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The *gua* Operon of *Escherichia coli* K-12: Evidence for Polarity from *guaB* to *guaA*

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Guanine auxotrophs of *Escherichia coli* K-12 were isolated after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, ethyl methane sulfonate, or the acridine mustard ICR 372. *guaA* (xanthosine 5'-monophosphate [XMP] aminase-less) mutants were distinguished from *guaB* (inosine 5'-monophosphate [IMP] dehydrogenase-less) mutants by their growth response to xanthine and by enzyme assay. Mutations were classified as base substitutions or frameshift on the basis of mutagen-induced reversion patterns. All *guaA* strains, including three frameshift mutants, produced derepressed levels of IMP dehydrogenase when cultured with a growth-limiting concentration of guanine. The *guaB* strains were of two types: (i) those producing derepressed levels of XMP aminase, and (ii) those producing basal levels of XMP aminase when grown under conditions of guanine starvation. In the *guaB* strains of the second type, the expression of the adjacent *guaA* gene is reduced. It is proposed that this pleiotropic effect of some *guaB* mutations is a result of polarity. The orientation of polarity suggests the gene order "operator"-*guaB*-*guaA*. Gel diffusion studies with IMP dehydrogenase antiserum showed that strains carrying polar *guaB* mutations do not produce cross-reacting material (CRM). The remaining *guaB* mutants were either CRM^+ or CRM^-. Mapping the mutations by three-factor crosses showed that polar and nonpolar *guaB* sites are clustered in a small genetic region cotransducible with *guaA*. The relative positions of the *guaB* mutational sites established that the polar mutations lie within the structural gene for IMP dehydrogenase.

In *Escherichia coli*, inosine 5'-monophosphate (IMP) is synthesized in 10 steps from phosphoribosylpyrophosphate. IMP is a common metabolic precursor for both adenine and guanine nucleotides (Fig. 1). Guanosine 5'-monophosphate (GMP) is synthesized in two stages from IMP. The first step is the oxidation of IMP to xanthosine 5'-monophosphate (XMP) by the reduced form of nicotinamide adenine dinucleotide (NAD^+)-linked enzyme IMP dehydrogenase (IMP: NAD^+ oxidoreductase, EC 1.2.1.14); XMP is subsequently converted to GMP by XMP aminase (XMP: ammonia ligase, adenosine 5'-monophosphate [AMP], EC 6.3.4.1). Mutations leading to a loss of either IMP dehydrogenase or XMP aminase activity occur at two linked loci, *guaB* and *guaA*, respectively, on the *E. coli* chromosome (15). The two loci form an operon such that the synthesis of the two enzymes is controlled coordinately (15). Evidence from studies with polar mutants suggests that the operator locus lies adjacent to *guaA*—hence the proposed order "operator"-*guaA*-*guaB* (16). Our results, however, are not consistent with this proposal. We have isolated a number of IMP dehydrogenase mutants that exert a pleiotropic-negative effect on the expression of the adjacent *guaA* structural gene. The results presented below indicate the gene order operator-*guaB*-*guaA*.

MATERIALS AND METHODS

Bacterial strains. *E. coli* K-12 strain W3110 was used as the prototrophic parental strain for the isolation of *gua* mutants. Stock cultures were maintained on Dorset egg slopes supplemented with guanine (20 μg/ml). The *gua* mutants used in this study are listed in Table 1.

Phage. Bacteriophage P1*kc* was used in transduction experiments, and was supplied by J. R. Guest, Department Microbiology, University of Sheffield, England.

Media. Defined salts medium (minimal medium) was that of Davis and Mingioli (3), containing glucose (final concentration: 2 mg/ml) as carbon source. Minimal agar medium was prepared by solidifying defined medium with 1% Ionagar no. 2 (Oxoid).

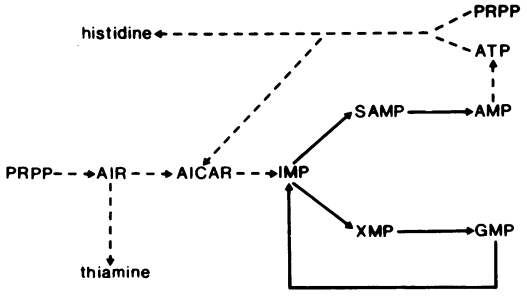

FIG. 1. *The purine nucleotide biosynthetic pathway of Escherichia coli. The intermediates of the purine pathway are abbreviated as follows: PRPP, 5-phosphoribosyl-1-pyrophosphate; AIR, 5-amino-imidazole ribonucleotide; AICAR, 5-amino-4-imidazolecarboxamide ribonucleotide; IMP, inosine 5'-monophosphate; XMP, xanthosine 5'-monophosphate; GMP, guanosine 5'-monophosphate; AMP, adenosine 5'-monophosphate; SAMP, succinyl-AMP; ATP, adenosine 5'-triphosphate.*

Purines, when required, were added to defined medium at a final concentration of 20 μg/ml. Thiamine was used at a final concentration of 5 μg/ml. L-G broth was prepared by adding guanine (20 mg/liter) to L broth (8). L-G agar was prepared by solidifying L-G broth with 1% agar (Difco). For the propagation of phage P1*kc*, $CaCl_2$ was added to L-G agar at a final concentration of 2.5 mM. The omission of guanine from L-G broth and L-G agar resulted in a 5- to 10-fold decrease in the phage titer when guanine auxotrophs were used as hosts for the propagation of P1*kc*. Nutrient broth was nutrient broth no. 2 (Oxoid; code CM67). Soft agar contained the following per liter: nutrient broth powder (Difco), 8 g; NaCl, 5 g; agar (Difco), 6 g; and $CaCl_2$, 2.5 mM final concentration.

Chemicals. Adenosine 5'-triphosphate (ATP) disodium salt, reduced glutathione, and β-NAD^+ were obtained from Boehringer Corporation (London) Ltd. XMP disodium salt was obtained from Koch-Light Laboratories Ltd. Freund complete adjuvant was obtained from Difco Laboratories. Ethyl methane sulfonate (EMS) was obtained from Eastman Kodak Co., and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) was from Ralph N. Emanuel Ltd. The frameshift mutagens ICR 191C (2-methoxy-6-chloro-9-[3-(2-chloroethyl)aminopropylamino] acridine dihydrochloride) and ICR 372 (2-methoxy-6-chloro-9-[3-(2-chloroethyl)aminopropylamino]-1-aza acridine dihydrochloride) were generous gifts from H. J. Creech, The Institute for Cancer Research, Philadelphia Pa. All other chemicals were obtained from British Drug Houses Ltd., Poole, Dorset, England.

