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Multiple Regulators Control Expression of the Entner-Doudoroff Aldolase (Eda) of *Escherichia coli*

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The *Escherichia coli eda* gene, which encodes the Entner-Doudoroff aldolase, is central to the catabolism of several sugar acids. Here, we show that Eda synthesis is induced by growth on gluconate, glucuronate, or methyl-β-D-glucuronide; phosphate limitation; and carbon starvation. Transcription of *eda* initiates from three promoters, designated P1, P2, and P4, each of which is responsible for induction under different growth conditions. P1 controls *eda* induction on gluconate and is regulated by GntR. P2 controls *eda* induction on glucuronate and galacturonate and is regulated by KdgR. P4 is active under conditions of phosphate starvation and is directly controlled by PhoB. In addition, CsrA activates Eda synthesis, apparently by an indirect mechanism that may be involved in the modest changes in expression level that are associated with carbon starvation. The complex regulation of *eda* is discussed with respect to its several physiological roles, which apparently accommodate not only sugar acid catabolism but also detoxification of metabolites that could accumulate during starvation-induced stress.

Sugar acids are metabolized via the Entner-Doudoroff and Ashwell pathways (1, 5, 8, 20). The Entner-Doudoroff aldolase, encoded by the *eda* gene, is necessary for growth on several mucus-derived sugar acids (20), which may define a niche for *Escherichia coli* colonization of the mammalian large intestine (4). Mutants lacking the key enzyme of the Entner-Doudoroff pathway, 6-phosphogluconate dehydratase (encoded by *edd*), are compromised in their ability to colonize the mouse intestine (4), as are *eda* mutants (29).

Eda cleavage of 2-keto-3-deoxy-6-phosphogluconate (KDPG) to pyruvate and triose-3-phosphate is a common step of hexonate and hexuronate catabolism (20). Eda has also been found to have a role in the degradation of 2-keto-4-hydroxyglutarate (KHG) to pyruvate and glyoxylate (16). Although this reaction has not been shown to occur in vivo, cleavage of KHG could serve in tricarboxylic acid (TCA) cycle regulation or, when operating in the reverse direction, in the detoxification of glyoxylate. Cloning and sequencing the genes encoding these functions revealed that KHG aldolase and Eda are the same enzyme (7, 18). However, this study is the first to extensively characterize *eda* regulation.

The *eda* gene is located immediately downstream of *edd*, at 40.5 min on the *E. coli* chromosome (Fig. 1) (6). Previously, primer extension analysis of the *eda* regulatory region revealed four putative *eda* promoters (7). P1 drives cotranscription of *edd* and *eda* and is induced by growth on gluconate (7); P1 appears to be controlled by GntR (20, 21, 24), although binding of GntR to the P1 regulatory region was not established prior to this work. A second promoter, P2, is located within the terminal one-third of the *edd* coding sequence, and the closely spaced third and fourth putative promoters (P3 and P4) are located at the distal end of the *edd* coding region (7). High basal expression is characteristic of *eda* (20). Although it is known that the P2, P3, and P4 promoters are inducible above basal levels, the specific inducers have not been established prior to this study (7). This organization of promoters suggests that regulation of *eda* transcription is complex. Hexuronates induce Eda, perhaps mediated at the transcriptional level through the actions of KdgR (22). Two dimensional gel electrophoresis revealed induced synthesis of Eda under conditions of phosphate limitation (31). Eda appears to be induced during carbon starvation or stationary phase, but these have not been distinguished with respect to control of Eda (17).

The objectives of this study were to characterize the promoters and conditions that initiate transcription of *eda*, as well as the regulatory factors and mechanisms involved. We show here that three functional promoters drive transcription of *eda* and that each promoter responds to different growth conditions. We show that P1 is induced by growth on gluconate, which is mediated by GntR. P2 is induced by growth on glucuronate, galacturonate, and methyl-β-D-glucuronide and is mediated directly by KdgR. Phosphate starvation results in induction of P4, which is directly mediated by PhoB. Eda levels are slightly elevated during carbon starvation but not stationary phase. The mRNA binding protein CsrA is also implicated in the control of Eda synthesis.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The *E. coli* strains, plasmids, and phages used in this study are listed in Table 1. The *eda*P1-*lacZ* fusions were created in *E. coli* P90C; other *eda-lacZ* fusions were created in *E. coli* W1485 Δ(*lac-pro*). *E. coli* DH5α was used for constructions and propagation of plasmids. *E. coli* strains were grown at 37°C, and growth was monitored with a Spectronic 601 spectrophotometer (Milton Roy Co.). Culture media used were Luria-Bertani medium (LB) (13), M63 minimal medium, MOPS (morpholinepropanesulfonic acid minimal medium; phosphate-replete medium contained 1.32 mM K_2HPO_4; phosphate-limiting medium contained 0.066 mM K_2HPO_4) (15), and Kornberg medium (12). Antibiotics were used at the follow-

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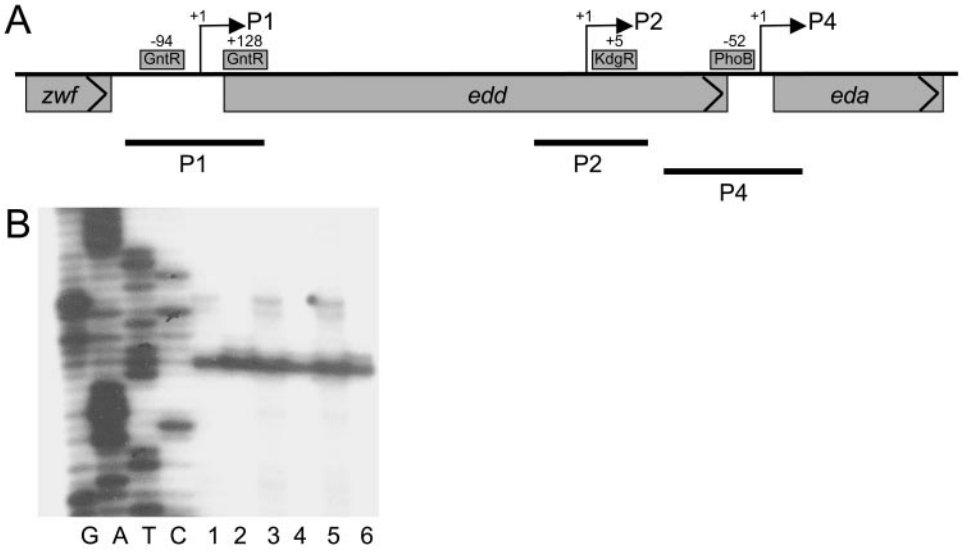

FIG. 1. (A) Genetic map of the *edd-eda* coding region. Arrows, transcription start sites confirmed by primer extension (P1, P2, and P4). Relative locations of operator sites (boxes) are shown above the line. DNA fragments used for *lacZ* fusions are shown below the line. P1, P1 operon fusion (*E. coli* NP304) and P1 operon fusion with mutant *gntO* site (*E. coli* NP305); P2, P2 operon fusion (*E. coli* BM106); P4, P4 protein (*E. coli* BM105) and operon (*E. coli* BM111) fusions. (B) Primer extension analysis of the 5′ end of the *eda* transcript originating from P4. Lanes G, A, T, and C, *eda* sequence ladder (generated with the same primer used for extension); lanes 1 and 2, RNA isolated from phosphate-starved cells; lanes 3 and 4, RNA isolated from glucose-starved cells; lanes 5 and 6, RNA isolated from stationary-phase cells. Lanes 1, 3, and 5 were extended with OmniScript reverse transcriptase; lanes 2, 4, and 6 were extended with Superscript reverse transcriptase.

ing final concentrations: kanamycin, 25 or 50 μg/ml; ampicillin, 100 μg/ml; chloramphenicol, 25 μg/ml; tetracycline, 25 μg/ml.

