAH KRYMDTYLNVY-KGY YFTGDGAGRDHDGYY  529
S. typh  RALDDRPSRLGSPGV PMYGYNVQLLNEVTG -EPCGINEKGMLVIE GPLPPGCIQTIWGDD ARFVKHYWSLFNRQV YATFDWGIRDAEGYY  488

          * ** *** *  **  ** * ***      *   ** *****      **   * *                              *   **    *
E. coli  WITGRVDDVLNVSGH RLGTAEIESALVAHP KIAEAAVVGIPHNIK GQAIYAYVTLNHG-- ---EEPSPELYAEVR NWVRKEIGPLATPDV  595
P. chry  WIRGRVDDVVNVSGH RLSTAEIEAALLEHP SVAEAAVVGIADELT GQAVNAFVSLKEG-- ---K-PTEQISKDLA MQVRKSIGPFAAPKA  613
S. typh  FILGRTDDVINIAGH RLGTREIEESISSYP NVAEVAVVGIKDALK GQVAVAFVIPKQSDT LADREAARDEENAIM ALVDNQIGHFGRPAH  578

                ********   **    *  *      **  *  **    *
E. coli  LHWTDSLPKTRSGKI MRRILRKIAAGDTSN LGDTSTLADPGVVEK LLEEKQAIAMPS--- -----  652
P. chry  VFVVDDLPKTRSGKI MRRILRKILSGEEDS LGDTSTLSDPSVVDK IIETVHSARQK---- -----  669
S. typh  VWFVSQLPKTRSGKM LRRTIQAICEGRDP- -GDLTTIDDPASLHK FARRSKNSRKPDKPR SATGZ  641
```

FIG. 8. Homology of PrpE to acetyl-CoA synthetases from *E. coli* (SWISS-PROT accession number P27550), *Penicillium chrysogenum* (*P. chry*) (SWISS-PROT accession number P36333), and *S. typhimurium* (*S. typh*) (GenBank accession number U51879). Asterisks indicate amino acids conserved in all three proteins.

for a lack of it, thus explaining the lack of a Prp phenotype of *prpE* mutants. The only indication that *prpE* may be part of the *prp* operon comes from analysis of the sequence between *prpD* and *prpE*. This region appears to lack a promoter sequence that could control *prpE* expression, suggesting that *prpE* expression may be controlled by the promoter upstream of *prpB*. Plasmid pPRP38 carried *prpE* under the control of a bacteriophage T7 promoter and was overexpressed by using plasmid pGP1-2 to provide T7 RNA polymerase. A soluble protein of ca. 70 kDa was detected by SDS-PAGE sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Coomassie blue staining (data not shown).

High levels of PrpB have a deleterious effect on growth of the cell. Plasmid pPRP15 (*prpB*$^+$) was placed into strain TR6583 (*prp*$^+$) to yield strain JE4015. Unlike TR6583, JE4015 poorly utilized propionate as a carbon and energy source. To investigate whether this phenotype was due to a gene dosage effect, the *prpB* gene was placed under the control of P_{BAD} (plasmid pPRP21) as described in Materials and Methods. The level of expression of any gene under P_{BAD} control is proportional to the amount of arabinose in the medium (10). To test the idea that high levels of PrpB were deleterious to cell growth, plasmid pPRP21 (P_{BAD} *prpB*$^+$) was moved into TR6583 to yield strain JE4016. The inability of strain JE4016 to grow on propionate correlated well with increasing concentrations of arabinose in the culture medium (Fig. 10A). The negative effect of increasing levels of PrpB was not evident until the concentration of arabinose in the medium reached 500 μM. At this concentration, the doubling time of the culture was increased 2.2-fold (from 5.8 to 12.8 h; [Fig. 10A]). A further increase in the doubling time (from 12.8 to 16 h) was observed with arabinose concentrations of >5 mM, with a substantial negative effect on the lag observed before the onset of exponential growth (Fig. 10A). These results indicated that either the amount of PrpB protein or its enzymatic activity was responsible for the observed effect on growth.

