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purF-lac Fusion and Direction of *purF* Transcription in *Escherichia coli*

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The *purF* locus codes for the first enzyme, glutamine phosphoribosylpyrophosphate amidotransferase, of the purine biosynthetic pathway. A strain of *Escherichia coli* K-12 was isolated in which the *lac* structural genes were fused to the control region of the *purF* locus. This *purF-lac* fusion was shown to respond to purine-specific regulatory signals. A plaque-forming lambda transducing phage bearing this *purF-lac* fusion was isolated. This phage was used to genetically determine the direction of transcription for the *purF* locus by two independent means. Results from both methods agreed that the direction of transcription of the *purF* locus was clockwise on the standard *Escherichia coli* K-12 genetic map.

The first complete nucleotide, IMP, of the purine biosynthetic pathway is formed via a series of 10 discrete steps from phosphoribosylpyrophosphate (Fig. 1). IMP is then converted by separate pathways into AMP and GMP. The first step of the pathway is catalyzed by the enzyme phosphoribosylpyrophosphate amidotransferase, whose gene is designated *purF*. This enzyme is subject to feedback inhibition by either IMP, AMP, or GMP separately and by AMP and GMP synergistically (16, 17).

The genetic loci which code for the enzymes of the purine pathway are scattered around the *E. coli* K-12 chromosome, with the exception of a three-gene operon which codes for the second (*purD*) and the last two genes (*purH*, *purJ*) of the IMP pathway (1). A cluster of purine genes, including those which comprise the two-gene operon for GMP synthesis (*guaAB*), exist in the 53- to 55-min interval on the recalibrated map (1, 19, 25). The *purF* gene, the subject of this paper, maps at 49.5 min and is cotransducible with *aroC*, which lies to the left at 50 min (1).

Although the intermediates and the enzymatic steps have been determined for the purine pathway, the nature of its regulation is only just beginning to be understood. The investigation into the regulatory properties of the purine biosynthetic pathway has been hampered by the scarcity and lability of substrates and the general inconvenience of the available assays to determine enzyme levels. Efforts to systematically isolate regulatory mutations by standard methods have been relatively unproductive, since resistance to available analog usually involved mutations at the site of conversion to the nucleotide form. The few available regulatory mutants were either fortuitously discovered (4) or were limited to effects on an episomal *purE-lac* fusion (9, 12).

To further elucidate the nature and mode of regulation of the purine biosynthetic pathway, we have undertaken an approach which should lead to a more thorough systematic analysis of the regulatory properties of this pathway. To circumvent the problem of enzyme assays, we have isolated fusions which place the lactose genes under the control of different purine control regions. Thus, we can assay β-galactosidase in place of the specific purine enzyme. This approach also allows us to systematically isolate a wide variety of regulatory mutants.

The *purF* locus encodes the enzyme catalyzing the initial step in the purine biosynthetic pathway, phosphoribosylpyrophosphate amidotransferase (EC 2.4.2.14). Our initial studies concerned this region and its means of control. In this paper we have applied the general procedure of Casadaban (6) for the isolation of a genetic fusion which places the *lac* genes under the control of the *purF* region. The existence of the *purF-lac* fusion allows us to assay β-galactosidase as a way to monitor the response of the *purF* gene to purine specific regulatory signals.

We have characterized this *purF-lac* fusion and have demonstrated that it responds to purine-specific control. In addition we have isolated a specialized lambda transducing phage bearing this *purF-lac* fusion which was used to determine the direction of transcription of the *purF* gene by two independent genetic means. Both methods agree that the direction of tran-

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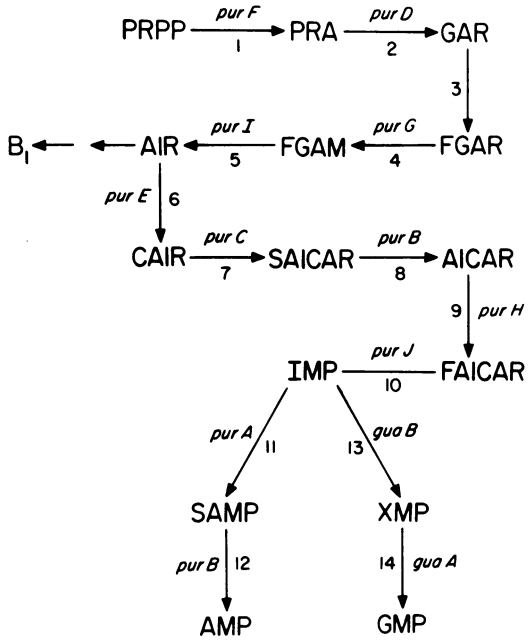