Mutagenesis and isolation of gua mutants. In all cases only one mutant of a particular class was retained from each mutagen-treated culture.

NG-induced mutations. Log-phase cultures in broth were treated with NG (final concentration: 50 μg/ml) for 15 min at 37 C. The mutagen-treated cultures were then washed in sterile saline (0.85% NaCl) and diluted 50-fold into minimal medium containing guanine (20 μg/ml). Growth in guanine medium allowed the phenotypic expression of guanine auxotrophs. After overnight incubation at 37 C, the cultures were washed twice in saline. The cultures were then diluted 50-fold into minimal medium containing sucrose (20% wt/vol), adenine (final concentration: 20 μg/ml), and thiamine. This medium allows growth of prototrophs and *pur* (biosynthesis of IMP and AMP) mutants, but not *gua* mutants. After

TABLE 1. *gua Mutants of E. coli K-12 W3110 used*

Strain no.	Mutagen[a]	Genotype
(i) *guaB* (IMP dehydrogenase-less) mutants		
PL1047	NG	*guaB29*
PL1051	NG	*guaB32*
PL1056	NG	*guaB37*
PL1063	NG	*guaB43*
PL1066	NG	*guaB46*
PL1072	NG	*guaB52*
PL1081	NG	*guaB61*
PL1087	NG	*guaB67*
PL1090	NG	*guaB70*
PL1096	NG	*guaB76*
PL1105	NG	*guaB85*
PL1106	NG	*guaB86*
PL1117	ICR	*guaB97*
PL1123	ICR	*guaB103*
PL1138	EMS	*guaB105*
(ii) *guaA* (XMP aminase-less) mutants		
PL1042	NG	*guaA24 pur-38*
PL1045	NG	*guaA27*
PL1053	NG	*guaA34*
PL1068	NG	*guaA48*
PL1073	NG	*guaA53*
PL1082	NG	*guaA62*
PL1089	NG	*guaA69*
PL1092	NG	*guaA72*
PL1099	NG	*guaA79*
PL1109	ICR	*guaA89*
PL1111	ICR	*guaA91*
PL1113	ICR	*guaA93*
PL1118	ICR	*guaA98*
(iii) *guaBguaA* double mutants		
PL1141 PL1063[b]	NG	*guaB43 guaA108*
PL1145 PL1087[b]	NG	*guaB67 guaA112*
PL1147 PL1106[b]	NG	*guaB86 guaA114*
PL1150 PL1123[b]	NG	*guaB103 guaA117*
PL1153 PL1138[b]	NG	*guaB105 guaA120*
PL1155 PL1072[b]	NG	*guaB52 guaA122*
PL1157 PL1096[b]	NG	*guaB76 guaA124*
PL1158 PL1105[b]	NG	*guaB85 guaA125*
PL1160 PL1117[b]	NG	*guaB97 guaA127*

[a] Abbreviations: NG, mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; ICR, mutagenesis with ICR 372; EMS, mutagenesis with ethyl methane sulfonate.

[b] Source.

incubation at 37 C for 3 h to allow depletion of guanine pools, penicillin was added to a final concentration of 2,000 U/ml. The cells were treated with penicillin for 90 min, centrifuged, and resuspended in aqueous penicillinase (final concentration: 100 U/ml). After treatment with penicillinase for 10 min at room temperature, viable counts were made on minimal agar containing guanine.

ICR 372-induced mutations. The method for obtaining ICR 372-induced mutants was based on that of Oeschger and Hartman (17). Cultures (10^8 cells/ml) were diluted 100-fold into minimal medium containing guanine (final concentration: 20 μg/ml) and ICR 372 (10 μg/ml). Cultures were incubated at 37 C until the cell density had reached about 10^9 cells/ml. The cells were then washed twice in sterile saline and diluted 50-fold into fresh minimal medium containing sucrose (20% wt/vol), adenine, and thiamine. Penicillin screening was for 24 h at 37 C.

EMS-induced mutations. The method for obtaining EMS-induced mutants was based on that of Loveless and Howarth (10). Log-phase cultures in nutrient broth were washed and suspended in 0.1 M sodium phosphate buffer, pH 7.0. EMS was added to a final concentration of 5% (vol/vol), and the cell suspensions were incubated for 30 min at room temperature. The cells were then washed in fresh buffer and diluted 50-fold into minimal medium containing guanine. After incubation at 37 C overnight, the cultures were washed and diluted 50-fold into minimal medium containing sucrose (20% wt/vol), adenine, and thiamine. Penicillin screening was for 16 h at 37 C.

Guanine-requiring mutants were recovered from mutagen-treated cultures by replica plating (7). Suitable dilutions of mutagen-treated and penicillin-enriched cultures were spread on plates of minimal agar supplemented with guanine. Colonies growing on the guanine supplemented medium were replicated onto minimal agar. Putative guanine auxotrophs were identified, picked from the master plate, and purified by two cycles of single-colony isolation on guanine medium. *guaB* mutants (IMP dehydrogenase-less) were distinguished from *guaA* mutants (XMP aminase-less) by their growth response to xanthine. *guaB* mutants grow on xanthine medium whereas *guaA* mutants do not. Enzyme deficiencies were confirmed by enzyme assay.

A number of *guaB guaA* double mutants were isolated from *guaB* mutants by NG mutagenesis. Cultures of NG-treated *guaB* mutants were enriched for *guaB guaA* double mutants by screening in medium containing penicillin and xanthine. The desired double mutants were detected by replica plating from guanine medium to xanthine medium. Mutants were purified by two cycles of single-colony isolation. *guaA guaB* double mutants were assayed for IMP dehydrogenase and XMP aminase, and in all cases the double mutants were shown to lack both these enzymes.

