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Genetic and Molecular Characterization of the *guaC-nadC-aroP* Region of *Escherichia coli* K-12

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The *guaC* (GMP reductase), *nadC* (quinolinate phosphoribosyltransferase), and *aroP* (aromatic amino acid permease) genes of *Escherichia coli* K-12 were located in the 2.5-min region of the chromosome (*mutT-guaC-nadC-aroP-aceE*) by a combination of linkage analysis, deletion mapping, restriction analysis, and plasmid subcloning. The *guaC* locus expressed a product of M_r 37,000 with a clockwise transcriptional polarity, and the GMP reductase activities of *guaC*$^+$ plasmid-containing strains were amplified 15- to 20-fold.

GMP reductase (NADPH:GMP oxidoreductase; EC 1.6.6.8) catalyzes the irreversible NADPH-dependent reduction of GMP to IMP (GMP + NADPH → IMP + NH_3^+ + $NADP^+$), and because it provides the only means of converting guanine nucleotides to adenine nucleotides, GMP reductase plays a pivotal role in the intracellular interconversion and balance of adenine and guanine nucleotides (21, 22). A simple lack of GMP reductase activity does not lead to purine auxotrophy, since the de novo biosynthesis of purine nucleotides via IMP remains intact. However, in purine auxotrophs blocked before the formation of IMP, a GMP reductase deficiency prevents the utilization of xanthine or guanine derivatives as purine sources. In *Escherichia coli*, GMP reductase is encoded by the *guaC* gene, which has been reported to lie clockwise of *azi* (19) and to be between the *mutT* and *nadC* loci (J. Gots, personal communication, cited in reference 1) at 2.5 min in the linkage map. The *guaC* gene is induced by GMP (10, 29), and its expression is also regulated by cyclic AMP (2), by the intracellular ratio of adenine and guanine nucleotides (3), and by glutamine and related analogs (9, 15). The GMP biosynthetic genes (*guaBA*) are located at 54 min in the *E. coli* linkage map and are regulated independently of *guaC* (24, 28).

The *nadC-aroP* region of the *E. coli* chromosome encoding quinolinate phosphoribosyltransferase and the aromatic amino acid permease was originally isolated as a 10.5-kilobase (kb) *Hin*dIII fragment in a pBR322 derivative (pGS15) during studies on the adjacent pyruvate dehydrogenase complex genes, *aceEF-lpd* (11–13). More recently, the *guaC* gene was subcloned from an RP4::Mu cointegrate carrying the *leu-mutT-guaC* region, and the *guaC* product was identified as a polypeptide of M_r 36,000 (26). Here, we report (i) the genetic and physical localization of the *guaC*, *nadC*, and *aroP* genes by deletion mapping and subcloning studies; (ii) evidence for the transcriptional polarity of *guaC* (clockwise); (iii) confirmation of the subunit size of GMP reductase (M_r 37,000); and (iv) a 15- to 20-fold amplification of GMP reductase activity obtained with a *guaC*$^+$ multicopy plasmid.

The bacterial strains, bacteriophages, and plasmids used are described in Table 1. The methods used for transduction and transformation and for the construction, isolation, and analysis of plasmids have been described elsewhere (12, 23, 25, 34). Strain ED8641 was the primary transformation host for plasmid construction, and strain AB2480 was used in the maxicell procedure (30). Strains were grown at 37°C in minimal medium (27) or rich medium (25) with appropriate supplements at the following final concentrations (in micrograms per milliliter): adenine, 35; hypoxanthine, 50; guanosine, 200; nicotinic acid, 5; ampicillin, 50; kanamycin, 25; tetracycline, 10. The minimal medium was supplemented with DL-5-methyltryptophan (20 μg/ml) and β-2-thienyl-DL-alanine (20 μg/ml) for testing the AroP phenotype, and the rich medium was supplemented with rifampin at 100 μg/ml for scoring the MutT phenotype (31). Cultures for enzymology studies were grown by inoculating 200 ml of medium with cells from a 5-ml preculture on the same growth medium to an optical density at 660 nm of 0.05 to 0.1 and by incubating until an optical density at 660 nm of 0.6 to 0.8 was reached. Ampicillin (50 μg/ml) was added to cultures of plasmid-containing strains. Crude extracts were prepared by sonication (33) and desalted (14) before GMP reductase (2, 22), IMP dehydrogenase (16), β-lactamase (20), and protein concentrations (6) were assayed.