Enzymes and chemicals. Restriction enzymes, DNA-modifying enzymes, including Superscript reverse transcriptase, *Taq* DNA polymerase, and Platinum *Taq* DNA polymerase, as well as IPTG (isopropyl-β-D-thiogalactopyranoside), X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), nucleoside triphosphates, and deoxynucleoside triphosphates were purchased from Invitrogen. Omniscript reverse transcriptase was purchased from QIAGEN. Media components and chemicals were purchased from Sigma Chemical and Fisher Scientific. Radionucleotides were purchased from NEN Life Science Products or ICN. Unlabeled oligonucleotide primers were purchased from Ransom Hill Biosciences. Fluorescein-labeled primers were purchased from Integrated DNA Technologies. Anti-Eda chicken antibodies were synthesized by Aves Labs using lyophilized Eda, which was a gift from Eric Toone.

Fusion constructions. DNA fragments used for *lac* fusion constructions are shown in Fig. 1. *eda-lacZ* transcriptional and translational fusions, in single copy on the chromosome as λ lysogens, were constructed by using the system of Simons et al. (28). The regions of DNA containing putative *eda* promoters were amplified by PCR with primers containing 5′ EcoRI and BamHI restriction sites. After digestion of the PCR products, they were ligated into pRS551 (for plasmids pBM106 and pBM111) or pRS552 (for plasmid pBM105). Clones were verified by restriction mapping and DNA sequencing. Fusion constructions were recombined with λRS88 and subsequently integrated into the λ *att* site on the chromosome. The copy numbers of constructions were checked with PCR by the method of Powell et al. (23). P1 transduction (14) was used to transduce the *csrA*::Kan^r mutation from *E. coli* TRI-5MG653 to strains carrying *eda-lacZ* fusions in a wild-type background. Transductants were screened for the *csrA* mutation by iodine staining (26).

β-Galactosidase assays. β-Galactosidase activity was determined from permeabilized cells as previously described (14) and is expressed in Miller units.

Western blot analysis. Samples for Western blot analysis (approximately 14 mg of cell biomass) were removed from the culture, harvested by centrifugation, and frozen at −20°C. Thawed cell pellets were resuspended in 500 μl of buffer (20 mM Tris-HCl, 1 mM EDTA, 5 mM β-mercaptoethanol [pH 8]) and disrupted by sonication. Samples (50 μg of total-cell protein) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (PAGE) (11). Proteins were transferred from PAGE gels to Immobilon-P membranes (Millipore) with a Bio-Rad transfer apparatus. Following transfer, membranes were baked at 80°C for 15 min or until dry in a vacuum oven, blocked by incubation for 1 h with 100 ml of PBST (10 mM sodium phosphate [pH 7.2], 0.9% sodium chloride, 0.05% Tween 20, 2.5 g of dry milk), and washed five times in PBST. Blocked membranes were incubated in 100 ml of PBST containing 50 μl of primary antibody (chicken anti-Eda) and washed as before, followed by incubation in 100 ml of PBST containing 8 mg of secondary antibody (horseradish peroxidase-labeled goat anti-chicken immunoglobulin Y) and another washing. Membranes were air dried and visualized by chemiluminescence with Western blotting detection reagents and ECL (Amersham Pharmacia Biotech) and exposure to Kodak Biomax MS film for 1 to 30 s.

DNA sequencing. DNA was prepared for sequencing by using the QIAprep Spin Miniprep kit (QIAGEN) and sequenced according to the manufacturer's instructions with either Sequenase, version 2.0 (USB/Amersham Life Science), for α-^{35}S-ATP sequencing or the double-stranded DNA cycle sequencing system (Invitrogen) for [α-^{32}P]ATP sequencing.

RNA isolation, Northern hybridization, and primer extension. Total RNA for northern hybridization and primer extension analysis was isolated by the hot-phenol method (19) from *E. coli* cultures grown to mid-exponential or stationary phase under the conditions described above. For mRNA half-life determinations, cells were grown overnight in 3 ml of Kornberg medium containing 0.5% glucuronate and then subcultured in 400 ml of Kornberg medium plus 0.5% glucuronate and grown at 37°C to an A_{600} of 0.5. Rifampin was added to the culture to a final concentration of 200 μg/ml, and samples were taken at 0 (control), 2, 4, 8, and 12 min postaddition. Samples (10 ml) were diluted 1:1 in RNA*later* (Ambion), and RNA was isolated with the QIAGEN RNA/DNA Maxi kit. Contaminating DNA was removed by treatment with RNase-free DNase (QIAGEN). For Northern hybridization, gels were loaded with 5 μg of total RNA and were treated as described previously (2, 30). Radioactively labeled RNA probes were synthesized with [α-^{32}P]UTP by using T7 RNA polymerase with pTC196 (HincII-digested) DNA as the template. The membranes were visualized by exposure to Kodak X-ray film at room temperature. Methods for transcript end mapping by primer extension were described previously (30).

Purification of KdgR. KdgR was prepared by using the His tag modification system from QIAGEN. An 840-bp fragment containing the complete *kdgR* coding sequence was amplified by PCR with primers that contained BamHI restriction sites at the 5′ end and ligated into pPCR-Script Amp (SK+) to create pBMExKS, and then the fragment was subcloned into pQE30 to create pBMExKQ. KdgR was overproduced and purified on a nickel-nitrilotriacetate column, as described previously (19). The protein contents of column fractions were quantified by using the method of Bradford (3).

Gel mobility shift assays for KdgR. The 115-bp DNA probe for the mobility shift assay was generated by PCR with primers flanking the promoter and

TABLE 1. Strains, plasmids, and phages

E. coli strain, plasmid, or phage	Relevant genotype	Source or reference
Strains		
DH5α	*lacZ ΔM15 recA*	Invitrogen
P90C	*ara Δ(pro-lac) thi*	28
TRI-5MG653	*csrA*::Kan^r Hfr *his thi str*-sΔ*(malA-asd)*	27
W1485	K-12 wild type	CGSC[a]
CT110	*kdgR*10 *relA*1 *spoT*1 *kdgT*110 *metB*1	CGSC
BW14087	ΔE3*(lac)X74 rpoS*(Am) *phoB*23	B. Wanner
BW13711	ΔE3*(lac)X74 rpoS*(Am)	B. Wanner
HT216	Δ*cya-851 ilv*::Tn*10 gntR*::Kan^r	19
W1485 Δ(*lac-pro*)	Δ(*lac-pro*) in wild-type background	Laboratory stock
BM105	*eda*P4-*lacZ* (protein) Δ(*lac-pro*) Kan^r	This study
BM106	*eda*P2-*lacZ* (operon) Δ(*lac-pro*) Kan^r	This study
BM111	*eda*P4-*lacZ* (operon) Δ(*lac-pro*) Kan^r	This study
BM105c	*eda*P4-*lacZ* (protein) Δ(*lac-pro*) Kan^r *csrA*::Kan^r Tet^r	This study
BM106c	*eda*P2-*lacZ* (operon) Δ(*lac-pro*) Kan^r *csrA*::Kan^r Tet^r	This study
NP304	*eda*P1-*lacZ* (operon)	N. Peekhaus
NP305	*eda*P1-*lacZ* (operon) *gntO* mutant	N. Peekhaus
BM304c	NP304 *csrA*::Kan^r Tet^r Kan^r	This study
Plasmids		
pQE30	Expression vector; His_6 affinity tag	QIAGEN
pRS551	*bla′ lacZ* + Kan^r Amp^r (operon fusion vector)	28
pRS552	*bla′ lacZ* + Kan^r Amp^r (protein fusion vector)	28
pPCR-Script Amp (SK+)	pBluescript derivative, contains SrfI in multicloning site	Stratagene
pCR-XL-TOPO	ColE1 ori; Kan^r Zeo^r *lacZ′ ccdB*	Invitrogen
pBM105	*eda*P4::*lacZ* in pRS552; Kan^r Amp^r	This study
pBM106	*eda*P2::*lacZ* in pRS551; Kan^r Amp^r	This study
pBM111	*eda*P4::*lacZ* in pRS551; Kan^r Amp^r	This study
pBMExKS	*kdgR* in pPCR-Script Amp (SK+); Amp^r	This study
pBMExKQ	*kdgR* subcloned from pBMExKS to pQE30; with His_6 tag	This study
pTC196	*eda* Amp^r	7
pBM204	*eda* P2 region cloned in pPCR-XL-TOPO	This study
Phages		
λRS88	*bla′ lacZ imm*^{434} *ind*	28
P1 (TRI-5MG653)	*csrA* Tet^r	T. Romeo