Mutagen-induced revertants. Overnight broth cultures of mutants were used directly to make lawns on minimal agar containing a trace amount of guanine (1 μg/ml). Mutagenic chemicals were added to the plates as follows. For NG, a single crystal of NG was placed at the center of the plate; ICR 191C and ICR 372 (50 μliters of a 1 mg/ml solution) were added to a sterile, filter paper disk (13 mm diameter) placed at the center of the plate. For EMS, 50 μliters of EMS was added to a sterile filter paper disk placed at the center of the plate. Plates were incubated at 37 C for 2 to 3 days. The presence of colonies growing around the zone of inhibition caused by the mutagen was taken as a positive reversion response to that mutagen.

Growth of cultures and preparation of cell extracts. Guanine-requiring mutants were grown overnight in 10 ml of minimal medium supplemented with guanine (final concentration: 20 μg/ml). Overnight cultures were diluted 10-fold into minimal medium supplemented with guanine (final concentration: 10 μg/ml) then grown with aeration at 37 C for 6 h by which time the cultures reached the mid-log phase of growth. Finally, cultures were diluted 10-fold into fresh minimal medium. Flasks were incubated in a warm air shaking incubator (L. H. Engineering Co. Mk V incubator) at 37 C for 16 h. The final cultures were supplemented with limiting amounts of guanine (final concentration: 4 μg/ml) to allow derepression of IMP dehydrogenase and XMP aminase synthesis. Fully repressed cultures were obtained when the guanine supplement exceeded 40 μg/ml. Wild-type bacteria were cultured under similar conditions except that guanine was omitted from the growth medium.

Cultures were harvested at 4 C by centrifugation at 10,000 × *g* for 10 min. The cells were washed twice in cold 0.02 M sodium phosphate buffer, pH 7.4, and were concentrated 100-fold during harvesting. The concentrated washed cells were sonically disrupted in a 100-W ultrasonic disintegrator (Measuring & Scientific Equipment, Ltd.) operating at 20 kHz. Cells were treated for 3 min and were cooled in an ice bath throughout. Cell debris was removed by centrifugation at 30,000 × *g* for 30 min at 4 C, and the cell extract was then dialyzed overnight at 4 C against 100 volumes of 0.02 M sodium phosphate buffer, pH 7.4.

The protein content of cell extracts was determined by the method of Lowry et al. (11). Crystalline bovine serum albumin (fraction V) was used as the protein standard.

Assay for IMP dehydrogenase. The assay for IMP dehydrogenase (EC 1.2.1.14) was based on the method described by Magasanik et al. (12). The reaction mixture contained the following in a total volume of 1.5 ml: tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.55), 100 μmol; KCl, 100 μmol; glutathione (reduced), 5 μmol; IMP, 1.5 μmol; NAD^+, 1.25 μmol, and cell extract (100–200 μg of protein). The reaction was carried out at 37 C and was stopped at various times up to 15 min by placing the tubes in boiling water for 3 min. The protein precipitate produced after boiling was removed by centrifugation. IMP was added to the control tube after boiling. The increase in optical density at 290 nm, due to the production of XMP, was measured in a Unicam SP500 spectrophotometer. The amount of XMP produced was calculated from a molar extinction coefficient for XMP of 4.6×10^3 at 290 nm, pH 7.6. Specific activity is expressed as micromoles of XMP produced per minute per milligram of protein.

Assay for XMP aminase. The assay for XMP aminase (EC 6.3.4.1) was based on the method described by Moyed and Magasanik (13) as modified by Udaka and Moyed (20). The assay mixture contained the following in a total volume of 1.0 ml: Tris-hydrochloride (pH 8.5), 100 μmol; $MgCl_2$, 15 μmol; $(NH_4)_2SO_4$, 80 μmol; ATP (pH 7.0), 4 μmol; XMP, 2 μmol; and cell extract (100–200 μg of protein). Tubes were incubated at 37 C, and the reaction was stopped at various times up to 15 min by the addition of 0.5 ml of 9% perchloric acid. XMP was added to the control tube after addition of perchloric acid. The protein precipitates were then removed by centrifugation. The increase in optical density at 290 nm due to the production of GMP was measured in a Unicam SP500 spectrophotometer. The amount of GMP produced was calculated from a molar extinction coefficient for GMP of 6×10^3 at 290 nm under the above conditions. The extinction of other purine nucleotides at 290 nm after acidification with perchloric acid is negligible. The specific activity is expressed as micromoles of GMP produced per minute per milligram of protein.

Preparation of antiserum to IMP dehydrogenase and detection of cross-reacting material (CRM). IMP dehydrogenase was partially purified from cell extracts of strain PL1068 by the method of Powell et al. (19). The mutant was grown under conditions of guanine starvation, resulting in a 40-fold increase in the level of IMP dehydrogenase. The fraction used for preparation of antiserum corresponded to fraction 5 in the method of Powell et al. (19).

Antibodies to *E. coli* IMP dehydrogenase were raised in 3-kg male rabbits. A 1-ml amount of enzyme fraction 5 (6 mg/ml of protein) was emulsified with 1 ml of Freund complete adjuvant and injected subcutaneously in 0.5-ml amounts in the neck and shoulder area. Rabbits received six doses at weekly intervals, the last two being booster doses of enzyme fraction without adjuvant. The rabbits were bled from the heart 10 days after the last injection.

The serum obtained from each rabbit was fractionated with ammonium sulfate (50% saturation), and the precipitated antibody fraction was collected by centrifugation. The antibody fraction was concentrated in 0.05 M potassium phosphate buffer, pH 7.4, containing 0.14 M NaCl, and dialyzed twice against 1-liter volumes of the same phosphate-buffered saline at 4 C.

A monospecific antibody preparation was obtained by treating the antibody fraction with crude cell-free extracts of a CRM^- *guaB* mutant (PL1063); sodium azide (0.2%) was added as a preservative. The antibody-PL1063 extract mixture was stored at 4 C overnight to allow complete precipitation. This procedure was repeated until there was no further precipitation and until the antibody preparation was judged monospecific by gel diffusion tests. The CRM character of PL1063 was previously determined as described by Lerner and Yanofsky (9).

