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A Common Regulator for the Operons Encoding the Enzymes Involved in D-Galactarate, D-Glucarate, and D-Glycerate Utilization in *Escherichia coli*

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Genes for D-galactarate (*gar*) and D-glucarate (*gud*) metabolism in *Escherichia coli* are organized in three transcriptional units: *garD*, *garPLRK*, and *gudPD*. Two observations suggested a common regulator for the three operons. (i) Their expression was triggered by D-galactarate, D-glucarate, and D-glycerate. (ii) Metabolism of the three compounds was impaired by a single Tn*5* insertion mapped in the *yaeG* gene (proposed name, *sdaR*), outside the D-galactarate and D-glucarate systems. Expression of the *sdaR* gene is autogenously regulated.

The genes encoding the enzymes in the D-glucarate and D-galactarate pathways have been identified in the *Escherichia coli* genome and found to be distributed in apparently three transcriptional units (Fig. 1) (12). One unit, located at min 60 and transcribed counterclockwise, is formed by three genes that encode a putative D-glucarate permease (b2789), a nonfunctional D-glucarate dehydratase-related protein (b2788), and the functional D-glucarate dehydratase (b2787). The other two units are located at min 70 and are divergently transcribed, one (clockwise) formed by the gene encoding D-galactarate dehydratase (*yhaG*) and the other (counterclockwise) formed by the genes for a putative D-galactarate permease (*yhaU*), 5-keto-4-deoxy-D-glucarate aldolase (*yhaF*), tartronate semialdehyde reductase (*yhaE*), and glycerate kinase (*yhaD*). Despite this recent detailed description of the gene structure and organization of these two metabolic systems, no information on their regulation was available. In this report we assign to a gene of unknown function the role of encoding a common regulator for D-galactarate, D-glucarate, and D-glycerate metabolism.

Coordinate regulation of D-galactarate, D-glucarate, and D-glycerate metabolism. Enzyme activities involved in the metabolism of D-galactarate and D-glucarate were determined in crude extracts of strain MC4100 (8) grown aerobically in minimal medium (6) with D-galactarate, D-glucarate, or their common intermediate metabolite D-glycerate. Activity levels of D-galactarate dehydratase (5), D-glucarate dehydratase (4), glycerate kinase (1), and tartronate semialdehyde reductase (9) showed that both enzyme systems were induced by growth on either of the carbon sources. D-Glycerate was the best inducer (Table 1).

Expression of the transcriptional units. To identify the functional promoters in the D-galactarate and D-glucarate systems, different fragments of the two gene clusters were fused to the *lacZ* gene of plasmid pRS550 or pRS551 (17) and introduced as a single copy in the genomic background of MC4100 as described by Elliott (10). Analysis of the β-galactosidase activities (14) showed the presence of promoter function only 5′ of the following genes: *yhaG* (proposed name, *garD*), *yhaU* (proposed name, *garP*), and b2789 (proposed name, *gudP*) (Fig. 1). A *garP*::Tn*5* insertion mutant (strain JA175) was obtained from strain MC4100 as described by Bruijn and Lupski (7). Impairment of D-glucarate and D-glycerate utilization in this mutant, which lacks the function of downstream genes in the *garPLRK* operon due to polarity effects, also indicated that no other promoter activity lies in this operon.

β-Galactosidase activities of Φ(*garD-lacZ*), Φ(*garP-lacZ*), and Φ(*gudP-lacZ*) were found to be induced in cultures grown in the presence of D-galactarate, D-glucarate, or D-glycerate compared to the basal levels obtained in glycerol. The activation was two- to threefold higher in D-glycerate than in D-galactarate or D-glucarate (Fig. 2). These results indicated a coordinate regulation of these three promoters. No induction was observed with other related sugars, such as D-glucuronic acid or D-galacturonic acid. The inducing capacity of any of the three compounds observed in strain JA175, which was unable to form D-glycerate from D-galactarate or D-glucarate, indicated that all of them acted as inducers. Induction by D-galactarate in strain JA175, which lacks galactarate permease, showed that this substrate is able to enter the cells by another permease, probably D-glucarate permease. This finding would be supported by the high similarity displayed by these two permeases (12). These results led us to explore if the three promoters were under the control of a common regulatory protein recognizing any of the three substrates as an inducer.