Failure to use propionate as a source of carbon and energy by a *prp*$^+$ strain in which PrpB was expressed at high levels was probably not due to the high level of PrpB, since the protein

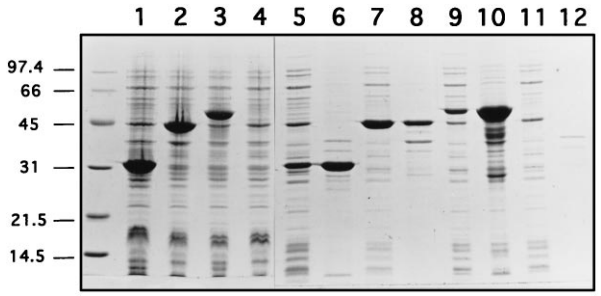

FIG. 9. Overexpression and solubility of PrpB, -C, and -D. Lanes: 1 to 3, levels of overexpression of PrpB, PrpC, and PrpD, respectively; 4, pT7-7 vector-only control; 5 to 12, solubility of the overexpressed proteins. Odd-numbered lanes show amounts of Prp protein remaining in the supernatant after centrifugation of cell extracts at 43,146 × *g* for 1 h at 4°C; even-numbered lanes show amounts of Prp proteins in the pellets. Abundant amounts of PrpB (lane 5), PrpC (lane 7), and PrpD (lane 9) remained soluble. Lanes 11 and 12 are pT7-7 vector-only controls. Sizes are indicated in kilodaltons.

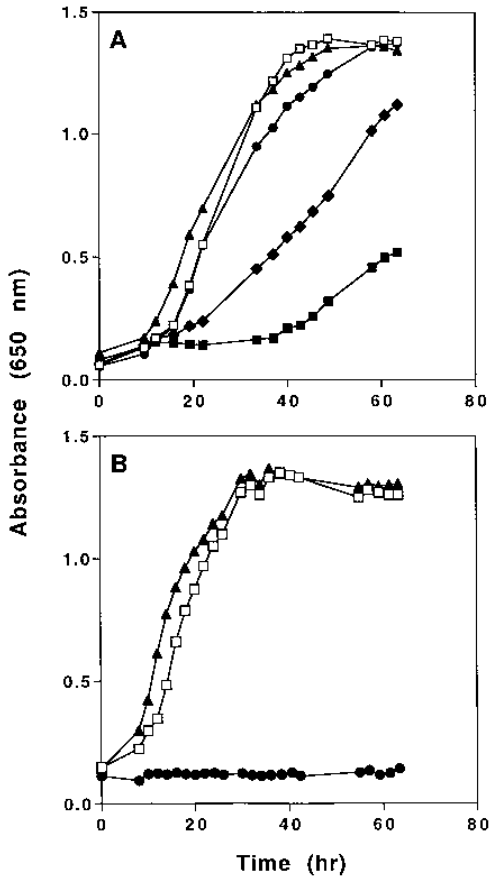

FIG. 10. Deleterious effect of the overexpression of *prpB* and complementation of *prpB* function. (A) Growth of TR6583 (*prp*$^+$) carrying plasmid pPRP21 (P_{BAD} *prpB*$^+$) on NCE medium supplemented with propionate, ampicillin, and various concentrations of arabinose. Symbols: open squares, control strain TR6583 carrying cloning vector pBAD30; triangles, medium lacking arabinose; circles, medium with 250 μM arabinose; diamonds, medium with 500 μM arabinose; squares, medium with 5 mM arabinose. (B) Complementation of *prpB* function by plasmid pPRP21 (P_{BAD} *prpB*$^+$). The medium used was NCE supplemented with propionate, ampicillin, and 500 μM arabinose. Symbols: squares, control strain TR6583 (*prp*$^+$) carrying cloning vector pBAD30; triangles, strain JE3946 (*prpB195*) carrying pPRP21 (P_{BAD} *prpB*$^+$); circles, JE3946 (*prpB195*) carrying cloning vector pBAD30.