Gel diffusion tests (18) were performed on microscope slides (25 by 76 mm). The gel contained 2% Ionagar (Oxoid) in Tris-buffered saline (5 mM Tris-hydrochloride, pH 7.3; 0.8% NaCl) with sodium azide (0.2%) added as a preservative. The melted gel was pipetted onto the microscope slides to a depth of 2 to 3 mm. Wells (2-mm diameter) were cut in the gel using a template, and the agar plugs were removed by suction. Six outer wells were arranged in a circle (10-mm diameter) about a single center well. The antibody preparation was added to the center well, and extracts of *guaB* mutants (grown under conditions of guanine starvation) were added to the outer wells. Gels were incubated at room temperature in a moist atmosphere. Precipitin lines were visible after 3 h, and the results were recorded after 6 h.

Preparation of P1kc lysates. A 0.8-ml amount of a late-log-phase culture of the transductional donor strain was mixed with 20 ml of soft agar held at 45 C. A 1.0-ml amount of phage P1*kc* (10^7 plaque-forming units [PFU]/ml) was then added to the soft agar, and the mixture was poured as overlays onto eight L-G agar plates. The plates were incubated at 37 C for 6 h, in which time confluent lysis was observed. The soft agar layers containing the bacteria and phage were homogenized on the plates in 3 ml of L-G broth per plate. The homogenized soft agar was then decanted and stored at 4 C overnight to allow elution of the phage. The soft agar was removed from the broth by low-speed centrifugation, and the supernatant fluid was shaken with chloroform. The chloroform layer was sedimented by centrifugation, and the supernatant fluid was decanted into a sterile bottle. The lysate was stored over a few drops of chloroform at 4 C.

Phage titers were determined in soft agar overlays using *Shigella dysenteriae* Sh as the assay organism. Titers of 10^{10} to 5×10^{10} PFU/ml were routinely obtained.

Transduction with bacteriophage P1kc. The method for transduction with phage P1*kc* was based on that of Lennox (8) as modified by J. R. Guest (personal communication).

The P1*kc* lysate was diluted to 10^{10} PFU/ml in L-G broth and aerated for 20 min at 37 C to remove chloroform. A 0.1-ml amount of the lysate was spread on a selection plate as a sterility check. An inoculum from an overnight broth culture of the transductional recipient strain was grown to 10^9 cells per ml in fresh L-G broth. The recipient bacteria were then centrifuged and concentrated 10-fold in L-G broth. A 0.5-ml amount of the recipient culture (5×10^9 bacteria total) was incubated for 30 min at room temperature with 0.1 ml of 50 mM $CaCl_2$. A 1.0-ml amount of lysate (10^{10} PFU/ml) was added to the recipient strain (multiplicity of infection = 2), and the mixture was incubated at 37 C for 30 min. L-G broth, instead of phage, was added to a second 0.5-ml sample of the recipient strain to act as a control. The cells were centrifuged and suspended in the original volume of saline (0.85% NaCl) containing 2.5 mM $CaCl_2$. One-tenth-milliliter samples of serial decimal dilutions were plated onto selection media.

RESULTS

Characterization of mutants. A preliminary classification of *gua* mutants was made by testing their growth response on media supplemented with various purines. Both *guaA* and

guaB mutants grow when supplemented with guanine; *guaB* mutants grow on xanthine medium, whereas *guaA* mutants do not. The final classification was made after checking the enzyme deficiency. In all cases *guaB* mutants lacked IMP dehydrogenase activity and *guaA* mutants lacked XMP aminase activity. The *gua* mutants used in this study are listed in Table 1.

guaB mutants were further classified according to their mutagen-induced reversion pattern (Table 2). All *guaB* mutants, except strains PL1072 (*guaB52*) and PL1138 (*guaB105*), were shown to revert spontaneously. Mutant PL1117 (*guaB97*) showed a relatively high spontaneous reversion rate which was not increased by any of the mutagens tested. One mutation, *guaB103*, was reverted strongly by the ICR mutagens and, to a lesser extent, by NG and EMS. Mutation *guaB61* demonstrated a weak response to the ICR mutagens. Mutants PL1117 (*guaB97*) and PL1123 (*guaB103*) were isolated after ICR mutagenesis and are tentatively classified as frameshift; the other *guaB* mutants have reversion patterns characteristic of a base substitution. Deletion or multisite mutants, which are stable to spontaneous and mutagen induced reversion, were not isolated.

Three of the *guaA* mutations (*guaA89*, *guaA91*, *guaA93*) were classified as frameshift on the basis of their mutagen-induced reversion pattern (Table 2).

Enzyme levels in guanine auxotrophs. *gua* mutants are defective in the biosynthesis of GMP from IMP and are consequently unable to convert adenine nucleotides to guanine nucleotides (Fig. 1). These mutants require guanine for growth but are able to synthesize adenine nucleotides de novo. These mutants were used to study the regulation of IMP dehydrogenase and XMP aminase synthesis by varying the amount of guanine available to the cell.

The derepressed levels of IMP dehydrogenase were determined in cell extracts of *guaA* mutants cultured under conditions of guanine starvation; similarly, the XMP aminase levels were determined in guanine-starved *guaB* mutants. All *guaA* mutants grown with growth-limiting guanine had derepressed levels of IMP dehydrogenase (Table 3). XMP aminase activity in *guaA* mutants was absent, or very much reduced, which is consistent with the observed growth on guanine only.

guaB mutants, however, could be divided into two classes with respect to the level of XMP aminase produced under conditions of guanine starvation (Table 4). One class of *guaB* mutant produced derepressed levels of XMP aminase when cultured with growth-limiting guanine. This situation is analogous to the production of IMP dehydrogenase by *guaA* mutants grown under similar conditions. The other class of *guaB* mutant produced low levels of XMP aminase when cultured in limiting

TABLE 2. *Reversion patterns of gua mutations*[a]