Map location of *guaC*. The gene order *mutT-guaC-nadC-aroP* was confirmed by linkage analysis and deletion mapping. Measurements of P1 cotransduction frequencies showed that the *guaC* mutation of strain TX282 is 70% linked to *nadC* and 35% linked to *ara*, whereas *nadC* is only 22% linked to *ara*. This finding suggested a gene order of *ara-guaC-nadC*. Further evidence from a three-factor cross with strains TX282 (donor; carrying *guaC200*) and TX366 (recipient; carrying *nadC8* and *mutT1*), in which the majority (85%) of Gua$^+$ Nad$^+$ transductants were Mut$^-$, confirmed the gene order *mutT-guaC-nadC*.

A selection of previously isolated strains with deletions in the *nadC-aroP* region (17, 18) were examined to determine whether any of these deletions affected the *guaC* gene. Because *guaC* strains exhibit a distinct growth phenotype only when the de novo pathway of purine synthesis is blocked, a *purD*::Tn*5* mutation was transduced into each deletion strain so that a lack of GMP reductase could be detected by the failure of exogenous guanosine to replace adenine or hypoxanthine as a source of purine. As shown in Fig. 1, the nutritional phenotypes indicated that three of the deletions affecting the *nadC-aroP* region (Δ*3*, Δ*10*, and Δ*32*)

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TABLE 1. Strains of *E. coli* K-12, phages, and plasmids used in this study

Strain or plasmid	Relevant genotype or phenotype	Source and reference(s)
AB2480	*pro thi uvrA6 recA13*	P. J. Emmerson
ED8641	*hsdR supE recA56 aux*	N. E. Murray
TX40	*Δlac ara*	32
TX276	*Δlac Δ(purM-upp)203 ara*	Spontaneous mutant of TX40
TX282	*guaC200 Δlac Δ(purM-upp)203 ara*	*guaC* mutant of TX276
TX366	*nadC8 mutT1 Δlac Δ(purM-upp) 203*	P1 transduction of TX282 with CGSC5179 (*nadC8*) and CGSC3599 (*mutT1*)
TX385	*purD212*::Tn*5 Δlac ara*	Mutagenesis of TX40 with Tn*5* (Kan^r)
TX549	*Δ(guaC-aceE)3 purD212*::Tn*5 thi*	Kan^r transductant of HΔ3; 17, 18
TX582	*Δ(guaC-aceE)3 purD212*::Tn*5 thi*(pGS89)	Transformation of TX549 with pGS89
TX609	*purD212*::Tn*5 thi*	GuaC$^+$ Nic$^+$ Ace$^+$ transductant of TX549
TX692	*purD212*::Tn*5 thi*(pGS89)	Transformation of TX609 with pGS89
JRG1736	*Δ(nadC-aroP)2 purD212*::Tn*5 thi*	Kan^r transductant of H Δ2; 17, 18
JRG1737	*Δ(guaC-aceF)10 purD212*::Tn*5 thi*	Kan^r transductant of H Δ10; 17, 18
JRG1738	*Δ(nadC-acE)17 purD212*::Tn*5 metB thy*	Kan^r transductant of K Δ17; 17, 18
JRG1739	*Δ(guaC-aceE)32 purD212*::Tn*5 metB thy*	Kan^r transductant of K Δ32; 17, 18
JRG1740	*Δ(nadC-aceE)35 purD212*::Tn*5 metB thy*	Kan^r transductant of C Δ35; 17, 18
JRG1741	*Δ(nadC-aceE)39 purD212*::Tn*5 metB thy*	Kan^r transductant of C Δ39; 17, 18
G76N	*λ srlλ1-2 shnλ3::nadC Δ(att-red) imm^{21} nin5*	13
G78N	*λ srlλ1-2 shnλ3::nadC Δ(att-red) cI857 nin5*	13
pBR322	Amp^r Tet^r	5
pGS15	Amp^r NadC$^+$ AroP$^+$ GuaC$^+$	12

inactivate the *guaC* gene, and this was confirmed by direct assay of GMP reductase. The original MutT, NadC, AroP, and Ace phenotypes were retained, and because the *nadC* genes of many *aroP-ace* deletion strains are unaffected (17, 18), the results fully support the gene order *mutT-guaC-nadC-aroP*.

