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GntP Is the *Escherichia coli* Fructuronic Acid Transporter and Belongs to the UxuR Regulon

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***Escherichia coli* has four gluconate transporters, GntP, GntU, GntT, and IdnT, which are members of the major facilitator superfamily. The physiological function of GntP was previously unknown and is the subject of this study. GntP is not induced by gluconate, and despite being located adjacent to genes involved in glucuronate catabolism, *gntP* does not encode a glucuronate transporter. Here we identify *gntP* as the gene which encodes the fructuronate transporter. We show that *gntP* is induced by fructuronate and is a new member of the UxuR regulon: *gntP* is derepressed in an *uxuR* strain, UxuR binds in vitro specifically to an operator site that overlaps the *gntP* promoter, and UxuR binding is eliminated by fructuronate. Transcription of *gntP* requires activation by cyclic AMP (cAMP)-cAMP receptor protein. A *gntP* mutant cannot grow on fructuronate but grows normally on glucuronate and gluconate. Thus, the UxuR regulon is a module of sugar acid catabolism whose physiological role is for growth on fructuronate. Glucuronate, because it proceeds through a fructuronate intermediate, must induce the UxuR regulon and must also induce the ExuR regulon, which encodes the glucuronate transporter, ExuT, and the first step in its catabolism, UxaC. Thus, hexuronate catabolism in *E. coli* requires both the ExuR and UxuR regulons, while fructuronate catabolism requires only the UxuR regulon.**

Escherichia coli grows on several hexonates and hexuronates (19), some that feed into the Entner-Doudoroff pathway (5, 7) and others that feed into the Ashwell pathway (1). The pathways involve at least 26 known genes in six regulons that have been studied extensively (19, 27). Still, there are some long-standing mysteries regarding the transport and catabolism of sugar acids by *E. coli*. In particular, we are interested in *gntP*, which was implicated as being important for colonization of the mouse intestine by a human commensal *E. coli* strain (30). *gntP* is one of four genes which encode gluconate transporters (20), but previous studies showed that it is not induced by gluconate (10).

The *gntP* gene is adjacent to and divergently transcribed from *uxuAB* (2, 10). The *uxuA* and *uxuB* genes are negatively regulated by the product of the downstream gene, *uxuR*, and are induced for growth on glucuronate (1, 2, 8, 23–27). The *gntP* gene is predicted to be induced by glucuronate (27), but this has not been established experimentally. However, GntP does not appear to be involved in glucuronate catabolism: GntP is not homologous to the known glucuronate transporters, ExuT and KdgT, and GntP does not appear to transport glucuronate (10). Since GntP does not transport glucuronate and is not induced by gluconate, we explored the possibility that GntP transports one of the other sugar acids that support growth of *E. coli*, i.e., fructuronate (17).

Fructuronate is the first intermediate of the glucuronate pathway and is the inducer of the UxuR regulon (8, 17, 21, 26). Growth on fructuronate does not require the hexuronate transporter, ExuT, but rather, depends on another transporter, the identity of which was unknown (17). Thus, we hypothesized that *gntP* encodes the *E. coli* fructuronate transporter.

Since fructuronate is not commercially available, we devised a way to biosynthesize it from glucuronate. This preparation was used to demonstrate that growth on fructuronate depends on functional GntP, strongly implicating GntP as the primary fructuronate transporter. We show here that *gntP* is induced by fructuronate, regulated by UxuR, and requires activation by cyclic AMP (cAMP)-cAMP receptor protein (CRP). Based upon these findings, we suggest that GntP functions to transport fructuronate and that the UxuR regulon alone is sufficient for the initial steps of fructuronate catabolism.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *E. coli* strains and plasmids used for this study are listed in Table 1. *E. coli* strains were grown at 37°C in Luria-Bertani broth (LB) (12) or MOPS (morpholinepropanesulfonic acid) minimal medium (16) with or without added carbohydrate (0.2%). Cell growth was monitored spectrophotometrically, at 600 nm.