Strain no.	Mutagen	Spontaneous reversion	Response to				Inferred class of mutation[b]
			NG	EMS	ICR191C	ICR372	
PL1047 *guaB29*	NG	+	++	+++	–	–	S
PL1051 *guaB32*	NG	+	+–	+–	–	–	S
PL1063 *guaB43*	NG	++	+	+++	–	–	S
PL1066 *guaB46*	NG	+–	+	–	–	–	S
PL1072 *guaB52*	NG	–	++	–	–	–	S
PL1081 *guaB61*	NG	++	+	++	+–	+–	S
PL1087 *guaB67*	NG	+	+	++	–	–	S
PL1090 *guaB70*	NG	+++	+	+	–	–	S
PL1096 *guaB76*	NG	+	+	++	–	–	S
PL1105 *guaB85*	NG	++	+	+++	–	–	S
PL1106 *guaB86*	NG	+++	+	++	–	–	S
PL1117 *guaB97*	ICR372	+++	–	–	–	–	F
PL1123 *guaB103*	ICR372	+++	+–	+	++	++	F
PL1138 *guaB105*	EMS	–	+	–	–	–	S
PL1109 *guaA89*	ICR372	+–	+–	–	+++	+++	F
PL1111 *guaA91*	ICR372	+–	–	–	++	+++	F
PL1113 *guaA93*	ICR372	+–	–	–	+++	+++	F

[a] Mutagen-induced reversion is recorded as an increase over and above the number of spontaneous revertants for each strain. Symbols: –, no reversion; +–, less than 10 colonies after prolonged incubation at 37 C; +, less than 10 colonies; ++, 10 to 50 colonies; +++, 50 to 100 colonies.

[b] S, Base substitution; F, frameshift.

TABLE 3. *IMP dehydrogenase and XMP aminase levels in guanine-starved guaA mutants*

Strain	Relative specific activities[a] of:	
	IMP dehydrogenase	XMP aminase
W3110[b]	1.0	1.0
PL1042 (*guaA24 pur-38*)[c]	45.0	0
PL1045 (*guaA27*)	40.0	0
PL1053 (*guaA34*)	29.0	0
PL1068 (*guaA48*)	44.5	0
PL1073 (*guaA53*)	45.0	0
PL1082 (*guaA62*)	42.0	0
PL1089 (*guaA69*)	38.0	0
PL1092 (*guaA72*)	44.0	0
PL1099 (*guaA79*)	39.0	0
PL1109 (*guaA89*)	19.0	0.1
PL1111 (*guaA91*)	30.0	0
PL1113 (*guaA93*)	30.0	0
PL1118 (*guaA98*)	39.5	0

[a] Relative to specific activities for strain W3110. IMP dehydrogenase = 1.2×10^{-2} μmol of XMP per min per mg of protein. XMP aminase = 1.5×10^{-2} μmol of GMP per min per mg of protein.

[b] Strain W3110 cultured in unsupplemented minimal medium.

[c] *pur-38* is probably a *purH* mutation because 5-amino-4-imidazole-carboxamide derivatives accumulate in the culture supernatant fluid.

TABLE 4. *IMP dehydrogenase and XMP aminase levels in guanine-starved guaB mutants*

Strain	Relative specific activities[a] of:	
	IMP dehydrogenase	XMP aminase
W3110[b]	1.0	1.0
PL1051 (*guaB32*)	0	19.7
PL1066 (*guaB46*)	0	14.0
PL1072 (*guaB52*)	0	9.0
PL1087 (*guaB67*)	0	17.3
PL1090 (*guaB70*)	0	13.0
PL1096 (*guaB76*)	0	11.5
PL1105 (*guaB85*)	0	18.0
PL1106 (*guaB86*)	0	18.7
PL1138 (*guaB105*)	0	24.0
PL1047 (*guaB29*)	0	1.1
PL1056 (*guaB37*)	0	0.54
PL1063 (*guaB43*)	0	2.4
PL1081 (*guaB61*)	0	3.0
PL1117 (*guaB97*)	0	0.85
PL1123 (*guaB103*)	0	2.37

[a] Relative to specific activities for strain W3110. IMP dehydrogenase = 1.2×10^{-2} μmol of XMP per min per mg of protein. XMP aminase = 1.5×10^{-2} μmol of GMP per min per mg of protein.

[b] Strain W3110 cultured in unsupplemented minimal medium.

guanine medium. The simplest explanation for this pleiotropic effect of some *guaB* mutations is that of polarity. These *guaB* mutations (strains PL1047, PL1056, PL1063, PL1081, PL1117, and PL1123) are probably polar and lead to a low-level expression of the adjacent *guaA* gene. Two of these polar *guaB* mutations (*B97* and *B103*) have been classified as frameshift, whereas the others are base substitution types (Table 2).

The levels of IMP dehydrogenase and XMP aminase could be repressed in *gua* mutants by raising the concentration of guanine in the culture medium. Representative examples are shown in Table 5. Enzyme levels were significantly repressed by guanine at a final concentration of 20 μg/ml. However, 30 to 40 μg of guanine per ml was required for repression to the basal level. IMP dehydrogenase activity was not detected in strain PL1068 cultured with 100 μg of guanine per ml.

Detection of CRM. The CRM character of *guaB* mutants was tested by gel diffusion on microscope slides. Results from a typical gel diffusion experiment are shown in Fig. 2. The CRM character of *guaB* mutants is recorded in Table 6. Polar *guaB* mutants are all CRM^-, whereas the nonpolar mutants are either CRM^+ or CRM^-. Out of a total of 14 *guaB* mutants examined, only 4 produce CRM. Extracts of mutants PL1072 (*guaB52*), PL1105 (*guaB85*), and PL1138 (*guaB105*) contained CRM antigenically identical to the wild-type IMP dehydrogenase. A spur was formed between the precipitin lines produced by PL1072 (*guaB52*) CRM and PL1096 (*guaB76*) CRM (Fig. 2). The spur is directed towards the well containing cell extract of strain PL1096, suggesting that the CRM produced by this mutant lacks some of the antigenic determinants present on the CRM produced by strain PL1072. Experiments with a crude antibody preparation demonstrated that the precipitated enzyme-antibody complex retained about 50% of the original enzyme activity.

