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Locating Essential *Escherichia coli* Genes by Using Mini-Tn*10* Transposons: the *pdxJ* Operon

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The mini-Tn*10* transposon (Δ16Δ17Tn*10*) confers tetracycline resistance. When inserted between a gene and its promoter, it blocks transcription and prevents expression of that gene. Tetracycline in the medium induces divergent transcription of the *tetA* and *tetR* genes within the transposon, and this transcription extends beyond the transposon in both directions into the bacterial genes. If the mini-Tn*10* inserts between an essential bacterial gene and its promoter, the insertion mutation can cause conditional growth which is dependent on the presence of tetracycline. Two essential genes in adjacent operons of *Escherichia coli* have been detected by screening for tetracycline dependence among tetracycline-resistant insertion mutants. These essential genes are the *era* gene in the *rnc* operon and the *dpj* gene in the adjacent *pdxJ* operon. The *pdxJ* operon has not been described previously. It consists of two genes, *pdxJ* and *dpj*. Whereas the *dpj* gene is essential for *E. coli* growth in all media tested, *pdxJ* is not essential. The *pdxJ* gene encodes a protein required in the biosynthesis of pyridoxine (vitamin B_6).

Conditional mutations facilitate the study of bacterial genes in two ways. They allow detection of physiological changes under nonpermissive conditions and provide the opportunity to select suppressor mutations. In addition to conditional lethal mutations of the thermosensitive type, conditional detects dependent on expression from heterologous promoters are also valuable for study. Chow and Berg (6) designed a transposition vector, Tn5tacl, to detect essential genes in *Escherichia coli* as conditionally lethal transposition mutations. Mutants generated depend on induction of the *tac* promoter from the transposon to express essential genes when their normal expression has been blocked by insertion of the transposon. We show here that a Tn*10* derivative can also be used to isolate a class of conditionally lethal mutations.

We have defined three genes of the *rnc* operon: *rnc*, *era*, and *recO* (31). These genes encode the proteins RNaseIII, a double-strand-dependent endoribonuclease (7); Era, a GTP-binding protein (5, 21); and RecO, required for DNA repair and RecF pathway recombination (25). Of these, only *era* is essential for bacterial growth (14, 31). We previously isolated an *E. coli* mutant in which a mini-Tn*10* (Δ16Δ17Tn*10*; 33) inserted in the leader sequence of the *rnc* operon caused the cell to become conditionally dependent on tetracycline for growth. This insertion mutation, *rnc-40*, caused *era* expression to be defective in the absence of tetracycline presumably because the mini-Tn*10* element was blocking normal transcription from the *rnc* promoter. We proposed that tetracycline, which induces the divergent promoters for the *tetA* and *tetR* genes of the mini-Tn*10* transposon (13), allowed transcription of these genes to continue beyond the Tn*10* element and into the *era* gene.

In this study, we have examined expression of *lacZ* fused to the *rnc* operon both with and without the *rnc-40* mini-Tn*10* insertion (A40). We have also isolated and examined several other mini-Tn*10* insertion mutations in the region of the bacterial chromosome near the *rnc* operon. One of these additional mini-Tn*10* insertions also confers a tetracycline-dependent (Tet^d) phenotype. This new tetracycline-dependent insertion has allowed us to define an operon adjacent to the *rnc* operon which also contains an essential gene.

MATERIALS AND METHODS

Materials. Restriction endonucleases and DNA-modifying enzymes were purchased from Bethesda Research Laboratories, Inc. (Gaithersburg, Md.); New England BioLabs, Inc. (Beverly, Mass.); and Boehringer Mannheim Biochemicals (Indianapolis, Ind.). Nick translation kits were purchased from Bethesda Research Laboratories or Boehringer Mannheim. DNA oligonucleotides were purchased from Midland Certified Reagent Co. (Midland, Tex.). The Sequenase kit was obtained from U.S. Biochemical Corp. (Cleveland, Ohio). Enzymes and kits were used as recommended by the manufacturers.

Ampicillin was used at a concentration of 50 μg/ml, and tetracycline was used at a concentration of 12.5 μg/ml. For tetracycline-dependent (Tet^d) strains, tetracycline was often used at concentrations of 1.0 to 2.5 μg/ml; the actual concentrations will be indicated for the experiments.

Bacterial and phage strains. Details of the *E. coli* K-12 strains and λ bacteriophage used are shown in Table 1. The genetic, bacteriological, and phage techniques used are described elsewhere, as are standard recipes for LB, TB, and M56 media (23, 29). P1 transductions were performed by using P1*vir* as described elsewhere (23), with the modification that for antibiotic selections, cells were spread on a nitrocellulose filter (BA85; Schleicher & Schuell, Inc., Keene, N.H.) on an LB plate. After 2 h of incubation, the filter was transferred to an LB-antibiotic plate.

Plasmids. Plasmids derived from pACS1 are shown in Fig.

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TABLE 1. Bacterial and phage strains

Strain	Genotype	Source
RB132	*galZXX*::Tn*10*Δ4HH104 (pNK217, Δ16Δ17Tn*10*)	33
N4903	*his ilv relA rpsL pgl*Δ8	31
HT41	N4903 *pdxJ8*::ΔTn*10*	This work
HT30	N4903 *era-13*::ΔTn*10*	31
W3110	*E. coli* K-12 prototroph	2
HT115	W3110 *rnc-14*::ΔTn*10*	31
HT120	W3110 *rnc-40*::ΔTn*10* (A40)	31
HT270	W3110 *era-13*::ΔTn*10*	W3110 + P1 · HT30
HT253	W3110 *pdxJ8*::ΔTn*10* (A8)	W3110 + P1 · HT41
HT210	W3110 *recO*::*kan*	31
TAP114	W3110 *lacI*^{q} *lacZ*ΔM15	T. A. Patterson
TAP106	*leu bio thi galK*(Am) *hsdR514 lac*Δ*U169 rpsL supE44* λ[(*int-ral*)Δ, *N*::*kan*, *cI*857 (*cro-bioA*)Δ]	5
TAP56	TAP106 [(*int-ral*)Δ, *N*$^{+}$ *cI*857 (*cro-bioA*)Δ]	T. A. Patterson
DLC712	TAP56 *pdxJ8*::ΔTn*10*	TAP56 + P1 · HT41
DLC713	TAP56 *rnc-14*::ΔTn*10*	TAP56 + P1 · HT115
λ		National Institutes of Health
λTD1	λ *rnc era recO pdxJ dpj*	31
λTD1 *imm*21		31
λTD1 *rnc40*::ΔTn*10 imm*21		This work
λTD1 *pdxJ8*::ΔTn*10 imm*21		This work