FIG. 1. *Biosynthetic pathway of purine nucleotides in E. coli. Corresponding structural genes are indicated for each step. The purF gene, the subject of this paper, codes for the first enzyme, glutamine phosphoribosylpyrophosphate amidotransferase. Details and discussion of other enzymes may be found in the references 8, 9, and 27. Abbreviations: PRPP, 5-phosphoribosyl-1-pyrophosphate; PRA, phosphoribosylamine; GAR, phosphoribosyl-glycinamide; F-GAR, phosphoribosyl-N-formyl-glycinamide; F-GAM, phosphoribosyl-N-formylglycinamidine; AIR, phosphoribosyl-5-aminoimidazole; C-AIR, phosphoribosyl-4-carboxy-5-aminoimidazole; 5-AICAR, phosphoribosyl-4-N-succino-carboxamide 5-aminoimidazole (succinyl-AICAR); AICAR phosphoribosyl-5-amino-4-imidazolecarboxamide; F-AICAR, phosphoribosyl-5-formamido-4-imidazole-carboxamide; IMP, inosine monophosphate; S-AMP, adenylosuccinic acid (succinyl-AMP); AMP, adenosine monophosphate; XMP, xanthosine monophosphate; GMP, guanosine monophosphate; B_1, thiamine.*

scription of the *purF* locus is clockwise on the standard *E. coli* K-12 genetic map.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. The bacterial strains employed in this study are derivatives of *E. coli* K-12. Bacterial strains and their sources or derivations are listed in Table 1. Strains were maintained on L-agar. Phage strains and their sources are listed in Table 2.

Media and growth conditions. The medium of Vogel and Bonner (26) supplemented with 0.5% glucose was used as a minimal growth medium. Necessary supplements were added at the following final concentrations; all amino acids at 50 μg/ml, all purines at 50 μg/ml except where noted, and all vitamins at 1 μg/ml or 0.1 μM. L-broth was employed as a rich medium (5). All carbon sources were added at a final concentration of 0.5%. MacConkey agar base (Difco) with 1% lactose was additionally supplemented with adenine at 100 μg/ml whenever this medium was used in the study of *pur-lac* fusions. 5-Bromo-4-chloro-3-indoyl-β-D-galactoside (X-gal) plates (15) were prepared with minimal glucose agar containing 40 μg of 5-bromo-4-chloro-3-indoyl-β-D-galactoside (X-gal) per ml. Growth of cultures in liquid medium was monitored by measuring the absorbance of each culture at 660 nm.

Mutagenesis. Mutagenesis with the bacteriophage Mu-1 *cts62 K1010* followed the method of Howe and

TABLE 1. *Bacterial strain list*

Strain	Genotype	Source or reference
CSH26	*ara* Δ(*gpt-pro-lac*) *thi*[a]	Cold Spring Harbor Laboratory
CU446	Δ(*lac*) *trp thi arg*	H. E. Umbarger (24)
AB2557	*aroC4 purF1 dsdA1 ilvD188 thi-1 mtl-2 xyl-7 malA1 lac tonA tsx rpsL supE44*	B. Bachmann
TX40	*ara* Δ*lac*	P1-mediated transduction of CSH26 with CU446 as donor
TX156	*ara* Δ*lac purF200*::Mu *cts62 K1010*	This paper
TX157	*ara* Δ*lac purF200*::Mu *cts62 K1010*:: λp1(209)	This paper
TX158	*ara* Δ*lac* φ[*purF200-lac*::λp1(209)][b]	This paper
TX191	*ara* Δ*lac aroC4*	P1-mediated transduction of TX158 with AB2557 as donor
TX192	*ara* Δ*lac aroC4 purF201*	Spontaneous *purF* mutant of strain TX191
TX193	*ara* Δ*lac aroC4 purF202*	Spontaneous *purF* mutant of strain TX191
TX251	*ara* Δ*lac aroC4 purF201* φ[*purF200-lac*: :λp1(209)]	This paper
TX252	*ara* Δ*lac aroC4 purF201* φ[*purF200-lac*: :λp1(209)] *purR203*	P1-mediated transduction of strain TX251

[a] The *thi* marker is presumed to be lost, since no thiamine requirement can be demonstrated.
[b] Fusion nomenclature is that of Bassford et al. (3), and genetic nomenclature is that of Bachmann et al. (1).