Mapping guaB mutational sites by three-factor crosses. Three-factor crosses were performed, by transduction with bacteriophage P1*kc*, to obtain an accurate order of the *guaB* mutational sites. *guaA guaB* double mutants were isolated from each *guaB* mutant so that pairs of *guaB* mutational sites could be ordered with respect to a third, *guaA*, reference mutation. The procedure for the isolation of double mutants was designed to select strains unable to convert xanthine to GMP. To eliminate the possibility that the mutation produced a block in the conversion of xanthine to XMP, each

TABLE 5. *Repression of IMP dehydrogenase and XMP aminase in guaA and guaB mutants*

Strain	Relative enzyme activities[a] of:							
	IMP dehydrogenase with guanine in culture medium (μg/ml)			XMP aminase with guanine in culture medium (μg/ml)				
	4	20	100	4	20	30	40	100
PL1068 (*guaA48*)	44.5	17.0	0					
PL1073 (*guaA53*)	45.0	17.6						
PL1082 (*guaA62*)	42.0	17.7						
PL1089 (*guaA69*)	38.0	18.7						
PL1092 (*guaA72*)	44.0	18.4						
PL1099 (*guaA79*)	39.0	18.4						
PL1051 (*guaB32*)				19.7	6.2	0.90		0.72
PL1066 (*guaB46*)				14.0	4.8			
PL1072 (*guaB52*)				9.0	5.3			
PL1087 (*guaB67*)				17.3	5.3			
PL1090 (*guaB70*)				13.0	1.8			
PL1096 (*guaB76*)				11.5	5.3			
PL1105 (*guaB85*)				18.0	6.6			
PL1106 (*guaB86*)				18.7	8.2	3.8	0.72	0.48

[a] Relative to a value of 1.0 obtained for strain W3110 grown in minimal medium. IMP dehydrogenase = 1.2×10^{-2} μmol of XMP per min per mg of protein. XMP aminase = 1.5×10^{-2} μmol of GMP per min per mg of protein.

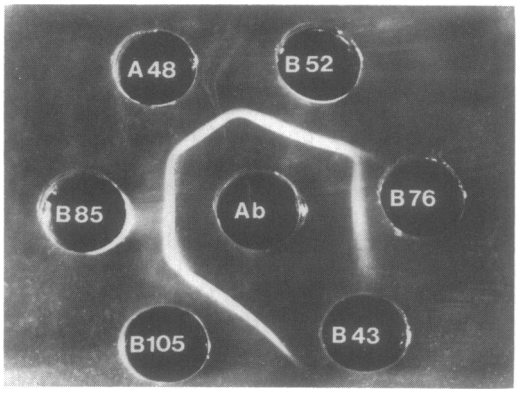

FIG. 2. *Agar gel diffusion of cell extracts prepared from various guaB strains. The center well (Ab) contained IMP dehydrogenase antiserum. The outer wells contained cell extracts from the following strains: A48, PL1068 (wild-type IMP dehydrogenase); B43, PL1063 (typical CRM mutant); B52, PL1072; B76, PL1096; B85, PL1105; B105, PL1138.*

double mutant was assayed for IMP dehydrogenase and XMP aminase. In each case both enzymes were found to be absent.

The usefulness of *guaA guaB* double mutants as recipients may be summarized as follows. (i) *guaB* mutant sites can be ordered with respect to a *guaA* marker (Fig. 3). (ii) Each cross is standardized by relating the number of wild-type recombinants to the total number of $guaA^+$ recombinants arising from the same cross. (iii) Reversion of the double mutants on minimal agar is negligible because of the double block in GMP biosynthesis, thus allowing the detection of low recombination frequencies.

TABLE 6. *Production of cross-reacting material (CRM) by guaB mutants as determined by gel-diffusion*

Mutant	Type	CRM production
PL1047 (*guaB29*)	Polar	CRM^-
PL1051 (*guaB32*)	Nonpolar	CRM^-
PL1063 (*guaB43*)	Polar	CRM^-
PL1066 (*guaB46*)	Nonpolar	CRM^-
PL1072 (*guaB52*)	Nonpolar	CRM^+
PL1081 (*guaB61*)	Polar	CRM^-
PL1087 (*guaB67*)	Nonpolar	CRM^-
PL1090 (*guaB70*)	Nonpolar	CRM^-
PL1096 (*guaB76*)	Nonpolar	CRM^+
PL1105 (*guaB85*)	Nonpolar	CRM^+
PL1106 (*guaB86*)	Nonpolar	CRM^-
PL1117 (*guaB97*)	Polar	CRM^-
PL1123 (*guaB103*)	Polar	CRM^-
PL1138 (*guaB105*)	Nonpolar	CRM^+

Each *guaB* mutant was crossed reciprocally with a second *guaB* mutant; the recipient *guaB* strain always carried a *guaA* mutation. The principle of the cross is outlined in Fig. 3. Wild-type recombinants were selected on minimal agar medium, and $guaA^+$ recombinants were selected on minimal agar supplemented with xanthine. The number of wild-type recombinants was expressed as a percentage of the total number of $guaA^+$ recombinants ($guaA^+$ $guaB^+$ and $guaA^+$ $guaB^-$). The results from a series of three-factor crosses are shown in Table 7. The values (percent $guaB^+$ $guaA^+/guaA^+$) obtained for a particular pair of reciprocal

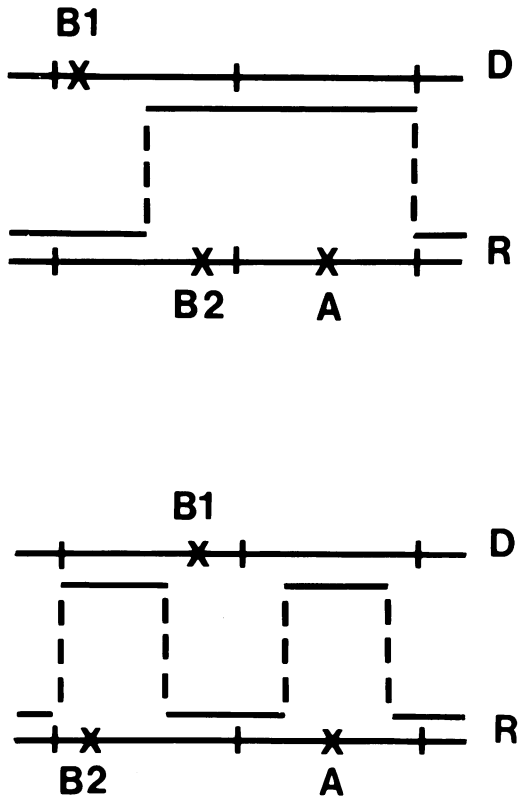

FIG. 3. *Principle of the three-factor cross. The higher ratio (B^+A^+/A^+) is given by the cross in which the recipient marker is between the donor marker and guaA. D and R refer to donor and recipient, respectively.*

crosses allowed the ordering of the two *guaB* mutations with respect to the *guaA* gene. The higher value was taken as the relative distance between the two *guaB* mutations. This value accounts for all wild-type recombinants arising from a minimum of two cross-over events, whereas wild-type recombinants resulting from four or more cross-over events are accounted for in the lower value. The former value is related, therefore, to the distance between the two *guaB* loci and does not depend on cross-over events between *guaB* and *guaA*. A genetic map of the IMP dehydrogenase structural gene was constructed using these values (Fig. 4). The values obtained allow only one order for the *guaB* mutant sites.