1, and their construction is described below. pACS1 (31) contains a 4.3-kb *Eco*RI bacterial fragment inserted in the *Eco*RI site of pBR322 (Fig. 1). pACS21 is pACS1 deleted from the *Bam*HI site indicated in Fig. 1 to the *Bam*HI site in pBR322. pACS3 is pACS1 deleted from the *Nru*I site in Fig. 1 to the *Nru*I site in pBR322. pDLC140 is pACS1 deleted from the *Sph*I site in Fig. 1 to the *Sph*I site in pBR322. pDLC132 is pACS1 deleted for the *Afl*II fragment indicated in Fig. 1. pDLC141 is pDLC140 deleted for the *Afl*II fragment indicated. pDLC142 is pACS1 deleted for the *Nsi*I fragment between the two *Nsi*I sites shown in Fig. 2. pACS42 is pACS1 deleted for the *Kpn*I fragment between the two *Kpn*I sites shown in Fig. 2. All of the preceding plasmids were made by cutting with the designated enzyme, diluting the DNA, and joining the cut ends with T4 DNA ligase. Ampicillin-resistant transformants were selected. pDLC145 is pACS1 deleted for the 4-nucleotide segment AGCT within the *Sac*I restriction site sequence (Fig. 2). This was done by cutting with *Sac*I and then adding T4 DNA polymerase in the presence of all four deoxynucleotide triphosphates to trim the 3′ protruding ends before joining them with T4 DNA ligase. pDLC149 and pDLC150 are pACS42 and pDLC145 deleted for the *Afl*II fragment analogously to pDLC132. All of the constructs shown in Fig. 1 were verified by appropriate restriction digest analysis to ensure that the proper DNA fragment had been lost. Only pDLC145 failed to be recut by the original enzyme used to create each respective deletion.

LacZ fusion plasmids used in this work are described in the text and in the legends to figures that illustrate them. These operon and protein fusion plasmids were constructed with the *lacZ* vectors made by Simons et al. (30) to detect promoters and measure promoter strength. Promoterless vectors pRS414 and pRS591 (30) were used to construct the protein fusions; plasmids pRS415 and pRS528 (30) were used to construct the operon fusions.

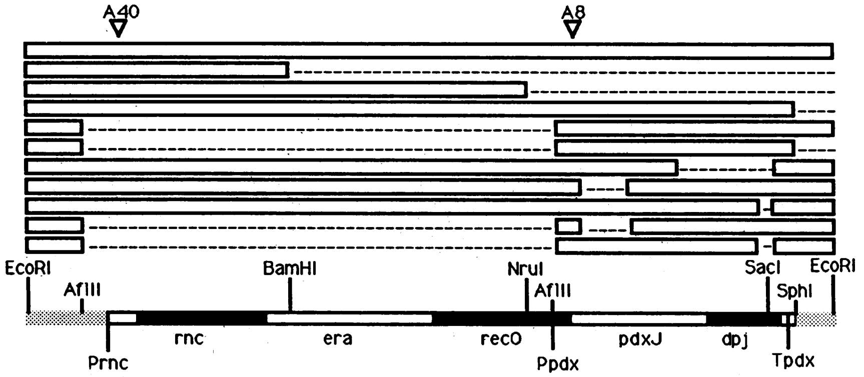

FIG. 1. The 4.3-kbp *Eco*RI fragment map of the *rnc* and *pdxJ* operons and plasmids. The genes *rnc*, *era*, *recO*, *pdxJ*, and *dpj* are indicated on the map below the open and filled rectangles that represent the sizes of the respective genes. The promoters p_{rnc} and p_{pdx} and terminator t_{pdx} are shown. The lightly stippled areas at each end of the *Eco*RI map are bacterial DNA segments outside the *rnc* and *pdxJ* operons. Restriction sites used to generate plasmids are shown above the genes or in Fig. 2. The set of rectangles above the map represents DNA of plasmids generated from a clone of this *Eco*RI fragment in pBR322. Plasmids are named at the right. For each plasmid indicated, the open rectangles represent DNA that is present, and the dashed line represents the region deleted. A40 and A8, indicated by triangles, represent the insertion sites for the mini-Tn*10* insertion. See Materials and Methods for details of each plasmid construction.

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     NruI
   1 TCGCGAAGAAAAAGGGTTTATCGCAAGCGTCGTTATCGACAATAAAACGTTCACCGGAAGGCAGTTAAAAGCGTTAAACGCACGGGAATTTCCTGAC

                                   -35           AflII      -10
  98 GCAGACACACTGCGCGCCGCGAAACGCTTTACCCGCATGGCGCTTAAGCCGTATCTTGGCGGTAAACCTTTAAAGAGCAGGGAACTGTTCCGGCAGT
                                 ━━━━━━              ━━━━━━

                                                                           A8  mini-Tn10
 195 TTATGCCTAAGCGAACGGTGAAAACACATTATGAATGATGAGGATTGTC ATG GCT GAA TTA CTG TTA GGC GTC AAC ATT GAC
                                                      1▶Met Ala Glu Leu Leu Leu Gly Val Asn Ile Asp

                                           KpnI              BamHI
 277 CAT ATC GCT ACG CTG CGC AAC GCG CGC GGT ACC GCT TAC CCG GAT CCG GTG CAG GCC GCG TTT ATT GCC GAG
 12▶His Ile Ala Thr Leu Arg Asn Ala Arg Gly Thr Ala Tyr Pro Asp Pro Val Gln Ala Ala Phe Ile Ala Glu

 349 CAG GCG GGA GCG GAC GGC ATT ACC GTG CAT TTA CGT GAA GAT CGC CGT CAC ATT ACT GAC CGC GAC GTG CGC
 36▶Gln Ala Gly Ala Asp Gly Ile Thr Val His Leu Arg Glu Asp Arg Arg His Ile Thr Asp Arg Asp Val Arg

 421 ATC CTG CGT CAG ACG CTG GAT ACC CGC ATG AAT CTG GAG ATG GCG GTG ACC GAA GAG ATG CTG GCG ATC GCC
 60▶Ile Leu Arg Gln Thr Leu Asp Thr Arg Met Asn Leu Glu Met Ala Val Thr Glu Glu Met Leu Ala Ile Ala

                                             KpnI
 493 GTT GAG ACG AAG CCA CAT TTT TGC TGC CTG GTA CCG GAA AAG CGT CAG GAA GTA ACA ACC GAA GGC GGC CTG
 84▶Val Glu Thr Lys Pro His Phe Cys Cys Leu Val Pro Glu Lys Arg Gln Glu Val Thr Thr Glu Gly Gly Leu

 565 GAT GTC GCA GGG CAG CGT GAC AAA ATG CGC GAT GCC TGC AAA CGT CTG GCA GAT GCC GGG ATT CAG GTT TCT
108▶Asp Val Ala Gly Gln Arg Asp Lys Met Arg Asp Ala Cys Lys Arg Leu Ala Asp Ala Gly Ile Gln Val Ser

 637 CTG TTT ATT GAC GCC GAT GAA GAG CAG ATC AAA GCT GCG GCA GAG GTT GGC GCA CCG TTT ATC GAG ATC CAC
132▶Leu Phe Ile Asp Ala Asp Glu Glu Gln Ile Lys Ala Ala Ala Glu Val Gly Ala Pro Phe Ile Glu Ile His