Bade (11). Bacteriophage at a multiplicity of infection of 1.0 was added to cultures that had been resuspended in 5 mM $MgSO_4$-$CaCl_2$.

After absorption for 20 min at 30°C, the cells mutagenized with Mu were washed with 0.85% NaCl, suspended in an appropriately supplemented minimal medium, and incubated at 30°C for 1 to 18 h to allow segregation of the mutants. Mu *c*25 (a clear plaque mutant) was sometimes added at a multiplicity of infection of 2.0 to kill any nonlysogens that might have escaped the first Mu infection. Mutagenesis with nitrous acid was performed by the method of Miller (15) on fresh stationary-phase cultures.

Transductions. The P1cm *clr*100 bacteriophage was used for generalized transductions by the method of Rosner (20). Specialized transductions with lambda phages were performed by the method of Shimada et al. (22).

Preparation of phage lysates. P1cm lysates were prepared by heat induction (20). Lambda lysates were prepared by lytic growth on strain TX40 in agar plate lysates. Mitomycin C (Sigma) was added at a final concentration of 1 μg/ml for the lytic growth of λ *purF-lac*-1. After lysis was complete, the lysates were treated with chloroform, clarified by centrifugation, and stored at 0 to 5°C in 0.01 M $MgSO_4$ over chloroform. Mu lysates were prepared and stored as described by Howe (10). Non-temperature-sensitive lambda lysogens were induced by adding mitomycin C at a final concentration of 1 μg/ml to an early log-phase culture of the lysogen. Incubation was continued at 37°C until lysis had occurred.

Enzyme assays. Crude extracts prepared by sonication were assayed for β-galactosidase by the method of Miller (15) and for phosphoribosylpyrophosphate amidotransferase by the procedure of Messenger and Zalkin (16). The protein concentration of crude extracts was determined by the procedure of Lowry et al. (14) or by the procedure of Ohnishi and Barr (18).

Ampicillin enrichment of auxotrophs. The ampicillin enrichment for purine auxotrophs was carried out by the method of Smith et al. (23). The counter-selection medium consisted of minimal medium supplemented wtih 0.5% glucose, 1 μg of thiamine per ml, and 0.1% casein hydrolysate (Difco).

Chemicals. 5-Bromo-4-chloro-3-indoyl-β-D-galactoside (X-gal) was purchased from Bachem, Inc. (Torrance, Calif.). All other fine chemicals were obtained from Sigma Chemical Co. (St. Louis, Mo.).

RESULTS

Isolation of Mu-induced purine auxotrophs.

Strain TX40 (Table 1) was subjected to Mu mutagenesis with Mu-1 *cts*62 *K*1010 as described in Materials and Methods. After two cycles of counter-selection with ampicillin, the surviving cells were scored for both purine auxotrophy and temperature sensitivity. The presumed Mu-induced purine auxotrophs were then characterized to determine the site of purine auxotrophy. This was determined by a combination of nutritional behavior, accumulation of intermediates, other observable phenotypes (27), and complementation analysis with either F′ episomes or other plasmids carrying known *pur* genes. Strains with Mu-1 insertions corresponding to all previously recognized *pur* lesions have been isolated.

Strains which had Mu inserted into the *purF* gene were identified by enzyme assay and by complementation with the F′129 episome (13) since the *purF* gene is separated from the other known *pur* loci. To establish that the purine auxotrophy was due to the insertion of a single Mu bacteriophage into *purF*, the presumed Mu-induced auxotrophs were transduced to prototrophy with a P1 lysate prepared from strain TX40. The resulting transductants were then scored for temperature sensitivity at 42°C. Strains that yielded 95% or greater temperature-resistant transductants were assumed to have Mu *cts*62 *K*1010 inserted at a single site on the chromosome. One strain that gave 98% (49/50) temperature-resistant transductants after transduction to prototrophy was retained as strain TX156 and used for the subsequent isolation of *purF-lac* fusions.

Isolation of λp1(209) and λp123(209) lysogens.