[a] CGSC, *E. coli* Genetic Stock Center.

pBM204 as the template. The PCR products were cleaned with the QIAquick PCR purification kit (QIAGEN) and labeled with [γ-^{32}P]ATP by using T4 polynucleotide kinase. Binding reaction mixtures (20 μl) contained labeled probe (0.025 pmol), reaction buffer (12 mM HEPES, 4 mM Tris-HCl [pH 8], 70 mM KCl, 1 mM EDTA, 10% glycerol, 1 μg of salmon sperm DNA, 4 μg of bovine serum albumin, 1 mM dithiothreitol), and KdgR in elution buffer. Reaction mixtures were incubated at room temperature for 30 min and then loaded onto 5% native polyacrylamide gels. Gels were dried on Whatman paper and exposed to X-ray film overnight.

Gel mobility shift assays for PhoB. The PhoB gel mobility shift assays employed a different protocol from that for KdgR. A fluorescein-labeled primer 138 bp upstream of the P4 transcription start site and an unlabeled primer 100 bp downstream were used to generate a fluorescein-tagged, 238-bp probe fragment by PCR with pBM105 as the template. The PCR product was purified with a QIAGEN QIAquick kit. PhoB was phosphorylated with acetyl phosphate solution (20 μg of PhoB, 50 mM Tris-HCl [pH 8], 53 mM $MgCl_2$, 50 mM acetyl phosphate) for 1 h at 37°C. Binding reaction mixtures typically contained 0.37 μg of fluorescein-labeled probe, buffer (10% glycerol, 10 mM Tris-HCl [pH 8], 0.05 mM EDTA, 2.64 mM $MgCl_2$, 0.25 mM $CaCl_2$, 1 μg of calf thymus DNA), and phosphorylated PhoB in a total volume of 20 μl. Reaction mixtures were incubated for 30 min at room temperature, loading buffer was then added, and the mixtures were electrophoresed on a 5% native polyacrylamide gel containing 2% glycerol. DNA-protein complexes were visualized directly by fluorescence with a Typhoon variable-mode imager (Molecular Dynamics).

RESULTS

Primer extension analysis of *eda* promoters. To determine the locations of promoters that are active under specific conditions, we mapped the 5′ ends of transcripts originating from P2, P3, and P4 in cells grown in conditions hypothesized to induce these promoters (Fig. 1). *E. coli* W1485 was grown in M63 minimal medium containing 0.4% glucuronate, and RNA was harvested during exponential growth. Primer extension analysis of cells grown on glucuronate revealed a transcription start site identical to that which was previously identified for high basal expression of the P2 promoter (7); the P2 transcription start site is located at position −353 with respect to the *eda* start codon (data not shown). Next, *E. coli* W1485 was grown under conditions of glucose or phosphate starvation and during the transition to stationary phase in Luria broth. Primer extension analysis of the P3-P4 promoter region revealed a single *eda* 5′ transcript end corresponding to the previously mapped *eda* P4 promoter transcription start site, which is located at position −26 with respect to the *eda* start codon (Fig. 1B). No transcript was mapped to the putative *eda* P3 promoter under any of the conditions tested. This result is in contrast to our previous report of two closely spaced transcript ends corresponding to P3 and P4 in cells grown on gluconate (7). Since it was not possible to separate the two putative promoters by an alternative strategy, i.e., subcloning, we could not rule out the existence of P3. However, since the present experiments revealed only P4 and since all of the transcrip-

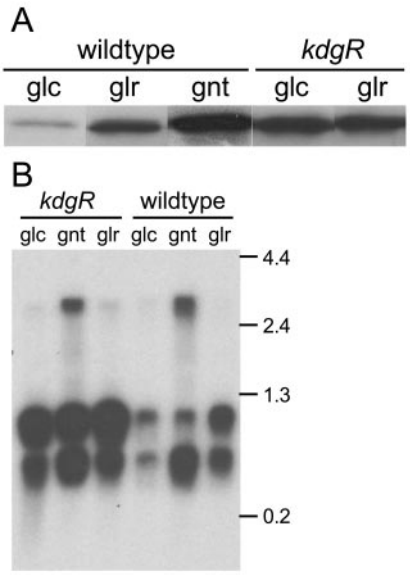

FIG. 2. Expression of Eda under various growth conditions. (A) Western blot of Eda in *E. coli* W1485 cells grown in M63 minimal glucose (glc), glucuronate (glr), or gluconate (gnt) medium. (B) Northern blot analysis of *eda* transcription in *E. coli* W1485 and *E. coli* CT110 (*gntR*) grown on glucose, glucuronate, or gluconate. Size markers in thousands of nucleotides are shown on the right.

tional regulation described below can be explained by a single promoter in this region, we conclude that P4 regulates *eda* expression during phosphate and carbon starvation.

P1 regulates induction by gluconate. Western analysis showed that Eda was produced in *E. coli* W1485 cells grown in M63 minimal medium containing 0.4% glucose and induced to higher levels when grown on gluconate (Fig. 2A). To determine which promoter was responsible for induction of Eda by gluconate, we compared levels of β-galactosidase production during growth on gluconate or glucose in cells carrying the various *eda-lacZ* promoter fusions. The transcriptional fusion to P1 (*eda*P1-*lacZ*) in *E. coli* NP304 was expressed at very low levels when cells were grown on glucose and induced 100-fold when cells were grown on gluconate (Table 2). No induction of *eda*P1-*lacZ* was observed in cells grown on glucuronate, galacturonate, 5-ketogluconate, *N*-acetylglucosamine, or *N*-acetylneuraminic acid; however, *eda*P1-*lacZ* was induced 25-fold when cells were grown on idonate and was induced 7-fold when cells were grown on 5-ketogluconate, conditions also known to induce the Entner-Doudoroff pathway (2). The P2 and P4 *eda* promoter fusion strains, when grown on gluconate, were not induced above the basal level of expression (Table 2). Induction of *eda* by gluconate was confirmed by Northern blot analysis (Fig. 2B), which showed increased expression of a 2,600-nucleotide (nt) transcript that was shown previously to correspond to P1 (7). Thus it is clear that P1 is responsible for induction of *eda* by gluconate, but not for high basal expression.