was successfully overexpressed to very high levels in strains growing in rich medium (Fig. 9, lanes 1 and 5). One possible explanation for the lack of growth on minimal propionate medium is that the substrate for PrpB may be a central metabolite required for other pathways. If so, high levels of PrpB may deplete the pool of such a metabolite, with the concomitant arrest of cell division. Alternatively, the product of the reaction catalyzed by PrpB may be toxic to the cell. Elucidation of the in vivo substrate for PrpB is needed to address these possibilities.

Summary. In summary, the *prp* locus at 8.5 Cs of the *S. typhimurium* LT2 chromosome is comprised of five genes (*prpR* and *prpBCDE*) organized as two divergently transcribed units. The *prpBCDE* genes are organized as an operon; PrpB, PrpC, and PrpD gene products are required for the catabolism of propionate in this bacterium; the requirement for PrpE is yet to be demonstrated. Homology of PrpR to the sigma-54 (RpoN) family of transcriptional activators and failure of an RpoN-deficient strain to grow on propionate support the idea that PrpR is likely responsible for the transcriptional activation of the *prpBCDE* operon. Our previous work on *prp* regulation suggested that a catabolite of propionate, not propionate itself, is the signal sensed by the regulator. Work aimed at the identification of this signal is in progress.

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ADDENDUM

The DNA sequence of *prpRBCDE* homologs in *E. coli* was recently reported (GenBank accession number U73857). The organization of the *E. coli prp* genes is the same as in *S. typhimurium*. All proteins share a high degree of identity (≥80%), strongly suggesting that these are the genes of *E. coli* involved in propionate catabolism.

REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, and E. W. Myers.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1989. Current protocols in molecular biology, vol. 1. Greene Publishing Associates and Wiley-Interscience, New York, N.Y.
3. **Bartolomé, B., Y. Jubete, E. Martínez, and F. de la Cruz.** 1991. Construction and properties of a family of pACYC184-derived cloning vectors compatible with pBR322 and its derivatives. Gene **102:**75–78.
4. **Benson, N. R., and B. S. Goldman.** 1992. Rapid mapping in *Salmonella typhimurium* with Mu*d*-P22 prophages. J. Bacteriol. **174:**1673–1681.
5. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. A manual for genetic engineering: advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Escalante-Semerena, J. C., M. G. Johnson, and J. R. Roth.** 1992. The CobII and CobIII regions of the cobalamin (vitamin B_{12}) biosynthetic operon of *Salmonella typhimurium*. J. Bacteriol. **174:**24–29.
7. **Evans, C. T., B. Sumegi, P. A. Srere, A. D. Sherry, and C. R. Malloy.** 1993. [^{13}C]propionate oxidation in wild-type and citrate synthase mutant *Escherichia coli*: evidence for multiple pathways of propionate utilization. Biochem. J. **291:**927–932.
8. **Fernández-Briera, A., and A. Garrido-Pertierra.** 1988. A degradation pathway of propionate in *Salmonella typhimurium* LT-2. Biochimie **70:**757–768.
9. **Fernández-Briera, A., and A. Garrido-Pertierra.** 1989. Studies with *Salmonella typhimurium* mutants defective in enzymes of the propionate metabolism. Curr. Microbiol. **19:**169–173.
10. **Guzmán, L.-M., D. Belin, M. J. Carson, and J. Beckwith.** 1995. Tight regulation, modulation, and high-level expression by vectors containing arabinose P_{BAD} promoter. J. Bacteriol. **177:**4121–4130.
11. **Halarnkar, P. P., J. H. Nelson, C. R. Heisler, and G. J. Blomquist.** 1985. Metabolism of propionate to acetate in the cockroach *Periplaneta americana*. Arch. Biochem. Biophys. **236:**526–534.
12. **Hammelman, T. A., G. A. O'Toole, J. R. Trzebiatowski, A. W. Tsang, D. Rank, and J. C. Escalante-Semerena.** 1996. Identification of a new *prp* locus required for propionate catabolism in *Salmonella typhimurium* LT2. FEMS Microbiol. Lett. **137:**233–239.