Since bacteriophage Mu can insert in either of two opposite orientations (11), strain TX156 was lysogenized with both λp1(209) and λp123(209) phages by the procedure of Casadaban (6). After isolation of the presumed lambda lysogens, the integration of the lambda phage at the site of the Mu insertion in *purF* was verified by transducing the presumed lysogen to prototrophy (Pur^+). The resulting transductants were scored for temperature sensitivity at 42°C and for sensitivity to lambda. Those transductants

TABLE 2. *Phage list*

Phage	Genotype	Source or reference
Mu-1	*cts*62 *K*1010	M. Howe (10)
Mu-1	*c*25	M. Howe
λp1(209)	*lac 'AYZO'-Δw209-trp 'AB'*::(+Mu)	M. J. Casadaban (6)
λp123(209)	*lac, 'AYZO'-Δw209-trp 'ABCDE'*::(−Mu)	M. J. Casadaban (6)
λh80(*int*) Δ-9		H. E. Umbarger
λ *vir*		H. E. Umbarger
λ *purF-lac*-1	*φ(lac 'AYZO'-purF200)*	This paper
P1cm	*clr*100	Rosner (20)

which were sensitive to both clear-plaque and virulent mutants of lambda were judged to be lambda free. The λp1(209) and λp123(209) lysogens of strain TX156 whose prototrophic transductants had lost both the Mu *cts*62 *K*1010 and lambda phage were assumed to have the appropriate lambda integrated at the site of the Mu *cts*62 *K*1010 insertion. These lysogens were retained for the isolation of *purF-lac* fusions.

Isolation of *purF-lac* fusions. Concentrated suspensions of the λp1(209) and λp123(209) lysogens of strain TX156 were spread on minimal lactose plates supplemented with a low concentration of xanthine (5 μg/ml). This amount of xanthine allowed the strains to grow without complete repression of the *purF* gene but still form colonies large enough to be detected. The plates were incubated at 42°C for 3 h before being shifted to 37°C. After incubation at 37°C for 24 to 72 h, the resulting colonies were characterized to determine those that were *purF-lac* fusions.

Characterization of potential *purF-lac* fusion strains. Only the λp1(209) lysogen of strain TX156 gave rise to colonies on the minimal lactose suboptimal xanthine plates. This strain was retained and designated strain TX157. To tentatively identify those colonies which had the *lac* genes fused to the *purF* control region, the colonies from the minimal lactose-xanthine plates were scored for inhibition by excess amounts of adenine (100 μg/ml) on minimal lactose plates. These were retained for further characterization as presumed candidates in which the *lac* genes were under *purF* control.

After single-colony isolation, the presumptive *purF-lac* fusion strains were further characterized. The β-galactosidase activity was determined under repressed (100 μg of adenine per ml) and derepressed (5 μg of xanthine per ml) conditions for these potential fusion strains as described in Materials and Methods. Only those strains that demonstrated purine control of the β-galactosidase activity were retained as true *purF-lac* fusions. Some strains were found that showed inhibition by 100 μg of adenine per ml on minimal lactose plates but did not exhibit purine control of β-galactosidase activity when assayed in liquid culture. Only 2 of 15 potential *purF-lac* fusion strains which exhibited inhibition by adenine on minimal lactose medium clearly showed repression and derepression of β-galactosidase activity in response to purine signals. Representative data are shown in Table 3. One such strain (TX157-4) which exhibited control was retained for further study and designated strain TX158. The genetic events that resulted in this *purF-lac* fusion are depicted in Fig. 2.

TABLE 3. *Characterization of some potential purF-lac fusion strains from strain TX157*

Isolate	β-galactosidase activity (nmol/min/mg of protein)		
	Repressed[a]	Derepressed[b]	Fold depressed
TX157-1	15.3	24.1	1.6
TX157-2	27.2	32.4	1.2
TX157-3	106.0	101.0	0.95
TX157-4	28.8	376.5	13.0

[a] Adenine (100 μg/ml).
[b] Xanthine (5 μg/ml).

Genetic characterization of strain TX158. To genetically characterize the location of the *lac* genes present in strain TX158 with respect to other *purF* mutations, a series of spontaneous *purF* mutations in strain TX191 (*purF*$^+$ *aroC*) were isolated after ampicillin enrichment as described in Materials and Methods. The strains were characterized as containing *purF* mutations by virtue of complementation by F'129.