Isolation and mapping of pleiotropic mutants. To search for a common regulator, 10^4 cells of strain MC4100 bearing Φ(*garD-lacZ*) were mutated by ethyl methanesulfonate (14) and screened for blue and white colonies on glycerol plates containing D-glucarate and 5-bromo-4-chloro-3-indolyl-β-D-galactoside. From among the white colonies we selected those that were unable to grow on D-glucarate, D-galactarate, or D-glycerate but able to grow on other carbon sources. These cells displayed no β-galactosidase activity and no detectable galactarate or glucarate dehydratase, tartronate semialdehyde reductase, or glycerate kinase activities when grown under any of the inducing conditions. The mutation in one of the isolates (strain JA176) was mapped by conjugation using the Hfr strain collection of Wanner (19). The 68% recombination with the Tn*10* marker of strain BW6156 and the 37% recombination with the Tn*10* marker present in strain BW6164 allowed us to

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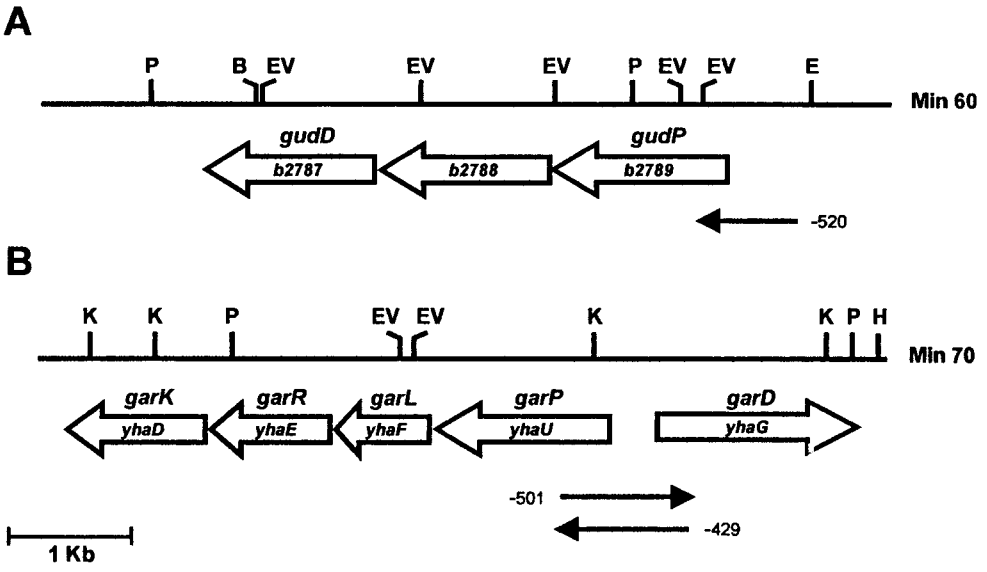

FIG. 1. Restriction map and gene organization of the *gud* (A) and *gar* (B) regions of the *E. coli* chromosome. Relevant restriction sites are shown along the *E. coli* DNA as follows: B, *Bam*HI; E, *Eco*RI; EV, *Eco*RV; H, *Hin*dIII; K, *Kpn*I; and P, *Pvu*II. Open arrows indicate the direction and extension of the genes involved in D-galactarate, D-glucarate, and D-glycerate metabolism. Genes are labeled inside the arrows according to the system of the *E. coli* Genome Project and outside by the names proposed in this work. Thin arrows correspond to the fragments fused to *lacZ* displaying promoter activity and are labeled by numbers that indicate the distance (in nucleotides) upstream of ATG.

locate the mutation in the genomic region of 95 to 5 min, mapping outside of the D-galactarate and D-glucarate loci. Fine mapping was performed by P1 transduction (14) with strain CAG12025 (15) (18% cotransduction), which located the mutation close to min 3.2.

Identification and cloning of the putative regulator. To identify the gene responsible for this pleiotropic effect, we obtained a Tn*5* insertion mutant (7) that mapped to the same locus and displayed the same phenotype as strain JA176. To rule out the possibility of multiple transposon insertions, this mutation was P1 transduced into the wild-type background of strain MC4100 (strain JA177). For Southern blot analysis, chromosomal DNA of strain JA177 was digested with restriction enzymes that had no recognition sites on the kanamycin resistance-coding region of the transposon. Using a Tn*5* probe, we identified a 5.5-kb *Bam*HI-*Eco*RV fragment, which was subsequently cloned into pBluescript. By sequencing the region adjacent to the Tn*5* joining site using as a primer an internal sequence of the transposon, the precise location of the Tn*5* insertion was determined at 20 nucleotides upstream of the stop codon of gene *yaeG*, which encodes a protein of 391 residues (accession no. AE000125, nucleotides 8952 to 10127) (3). In order to confirm that the above-described mutation of strain JA176 also affected the *yaeG* gene, the corresponding region was amplified by PCR, using primer A1 (CGGAATTC GCGACGGTAAGCAGGTT) and primer A2 (TGCGGATC CTCCTCGACGACTTACG). The fragment was cloned into pBluescript, making use of the *Bam*HI and *Eco*RI sites of the primers (underlined). After sequencing of several clones, the mutation was identified in all as a C→T transition resulting in a replacement of Pro297 by a Ser in the encoded protein. This gene is likely to be a regulator of these systems and is given here the name *sdaR* for sugar diacid regulator. Furthermore, computer analysis permitted us to classify the *sdaR* gene product within a set of proteins that present a conserved helix-turn-helix motif, including other gene expression regulators such as XylR (13). In the case of the SdaR protein this motif was identified close to the carboxy-terminal end, between amino acid residues 343 and 367. We would like to point out that T-fasta analysis of SdaR yielded a high percentage of identity (95.9% in a 170-amino-acid overlap) with some protein sequences encoded in *Homo sapiens* chromosome 5. This unusually high identity has to be explained by an incorrect entry in GenBank, as the same identity was found in comparing the nucleotide sequences.