13. **Hidaka, T., M. Hidaka, and H. Seto.** 1992. Studies on the biosynthesis of bialaphos (SF-1293). 14. Nucleotide sequence of phosphoenolpyruvate phosphomutase gene isolated from a bialaphos producing organism, *Streptomyces hygroscopicus*, and its expression in *Streptomyces lividans*. J. Antibiot. **45:** 1977–1980.
14. **Hidaka, T., S. Imai, O. Hara, H. Anzai, T. Murakami, K. Nagaoka, and H. Seto.** 1990. Carboxyphosphoenolpyruvate phosphomutase, a novel enzyme catalyzing C-P bond formation. J. Bacteriol. **172:**3066–3072.
15. **Hong, J.-S., and B. N. Ames.** 1971. Localized mutagenesis of any specific small region of the bacterial chromosome. Proc. Natl. Acad. Sci. USA **68:** 3158–3162.
16. **Joshi, A. K., V. Baichwal, and G. Ferro-Luzzi Ames.** 1991. Rapid polymerase chain reaction amplification using intact bacterial cells. BioTechniques **10:** 42–44.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
18. **Martínez, E., B. Bartolomé, and F. de la Cruz.** 1988. pACY184-derived cloning vectors containing the multiple cloning site and *lacZ*α reporter gene of pUC8/9 and pUC18/19 plasmids. Gene **68:**159–162.
19. **McKie, N., N. H. Keep, M. L. Patchett, and P. F. Leadlay.** 1990. Adenosylcobalamin-dependent methylmalonyl-CoA mutase from *Propionibacterium*

shermanii. Biochem. J. **269:**293–298.
20. **Merrick, M. J.** 1993. In a class of its own—the RNA polymerase sigma factor σ^{54} (σ^{N}). Mol. Microbiol. **10:**903–909.
21. **Miyakoshi, S., H. Uchiyama, T. Someya, T. Satoh, and T. Tabuchi.** 1987. Distribution of the methylcitric acid cycle and β-oxidation for propionate catabolism in fungi. Agric. Biol. Chem. **51:**2381–2387.
22. **O'Toole, G. A., and J. C. Escalante-Semerena.** 1993. *cobU*-dependent assimilation of nonadenosylated cobinamide by *cobA* mutants of *Salmonella typhimurium*. J. Bacteriol. **175:**6328–6336.
23. **O'Toole, G. A., and J. C. Escalante-Semerena.** 1995. Purification and characterization of the bifunctional CobU enzyme of *Salmonella typhimurium* LT2. Evidence for a CobUGMP intermediate. J. Biol. Chem. **270:**23560–23569.
24. **Pollack, S. J., S. Freeman, D. L. Pompliano, and J. R. Knowles.** 1992. Cloning, overexpression and mechanistic studies of carboxyphospho*enol*-pyruvate mutase from *Streptomyces hygroscopicus*. Eur. J. Biochem. **209:**735–743.
25. **Pronk, J. T., A. van der Linden-Beuman, C. Verduyn, W. A. Scheffers, and J. P. van Dijken.** 1994. Propionate metabolism in *Saccharomyces cerevisiae*: implications for the metabolon hypothesis. Microbiology **140:**717–722.
26. **Rondon, M. R., A. R. Horswill, and J. C. Escalante-Semerena.** 1995. DNA polymerase I (Poll) function is required for the utilization of ethanolamine, 1,2-propanediol, and propionate by *Salmonella typhimurium* LT2. J. Bacteriol. **177:**7119–7124.
27. **Rondon, M. R., R. Kazmierczak, and J. C. Escalante-Semerena.** 1995. Glutathione is required for maximal transcription of the cobalamin biosynthetic and 1,2-propanediol utilization (*cob*/*pdu*) regulon and for the catabolism of ethanolamine, 1,2-propanediol, and propionate in *Salmonella typhimurium* LT2. J. Bacteriol. **177:**5434–5439.