P2 regulates induction by hexuronates. Western blots showed that Eda was induced in *E. coli* W1485 cells grown on M63 minimal medium containing 0.4% glucuronate (Fig. 2A) or methyl-β-D-glucuronide (data not shown), which is another substrate of the Ashwell pathway. To determine which promoter was responsible for glucuronate-dependent Eda induction, β-galactosidase activity in various *eda-lacZ* fusion strains was measured. The *eda*P2-*lacZ* operon fusion in *E. coli* BM106 was induced 2.5-fold in cells grown on glucuronate, compared to induction in cells grown on glucose, and 1.7-fold in cells grown on galacturonate (Table 2). The *eda*P2-*lacZ* fusion was not induced by gluconate, 5-ketogluconate, *N*-acetylglucosamine, *N*-acetylgalactosamine, *N*-acetylneuraminic acid, or glycerol. Expression of *eda*P2-*lacZ* in cells grown on glucuronate with added glucose, but not with added gluconate, was repressed slightly compared to that in cells grown on glucuronate alone (Table 2). This suggests that *eda* P2 is subject to catabolite repression or is simply not induced to the same extent when alternative substrates are available, in keeping with the preference of *E. coli* for glucose over glucuronate (22). Induction of *eda* by glucuronate was confirmed by Northern blot analysis (Fig. 2B), which showed increased expression of a 1,000-nt transcript that was shown previously to correspond to P2 (7). From these results we conclude that P2 is responsible for induction of *eda* by hexuronates.

TABLE 2. Carbon source-dependent regulation of *eda-lacZ* fusions

Substrate[a]	β-Galactosidase activity (Miller units) for:		
	P1 fusion[b]	P2 fusion[c]	P4 fusion[d]
Glucose	10 +/− 6	174 +/− 46	173 +/− 58
Gluconate	1034 +/− 148	186 +/− 61	174 +/− 47
Glucuronate	13 +/− 7	440 +/− 98	177 +/− 45
Galacturonate	16 +/− 7	290 +/− 89	119 +/− 41
Glucose + gluconate	818 +/− 53	ND[e]	ND
Glucose + glucuronate	12 +/− 6	322 +/− 50	ND
Gluconate + glucuronate	904 +/− 160	297 +/− 110	ND
Idonate	244 +/− 59	ND	122 +/− 20
5-Ketogluconate	68 +/− 58	194 +/− 80	145 +/− 79
N-Acetylglucosamine	15 +/− 7	76 +/− 18	118 +/− 12
N-Acetylneuraminic acid	5 +/− 6	75 +/− 23	156 +/− 36
Glycerol	ND	78 +/− 16	171 +/− 44

[a] 0.2% carbon source in M63 minimal medium.
[b] *eda*P1-*lacZ* fusion, *E. coli* NP304.
[c] *eda*P2-*lacZ* fusion, *E. coli* BM106.
[d] *eda*P4-*lacZ* fusion, *E. coli* BM111.
[e] ND, not determined.

P4 regulates induction by phosphate starvation. Previously, two-dimensional gel electrophoresis of protein gels indicated that Eda is induced when *E. coli* was starved for phosphate (31). To directly determine if *E. coli* W1485 synthesizes larger amounts of Eda under phosphate-limiting conditions, cells were grown overnight in MOPS minimal medium containing 1.32 mM K_2HPO_4 and then transferred to the same medium containing 0.066 mM K_2HPO_4. The culture began to starve for phosphate after approximately 2.6 h. Western blots showed increased production of Eda upon phosphate starvation (Fig. 3A). To determine which promoter was responsible for *eda* induction upon phosphate starvation, the *eda-lacZ* fusions were tested under this condition; in this experiment the culture began to starve for phosphate after approximately 3.5 h. *E. coli* BM111, containing the *eda*P4-*lacZ* operon fusion, was induced two- to threefold after 2 h of phosphate starvation (Fig. 3B). Addition of phosphate to starved cells in stationary phase restored exponential growth of the culture and reduced *eda*P4-*lacZ* expression to prestarvation levels (data not shown). The

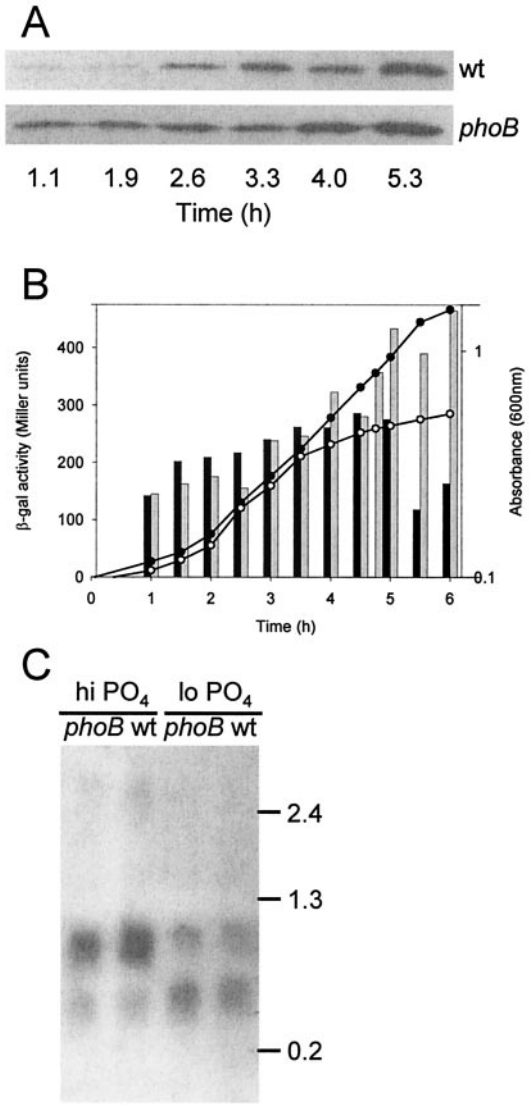

FIG. 3. Induction of Eda during phosphate starvation. (A) Western blot analysis of Eda synthesis in *E. coli* BW14087 (*phoB*) and *E. coli* BW13711 (wild type [wt]; isogenic *phoB*$^+$). Cells were grown in phosphate-limited minimal medium and harvested at the indicated times. (B) β-Galactosidase activity of an *eda-lacZ* fusion (*E. coli* BM111; P4 operon fusion). Black circles, growth on phosphate-replete medium; black bars, *eda-lacZ* fusion expression under these conditions; open circles, growth of cells on phosphate-limited medium; gray bars, *eda-lacZ* fusion expression under these conditions. A_{600} is shown in logarithmic scale. (C) Northern blot analysis of *eda* transcription in *E. coli* BW14087 (*phoB*) and *E. coli* BW13711(wt) grown on phosphate-replete (hi PO_4) or -limited (lo PO_4) medium. Size markers in thousands of nucleotides are shown on the right.

P1 and P2 fusions were not induced by phosphate starvation (data not shown). Induction of *eda* by phosphate starvation was confirmed by Northern blot analysis (Fig. 3C), which showed increased expression of a 750-nt transcript that was shown previously to correspond to P4 (7). Thus, we conclude that *eda* P4 is responsible for the induction of *eda* that occurs upon starvation for phosphate.

Eda is modestly induced by starvation, but not by stationary phase. To test the effect of starvation on Eda expression, *E. coli* W1485 was grown in MOPS-glucose minimal medium and Eda synthesis was measured by Western blotting. M63 minimal medium containing 0.2% glucose is designed such that *E. coli* is starved for both carbon and nitrogen (ammonia) when growth ceases (15). Eda levels were elevated upon exhaustion of 0.2% glucose (Fig. 4A and B). To test whether this increase was due to exhaustion of glucose or nitrogen, the experiment was repeated with 0.1% glucose, conditions which do not starve the cells for nitrogen; the same result was obtained (Fig. 4B). To examine Eda synthesis during non-starvation-induced stationary phase, *E. coli* W1485 was grown in five concentrations of LB (Fig. 4C). Cells grown in diluted concentrations of LB would be expected to enter stationary phase upon starvation for a limiting nutrient, while cells grown in more-concentrated LB would enter stationary phase for reasons other than