The relative order of the *purF* mutations was determined by the use of three-factor crosses in which *aroC* was the outside marker. After preliminary genetic analysis of several *purF* mutations, two mutations were found that mapped on separate sides of the *purF-lac* fusion in strain TX158. Since the Mu insertions and subsequent lambda prophage and *lac* genes behave as point mutations in genetic crosses (2), the *purF* mutations were mapped against the *lac* genes inserted in the *purF* structural genes of strain TX158. The data from the genetic crosses used to order the two *purF* mutations with respect to *purF200* (*lac* fusion) of TX158 are presented in Table 4. An examination of the data reveals that the gene order *aroC4-purF202-purF200(lac)-purF201* is the best interpretation consistent with the data. This is based on the fact that in the reciprocal crosses shown, the one that gives the reduced cotransduction between *purF* and *aroC* will be the one in which the mutant *purF* allele of the donor is in the middle. The exact position of the *lac* region cannot be determined from this experiment and will be examined below.

The isolation of *purF* mutations that lie to either side of the *purF-lac* fusion in strain TX158 allows us to conclude that the deletion event which fused the *lac* genes to the *purF* structural region did not extend into or beyond the *purF* control region (Fig. 2).

Isolation of a λ phage carrying the *purF-lac* fusion. The deletion event which placed the *lac* genes under the control of the *purF* control region should not have affected the λ prophage inserted in *purF*, since the prophage lies distal

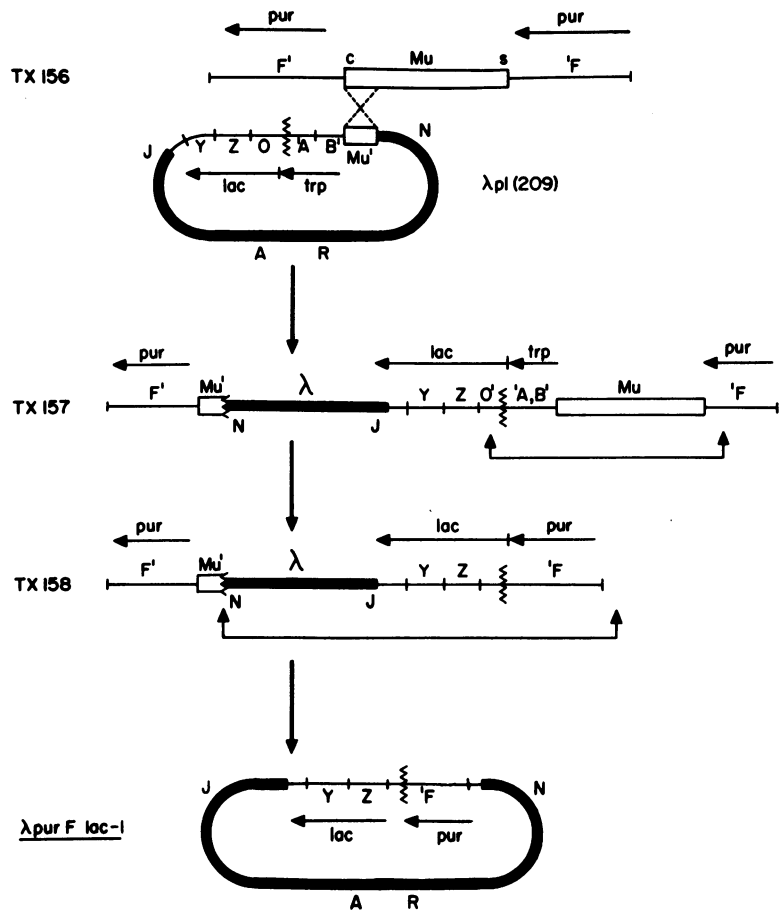

FIG. 2. *Isolation of purF-lac fusions and a lambda phage carrying the purF-lac fusion. Strain TX156 has the Mu cts phage inserted into the purF gene. Lysogenization of strain TX156 with the λp1(209) phage yielded strain TX157 in which the lac genes are inserted in the correct orientation with respect to purF. Deletion of the Mu phage in strain TX157 yielded strain TX158 containing the purF-lac fusion. From this strain, a plaque-forming lambda phage carrying the purF-lac fusion was isolated after induction with mitomycin C as described in the text.*

TABLE 4. *Genetic characterization of the purF-lac fusion (purF200-lac) in strain TX158*