 709 ACC GGT TGC TAT GCT GAT GCC AAA ACT GAC GCC GAA CAG GCG CAA GAG CTG GCG CGT ATC GCC AAA GCC GCG
156▶Thr Gly Cys Tyr Ala Asp Ala Lys Thr Asp Ala Glu Gln Ala Gln Glu Leu Ala Arg Ile Ala Lys Ala Ala

 781 ACC TTT GCC GCA AGC CTC GGT CTG AAA GTT AAC GCC GGA CAC GGT CTG ACC TAT CAC AAC GTG AAA GCC ATT
180▶Thr Phe Ala Ala Ser Leu Gly Leu Lys Val Asn Ala Gly His Gly Leu Thr Tyr His Asn Val Lys Ala Ile

                         NsiI
 853 GCC GCC ATC CCT GAG ATG CAT GAA CTG AAT ATC GGT CAT GCC ATT ATT GGT CGT GCA GTG ATG ACC GGA CTG
204▶Ala Ala Ile Pro Glu Met His Glu Leu Asn Ile Gly His Ala Ile Ile Gly Arg Ala Val Met Thr Gly Leu

 925 AAA GAT GCG GTG GCA GAA ATG AAG CGT CTG ATG CTG GAA GCG CGT GGC TA ATG GCA ATA TTA GGT TTA GGC ACG
228▶Lys Asp Ala Val Ala Glu Met Lys Arg Leu Met Leu Glu Ala Arg Gly  1▶Met Ala Ile Leu Gly Leu Gly Thr

 999 GAT ATT GTG GAG ATC GCT CGC ATC GAA GCG GTG ATC GCC CGA TCC GGT GAT CGC CTG GCA CGC CGC GTA TTA
  9▶Asp Ile Val Glu Ile Ala Arg Ile Glu Ala Val Ile Ala Arg Ser Gly Asp Arg Leu Ala Arg Arg Val Leu

1071 AGC GAT AAC GAA TGG GCT ATC TGG AAA ACG CAC CAC CAG CCG GTG CGT TTT CTG GCG AAG CGT TTT GCT GTG
 33▶Ser Asp Asn Glu Trp Ala Ile Trp Lys Thr His His Gln Pro Val Arg Phe Leu Ala Lys Arg Phe Ala Val

                                             BamHI
1143 AAA GAA GCC GCA GCA AAA GCG TTT GGC ACC GGG ATC CGC AAT GGT CTG GCG TTT AAT CAA TTT GAA GTA TTC
 57▶Lys Glu Ala Ala Ala Lys Ala Phe Gly Thr Gly Ile Arg Asn Gly Leu Ala Phe Asn Gln Phe Glu Val Phe

         SacI
1215 AAT GAT GAG CTC GGC AAA CCA CGG CTA CGG CTA TGG GGC GAG GCA TTA AAA CTG GCG GAA AAG CTG GGC GTT
 81▶Asn Asp Glu Leu Gly Lys Pro Arg Leu Arg Leu Trp Gly Glu Ala Leu Lys Leu Ala Glu Lys Leu Gly Val

         NsiI
1287 GCA AAT ATG CAT GTA ACG CTG GCA GAT GAG CGG CAC TAT GCT TGT GCC ACG GTA ATT ATT GAA AGT TAAAAATA
105▶Ala Asn Met His Val Thr Leu Ala Asp Glu Arg His Tyr Ala Cys Ala Thr Val Ile Ile Glu Ser

                                                                                    SphI
1361 ATACCGTTCTCTTTAAAAAGTAAAACCCCCGAACTGGTAAGGAACGGGGGTTTTACTTTTTAACTTTAATTTTGTGCAGCATGC
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FIG. 2. Nucleotide and deduced amino acid sequences of *pdxJ* and *dpj*. Nucleotides are numbered from 1 to 1444. Relative to the maps in Fig. 1, nucleotide 1 corresponds to nucleotide position 2676 from the left *Eco*RI site, and nucleotide 1444 corresponds to nucleotide position 4119 from the left *Eco*RI site. The recognition sites for restriction enzymes are in boldface letters. Amino acids of the gene products are numbered and indicated below their codons. *pdxJ* translation starts with the AUG at nucleotide position 244, and we assume that *dpj* starts at the AUG at nucleotide position 975. The two tandem translation stop codons UGA UGA of the *recO* gene are indicated by shadowed letters upstream of the *pdxJ* initiation codon. The *pdx* promoter is indicated by its −35 and −10 sites. Other *pdx* genes (*pdxA* and *pdxB*) each contain a sequence in the translation initiation region that is indicated by boldface italics (26). The target sequence of 9 nucleotides where the A8 insertion occurred is designated. The inverted repeat of a proposed rho-independent terminator is shown by inverted arrows. There is one discrepancy between this sequence and one published for the *recO* gene (25): at position 315 in our sequence, Morrison et al. found two Cs, whereas we find three Cs.

λTD1::ΔTn*10* insertions. Phage clone λTD1 was grown on the defective Tn*10* transposon Δ16Δ17Tn*10* (ΔTn*10*) donor strain RB132. Approximately 1 in 10^6 phage from such infections of RB132 carries a stable ΔTn*10* insertion in its DNA. These rare phage with ΔTn*10* inserts were not detected directly; instead, the population of phage was used to transduce *E. coli* to tetracycline resistance as described previously (31).

Briefly, to transfer the ΔTn*10* from the phage to the chromosome of *E. coli*, an overnight culture of the nonlysogenic strain N4903 was infected with the population of λTD1 phage prepared on RB132. To reduce phage viability and thereby reduce cell killing, the phage for this infection were preirradiated with UV light (approximately 6 × 10^3 ergs/mm^2; Blak-Ray J-225 shortwave UV meter). Infected cultures of N4903 were grown on LB-tetracycline plates to select Tet^r colonies in which the ΔTn*10* was recombined to the chromosome. Simple lysogens of λTD1 cannot be formed because the phage is defective for the λ repressor.

For sequencing of the A8 insertion, the mutation in the chromosome was recombined into λTD1*imm*21 and then subcloned to pUC19 as previously described (31).

Physical mapping of the ΔTn*10* insertions. Bacterial DNA was isolated as described elsewhere (29). Southern blots of the bacterial DNA digested with various restriction endonucleases were hybridized to nick-translated phage or plasmid probes containing DNA from pACS1. The ΔTn*10* insertions were mapped by using *Bam*HI, *Eco*RI, *Hin*dIII, and *Pst*I digests of chromosomal DNA from the insertion strains.

DNA sequencing. Sequencing was performed with the Sequenase kit (U.S. Biochemical Corp.) and [^{32}P]dATP using minipreparation plasmid DNA. Precise location of the mini-Tn*10* insertion A8 was determined by sequencing from synthetic oligonucleotides (5'GAAGGAACGTCAATTCCCAT-3' and 5'CTAAGGCAGACCAACCATTTG-3') hybridized within the left and right ends, respectively, of the mini-Tn*10* element cloned in pUC19, thereby priming synthesis into the flanking *E. coli* DNA (31). The DNA sequences of *pdxJ* and *dpj* were determined from both strands of plasmid pACS1 by using a series of synthetic oligonucleotide primers. DNA sequence analysis was carried out with programs supplied by the University of Wisconsin.