Cloning and complementation analysis in the *guaC-nadC-aroP* region. Plasmid pGS15 was originally derived by subcloning the 10.5-kb *Hin*dIII insert from a λ *nadC* transducing phage (λG76N) into pBR322 (12). It was found to restore the GuaC$^+$, NadC$^+$, and AroP$^+$ phenotypes to Amp^r transformants of the deletion strain TX549 [*Δ(guaC-aceE)*]. A series of plasmids containing different subsegments of the bacterial fragment (H_1-H_2) that was constructed to locate the *guaC*, *nadC*, and *aroP* genes and to identify their products is shown in Fig. 2. Several deletion derivatives of pGS15 were generated by treatment with *Bgl*II (pGS90), *Bam*HI (pGS85), or *Eco*RI (pGS84) and religation (Fig. 2). In the case of pGS89, pGS15 was digested with *Bam*HI and *Eco*RI (plus *Hin*dIII), followed by religation so that the 3.0-kb *Eco*RI-*Bam*HI fragment (R_1-B_1) was recloned between the corresponding sites in pBR322 with the complete elimination of *tet* promoter DNA (Fig. 2). Other deletion derivatives were obtained from pGS85 with *Sph*I (pGS135) or from pGS89 with *Bgl*II (pGS136) or *Sph*I (pGS138). Plasmid pGS137, containing the 1.7-kb *Bgl*II fragment (Bg_1-Bg_2) cloned with unknown polarity in the *Bam*HI site of pBR322, was recovered from a ligation mixture containing *Bgl*II (plus *Eco*RI)-digested pGS89 and *Bam*HI-digested pBR322 (Fig. 2). Plasmid pGS139 was likewise constructed from mixed digests of pGS89 (*Sph*I plus *Pst*I) and pBR322 (*Sph*I), so that the hybrid 2.2-kb *Sph*I fragment (Sp_1-Sp_{vector}) was recloned with an orientation defined by *Bam*HI digestion. Finally, pGS140 was constructed by isolating the 2.7-kb *Bam*HI-*Sph*I fragment (B_1-Sp_2) from pGS85 and recloning it in pBR322. In every case, the ability to confer the Amp^r phenotype was retained and the *tet* gene of the vector was inactivated, though three plasmids retained *tet* promoters acting on the bacterial inserts with a clockwise (pGS139 and pGS140) or undefined (pGS137) transcriptional polarity.

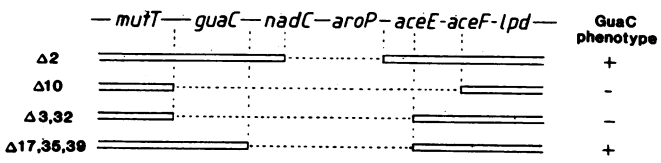

FIG. 1. Map showing the approximate positions of the deletions in seven spontaneous auxotrophic *aroP* mutants having NadC$^-$ Ace$^+$ Ace$^-$ phenotypes (derived from the research of Langley and Guest [18]). The GuaC, NadC, AroP, and Ace phenotypes were tested nutritionally in *purD212*::Tn*5* derivatives (Table 1).

Amp^r transformants of TX549 [*Δ(guaC-aceE) purD*::Tn*5*] containing each of the plasmids were tested for their GuaC, NadC, and AroP phenotypes, as well as Pur and Kan, to ensure that the *purD212*::Tn*5* mutation was retained. The results indicated that the *guaC* gene is located in the 3.0-kb *Eco*RI-*Bam*HI fragment (R_1-B_1) of pGS89 and that it spans the *Sph*I site (Sp_1) because neither pGS138 nor pGS139 conferred the GuaC$^+$ phenotype (Fig. 2). The *guaC* locus also appeared to span one or both of the *Bgl*II sites (Bg_1 or Bg_2) because pGS137 was Gua$^-$ (Fig. 2). This location is not entirely consistent with that proposed by Moffat and Mackinnon (26), since their restriction map of the *guaC* region is different. They located *guaC* within a 1.6-kb *Eco*RI-*Bgl*II-*Sph*I segment which is larger than the 0.9-kb R_1-Bg_1-Sp_1 segment shown in Fig. 2. In pGS138, the latter segment confers a GuaC$^-$ phenotype, indicating that the *guaC* locus extends beyond the *Sph*I site. These discrepancies may be because Moffat and Mackinnon used a single clone derived from a partial *Sau*3A digest and a recombination-proficient strain (rather than a recombination-deficient or a deletion strain) for plasmid complementation (26). The properties of other Amp^r transformants showed that the *nadC* gene spans the other *Sph*I site (Sp_2), because pGS85 confers a NadC$^+$ phenotype but pGS135 and pGS140 do not, and that the *aroP*