Donor	Recipient	Selected marker	Scored marker	% Cotransduction	Suggested gene order
TX191 *aroC4*	TX158 (*purF200-lac*)	*purF*$^+$	*aroC*	23.8 (24/101)	*aroC4-(purF200-lac)*
TX158 (*purF200-lac*)	TX192 *purF201-aroC4*	*purF*$^+$	*aroC*$^+$	15.6 (24/154)	*aroC4-(purF200-lac)-purF201*
TX192 *purF201 aroC4*	TX158 (*purF200-lac*)	*purF*$^+$	*aroC*	24.2 (96/396)	
TX158 (*purF200-lac*)	TX193 *purF202 aroC4*	*purF*$^+$	*aroC*$^+$	44.9 (62/138)	*aroC4-purF202-(purF200-lac)*
TX193 *purF202 aroC4*	TX158 (*purF200-lac*)	*purF*$^+$	*aroC*	7.4 (2/27)	

to the *lac* genes (Fig. 2). Thus, the isolation of a plaque-forming lambda transducing phage carrying the *purF-lac* fusion is both feasible and desirable.

Strain TX158 was induced with mitomycin C as described in Materials and Methods. The resulting mixed lysate was titrated on strain TX40 on minimal glucose X-gal plates to detect

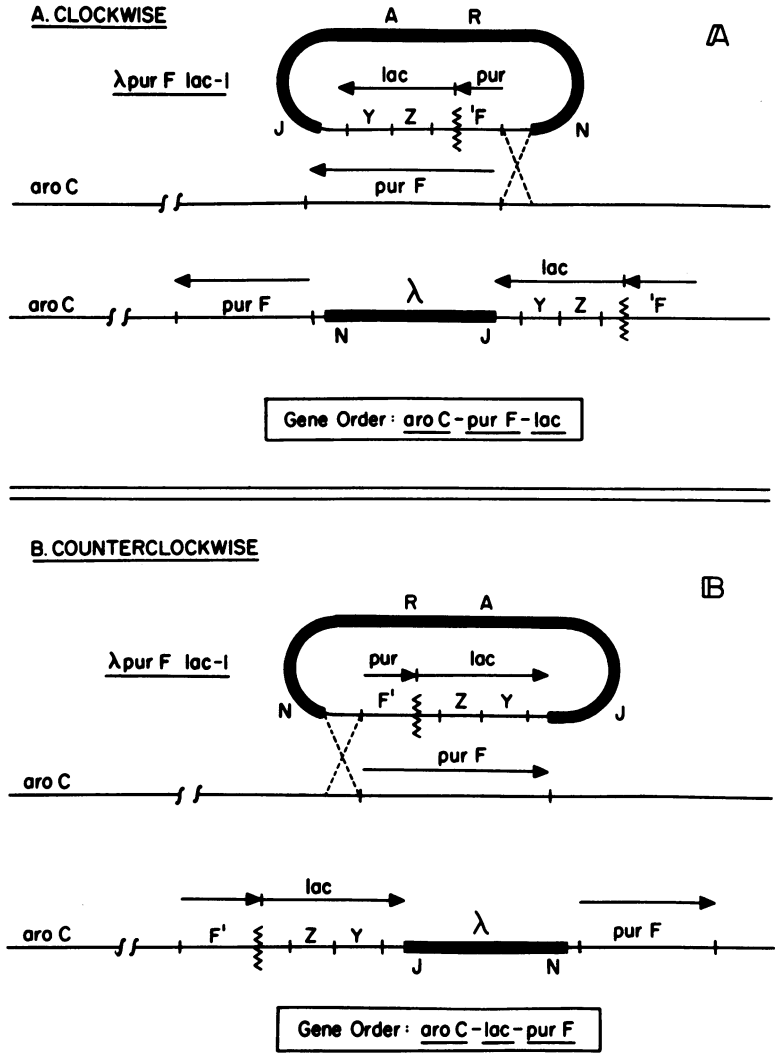

FIG. 3. *Integration of the λ purF-lac-1 phage in the purF region. This produces a unique gene order which is dictated by the direction of transcription of the purF gene. If the direction of transcription is clockwise (A), the resulting gene order will be aroC-purF-lac. If the direction of transcription is counterclockwise (B), the gene order will be aroC-lac-purF. Thus, the direction of transcription can be inferred by determining the gene order as described in the text.*

the rare plaque-forming phage which carried the *purF-lac* fusion. Blue plaques were picked and propagated on strain TX40 until a single blue plaque yielded a purified phage lysate of a λ *purF-lac* phage. The phages were designated λ *purF-lac A*, *B*, *C*, etc. and propagated until a high-titer lysate (ca. 10^{10} φ/ml) was achieved for each lysate.