Expression and purification of 27-kDa protein. TAP56 (pZSZ1) was grown in LB medium (50 ml) containing ampicillin at 32°C to an optical density at 650 nm of 0.5 and transferred to 42°C for 2 h. Cells were harvested and lysed, and crude extracts were prepared as described by Chen et al. (5). The ~27-kDa protein (p27) was localized by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) analysis to the supernatant fraction after low-speed centrifugation.

A 450-μl portion of the supernatant was added to an equal volume of saturated guanidine hydrochloride, and the solution was then brought to pH 2 with the addition of 20% trifluoroacetic acid (TFA). The slightly turbid mixture was added to a Waters C-18 reverse-phase column in an LKB high-pressure liquid chromatography system. The column was washed with 0.05% aqueous TFA at 0.5 ml/min until the initial salt peaks were eluted. The column was then subjected to a 0 to 60% gradient of 0.05% TFA in acetonitrile at 1 ml/min over 60 min. Fractions were collected manually. Aliquots of protein peaks of interest were lyophilized and used for SDS-PAGE, amino acid composition, and amino-terminal-sequence analysis.

Amino acid composition analysis. Protein from aliquots containing the 27-kDa protein (p27) were hydrolyzed with 6 N HCl at 110°C for 24, 48, and 72 h in vacuo in glass ignition tubes. The samples were then analyzed on a Beckman model 6300 amino acid analyzer.

Amino-terminal-sequence analysis. The protein p27 was subjected to Edman degradation in an Applied Biosystems model 470A gas-phase Sequenator, and phenylthiohydantoin derivatives of amino acids were identified and quantitated on-line with the model 120A analyzer.

Trypsin digestion. Protein was dissolved in 0.5 ml of 0.1 M NH_4HCO_3 (pH 8), and trypsin was added to give a protein/trypsin ratio of 100:1. The reaction was allowed to proceed for 24 h at 37°C. The reaction was stopped by the addition of aqueous TFA to give a pH of 2. The mixture was then applied to a Waters C-18 column, and a 0 to 60% gradient of 0.05% TFA in acetonitrile developed over 2 h. Peptide peaks were collected manually, and aliquots were taken for amino acid analysis.

Galactokinase measurements. The *galK* termination vector used in this work was pMZ240, which is derived from vector pKG1800 described by McKenney et al. (22) by a modification made by Zuber et al. (34) in which the *gal* promoter on pKG1800 was replaced by the *lac* promoter. Conditions for cell growth and measurement of galactokinase units have been described elsewhere (34).

Nucleotide sequence accession number. The nucleotide sequence reported here has been submitted to GenBank and has received the accession number M76470.

RESULTS

Localized mini-Tn*10* transposon mutagenesis. Phage λTD1, which contains the *rnc* operon in a 14.5-kbp DNA segment of the *E. coli* chromosome, was mutagenized with the mini-Tn*10* transposon Δ16Δ17Tn*10* (see Materials and Methods; 31). Briefly, λTD1 was used to infect strain RB132, which contains the mini-Tn*10* transposon encoding tetracycline resistance. RB132 also expresses transposase at high levels. The lysate from such an infection contained many phage which carried mini-Tn*10*. This lysate was used to infect strain N4903 in order to transfer the mini-Tn*10* insertions in the bacterial DNA segment of the phage to the bacterial chromosome by homologous recombination. The recombinant bacteria form tetracycline-resistant (Tet^r) colonies. We found two types of Tet^r colonies: those that grew with or without tetracycline and those that grew only when tetracycline was present. The latter we have called tetracycline dependent (Tet^d). Concentrations of tetracycline as low as 1.0 μg/ml can satisfy the growth requirement of the Tet^d colonies, and a detoxified form of chlortetracycline can be used to replace tetracycline (31). We have studied two of these Tet^d mutants in detail. For our initial discussions, the insertion mutations will be referred to as A40 and A8.

Control of *rnc* operon expression by the A40 insertion. The A40 mutation is located in the leader sequence of the *rnc* operon (Fig. 1). In the absence of tetracycline, this insertion prevents expression of the *era* gene, which is essential for *E. coli* growth (31). To determine how expression of *era* is controlled by A40, we fused *lacZ* to the *era* gene and measured levels of β-galactosidase. Without the A40 insertion, β-galactosidase expression was dependent on the promoter for the *rnc* operon (Table 2, line 1 versus line 2). The presence of the A40 insertion blocked *lacZ* transcription from this *rnc* promoter (Table 1, line 1 versus line 3). In the presence of tetracycline, the expression of β-galactosidase in the insertion mutants was increased severalfold (Table 2, lines 3 and 4). This induction occurred even when the *rnc*

TABLE 2. Effect of mini-Tn*10* insertion A40 on expression of *era-lazZ* fusions

Protein fusion			Operon fusion		
Plasmid[a]	Amt (U) of β-galactosidase		Plasmid	Amt (U) of β-galactosidase	
	−Tet	+Tet		−Tet	+Tet
pCF14	2,683	ND	pCF140	6,240	ND
pCF22	29	ND	pCF220	242	ND
pCF03	38	133	pCF030	82	717
pCF01	15	92	pCF010	250	881
pRS591	<10	<10	pRS528	58	48

[a] The indicated *lacZ* fusion plasmids are described in Fig. 3. TAP114 carrying the indicated plasmids was grown to 2 × 10^8/ml in LB medium plus ampicillin either with 2.5 μg of tetracycline per ml (+Tet) or without tetracycline (−Tet). ND, not determined. Tetracycline has no effect on expression of β-galactosidase from pCF14 or pCF140 in other strains containing a copy of Tn*10* on the bacterial chromosome (data not shown).

promoter was deleted. Thus, when the A40 insertion is present, transcription of *era* derives only from the mini-Tn*10* and is tetracycline dependent. However, this level of tetracycline-dependent expression from the insertion is still considerably less than that from the *rnc* promoter.

TABLE 3. Complementation of A40 and A8 insertion defects

Plasmid[a]	−Tetracycline[b]		Pyridoxine[c] (A8)
	A40	A8	
pACS1	+	+	+
pACS21	−	−	−
pACS3	+	−	−
pDLC140	+	+	+
pDLC132	−	+	+
pDLC141	−	+	+
pDLC142	+	−	−
pACS42	+	+	−
pDLC145	+	−	+

[a] Strains HT120 (A40) and HT253 (A8) carrying the respective plasmids were grown overnight in LB with ampicillin and tetracycline (2.5 μg/ml). The cultures were centrifuged, and the cell pellet was washed and suspended in 10 mM $MgSO_4$.

[b] Dilutions of the cultures were spotted for isolated colonies on LB-ampicillin plates. + or −, growth or no growth, respectively, in the absence of tetracycline.