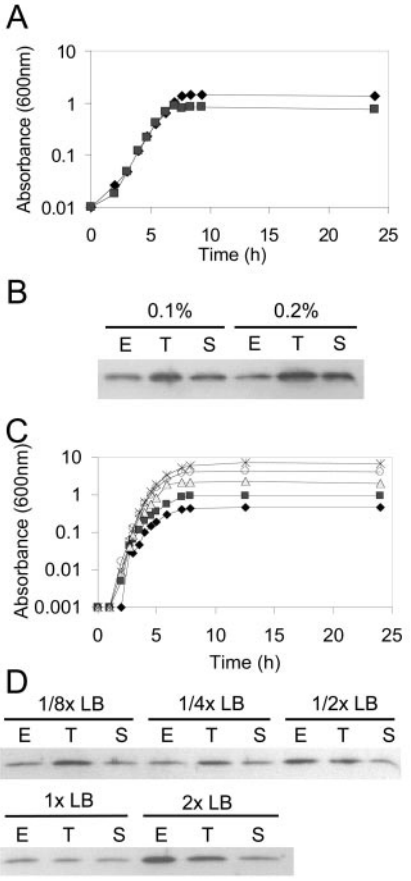

FIG. 4. Synthesis of Eda under carbon starvation and stationary-phase conditions. (A) Growth of *E. coli* W1485 in M63 minimal medium supplemented with 0.1% (squares) or 0.2% (diamonds) glucose. (B) Eda synthesis in *E. coli* W1485 as measured by Western blot analysis of cells harvested during exponential phase (E) or stationary phase (S; 2 h after inflection of growth curve) or during the transition into stationary phase (T; at inflection of the growth curve). (C) Simulation of non-starvation-induced stationary phase by growth of *E. coli* W1485 in one-eighth-strength (diamonds), one-fourth-strength (squares), one-half-strength (triangles), 1× (circles), or 2× (asterisks) LB. (D) Western blot analysis of Eda synthesis in *E. coli* W1485 under non-starvation-induced stationary-phase conditions during exponential phase, stationary phase (5 h after inflection of the growth curve), or the transition into stationary phase (at inflection of the growth curve).

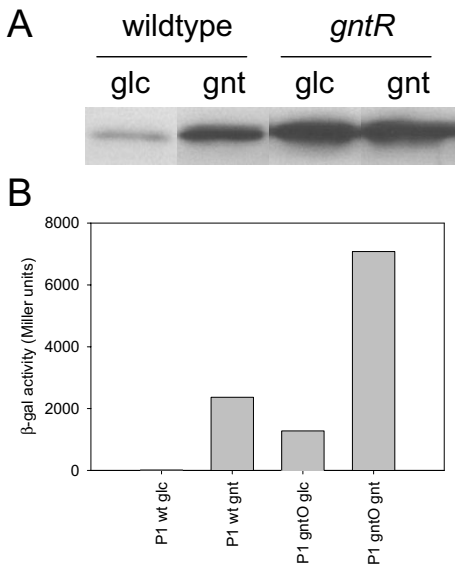

FIG. 5. GntR control of transcription from the *eda* P1 promoter. (A) Western blot analysis of Eda synthesis in *E. coli* W1485 (wild type) or *E. coli* HT216 (*gntR*) grown in glucose (glc) or gluconate (gnt) minimal medium. (B) Transcription of the *eda*Pl-*lacZ* fusion in *E. coli* NP304 (wild type [wt]) or NP305 (*gntO*) grown on glucose or gluconate.

starvation, perhaps as a result of the toxic buildup of metabolic by-products (17). Eda synthesis in cells grown on one-eighth- or one-fourth-strength LB increased slightly during the transition to stationary phase brought on by starvation (Fig. 4D). There was no increase in Eda production by cells during non-starvation-induced stationary phase when grown on one-half-strength, 1×, or 2× LB. Thus, there appears to be a correlation between starvation and Eda induction, but no correlation between Eda expression and stationary phase was established.

GntR controls transcription from the *eda* P1 promoter. To determine if the gluconate repressor protein GntR regulates Eda induction, Eda synthesis by the *gntR* mutant strain *E. coli* HT216 was monitored. Levels of Eda were high when cells were grown on either glucose or gluconate, indicating that Eda synthesis is derepressed in *E. coli* HT216 (Fig. 5A). The *eda* P1 promoter region contains two putative GntR binding sites (Fig. 1), which have a consensus sequence of ATGTTACCGGTA ACAT (21, 24). To determine if GntR binding is important for regulating *eda* expression, *eda*P1-*lacZ* containing a TTAC-to-CGCA mutation in the left half-site of the downstream GntR binding site was tested. The P1 operator mutation resulted in 145-fold derepression in cells grown on glucose and 2.6-fold derepression in cells grown on gluconate by comparison to results for the wild-type fusion (Fig. 5B). Interestingly, the downstream P1 operator site mutant remained inducible by gluconate (approximately threefold), probably because the upstream GntR binding site also contributed to repression by GntR, which was not tested. We previously showed that both operators in the *gntT* promoter region exert control over *gntT* transcription (19). In summary, these results indicate that transcription of *eda* from the P1 promoter is under negative control by GntR.

KdgR controls transcription from the *eda* P2 promoter. To determine if KdgR controls Eda synthesis, *E. coli* CT110, a *kdgR* mutant, was grown in minimal medium containing 0.4% glucose or glucuronate. In the wild-type strain, Eda was induced by growth on glucuronate. In the *kdgR* mutant, Eda was produced at high levels on both glucose and glucuronate (Fig. 2A). Transcription of *eda* in *E. coli* W1485 and *E. coli* CT110 grown in minimal medium containing 0.4% glucose or glucuronate was measured by Northern blot hybridization (Fig. 2B). The 1,000-nt transcript initiated from the P2 promoter was present under all conditions. This transcript was derepressed in *E. coli* CT110 grown on glucose and induced by glucuronate in the wild type. These data indicate that *eda* P2 has a high basal level of transcription, is induced by glucuronate, and is repressed by KdgR. A 750-nt transcript was also present under all growth conditions, and its abundance tended to correlate with that of the 2,600- and 1,000-nt transcripts, depending on which one was induced (Fig. 2B). This suggests that the 750-nt band in Northern blots may result, at least in part, from the processing of longer transcripts initiated from upstream promoters, although a band of this size can also result from transcription initiation from P4, which is not induced by hexuronates (Table 2).

Gel mobility shift analysis was used to determine if *eda* is regulated directly by KdgR binding. Purified KdgR retarded the mobility of a labeled DNA probe corresponding to the *eda* P2 promoter region, which contains the putative KdgR binding right half-site GTGTTTCAAA (Fig. 1), a close match to the consensus sequence, AAATGAAACAnTGTTTCATTT (24) (Fig. 6A). The *eda* probe was shifted with as little as 1.4×10^{-3} pmol of KdgR and was shifted completely by 1.4 pmol of KdgR. A probe containing a GTTT-to-GGGT mutation in the right half-site was not shifted by KdgR (data not shown). These results indicate that KdgR binds to the KdgR right half-site in the *eda* P2 promoter region.