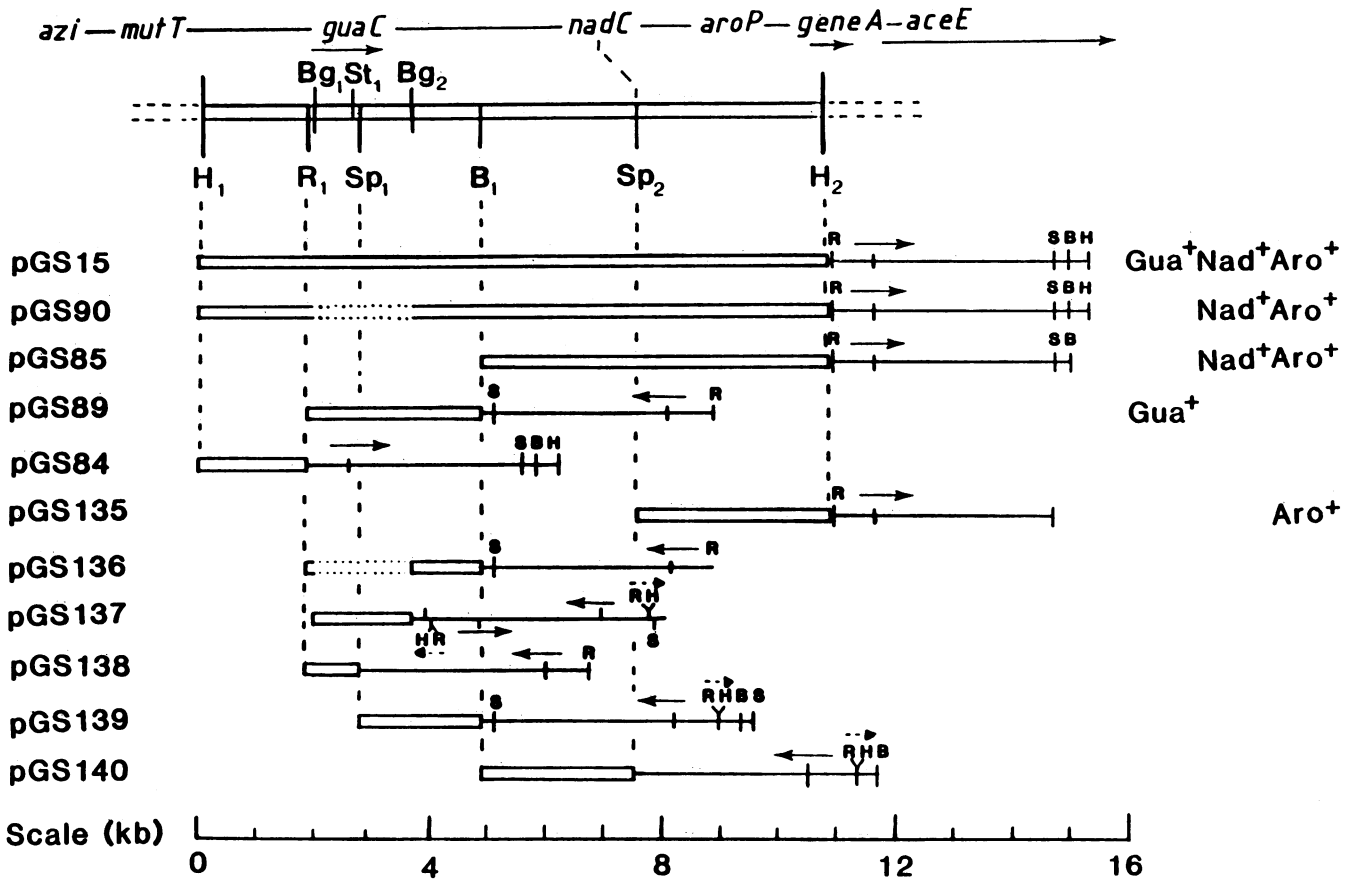

FIG. 2. Simplified restriction map of the *nadC* (2.5-min) region of the *E. coli* chromosome showing the segments of DNA cloned in a series of pBR322-derived plasmids. The positive phenotypes conferred by the plasmids are indicated, and the positions and polarities of the putative *guaC* gene and other genes are marked. Bacterial DNA is denoted by open bars, and single lines represent vector DNA. The scale drawings show the targets for B, *Bam*HI; Bg, *Bgl*II; R, *Eco*RI; H, *Hin*dIII; S or Sp, *Sph*I; St, *Sst*I; all were numbered with subscripts according to the method of Guest et al. (12). Additional restriction sites in this region are listed in reference 12. The relative orientation of bacterial and vector DNA can be deduced from the vertical bar and arrowhead, which denote the *Pst*I site in the vector and the polarity of the β-lactamase gene, respectively, and from the other restriction sites in the vector. Where present, the *tet* promoter is indicated by the dashed arrow.