[c] Aliquots (100 μl each) of diluted cultures were spotted on minimal glucose plates with 0.2% Casamino Acids and 1.0 μg of tetracycline per ml. +, growth of colonies in the spots similar to growth on control plates with pyridoxine added; −, no growth.

Defects caused by the A8 insertion. Restriction digests and Southern blot hybridization analyses mapped the A40 insertion to the leader sequence of the *rnc* operon (31) and the A8 insertion to near the end of the *recO* gene (data not shown). In the mutant with the A40 insertion, the divergent *tetR* and *tetA* genes present in the element are arranged with *tetR* in the same orientation as *rnc* and *era*. In the mutants with the A8 insertion, the *tetR* and *tetA* genes are inverted relative to A40 (data not shown). Since both the *tetA* and *tetR* promoters are activated by tetracycline, the Tet^d phenotype of the A8 mutant strain may reasonably be explained by a mechanism analogous to that of the A40 mutant strain in which the *tetR* promoter is thought to direct *era* expression. Therefore, we postulated that an essential gene is positioned beyond A8 relative to *recO* and is dependent on transcription from the *tetA* promoter for its expression.

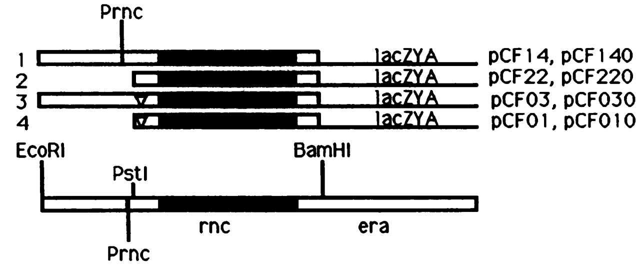


FIG. 3. Gene and operon *lacZ* fusions at the *Bam*HI site in *era*. Plasmids are indicated above the map as in Fig. 1. The region beyond the *Bam*HI site in *era* is replaced by the *lac* fusions (represented by *lacZYA*) in all the plasmids. In the constructs on lines 1 and 2, the *rnc-105* mutation is present, and in the constructs on lines 3 and 4, the A40 insertion is present in the leader. It is indicated by the inverted triangle. The *rnc* gene (black rectangle) and promoter p_{rnc} are shown. Constructs 2 and 4 lack the *rnc* promoter. Construct 1 was created by using the *Eco*RI-to-*Bam*HI fragment of pACS105 (pACS1 *rnc-105*) containing the *rnc* gene to replace the *Eco*RI-*Bam*HI fragment of the gene and operon fusion vectors pRS591 and pRS528 (30). Construct 2 was created by using the *Pst*I-to-*Bam*HI fragment of pACS105 containing the *rnc* gene. This fragment was first placed between *Pst*I and *Bam*HI sites in the pUC19 multicloning site, and then the fragment was reisolated by cutting it with *Eco*RI and *Bam*HI, thereby adding a few nucleotides of the multicloning site that exists between the *Eco*RI and *Pst*I sites. This *Eco*RI-to-*Bam*HI fragment was used to replace the *Eco*RI-*Bam*HI fragment of the fusion vectors. Note that the plasmid constructs containing the mini-Tn*10* are toxic to the cell in the presence of tetracycline at concentrations above 2.5 μg/ml. This toxicity is caused by the produce of *tetA*, which is overproduced and affects the cell membrane (32).

The A8 and A40 insertions each require tetracycline for growth. Since pACS1 complemented Tet^d of both A40 and A8 strains (Fig. 1 and Table 3), we tested several other deletion derivatives of pACS1 for complementation. As determined previously (31), the Tet^d defect caused by A40 was complemented by plasmids carrying an intact *era* gene. The A8 mutation was complemented for Tet^d by all the plasmids in which the entire distal reading frame (*dpj* in Fig. 1) downstream of the A8 insertion (pDLC140, pDLC141, pACS42) was intact. In other words, a strain with the A8 insertion could grow without tetracycline if it contained a plasmid with an intact copy of the second open reading frame. Thus, the distal gene, like *era*, is vital for *E. coli* growth in all media that we have tested.

Unlike the strain with the A40 insertion, the strain with the A8 insertion failed to grow even in the presence of tetracycline on M56 minimal medium supplemented with glucose or any of several other carbon sources tested (data not shown). Casamino Acids did not satisfy the growth requirement, but a vitamin mixture permitted growth. Further testing indicated that pyridoxine was required for growth.

We used the plasmids described above to test for complementation of the pyridoxine requirement, i.e., for growth on minimal medium with tetracycline. The A8 defect on minimal plates with tetracycline present was complemented by those plasmids containing the first gene, i.e., *pdxJ* in Fig. 1 (pDLC140, pDLC141, pDLC145). Therefore, the disruption of this gene by the A8 insertion causes the pyridoxine requirement.

We had also noticed that λ formed clear plaques on lawns of strains with the A8 insertion. When pyridoxine or yeast extract was added to the TB-tetracycline plates used for λ plaque tests, λ formed turbid plaques on the A8 insertion

TABLE 4. Complementation of *pdx* mutants

Strain[a]	Allele[b]	Growth with plasmids[c]:			
		None	pDLC132	pDLC149	pDLC150
WG1	*pdx*$^+$	+	+	+	+
WG1027	*pdxJ151*	−	+	−	+
WG1050	*pdxJ161*	−	+	−	+
WG1223	*pdxJ178*	−	+	−	+
WG1229	*pdxK180*	−	+	−	+
WG1280	*pdxJ186*	−	+	−	+
WG1335	*pdxK191*	−	+	−	+
HT253	*pdxJ8*::ΔTn*10*	−	+	−	+

[a] Strains were plated for isolated colonies on M56 glucose with Casamino Acids added. All strains grew on this medium when supplied with pyridoxine. Strains WG2(*pdxH2*), WG3(*pdxB3*), WG25(*pdxA25*), WG73(*pdxA73*), WG1145 (*pdxF*), and WG1468(*pdxB*) were also tested and failed to be complemented by pDLC132. All WG strains are *E. coli* B derivatives of WG1, a prototrophic strain (9).

[b] Strains with *pdxK* mutations are leakier than *pdxJ* for growth in the absence of pyridoxine (9).

[c] +, complete complementation for growth as with pyridoxine added; −, no or very reduced growth relative to the pyridoxine control. All plasmids are deleted for the *Afl*II fragment and part of the *pdx* promoter, but enough residual promoter activity remains to complement at high copy number (Table 6, plasmid pDLC135). The *Afl*II deletion removes an *Eco*B restriction site of pACS1.