DISCUSSION

The results presented support the finding of Nijkamp and De Haan (15) that certain mutants of *E. coli* blocked in the conversion of IMP to GMP produce derepressed levels of either IMP dehydrogenase or XMP aminase when growth is limited by guanine starvation. Repression of the two enzymes by the addition of increasing concentrations of guanine to the culture medium suggests that guanine (or a derivative) is acting as a corepressor.

The double mutant PL1042 (*guaA24 pur-38*) blocked in the biosynthesis of IMP and in the conversion of IMP to GMP, also produced a derepressed level of IMP dehydrogenase when grown with limiting guanine. *pur-38*, a revertant of this double mutant with a nonspecific requirement for purines likewise was derepressed for both IMP dehydrogenase and XMP aminase under conditions of either guanine or adenine depletion (P. R. Lambden Ph.D. thesis, Southampton University, England, 1973). These results are contrary to the behavior of similar mutants described by Nijkamp (14).

Several *guaB* (IMP dehydrogenase-less) mutations were pleiotropic negative and resulted in low levels of XMP aminase under growth conditions which normally led to enzyme derepression. The simplest explanation for this behavior is that of polarity in an operon operator-*guaB-guaA*. The absence of CRM to IMP dehydrogenase antiserum in extracts of five strains with pleiotropic *guaB* mutations supports this conclusion. By contrast, about half of the strains carrying nonpolar mutations were CRM^+. Mutation *guaB52* shows an apparent weak polar effect, but unlike the other polar mutations is CRM^+. The polar mutations may therefore represent types which lead to chain termination with production of an incomplete polypeptide. Two of the polar mutations (*guaB97* and *guaB103*) were induced with ICR 372 and are classified as frameshift on the basis of their reversion patterns. Mutation *guaB97* had a relatively high spontaneous reversion rate which was not increased by any of the mutagens tested. Mutations of this type have been previously classified as frameshifts (4). A number of workers have reported that the base substituting mutagens NG and DES occasionally cause the reversion of frameshift mutations by deleting a G-C base pair (21). Mutation *guaB103* was reverted by NG and EMS and is therefore tentatively classified as a "+1" frameshift.

Nijkamp and Oskamp (16) identified a number of suppressible mutations in *guaA* that had a polar effect on *guaB*, suggesting a gene order operator-*guaA*-*guaB*. However, polarity in the *gua* operon in the direction *guaB* to *guaA* places *guaB*, the gene for the first enzyme of the unique pathway of GMP biosynthesis, adjacent to the operator. In this respect, the *gua* operon resembles the *trp* operon, and the *his* operon in

Salmonella typhimurium. *guaA89* has an apparent pleiotropic effect but probably represents a leaky mutation. Leaky *guaA* revertants (slow growth on unsupplemented minimal medium) have been isolated and have XMP aminase activities less than the wild-type level and also reduced derepression of IMP dehydrogenase (P. R. Lambden, unpublished observations).

An alternative explanation for the pleiotropic behavior of certain of the *guaB* mutations is that of a pseudo- or anti-polar effect (1) on an operator proximal *guaA* gene. XMP aminase may be active only when complexed with IMP dehydrogenase. Some *guaB* mutants, particularly CRM^- and frameshift types, would therefore be expected to lack a suitable species of IMP dehydrogenase for formation of this active complex. Similar reasoning has been applied to anti-polar mutations in *trpB* (1) and *purH* (4) in *S. typhimurium*. This explanation would not rule out the possibility of genuine polar *guaA* mutations. However, no such mutations were detected in this study; all *guaA* mutants were

TABLE 7. *Data from reciprocal three-factor crosses*[a]

Donor	Recipient	Total transductants		B^+A^+/A^+ (%)	Inferred order
		guaB$^+$*guaA*$^+$	*guaA*$^+$		
guaB67	*guaB67 guaA112*	0	57,200	0	
guaB76	*guaB76 guaA124*	0	23,300	0	
guaB97	*guaB97 guaA127*	0	15,700	0	
guaB52	*guaB52 guaA122*	0	16,100	0	
guaB103	*guaB103 guaA117*	0	25,000	0	
guaB85	*guaB85 guaA125*	0	195,000	0	
guaB105	*guaB105 guaA120*	0	104,000	0	
guaB86	*guaB86 guaA114*	0	28,000	0	
guaB86	*guaB52 guaA122*	113	22,650	0.50	*B52-B86-guaA*
guaB52	*guaB86 guaA114*	935	24,700	3.80	
guaB43	*guaB52 guaA122*	55	6,100	0.90	*B43-B52-guaA*
guaB52	*guaB43 guaA108*	10	15,500	0.065	
guaB97	*guaB52 guaA122*	35	9,850	0.36	*B97-B52-guaA*
guaB52	*guaB97 guaA127*	205	77,000	0.27	
guaB103	*guaB52 guaA122*	50	19,650	0.25	*B52-B103-guaA*
guaB52	*guaB103 guaA117*	752	51,300	1.46	
guaB67	*guaB52 guaA122*	1,175	25,200	4.66	*B67-B52-guaA*
guaB52	*guaB67 guaA112*	142	63,500	0.22	
guaB105	*guaB52 guaA122*	97	39,100	0.25	*B52-B105-guaA*
guaB52	*guaB105 guaA120*	1,127	58,450	1.92	
guaB85	*guaB52 guaA122*	87	13,050	0.67	*B52-B85-guaA*
guaB52	*guaB85 guaA125*	2,500	162,000	1.54	
guaB76	*guaB52 guaA122*	660	21,900	3.0	*B76-B52-guaA*
guaB52	*guaB76 guaA124*	52	16,900	0.31	
guaB86	*guaB105 guaA120*	1,247	100,600	1.24	*B105-B86-guaA*
guaB105	*guaB86 guaA114*	1,372	85,500	1.61	
guaB105	*guaB85 guaA125*	1,643	419,000	0.40	*B85-B105-guaA*
guaB85	*guaB105 guaA120*	335	74,000	0.45	
guaB85	*guaB103 guaA117*	235	34,750	0.68	*B103-B85-guaA*
guaB103	*guaB85 guaA125*	2,280	242,000	0.94	
guaB97	*guaB43 guaA108*	2	5,600	0.036	*B43-B97-guaA*
guaB43	*guaB97 guaA127*	220	26,000	0.85	
guaB43	*guaB76 guaA124*	100	11,000	0.91	*B76-B43-guaA*
guaB76	*guaB43 guaA108*	292	11,700	2.50	
guaB76	*guaB67 guaA112*	2,830	306,500	0.92	*B67-B76-guaA*
guaB67	*guaB76 guaA124*	330	13,000	2.54	
guaB67	*guaB86 guaA114*	3,810	40,500	9.4	*B67-B86-guaA*
guaB86	*guaB67 guaA112*	460	38,400	1.2	
guaB76	*guaB103 guaA117*	1,032	22,200	4.65	*B76-B103-guaA*
guaB103	*guaB76 guaA124*	5	13,400	0.037	
guaB97	*guaB105 guaA120*	475	20,800	2.30	*B97-B105-guaA*
guaB105	*guaB97 guaA127*	1,200	79,600	1.50	