28. **Rosenberg, L. E., and W. A. Fenton.** 1989. Disorders of propionate and methylmalonate metabolism, p. 822–844. *In* C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (ed.), The metabolic basis of inherited disease, 6th. ed. McGraw-Hill, New York, N.Y.
29. **Sanderson, K. E., A. Hessel, and K. E. Rudd.** 1995. Genetic map of *Salmonella typhimurium*, edition VIII. Microbiol. Rev. **59:**241–303.
30. **Schmieger, H.** 1971. A method for detection of phage mutants with altered transduction ability. Mol. Gen. Genet. **100:**378–381.
31. **Schmieger, H., and H. Bakhaus.** 1973. The origin of DNA in transducing particles of P22 mutants with increased transduction frequencies (HT-mutants). Mol. Gen. Genet. **120:**181–190.
32. **Seidel, H. M., S. Freeman, H. Seto, and J. R. Knowles.** 1988. Phosphonate biosynthesis: the isolation and characterization of phosphenolpyruvate phosphomutase, the enzyme responsible for the formation of a carbon-phosphorous bond. Nature **335:**457–458.
33. **Suh, S.-J., and J. C. Escalante-Semerena.** 1995. Purification and initial characterization of the ATP:corrinoid adenosyltransferase encoded by the *cobA* gene of *Salmonella typhimurium.* J. Bacteriol. **177:**921–925.
34. **Tabor, S.** 1990. Expression using the T7 RNA polymerase/promoter system, p. 16.2.1.–16.2.11. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology, vol. 2. Wiley Interscience, New York, N.Y.
35. **Trzebiatowski, J. R., G. A. O'Toole, and J. C. Escalante-Semerena.** 1994. The *cobT* gene of *Salmonella typhimurium* encodes the NaMN:5,6-dimethylbenzimidazole phosphoribosyl transferase responsible for the synthesis of N^1-(5-phospho-α-D-ribosyl)-5,6-dimethylbenzimidazole, an intermediate in the synthesis of the nucleotide loop of cobalamin. J. Bacteriol. **176:**3568–3575.
36. **Tsai, S. P., R. J. Hartin, and J. Ryu.** 1989. Transformation in restriction-deficient *Salmonella typhimurium* LT2. J. Gen. Microbiol. **135:**2561–2567.
37. **Tsang, A., and J. C. Escalante-Semerena.** Unpublished results.
38. **Tsang, A. W., and J. C. Escalante-Semerena.** 1996. *cobB* function is required for catabolism of propionate in *Salmonella typhimurium* LT2: evidence for existence of a substitute function for CobB within the 1,2-propanediol utilization (*pdu*) operon. J. Bacteriol. **178:**7016–7019.
39. **Vagelos, P. R.** 1959. Propionic acid metabolism. IV. Synthesis of malonyl coenzyme A. J. Biol. Chem. **234:**490–497.
40. **Vagelos, P. R., and J. M. Earl.** 1959. Propionic acid metabolism. III. β-Hydroxypropionyl coenzyme A and malonyl semialdehyde coenzyme A, intermediates in propionate oxidation by *Clostridium kluyveri*. J. Biol. Chem. **234:**2272–2280.
41. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithase of *Escherichia coli*: partial purification, and some properties. J. Biol. Chem. **218:**97–106.
42. **Wegener, W. S., H. C. Reeves, R. Rabin, and S. J. Ajl.** 1968. Alternate pathways of metabolism of short-chain fatty acids. Bacteriol. Rev. **32:**1–26.
43. **Wurtele, E. S., and B. J. Nikolau.** 1990. Plants contain multiple biotinenzymes: discovery of 3-methylcrotonyl-CoA carboxylase, propionyl-CoA carboxylase and pyruvate carboxylase in the plant kingdom. Arch. Biochem. Biophys. **179:**179–186.
44. **Youderian, P., P. Sugiono, K. L. Brewer, N. P. Higgins, and T. Elliott.** 1988. Packaging specific segments of the Salmonella chromosome with locked-in Mu *d*-P22 prophages. Genetics **118:**581–592.