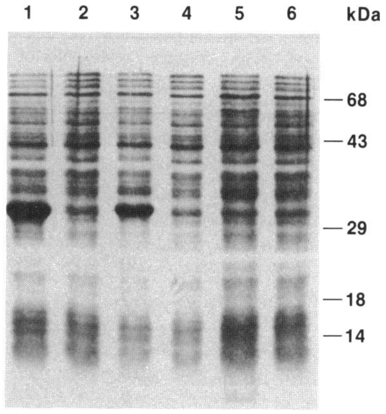


FIG. 5. High-level expression of *pdxJ*. The N^+ TAP56 (lanes 1, 3, and 5) and *N* mutant TAP106 (lanes 2, 4, and 6) strains carrying plasmids pCE21 (lanes 1 and 2), pZSZ1 (lanes 3 and 4), and vector pJLA16 (lanes 5 and 6) were grown to an optical density at 650 nm of 0.4 at 32°C in LB-ampicillin (50 µg/ml). Cultures were shifted to 42°C for 2 h. Cells were prepared, lysed in SDS-containing buffers, and electrophoresed on a polyacrylamide gel. The arrow indicates the PdxJ protein. Under these conditions, it runs slightly slower than a 29-kDa marker band.

mutant. Thus, the clear-plaque phenotype observed was most likely caused by a deficiency of pyridoxine in TB medium which resulted in poor cell growth. Poor cell growth is known to reduce the turbidity of λ plaques (20).

The *pdxJ* and *pdxK* genes were previously mapped at min 55 on the *E. coli* chromosome (1, 2, 9) very close to *rnc*. These mutants require pyridoxine for growth. Six strains with *pdxJ* or *pdxK* point mutations (9) could be complemented for growth in the absence of pyridoxine by pDLC132 and pDLC150 (Table 4). Other *pdx* genes, located at other sites on the chromosome, are not complemented by these plasmids (Table 4). Therefore, the *pdxJ* and *pdxK* mutations are likely to represent different alleles of the same gene that differ only in their leakiness, as suggested previously (9). In keeping with the genome map (1, 2), we have named the gene inactivated by A8 *pdxJ*. We have named the distal gene *dpj*, since it is downstream of *pdxJ* in the operon. We have no results that indicate that *dpj* is involved in pyridoxine biosynthesis.

Nucleotide sequence of the *pdxJ* operon and insertion site of A8. Figure 2 shows the nucleotide sequence that we determined for the region from a *Nru*I site in *recO* to a *Sph*I site near the right end of the *Eco*RI fragment (Fig. 1). Both DNA strands were sequenced by using several synthetic oligonucleotides to prime dideoxy-chain termination reactions. Analysis of the sequence revealed two adjacent open reading frames that nearly span the region from beyond *recO* to the *Sph*I site (Fig. 2). Both open reading frames use the same template DNA strand that encodes the *rnc* operon genes, and the initiating methionine codon for the first reading frame begins 9 bp beyond *recO*. We believe that this is the *pdxJ* gene and that the open reading frame in which the *Sac*I site lies is *dpj*.

The *pdxJ* gene potentially encodes a protein of 243 amino acids (26,384 Da), and the *dpj* gene encodes a protein of 126 amino acids (14,052 Da). From their amino acid compositions, isolectric points of 5.9 and 10.0 were predicted, respectively. Both genes use rare codons characteristic of other genes in *E. coli* that are expressed at low levels, including *pdxA* and *pdxB* (26, 28).

We determined the precise location of the A8 insertion by sequencing a subclone in pUC19 (see Materials and Methods). The insertion site is 36 bp beyond the 3′ end of *recO* and within the *pdxJ* reading frame (Fig. 2). The insertion target sequence TGTTAGGCG was 9 nucleotides in length and was repeated at each border of the mini-Tn*10*, as expected for Tn*10* transposition (15).

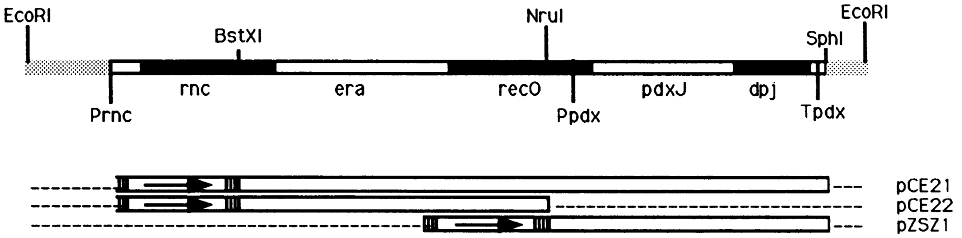


FIG. 4. Genes in the *rnc* and *pdxJ* operons were placed downstream of the λ p_L promoter. pCE21 was made by replacing the *Eco*RI-to-*Bst*XI fragment of pDLC140 (Fig. 1) with the *Eco*RI-*Bst*XI fragment of pJL6 containing the p_L promoter (5, 18). pCE22 was made by replacing the same fragment but in pACS3. pZSZ1 was made by replacing the *Eco*RI-to-*Nru*I fragment of pDLC140 with the *Eco*RI-to-*Nru*I fragment of pJLA16 containing the p_L promoter (19). The p_L promoter regions are indicated by horizontal arrows inside closely spaced vertical bars.

TABLE 5. Amino acid composition of p27

Amino acid	No. of residues	
	Found[a]	Predicted[b]
Cysteine	ND	4
Aspartic acid	23	16
Asparagine		6
Threonine	13	15
Serine	7	2
Glutamic acid	30	20
Glutamine		9
Proline	7	6
Glycine	17	17
Alanine	31	40
Valine	15	15
Methionine	6	8[c]
Isoleucine	14	18
Leucine	21	21
Tyrosine	4	3
Phenylalanine	6	5
Histidine	7	9
Lysine	13	11
Arginine	15	17
Tryptophan	ND	0

[a] Based on 24-, 48-, and 72-h hydrolyses. ND, not determined.

[b] From Fig. 2.

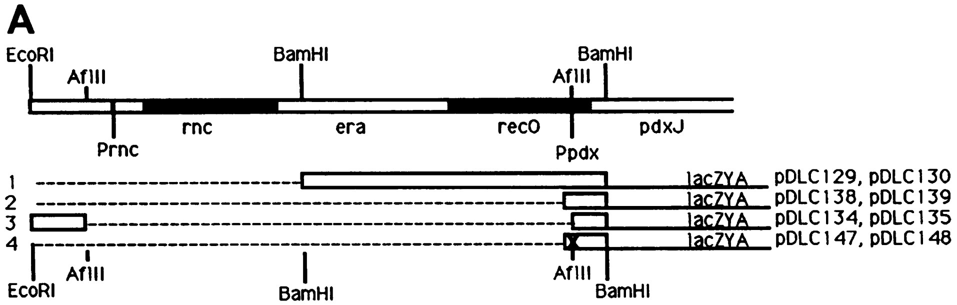
[c] The amino-terminal methionine has not been included.

TABLE 6. Mapping the *pdxJ* operon promoter with *pdxJ-lacZ* fusions[a]

Gene fusion		Operon fusion	
Plasmid	Amt (U) of β-galactosidase	Plasmid	Amt (U) of β-galactosidase
pDLC129	2,838	pDLC130	5,544
pDLC138	2,742	pDLC139	3,077
pDLC134	113	pDLC135	431
pDLC147	58	pDLC148	259
pDLC414	<10	pRS415	23

[a] The *lacZ* fusion plasmids are described in detail in Fig. 6. TAP56 carrying the respective plasmids was grown overnight in LB medium plus ampicillin. Fresh cultures were inoculated in the same medium at a 1:100 dilution and grown to 2×10^8 to 4×10^8 cells per ml.