The stability of various λ*purF-lac* phages was determined by titrating the lysates on strain TX40 on X-gal plates after several propagations under nonselective conditions. The ratio of white plaque (revertants) to blue plaques gives an index of stability for each phage. One such phage that appeared stable was designated λ*purF-lac*-1 and retained for further study. The excision event that is assumed to have led to the formation of λ *purF-lac*-1 is shown in Fig. 2.

The original selection criteria for these phages were based on the presence of the *lacZ* gene and not necessarily on the presence of the *purF* control region. The complete characterization of λ *purF-lac*-1 will be reported elsewhere (Smith, Kamholz, and Gots, manuscript in preparation), but preliminary characterization indicates that at a minimum it carries the structural DNA

between the *lac* genes and the *purF* control region and probably the control region as well.

Determination of the direction of transcription of the *purF* gene. By employing the λ *purF-lac*-1 phage described above, it is possible to genetically determine the direction of transcription of the *purF* locus by two independent means.

Since the λ *purF-lac*-1 phage carries the *purF* structural DNA between the *lac* genes and the *purF* control region, it should complement or recombine with various *purF* mutations that lie in this region. The genetic characterization of the *purF-lac* fusion described above has revealed the existence of two mutations that map to either side of the *lac* genes (Table 4). Thus, since the λ *purF-lac*-1 phage should carry *purF* structural DNA from only one side of the *lac* genes, it should complement or recombine with only one of the *purF* mutations and not the other.

Accordingly, the λ *purF-lac*-1 phage was used in an attempt to transduce strains TX192 (*purF201*) and TX193 (*purF202*) to prototrophy (Pur^+). The λ *purF-lac*-1 transduced only strain TX192(*purF201*) to Pur^+ but not TX193(*purF202*). The recombination of strain TX192(*purF201*) indicates that the λ*purF-lac*-1 phage carries the DNA necessary to complement or recombine with the *purF201* mutation. Since the relative order of these mutations with respect to the *lac* genes and *aroC* has been determined (Table 4), the results indicate that the *purF* control region lies to the *aroC* distal side of the *lac* genes. Thus, from these data, the direction of transcription of the *purF* gene can be inferred to be clockwise.

Another independent means of genetically determining the direction of transcription also involves the use of the λ *purF-lac*-1 phage described above. Since the λ *purF-lac*-1 phage lacks an attachment site (6) and the strains involved in this study contain *lac* deletions, the only region of homology after infection is the *purF* DNA carried in common between the chromosome and the λ *purF-lac*-1 phage. Depending on the direction of transcription, the λ*purF-lac*-1 phage will reintegrate in either of two arrangements in relation to the *purF* gene and the *aroC* locus. The two possible gene arrangements are shown in Fig. 3. As can be seen, a unique gene order is generated for the *purF*, *lac*, and *aroC* genes, depending on the absolute direction of transcription of *purF*. The gene order can be determined by analysis of three-factor crosses and linkage tests. Thus, the direction of transcription of the *purF* locus can be inferred by determining the gene order present in the resulting lysogens.

To provide a *purF* mutation for use in the ordering of the *lac* and *purF* genes with respect to *aroC*, the λ *purF-lac*-1 phage was used to lysogenize strain TX192 (*purF201 aroC4*). Transductants were selected by plating on minimal lactose plates supplemented with suboptimal amounts of xanthine (5 μg/ml). The transductants were scored for purine auxotrophy since both Pur^+ and Pur^- transductants can result from the integration of the λ *purF-lac*-1 phage. Several Pur^- transductants were isolated from single colonies twice under nonselective conditions and retested for the presence of the *lac* genes and purine auxotrophy to verify that the integration of the lambda phage was stable.

Additionally, to facilitate the complete recovery of transductants where *purF201* was an unselected marker, a regulatory mutation (*purR203*) that allowed high expression of the *lac* genes in the presence of repressing amounts of purines was introduced by P1 transduction. This and other regulatory mutations will be described in detail elsewhere (Smith and Gots, manuscript in preparation). Since regions of homology exist which make it possible for the *lac* genes and the lambda phage to be lost by recombination (Fig. 3), a selection was made for Lac^+ during most of the three-factor crosses. To avoid the problems of zygotic induction, the λ *purF-lac*-1 lysogen of strain TX192 was always used as the recipient, whereas the donor was always lambda-free.