The gene *sdaR* was amplified by PCR from strain MC4100 using primers A1 and A2 and cloned in pBluescript, yielding pRM1. Cells of strain JA176 or JA177 harboring pRM1 were able to grow on D-glucarate, D-galactarate, or D-glycerate, indicating that the SdaR protein restored the wild-type phenotype. According to these results the common regulator was

TABLE 1. Enzyme activities involved in D-galactarate, D-glucarate, and D-glycerate metabolism in strain MC4100

Enzyme	Activity (mU/mg) under growth condition			
	Glycerol	D-Galactarate	D-Glucarate	D-Glycerate
Galactarate dehydratase	<5	20	15	40
Glucarate dehydratase	<5	40	35	90
Glycerate kinase	<5	165	130	280
Tartronate semialdehyde reductase	<5	190	170	315

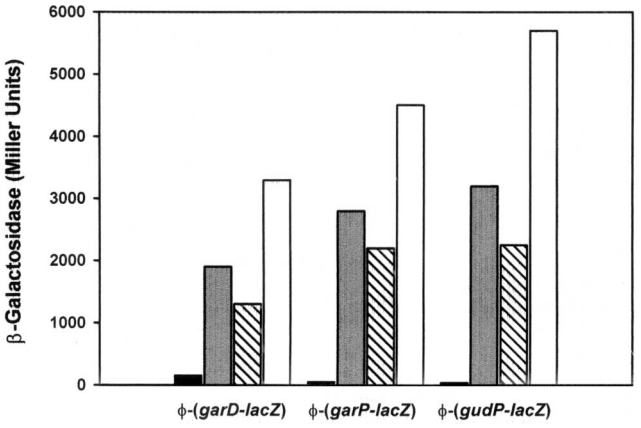

FIG. 2. Expression of Φ(*garD-lacZ*), Φ(*garP-lacZ*), and Φ(*gudP-lacZ*) in strain MC4100. β-Galactosidase activities were determined in cultures grown on glycerol with the addition of the following carbon sources: none (solid bars), D-galactarate (grey bars), D-glucarate (hatched bars), and D-glycerate (open bars).

likely to be an activator, the lack of which would give a pleiotropism-negative phenotype for the three sugars.

Regulation of *sdaR* expression. Control of *sdaR* expression was studied by fusing its putative promoter region to *lacZ* in the genetic background of strain MC4100. The fragment obtained by PCR using primer A3 (CGGAATTCGGCACTCA ACATTCAGC) and primer A4 (TGCGGATCCAGTACCAG CAATGCAC) extended 188 bp upstream of the *sdaR* GTG start codon proposed in the *E. coli* genome (3). β-Galactosidase activities showed that *sdaR* was expressed at a basal level of 35 U in glycerol cultures, which reached values of 350 U in the presence of D-glucarate or D-galactarate and of 950 U in the presence of D-glycerate. Lack of a functional *sdaR* gene product abolished the activation of the *sdaR-lacZ* fusion by the three compounds, indicating that the expression of the regulator gene is mediated by its own product.

Of the possible models of coupled expression of regulator and effector genes (11), those involving negative controls would be inconsistent with the absence of activation of Φ(*sdaR-lacZ*) or Φ(*garP-lacZ*) in the knockout mutants of the *sdaR* gene. Autogenous regulation of *sdaR* in response to the inducers must be explained by positive regulation as described for the *rhaSR* genes of *E. coli* (18), the *eutR* gene of *Salmonella enterica* serovar Typhimurium (16), or the *moaR* gene of *Klebsiella aerogenes* (2). In the presence of the inducing molecules, low residual levels of SdaR are activated, thereby increasing its own expression. Under these conditions the rise in the regulator concentration would balance the equilibrium between the inactive and the active forms of the activator towards a further regulator activation, which would in turn enhance expression of the three structural operons.

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