Expression of the *pdxJ* operon from the λ p_L promoter. Previously, we had made fusions of the p_L promoter of phage λ to the *rnc* and *era* genes and expressed the products of these genes at high levels (5). In similar experiments, we have looked for p_L-promoted expression of the genes beyond *recO*. pCE21 and pZSZ1 (Fig. 4) produced a 27-kDa protein (p27) from the p_L promoter but only in cells containing λ N protein; i.e., no 27-kDa protein was produced in the



B

		% ß-gal units	
		Protein	Operon
1	AAACGCT**TT**ACCCGCATGGCGCTTAAGCCG**TAT**CT**T**GGCGGTAAACCTTT	100	100
2	<u>ATT</u>CGCT**TT**ACCCGCATGGCGCTTAAGCCG**TAT**CT**T**GGCGGTAAACCTTT	97	56
3	<u>CGAATGGCCGACTGGTCT</u>GCGCTTAAGCCG**TAT**CT**T**GGCGGTAAACCTTT	4	8
4	<u>GCTTTACCCGCATGGCGCTTAA</u>TTAAGCCG**TAT**CT**T**GGCGGTAAACCTTT	2	5

TTGaca 17 nucleotides TAtaaT ⟶
-35 -10

FIG. 6. Defining the *pdx* promoter by gene and operon *lacZ* fusions at the *Bam*HI site in *pdxJ*. (A) In construct 1, the *Bam*HI fragment (open rectangle) was isolated and inserted in the orientation shown at the *Bam*HI site of the *lacZ* vectors pRS414 and pRS415 (30), creating pDLC128 and pDLC130, respectively. In construct 3, the *Eco*RI-to-*Bam*HI fragment from pDLC132 (Fig. 1), was used to generate pDLC134 and pDLC135 by replacing the *Eco*RI-to-*Bam*HI fragments from pRS414 and pRS415, respectively. A 19-bp synthetic DNA, CGCTTTAC CCGCATGGCGC, was created from complementary oligonucleotides. This synthetic DNA had single-stranded *Eco*RI- and *Afl*II-compatible ends. It was used to replace the *Eco*RI-to-*Afl*II fragments of pDLC134 and pDLC135, creating pDLC138 and pDLC139. pDLC147 and pDLC148 are pDLC138 and pDLC139 but with the *Afl*II site cut and then repaired with Klenow enzyme to add the 4 nucleotides TTAA (shown as an X). The sequence of each of these regions at the *Afl*II site is shown in panel B. (B) The numbered sequences represent each construct shown in panel A. Line 1 is entirely wild-type sequence for this region. Lines 2 and 3 represent deletion derivatives in which wild-type sequence is deleted from the left. The underlines indicate nucleotide sequences changed relative to the wild-type sequence. Line 4 represents the same plasmid and sequence as in line 2 except that the *Afl*II site between the −35 and −10 regions has been cut and then filled in with Klenow enzyme to add 4 nucleotides (TTAA). This shifts the upstream sequence relative to the promoter. Below the sequences is the consensus sequence for the −35 and −10 regions of a promoter with the optimal 17-nucleotide spacing (27). The most-conserved and most-important nucleotides in the −35 and −10 hexamers are in capital letters, and the less-conserved nucleotides are in lowercase letters. Within each of the four sequences, nucleotides in the appropriate −35 and −10 regions that match the consensus are shown in boldface type. The arrow seven nucleotides beyond the −10 region represents the proposed start and direction of transcription for the promoter. Promoter activity is normalized relative to the 100% β-galactosidase values for the protein (pDLC129) and operon (pDLC130) fusions, which are 2,838 and 5,544 U, respectively.

N mutant strain, TAP106 (Fig. 5). This N effect will be analyzed in the Discussion. pCE22 failed to express the 27-kDa protein (data not shown). pCE22 is deleted for both *pdxJ* and *dpj*. Since *pdxJ* can encode a 27-kDa protein, we considered it to be the source of the expressed protein. No other protein in the size range (~14 kDa) expected for *dpj* was found in the expressed cells.

We expressed the 27-kDa protein in TAP56 containing pZSZ1 and prepared crude extracts by freeze-thaw lysis of the cells. The 27-kDa protein, which made up more than 40% of the total cellular protein, was in the supernatant after a low-speed centrifugation. This supernatant was then fractionated by reverse-phase liquid chromatography, and the major protein peak (p27) was subjected to amino-terminal-sequence analyses as well as composition analysis. The amino-terminal sequence of 18 amino acids matched that deduced from the coding sequence except that methionine, the first amino acid residue in the predicted sequence, was absent in the protein. Two internal peptides were purified from a tryptic digest of the protein. Their amino acid sequences corresponded with the proposed protein sequence from positions 33 to 44 and from positions 134 to 146.

The amino acid composition of p27 is given in Table 5 and compared with the predicted amino acid composition. Overall, the agreement is quite good. Serine levels are high, but they could be due to contamination of free amino acid. The only discrepancy is that the level of alanine is somewhat low.

The *pdx* promoter and terminator. Protein and operon fusions of *pdxJ* to *lacZ* at the *Bam*HI site in *pdxJ* were constructed to detect and map the promoter for this gene. Differences in β-galactosidase levels between different deletion mutants indicated that an active promoter was located at the *Afl*II site just upstream of *pdxJ* (Fig. 6; Table 6). The *Bam*HI fragment containing the entire *recO* gene was joined to *lacZ* in both protein (pDLC129) and operon (pDLC130) fusions. We note that neither of these fusions complemented the *recO* mutant strain HT210 for UV^r, indicating that the *recO* (p_{rnc}) promoter had been deleted. However, both fusion plasmids yielded high levels of β-galactosidase activity, indicating the presence of a promoter on the *Bam*HI fragment. Plasmids bearing only the *Afl*II-to-*Bam*HI fragment fused to *lacZ* (pDLC134 and pDLC135) produce greatly reduced levels of β-galactosidase. This indicates that the promoter for *pdxJ* lies in the interval between the *Bam*HI and *Afl*II sites missing in pDLC134 and pDLC135. High β-galactosidase levels were restored by replacing 19 bp of wild-type sequence upstream of the *Afl*II site (pDLC138 and pDLC139). This result suggested that the upstream boundary (the −35 region) of the promoter was within the replaced 19-bp region (Fig. 6B, line 2). If this 19 bp defines the −35 region, then the *Afl*II site is located in the 17-bp spacer between the −35 and −10 regions of the promoter. When the *Afl*II site of pDLC138 or pDLC139 was mutated by insertion of 4 bp, the promoter became inactive (Table 6, line 4). We believe, therefore, that the −35 and −10 positions indicated in Fig. 3 and 6B define the promoter for the *pdxJ* operon.