gene resides within the 3.1-kb *Sph*I-*Hin*dIII fragment (Sp_2-H_2) of pGS135 (Fig. 2).

Expression of GMP reductase from multicopy plasmids. The expression of GMP reductase from the smallest *guaC*$^+$ multicopy plasmid (pGS89) was tested in *guaC*$^+$ and Δ*guaC* hosts under inducing and noninducing growth conditions (Table 2). Under noninducing conditions, expression was elevated approximately 15-fold and only increased a further 1.3-fold under inducing conditions. This almost constitutive expression contrasts with the threefold amplification and normal regulation (fivefold induction) that has been observed with a ColE1-*guaC*$^+$ plasmid, pLC37-40 (15). The differences may simply be due to the higher copy number of pGS89, which would not only lead to higher enzyme activi-

TABLE 2. GMP reductase expression from a multicopy plasmid

Strain	Relevant genotype	Sp act (nmol/min per mg of protein)[a] of:			
		IMP dehydrogenase		GMP reductase	
		Noninducing[b]	Inducing[c]	Noninducing[b]	Inducing[c]
TX549	Δ*guaC*	2.5	ND[d]	NAD[d]	ND
TX582	Δ*guaC*(pGS89)*guaC*$^+$	11.9	5.2	47.0	73.5
TX609	*guaC*$^+$	8.3	7.5	3.5	9.5
TX692	*guaC*$^+$(pGS89)*guaC*$^+$	4.2	2.3	54.0	71.0

[a] β-Lactamase activity was used to normalize GMP reductase activity due to copy number variation of plasmid pGS89 between growth conditions in strains TX582 and TX692. Strain TX582 had specific activities of 4.7 and 3.2, whereas strain TX692 had specific activities of 3.6 and 2.6 μmol/min per mg of protein with noninducing and inducing conditions, respectively. IMP dehydrogenase activity was not normalized.

[b] Growth in minimal medium supplemented with 0.1% Casamino Acids and hypoxanthine (2, 3).

[c] Growth in minimal medium supplemented with 0.1% Casamino Acids and guanosine (2, 3).

[d] Abbreviations: ND, not done; NAD, no activity detected.

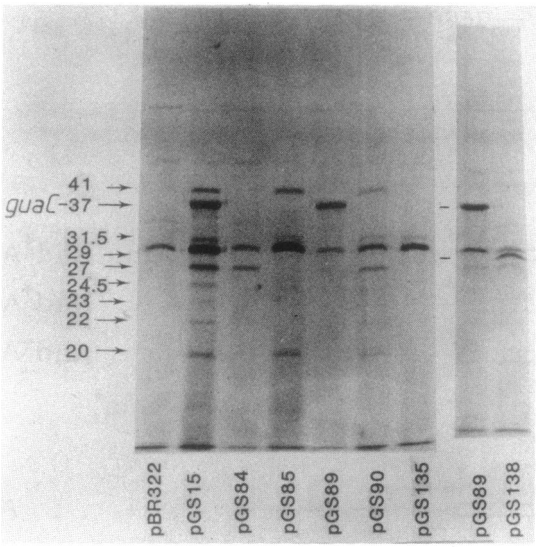

FIG. 3. Autoradiogram of [^{35}S]methionine-labeled polypeptides detected by the maxicell procedure with different plasmids. The molecular weights of the polypeptides are shown in thousands, and the putative *guaC* gene product is indicated. No polypeptides of bacterial origin were detected with pGS136, pGS137, or pGS139, and pGS140 expressed the 22,000-, 24,500-, and 41,000-M_r polypeptides.

ties but also to derepressed (constitutive) expression because of the titration of a negatively acting repressor by multiple copies of the *guaC* operator. Under the same conditions, the synthesis of IMP dehydrogenase was not significantly affected (Table 2), supporting the view that the *guaBA* operon and the *guaC* gene are not coregulated (10, 15, 22).