The results of the genetic crosses used to determine the relative order of the *purF*, *lac*, and *aroC* loci are presented in Table 5. From the results of these crosses, it is apparent that the *lac* genes lie outside the *purF201 aroC4* interval in strain TX252. This can be inferred not only from the linkage data but also the gene order data (Table 5). Since the *purF201-aroC4* linkage is equal in strains TX192 and TX252, the conclusion may be drawn that *lac* is outside of the *aroC-purF* interval, giving the apparent gene order of *aroC4-purF201-lac*. The data from the three-factor crosses shown in Table 5 are also consistent with a gene order of *aroC-purF-lac*. Thus, from this apparent gene order, the direction of transcription can be inferred to be clockwise. This result is in agreement with the clockwise direction of transcription inferred from the recombination experiments with λ *purF-lac*-1.

DISCUSSION

This study was undertaken to begin the elucidation of the mechanisms of control and regulation of the purine biosynthetic pathway. To-

TABLE 5. *Linkage analysis of the (purF-lac) fusion with respect to purF and aroC in strain TX252*[a]

Donor	Recipient	Selected markers	Scored markers	% Cotransduction
TX40 *aroC*$^+$ *purF*$^+$	TX192 *purF201 aroC4*	*purF*$^+$	*aroC*$^+$	48 (24/50)
		aroC$^+$	*purF*$^+$	54 (27/50)
TX40 *aroC*$^+$ *purF*$^+$	TX252 *purF201 aroC4 purR* λ *purF-lac*-1 lysogen	*purF*$^+$	*aroC*$^+$	42 (39/100)
		aroC$^+$	*purF*$^+$	57 (113/200)
		purF$^+$ *lac*$^+$	*aroC*$^+$	39 (39/100)
		aroC$^+$ *lac*$^+$	*purF*$^+$	38 (38/100)

[a] Suggested gene order: *aroC4-purF201-lac*.

wards this end and for the eventual isolation of regulatory mutants, strains were isolated in which the *lac* genes are fused to the *purF* control region. The results presented here demonstrate that the *purF-lac* fusion responds to purine control as evidenced by the fact that the *lac* genes respond to purine control signals (Table 3).

Since the deletion event that fused the *lac* genes to the *purF* control region left the lambda phage intact (Fig. 2), it was possible to isolate a specialized lambda transducing phage that carried the *purF-lac* fusion. This phage has been used to genetically determine the direction of transcription by two independent means. The recombination analysis with the λ *purF-lac*-1 phage and the *purF* point mutations is analogous to deletion mapping since only the DNA from one side of the *lac* genes can be present on the phage (Fig. 2). This same principle has been used by Saint-Girons to map various mutations in the *thr* operon, including operator constitutive mutations (21). Whether the *purF* prototrophs that are seen are the result of complementation or recombination has not yet been resolved. The analysis of *purF* mutations for complementation groups has been undertaken in an effort to address this point. However, analysis of a significant number of *purF* mutants of *Salmonella* by abortive transduction did not reveal more than one complementation group (J. S. Gots, unpublished data). Support for the concept of single *purF* locus is also provided by the fact the *purF* enzyme has been purified to homogeneity and shown to consist of identical subunits (16).

The second genetic means of determining the direction of transcription depends on the reintegration of the λ *purF-lac*-1 back into the bacterial chromosome. This reintegration will generate a gene order that is dependent upon the direction of transcription. Hence, by determining the gene order, the direction of transcription can be inferred. This technique has been used to determine the direction of transcription of the *argA* gene (7).

Both methods independently agree that the direction of transcription of the *purF* gene is clockwise on the standard *E. coli* K-12 map. These two methods can be used to determine the direction of transcription of any gene to which the *lac* genes have been fused. A variation of this technique can be employed for any single gene operon for which a specialized transducing phage and a suitable outside marker exist. However, the results would probably have to be based on the analysis of linkage tests.

The *purF-lac* fusion strain (TX158) has also provided a useful approach in the isolation of purine regulatory mutants which are presently undergoing characterization and analysis in an effort to further the understanding of the control of the purine biosynthetic pathway and its regulatory elements.

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