We have also tested pDLC129 and pDLC130 in TAP56, TAP56 *rnc-14*, and TAP56 *pdxJ8* strains and found no difference in expression between these strains (data not shown). Thus, we see no regulatory control on *pdxJ* by the *rnc* and *pdxJ* gene products.

A transcription terminator beyond *dpj*. Between the end of *dpj* and the *Sph*I site is an extensive inverted repeat sequence which we show in Fig. 2 by inverted arrows. This site has characteristics of a rho-independent terminator, namely, an RNA loop between a GC-rich stem segment followed by a uridine stretch in the RNA. Since the inverted repeat in this region includes both the GC-rich stem and the adjacent uridine segment, the symmetric DNA sequence may be capable of terminating transcription in both orientations (11).

We placed a 150-bp DNA fragment (*Nsi*I to *Sph*I in Fig. 2) containing the inverted repeat in a transcription termination test plasmid between the *lac* promoter and a reporter *galK* gene encoding galactokinase. Without the fragment inserted, the vector produced 1,087 U of galactokinase. Twenty-one units and 90 U were made with the proposed terminator fragment inserted in the normal and inverted orientations, respectively. Thus, it is an effective terminator in both orientations.

DISCUSSION

We have demonstrated that in addition to its ability to transpose, the mini-Tn*10* element Δ16Δ17Tn*10* (33) has two properties that allow detection of essential genes in *E. coli*. First, when the element inserts in a transcribed region, it causes a strong polarity effect and prevents gene expression from the natural promoter. Second, when tetracycline is present in the medium, the two divergent genes within the transposon are induced for expression (13) and the resulting transcriptions of *tetA* and *tetR* extend beyond both ends of the element into adjacent bacterial sequences. Thus, while blocking expression of downstream genes from the bacterial promoter, the mini-Tn*10* can conditionally activate those downstream genes following induction with tetracycline. If the downstream genes are essential for growth, the mutants may grow conditionally upon the addition of tetracycline. In this manner, the mini-Tn*10* insertions have allowed us to find two essential genes, *era* and *dpj*. Although the normal Tn*10* transposable element causes polarity (16), there are no reports that its tetracycline-induced transcripts extend beyond its boundaries into bacterial genes as the mini-Tn*10* transcripts do.

We suspect that not all genes will be expressed at functional levels from a mini-Tn*10* insert, especially those genes which require high levels of expression. Both *era* and *dpj* contain rare codons characteristic of *E. coli* genes that are expressed at low levels. Our measurements of Era protein in the cell substantiated its low level (3). The level of tetracycline-dependent Era expressed from A40 is clearly less than that from the natural *rnc* promoter (Table 2); however, it is still sufficient to allow cell growth. We note that transcription from the A40 element should include both *rnc* and *era*, since A40 is located upstream of both genes, yet the cells remain phenotypically Rnc$^-$. We presume that the cell is making reduced levels of RNase III that are below a threshold required for an Rnc$^+$ phenotype. Thus, *rnc* is an example of a gene that cannot be restored to a normal phenotype by transcription from the mini-Tn*10*. Still, we believe that the mini-Tn*10* will be useful for detecting genes, both essential and nonessential, that are expressed at low levels.

Takiff et al. (31) isolated a mini-Tn*10* insertion (A14) within the *rnc* gene. This strain is not Tet^d. We have examined *era-lacZ* fusions of this A14 mutant and found that its expression is higher than that found for the strain with the A40 insertion and that expression is constitutive, i.e., not inducible by tetracycline. Sequence analysis of the junction with the mini-Tn*10* and *rnc* revealed that a weak promoter was probably created. It is composed of a poorly conserved −35 element (TAGGGG) that is present in the inverted repeat at the ends of the mini-Tn*10* and spaced 17 bases from a −10 sequence (TACACT) present in the *rnc* gene adjacent and just downstream of the insertion.

The gene *pdxJ* is inactivated by the mini-Tn*10* insertion A8, and *dpj* is not expressed because of transcriptional polarity. Since *dpj* is essential for growth, cells with the A8 insertion grow only with tetracycline present or if carrying a plasmid that contains an intact *dpj* gene. The function of the *dpj* product remains unknown. The *dpj* gene is known only by its codons, amino acid sequence, and operon relationship with *pdxJ*. *dpj* encodes a highly basic protein of 14.0 kDa. Comparison of its amino acid sequence with those in the translated genetic data base failed to reveal any significant similarities with other gene products.

The *pdxJ* gene encodes a protein of 26.4 kDa that functions in the biosynthesis of pyridoxal phosphate. The A8 insertion interrupts this gene and causes a cellular requirement for pyridoxal or pyridoxine (8, 10). Lam et al. in the accompanying paper (17) suggest that PdxJ may take part in the synthesis of pyridoxine by the condensation of 1-D-deoxyxylulose and 4-hydroxythreonine.

The *rnc* operon consists of three genes: *rnc*, *era*, and *recO*. Three observations support the proposal that transcription of *recO* is dependent on the *rnc* promoter. First, insertion mutations in *rnc* and *era* between p_{rnc} and *recO* are polar on *recO*. These polar mutations cause the cell to become sensitive to UV irradiation, a phenotype of *recO* mutants (25, 31). The *Bam*HI fragment that includes *recO* and is found in pDLC129 and pDLC130 (Fig. 6) does not complement the *recO* mutant strain HT210. This indicates that the *recO* promoter is upstream of Era. Complementation of *recO* seen previously with this *Bam*HI fragment (25) may have been caused by transcription from plasmid promoters flanking the fragment. The *lacZ* plasmid vectors used here were constructed to minimize interference by endogenous plasmid promoters (30). Finally, transcription from the *rnc* promoter is reduced as it enters *recO*; only about 1 in 10 transcripts continues through the *recO* gene (3).

The *pdxJ* operon promoter is located within the *recO* gene, and the *pdxJ* ribosome-binding and initiation site overlaps with the translation termination codons of *recO*. We have not found a typical rho-independent terminator (4) for *rnc* operon transcription in the 100 nucleotides of sequence beyond *recO*. However, we believe for the following reasons that there is a strong terminator, perhaps a rho- or other factor-dependent terminator, in this region. Transcription initiated from the powerful λ p_L promoters in plasmids pCE21 and pZSZ1 fails to produce excess PdxJ protein. However, with the same p_L fusions, large amounts of PdxJ are made when the λ *N* gene product is provided (Fig. 4). *N* causes RNA polymerases that initiate at p_L to become insensitive to terminators; i.e., *N* causes transcription antitermination (12). We assume that the terminator that blocks transcription from p_L under N$^-$ conditions is at the natural end of the *rnc* operon within *pdxJ*. If this is correct, we are left with the problem of how the promoter located just upstream of *pdxJ* in *recO* can bypass this terminator. We speculate that the answer may lie in the proximity of the *pdxJ* promoter to the proposed transcription terminator, i.e., that it may be too close. For rho-dependent termination to be effective, the terminator must be at least 100 nucleotides from the promoter (24, 34).

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