PhoB-dependent regulation of P4. Many proteins induced in response to phosphate starvation are under the control of the two-component PhoB-PhoR regulatory system. Since Eda is apparently induced by phosphate starvation (Fig. 3), we sought to determine if Eda synthesis is under PhoB control. Eda levels in Western blots of *E. coli phoB* mutant strain BW14087 were compared with levels in blots of the isogenic wild-type strain, *E. coli* BW13711. Both strains were grown overnight in MOPS-glucose minimal medium containing 1.32 mM K_2HPO_4 and then subcultured by dilution into MOPS-glucose minimal medium containing 0.066 mM K_2HPO_4. Cells grown in phosphate-limiting conditions began to starve after 2.6 h and grew more slowly thereafter (data not shown). Western blot analysis revealed higher levels of Eda in the *phoB* mutant prior to starvation and at all times during growth than in the isogenic wild-type strain (Fig. 3A). Derepression of Eda synthesis in the *phoB* mutant suggests that PhoB may act as a repressor of *eda* transcription in a fashion that could be described as exponential silencing. Northern analysis of cells starved for phosphate showed slightly larger amounts of the 750-nt transcript originating from *eda* P4 in the *phoB* mutant than in the wild-type strain and a higher ratio of the 750- to 1,000-nt transcripts (Fig. 3C). Examination of the DNA sequence of the *eda* P4 region revealed a putative PHO box (Fig. 1), CTTGCGTGAAAAA CTGTCCG, upstream of the *eda* P4 promoter (32). Gel mo-

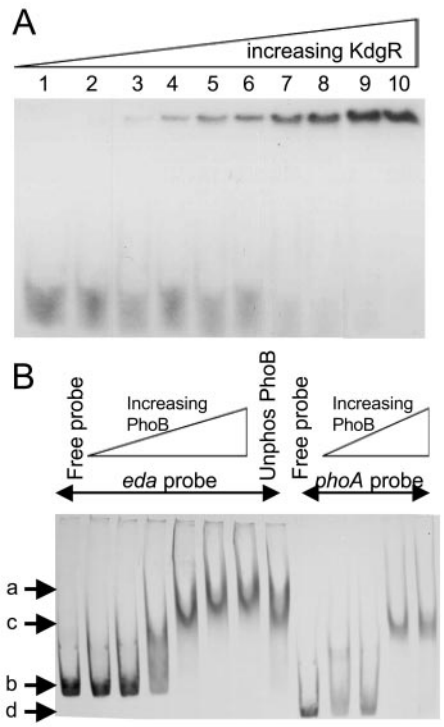

FIG. 6. (A) KdgR binding to the *eda* P2 promoter region. Shown is a gel mobility shift assay of the binding of recombinant KdgR to a radioactively labeled DNA fragment containing *eda* P2 and the putative KdgR binding site. Top band, bound probe; bottom band, free probe. Lanes 1 to 10, 0, 0.07, 0.14, 0.21, 0.28, 0.35, 0.49, 0.63, 1.41, and 2.11 pmol of KdgR, respectively. (B) PhoB binding. Shown is a gel mobility shift assay of PhoB binding to the *eda* and *phoA* regulatory regions. Lanes 1 to 13, 0, 1.99, 8.3, 24.9, 49.8, 58.1, 66.4, 49.8, 0, 1.66, 24.9, 58.1, and 59.1 pmol of PhoB, respectively. Arrows: a, bound-*eda* probe; b, free-*eda* probe; c, bound-*phoA* probe; d, free-*phoA* probe.

bility shift assays with a DNA probe containing the *eda* P4 promoter region confirmed binding by purified, phosphorylated PhoB (Fig. 7B). The affinity of PhoB for its binding site at P4 was comparable to the affinity of PhoB for its binding site at *phoA* (Fig. 6B). This experiment also showed that unphosphorylated PhoB can bind. From these results we conclude that the modest induction of Eda associated with phosphate starvation is mediated by negative transcriptional control by PhoB.

CsrA exerts indirect negative control of Eda levels. CsrA is a global regulatory protein that controls a variety of genes, including those encoding many central metabolism enzymes. To determine if CsrA regulates *eda*, synthesis of Eda in wild-type and *csrA* mutant strains grown in LB with or without gluconate or glucuronate was measured. All cultures grew at similar rates and reached similar final densities (data not shown). Western blots showed decreased Eda production in the *csrA* mutant, *E. coli* BM106c, by comparison to that by the parent strain, *E. coli* BM106, upon transition to stationary phase (Fig. 7A). Since CsrA affects the mRNA stability of target genes (26), we investigated the turnover rate for the *eda* transcript (Fig. 7B). There was no significant difference between the turnover rates for *eda* transcripts in *E. coli* BM106c and *E. coli* BM106 grown on rich (Kornberg's) medium containing 0.4% glucuronate. To determine if CsrA had an effect on the transcription of *eda* from any of its promoters, β-galactosidase activity in reporter strains with *csrA* mutant or wild-type backgrounds was assayed. Expression of the *eda*P1-*lacZ* fusion was decreased 1.6-fold in the *csrA* mutant strain grown on gluconate, compared to that in the wild-type (*csrA*$^+$) strain (Fig. 7C). Expression of the *eda*P2-*lacZ* fusion was decreased 1.8-fold in the *csrA* mutant strain grown on glucuronate (Fig. 7D). Expression of the *eda*P4-*lacZ* fusion was also decreased 1.8-fold in the *csrA* mutant strain grown on glucose (Fig. 7E). In addition, *E. coli csrA* strain BM106c (Fig. 6D) did not induce *eda* during the transition to stationary phase. Taken together, these results indicate that CsrA does not affect the turnover of *eda* transcripts but does have a generally positive effect on *eda* transcription.

DISCUSSION

The first goal of this study was to establish the number of promoters that drive the transcription of *eda*. We obtained evidence for three functional promoters located in the *edd-eda* region, designated P1, P2, and P4, and conclude that the previously identified P3 is not a functional promoter. The second goal of this study was to establish which promoters are responsible for *eda* expression under inducing conditions. Our results indicate that P1 is induced by growth on gluconate and idonate, P2 is induced by growth on hexuronates, and P4 is induced by phosphate starvation. Starvation for carbon, but not the transition to stationary phase, slightly increased Eda synthesis. The third goal of this study was to identify the regulatory factors that control *eda* transcription. We obtained evidence that P1 is under negative control of GntR, P2 is under negative control of KdgR, and P4 is under negative control of PhoB. CsrA appears not to affect *eda* transcript stability but appears to activate *eda*, since *eda* transcription was 1.6- to 1.8-fold lower in a *csrA* background. The final goal of this study was to characterize the mechanism of regulation of the three functional promoters. GntR regulates *eda* by binding to GntR binding sites in the P1 regulatory region. Likewise, KdgR and PhoB appear to directly affect expression from P2 and P4, respectively, by DNA binding. CsrA appears to activate *eda* expression but does so by an unknown mechanism. The convergence of these control systems on *eda* expression suggests multiple physiological roles for Eda.

Previously, we mapped four putative promoters in three locations within the *edd-eda* region (7). The results reported here indicate that P1, P2, and P4 drive the transcription of *eda* (Fig. 1). P1 is located 109 bp upstream of the *edd* start codon. Results from primer extension, Northern hybridization, and reporter fusions indicate that P1 is solely responsible for the induction of *eda* in cells grown on gluconate and idonate and drives transcription of the 2,600-nt polycistronic *edd-eda* transcript (Fig. 1). P2 is located 353 bp upstream of the *eda* start codon, within the *edd* coding sequence. The results presented here show that the 1,000-nt *eda* transcript originates from P2, which is solely responsible for the two- to threefold induction by glucuronate and galacturonate (Fig. 1). Finally, previous results from gluconate-grown cells indicated the presence of two closely spaced promoters, P3 and P4 (7). Primer extension analysis was used to dissect this regulatory region and showed