Maxicell studies and transcriptional polarity of *guaC*. The maxicell procedure (30) was used to detect the polypeptides expressed from pGS15 and all of its derivatives to correlate the products with specific DNA fragments and functions. A total of nine bacterial polypeptides of M_r 41,000, 37,000, 31,500, 29,000 (often masked by β-lactamase), 27,000, 24,500, 23,000, 22,000 and 20,000 were expressed from pGS15 (Fig. 3). The M_r 37,000 polypeptide was identified as a product of the *gua* locus because it alone was expressed by the Gua$^+$ plasmid (pGS89) but not by any of the Gua$^-$ plasmids (pGS136 to pGS139) containing subfragments of the pGS89 insert (Fig. 2) and because it undoubtedly corresponded to the M_r 36,000 polypeptide previously correlated with *guaC* by Tn*5* mutagenesis (26).

Several lines of evidence indicated that the transcriptional polarity of the *guaC* locus is clockwise, i.e., left to right (Fig. 2). First, it was observed that pGS138 produced a unique polypeptide of M_r 28,500, which is probably a truncated *guaC* product, because no other polypeptides were detected with pGS89 or its subset of plasmids, pGS136 to pGS139 (Fig. 2 and 3). The simplest explanation that is consistent with the coding capacities of the plasmids is that expression of the *guaC* locus originates close to the *Eco*RI site (R_1) and extends rightward across the entire *Bgl*II-*Sph*I fragment, Bg_1-Sp_1 (Fig. 2). Further evidence came from studies in which a series of *lacZY-kan* fusion cassettes representing three possible reading frames (A. A. Tiedeman and J. M. Smith, unpublished observations) were inserted at the *Sst*I site of pGS89 (Fig. 2). The orientation of the cassette in a putative *gua-lacZY* fusion (Lac$^+$ on complex medium with guanosine and X-Gal [5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside]) confirmed that transcription is polarized rightward at the *Sst*I site. It should also be noted that insertion of the cassette resulted in the inactivation of the GuaC$^+$ phenotype of pGS89, and this inactivation is consistent with the location of the *Sst*I site in the *guaC* locus. It also is highly probable that the M_r 37,000 polypeptide is identical to one of M_r 36,500 that was previously identified as the product of a gene of clockwise polarity in postinfection labeling studies with λG78N, as λ *nadC* transducing phage (11).

It was also concluded that the M_r 27,000 and 22,000 polypeptides originate from the leftmost 1.8-kb *Hin*dIII-*Eco*RI fragment (H_1-R_1) and that the M_r 31,500 and 24,500 polypeptides derive from the rightmost 3.2-kb *Sph*I-*Hin*dIII *aroP* region (Sp_2-H_2), though neither corresponds to the *aroP* gene product (M_r 37,000), which is only expressed under inducing conditions (8). The remaining polypeptides (M_r 41,000, 23,000, and 20,000) are encoded by the central region (containing B_1 and Sp_2) and probably include the product of the *nadC* gene, which spans the *Sph*I site, Sp_2 (Fig. 2).

The relative positions of the *guaC*, *nadC*, *aroP*, and *ace* genes have been deduced by genetic analysis in the 2.5-min region of the *E. coli* linkage map and further defined by molecular analysis of the corresponding 10.5-kb *Hin*dIII DNA fragment. A minimum of nine polypeptides were expressed from this fragment, and the identity of one (M_r 37,000), the *guaC* gene product (GMP reductase), was confirmed (26). In addition, the *guaC* gene (ca. 1 kb in size) was located in the 10.5-kb DNA fragment as a region spanning the Bg_1-Sp_1 segment, and its transcriptional polarity was deduced from the properties of a truncated *guaC* product and a *guaC-lac* fusion (Fig. 2). The *nadC* gene appeared to span the *Sph*I restriction site (Sp_2), and the precise location of the *aroP* gene in the Sp_2-H_2 fragment has now been defined with pGS85 (8). The 10.5-kb fragment clearly contains several unidentified genes. One possibility is a guanine transport gene (*guaP*), which is reported to map in the *mutT-nadC* region of *Salmonella typhimurium* (4). However, the existence of a distinct *guaP* locus has been questioned (7), and guanine transport is unaffected in two representative *guaC* deletion strains, Δ*3* and Δ*10* (7; K. Burton, personal communication). The availability of a plasmid (pGS89) expressing high levels of GMP reductase should be very useful for further studies on the enzyme and for elucidating the complexities of the mechanism regulating its expression.

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