[a] Where necessary, the total numbers of transductants in the cross were calculated from 10- or 100-fold plate dilutions.

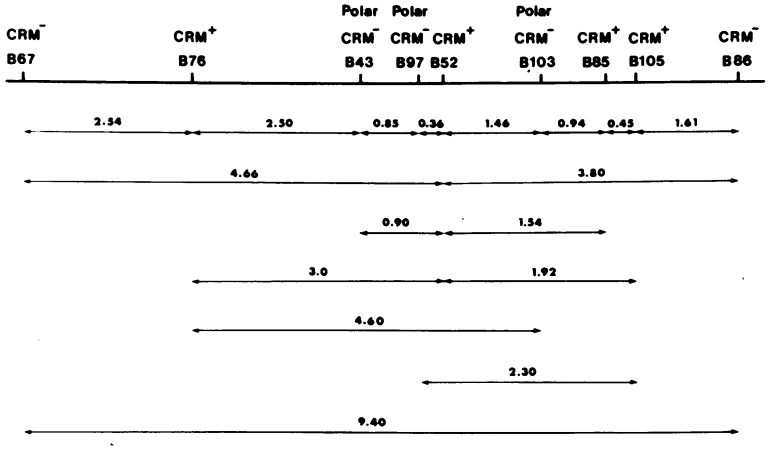

FIG. 4. *Genetic map of the IMP dehydrogenase structural gene obtained by three-factor crosses.*

nonpolar, including those classified as frameshift.

Three-factor crosses gave the relative positions of the polar and nonpolar *guaB* mutations. The order of the mutant sites establishes that the pleiotropic-negative (polar) mutations lie within the structural gene for IMP dehydrogenase. This supports the direction of polarity of the *gua* operon, operator-*guaB*-*guaA*, as deduced from enzyme assays and from immunological methods. Some of the reciprocal crosses gave rise to similar recombination frequencies; therefore the order inferred for the relevant loci cannot be considered reliable. Each *guaB* recipient carried a different *guaA* mutation because it was not possible to construct a series of *guaB* strains with the same *guaA* marker. No methods are available in practice for selecting *guaA*⁻ *guaB*⁻ double mutants from a mixture with either *guaA*⁻ *guaB*⁺ or *guaA*⁺ *guaB*⁻ cells.

The recombination frequencies measured for the *guaB* mutations are not absolute values but must necessarily be underestimates because the *guaA*⁻ *guaB*⁺ class of recombinant could not be selected. This problem could be overcome by constructing a map of *guaB* using a third marker, other than *guaA*, to standardize the cross. This method would be a two-factor cross if the third marker is not cotransducible with *gua* and a reliable order of sites could not be deduced. The frequency of recombination between *guaB67* and *guaB86*, which lie at the extremities of the map, is of the order of 10%. Comparing this value with those obtained in mapping the *trpA* gene (5) gives a molecular weight of approximately 80,000 for the protein determined by *guaB*. The estimated molecular weight of the *guaB* product is probably low because the size of the gene is defined by the distance between the two most widely spaced *guaB* sites mapped to date. It is probable that other *guaB* mutations lie outside the present genetic map.

Two nonpolar, CRM⁻, mutations have been mapped. These mutations (*guaB67* and *guaB86*) are at the extremities of the map, which raises the possibility that the N and C termini of IMP dehydrogenase are important for its antigenic structure. The mutations that lie between *guaB67* and *guaB86* are all CRM⁺, with the exception of the CRM⁻ polar mutations.

An interesting possibility is that the *guaB* mutations fall into two or more complementation groups. Recently Hannon and Levin (Bacteriol. Proc., p. 129, 1971) demonstrated complementation in vivo and in vitro between some *guaB* mutants of *S. typhimurium*.

The reason for the contradictory data on the direction of polarity in the *gua* operon is not clear but may reflect fundamental differences between the parental strain used for isolation of the *gua* mutants. The *gua* mutants isolated by Nijkamp and Oskamp (16) were derived from *E. coli* strain H865. This strain has a long lineage and was derived from a wild-type strain of *E. coli* through several cycles of X-ray and ultraviolet treatment. X rays can cause considerable chromosome damage resulting in double-strand breaks in DNA (6). *E. coli* W3110 used in this study has not been subjected to harsh mutagenic treatment and has a relatively short lineage. The differences in orientation of polarity in the *gua* operons in these two strains may be explained by a transposition of the *guaA* and *guaB* genes at some stage in the history of strain H865, perhaps as a result of mutagen treatment. The transposition of genes in bacteria is

not unknown. A number of *E. coli* strains have been isolated in which the *lac* genes have been transposed from their normal position on the chromosome (2). A simple inversion of the two *gua* genes, with respect to a fixed operator locus, could not account for the different polarities because the "sense" strand of the deoxyribonucleic acid would not be in the correct orientation.

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