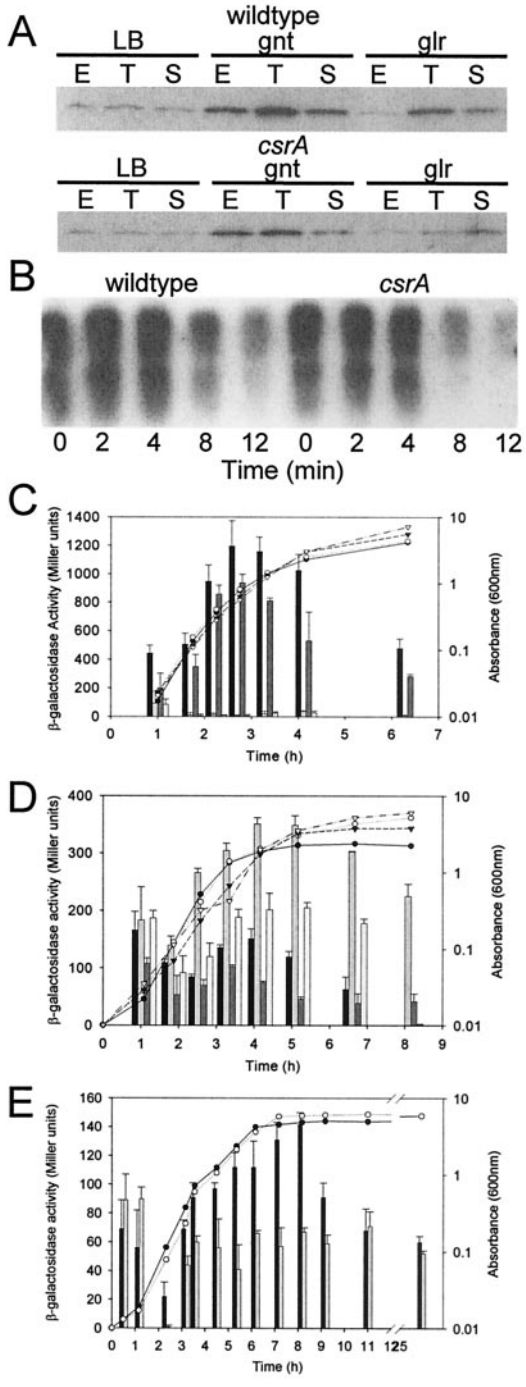

FIG. 7. CsrA control of *eda* expression. (A) Synthesis of Eda in *E. coli* BM106 (wild type) and BM106c (*csrA*) grown in LB, LB plus gluconate (gnt), or LB plus glucuronate (glr). Cells were harvested during exponential phase (E), stationary phase (S), or the transition from exponential to stationary phase (T). (B) Northern blot analysis of *eda* transcripts following rifampin treatment of *E. coli* BM106 (wild type) or BM106c (*csrA*). Cells were harvested at the indicated times. (C) β-Galactosidase activity and growth of *eda*P1-*lacZ* fusion strains *E. coli* NP304 (*csrA*$^+$) grown on glucose (black bars and open circles) and gluconate (dark gray bars and filled circles) and *E. coli* BM304c (*csrA*) grown on glucose (dark gray bars and open inverted triangles) and gluconate (light grey bars and filled inverted triangles). (D) β-Galactosidase activity and growth of *eda* P2-*lacZ* fusion strains *E. coli* BM106 (*csrA*$^+$) grown on glucose (black bars and open circles) and glucuronate (light gray bars and filled circles) and *E. coli* BM106c (*csrA*) grown on glucose (dark gray bars and open inverted triangles) and glucuronate (light grey bars and filled inverted triangles). (E) β-Galactosidase activity and growth of *eda*P4-*lacZ* fusion strains *E. coli* BM105 (*csrA*$^+$) grown on glucose (black bars and open circles) and *E. coli* BM105c (*csrA*) grown on glucose (gray bars and filled circles).

that P4 is active under phosphate and carbon starvation conditions (Fig. 1). No primer extension product corresponding to P3 was found under the conditions tested (Fig. 1). P4 is located in the intergenic region between *edd* and *eda*, 26 bp upstream of the *eda* start codon. Both P2 and P4 are responsible for the high basal level of *eda* expression. Northern analysis showed that a 750-nt transcript is expressed in cells grown on gluconate (Fig. 2). Since this transcript increases when upstream promoters are highly expressed, it seems likely that there is a transcript-processing site in this region, thus complicating the interpretation of Northern blots. However, β-galactosidase assays of *eda-lacZ* fusions showed there are no gluconate-inducible promoters in this region. Thus, we conclude that the *eda* P4 promoter is responsible for inducing a 750-nt transcript under phosphate starvation conditions.

In this study we identified three transcription factors that directly affect the transcription of *eda*. The first is GntR, a negative regulator of P1 (20). Our results confirm that Eda synthesis is derepressed in a *gntR* strain (Fig. 5A). There are two putative GntR binding sites located adjacent to P1 (21, 24), but these had not been previously analyzed. Mutation of the downstream GntR binding site caused derepression of the *eda*P1-*lacZ* fusion (Fig. 5B). GntR binding and the effector molecule gluconate most likely regulate P1 by a mechanism analogous to that described for *gntT* (19). Thus, *eda* is confirmed to be a member of the GntR regulon (20). The second factor affecting *eda* transcription is KdgR. Derepression of *eda* in a *kdgR* strain (Fig. 2) and the binding of KdgR to the P2 promoter in vitro (Fig. 6A) are in keeping with the role of the KdgR regulon in hexuronate catabolism (20, 22).

A third factor found to affect *eda* transcription is PhoB. *E. coli* W1485 induces Eda in response to phosphate limitation (Fig. 3), and the P4 promoter region is bound by PhoB in vitro (Fig. 6B). Phosphate starvation causes the sensor kinase PhoR to phosphorylate the response regulator PhoB, which in turn activates phosphate starvation genes (32). Although PhoB typically acts as a transcriptional activator, it is a repressor of *eda* expression (Fig. 3). Also, in contrast to typical PhoB-dependent control, we observed the binding of the P4 promoter region by unphosphorylated PhoB. While unphosphorylated PhoB possesses DNA binding capability, phosphorylated PhoB dimerizes, which increases its affinity for PhoB binding sites (9). Under phosphate-replete conditions, unphosphorylated, monomeric PhoB may bind to its P4 operator to repress *eda* expression. If phosphate becomes limiting, phosphorylated PhoB dimers may have a higher affinity for other PhoB binding sites and thereby derepress the *eda* P4 promoter. Negative control of target genes by PhoB, although rare, has been observed by others (Barry Wanner, personal communication). While the mechanism of Eda regulation by PhoB remains to be elucidated, it is clear that Eda is induced by phosphate limitation in a PhoB-dependent manner.

We found that, in addition to induction by phosphate starvation, Eda was induced by carbon starvation in minimal glucose medium and in dilute LB (Fig. 4). The results suggest that carbon starvation is the inducing signal and not entry into stationary phase per se, since Eda was not induced when growth ceased in higher-strength LB. The mechanism underlying Eda induction by carbon starvation was not established, but it is interesting to speculate that CsrA might be involved. CsrA, the carbon storage regulator, was originally identified as a regulator of glycogen biosynthesis, although later it was found that CsrA regulates gluconeogenesis, flagellum production, cell surface properties, and motility, indicating that it functions as a global regulator (25). It is also clear that CsrA is an activator of several glycolytic enzymes (25, 27). In keeping with this role, Eda expression in a *csrA* mutant strain was low. In addition, Eda was no longer induced during the transition to stationary phase in the *csrA* strain (Fig. 7). The mechanism underlying this control of *eda* transcript levels did not appear to involve CsrA-dependent modulation of transcript stability, as it was the same in wild-type and *csrA* strains, which suggests that CsrA activates *eda* indirectly.

Finally, we consider the several physiological roles of Eda (KDPG aldolase) which are suggested by its regulation. Since Eda is required for growth on hexonates and hexuronates, its induction by these sugar acids and inclusion of *eda* in both the GntR and KdgR regulons are easily understood. This dual control facilitates the induction of Eda only for growth on hexuronates, which is mediated by KdgR and does not require Edd, which is only necessary for converting 6-phosphogluconate to KDPG; coordinate induction of Edd and Eda for growth on hexonates is controlled by GntR. It may also be important that Eda does not vary in expression by more than threefold and is constitutively produced. It has been proposed that the high basal level of Eda is a mechanism to protect the cell from accumulation of toxic metabolites, such as KDPG (the substrate of Eda), which is formed by sugar acid catabolism (10), as well as glyoxylate, which is formed as a by-product of the TCA cycle (16, 18). In this respect, Eda might be regarded as a member of the glucose starvation stimulon, which includes coordinate repression of the TCA cycle and activation of glycolysis (17). The role of Eda in detoxification may explain why it is expressed at high basal levels; this might also explain why it is induced by starvation for carbon and phosphate. Increased Eda synthesis during phosphate limitation may supply phosphorylated metabolites where they are needed by draining the cytoplasmic pool of KDPG. In other words, imbalances in metabolism created by starvation for carbon or phosphate may lead to accumulation of toxic metabolites that are substrates for degradation by Eda. In this way, Eda may function as a stress response protein, in addition to its role in sugar acid catabolism.

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REFERENCES

1. **Ashwell, G.** 1962. Enzymes of glucuronic and galacturonic acid metabolism in bacteria. Methods Enzymol. **5:**190–208.
2. **Bausch, C., N. Peekhaus, C. Utz, T. Blais, E. Murray, T. Lowary, and T. Conway.** 1998. Sequence analysis of the GntII (subsidiary) system for gluconate metabolism reveals a novel pathway for L-idonic acid catabolism in *Escherichia coli.* J. Bacteriol. **180:**3704–3710.
3. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
4. **Chang, D. E., D. J. Smalley, D. L. Tucker, M. P. Leatham, W. E. Norris, S. J. Stevenson, A. B. Anderson, J. E. Grissom, D. C. Laux, P. S. Cohen, and T. Conway.** 2004. Carbon nutrition of *Escherichia coli* in the mouse intestine. Proc. Natl. Acad. Sci. USA **101:**7427–7432.
5. **Conway, T.** 1992. The Entner-Doudoroff pathway: history, physiology and molecular biology. FEMS Microbiol.Rev. **9:**1–27.
6. **Conway, T., K. C. Yi, S. E. Egan, R. E. Wolf, Jr., and D. L. Rowley.** 1991. Locations of the *zwf*, *edd*, and *eda* genes on the *Escherichia coli* physical map. J. Bacteriol. **173:**5247–5248.
7. **Egan, S. E., R. Fliege, S. Tong, A. Shibata, R. E. Wolf, Jr., and T. Conway.** 1992. Molecular characterization of the Entner-Doudoroff pathway in *Escherichia coli*: sequence analysis and localization of promoters for the *edd-eda* operon. J. Bacteriol. **174:**4638–4646.
8. **Entner, N., and M. Doudoroff.** 1952. Glucose and gluconic acid oxidation of *Pseudomonas saccharophila.* J. Biol. Chem. **196:**853–862.
9. **Fiedler, U., and V. Weiss.** 1995. A common switch in activation of the response regulators NtrC and PhoB: phosphorylation induces dimerization of the receiver modules. EMBO J. **14:**3696–3705.
10. **Fuhrman, L. K., A. Wanken, K. W. Nickerson, and T. Conway.** 1998. Rapid accumulation of intracellular 2-keto-3-deoxy-6-phosphogluconate in an Entner-Doudoroff aldolase mutant results in bacteriostasis. FEMS Microbiol. Lett. **159:**261–266.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
12. **Liu, M. Y., H. Yang, and T. Romeo.** 1995. The product of the pleiotropic *Escherichia coli* gene *csrA* modulates glycogen biosynthesis via effects on mRNA stability. J. Bacteriol. **177:**2663–2672.
13. **Luria, S. E., and M. Delbruck.** 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28:**491–511.
14. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
15. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119:**736–747.
16. **Nishihara, H., and E. E. Dekker.** 1969. A stereospecific 2-keto-4-hydroxyglutarate aldolase from *Escherichia coli.* Biochim. Biophys. Acta **185:**255–257.
17. **Nystrom, T.** 1994. The glucose-starvation stimulon of *Escherichia coli*: induced and repressed synthesis of enzymes of central metabolic pathways and role of acetyl phosphate in gene expression and starvation survival. Mol. Microbiol. **12:**833–843.
18. **Patil, R. V., and E. E. Dekker.** 1992. Cloning, nucleotide sequence, overexpression, and inactivation of the *Escherichia coli* 2-keto-4-hydroxyglutarate aldolase gene. J. Bacteriol. **174:**102–107.
19. **Peekhaus, N., and T. Conway.** 1998. Positive and negative transcriptional regulation of the *Escherichia coli* gluconate regulon gene *gntT* by GntR and the cyclic AMP (cAMP)-cAMP receptor protein complex. J. Bacteriol. **180:** 1777–1785.
20. **Peekhaus, N., and T. Conway.** 1998. What's for dinner?: Entner-Doudoroff metabolism in *Escherichia coli.* J. Bacteriol. **180:**3495–3502.
21. **Porco, A., N. Peekhaus, C. Bausch, S. Tong, T. Isturiz, and T. Conway.** 1997. Molecular genetic characterization of the *Escherichia coli gntT* gene of GntI, the main system for gluconate metabolism. J. Bacteriol. **179:**1584–1590.
22. **Pouyssegur, J. M., and F. Stoeber.** 1974. Genetic control of the 2-keto-3-deoxy-D-gluconate metabolism in *Escherichia coli* K-12: *kdg* regulon. J. Bacteriol. **117:**641–651.
23. **Powell, B. S., M. P. Rivas, D. L. Court, Y. Nakamura, and C. L. Turnbough, Jr.** 1994. Rapid confirmation of single copy lambda prophage integration by PCR. Nucleic Acids Res. **22:**5765–5766.
24. **Rodionov, D. A., A. A. Mironov, A. B. Rakhmaninova, and M. S. Gelfand.** 2000. Transcriptional regulation of transport and utilization systems for hexuronides, hexuronates and hexonates in gamma purple bacteria. Mol. Microbiol. **38:**673–683.
25. **Romeo, T.** 1998. Global regulation by the small RNA-binding protein CsrA and the non-coding RNA molecule CsrB. Mol Microbiol. **29:**1321–1330.
26. **Romeo, T., M. Gong, M. Y. Liu, and A. M. Brun-Zinkernagel.** 1993. Identification and molecular characterization of *csrA*, a pleiotropic gene from *Escherichia coli* that affects glycogen biosynthesis, gluconeogenesis, cell size, and surface properties. J. Bacteriol. **175:**4744–4755.
27. **Sabnis, N. A., H. Yang, and T. Romeo.** 1995. Pleiotropic regulation of central carbohydrate metabolism in *Escherichia coli* via the gene *csrA.* J. Biol. Chem. **270:**29096–29104.
28. **Simons, R. W., F. Houman, and N. Kleckner.** 1987. Improved single and

multicopy *lac*-based cloning vectors for protein and operon fusions. Gene **53:**85–96.
29. **Sweeney, N. J., D. C. Laux, and P. S. Cohen.** 1996. *Escherichia coli* F-18 and *E. coli* K-12 *eda* mutants do not colonize the streptomycin-treated mouse large intestine. Infect. Immun. **64:**3504–3511.
30. **Tong, S., A. Porco, T. Isturiz, and T. Conway.** 1996. Cloning and molecular genetic characterization of the *Escherichia coli gntR*, gntK, and *gntU* genes of GntI, the main system for gluconate metabolism. J. Bacteriol. **178:**3260–3269.
31. **VanBogelen, R. A., E. R. Olson, B. L. Wanner, and F. C. Neidhardt.** 1996. Global analysis of proteins synthesized during phosphorus restriction in *Escherichia coli.* J. Bacteriol. **178:**4344–4366.
32. **Wanner, B. L.** 1996. Phosphorous assimilation and control of the phosphate regulon, p. 1357–1381. *In* F. C. Neidhardt, R. Curtiss III, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.