Construction of mutants. Transduction with bacteriophage P1 was described previously (11). *E. coli* I57 (26) served as the source of the *uxuR* allele. *E. coli* RH74 (13) was the source of the Δ*cya851* allele. RH77 (13) was the source of the Δ*crp-zhd732*::Tn*10* allele. All other mutants were constructed by allelic replacement and antibiotic resistance markers were excised as described previously (6). All mutations were verified by DNA sequencing of genomic DNA preparations.

Construction of *lacZ* chromosomal fusions. The *gntP* promoter region (720 bp) was amplified using primers 5′-GCGGATCCACCACCCAGAGAATGTTAAG CA-3′ and 5′-GCGGATCCGTATTCGAGGTCAGTACGGGTC-3′ and cloned into pRS551 (operon fusion vector) to make plasmid pCU110 (29). The plasmid construction was confirmed by restriction analysis. Single-copy chromosomal fusions were made by homologous recombination with the lambda phage λRS88 and integration of the lysogenic phage into the chromosome to create a single-copy *gntP-lacZ* fusion in the wild-type background, *E. coli* CU110. The copy number was confirmed as described previously (22). The same method was used

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TABLE 1. Plasmids and strains used

Strain or plasmid	Relevant genotype	Source or reference
E. coli strains		
MG1655Str^r	K-12 wild type (CGSC 7740) Str^r	15
W1485	K-12 wild type (CGSC 5024)	CGSC[a]
ABN11	MG1655Str^r Δ*uxuB*::*Kan*^{r}	This study
ABN12	MG1655Str^r Δ*gntP*::*Kan*^{r}	This study
ABN13	MG1655Str^r Δ*uxaC*::*Kan*^{r}	This study
ABN14	MG1655Str^r Δ*uxuB*::*Cam*^{r} Δ*uxaB*::*Kan*^{r}	This study
ABN15	MG1655Str^r Δ*uxaC*::*Cam*^{r} Δ*gntP*::*Kan*^{r}	This study
CU110	W1485 *gntP-lacZ*	This study
CU111	W1485 *uxuR gntP-lacZ*	This study
CU112	W1485 Δ*cya gntP-lacZ*	This study
CU113	W1485 Δ*crp gntP-lacZ*	This study
I57	*uxuR*	J. Robert-Boudouy
RH74	MC4100 Δ*cya851 ilv*::Tn*10*	R. Hengge-Aronis
RH77	MC4100 Δ*cya851*Δ*crp-zhd732*::Tn*10*	R. Hengge-Aronis
Plasmids		
pABN13	*uxaC*	This study
pQE30	Expression vector, His_6 affinity tag	18
pCU107	*uxuR*	This study
pCU108	*gntP* promoter, mutated operator	This study
pCU109	*gntP* promoter, mutated operator	This study
pCU110	*gntP* promoter 720-bp fragment	This study

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

to generate *gntP-lacZ* single-copy chromosomal fusions in *E. coli* CU111, CU112, and CU113.

β-Galactosidase assays. β-Galactosidase activity was determined as described previously and is expressed in Miller units (14). Reported values are the average of three independent measurements.

RNA isolation and Northern blot analysis. Total RNA was isolated by the hot phenol method (18) from *E. coli* W1485 grown to mid-log phase (optical density, 0.6). Contaminating DNA was removed by treatment with RNase-Free DNase (QIAGEN). RNA samples were electrophoresed on a 1.5% agarose-formaldehyde denaturing gel. RNA was transferred overnight onto a Nytran membrane using 20× SSC buffer (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). After prehybridization, hybridization with the radiolabeled probe was carried out overnight at 65°C. To synthesize radiolabeled RNA probes, internal fragments of *gntP*, *uxuA*, *uxuB*, and *uxuR* were cloned into pBluescript and confirmed by sequencing. The plasmids were linearized, and RNA was synthesized using T3 RNA polymerase and [α-^{32}P] UTP. The membranes were washed, dried, and visualized by exposure to Kodak X-ray film at room temperature.

QPCR. Transcript levels were confirmed by quantitative PCR (QPCR). Cells were grown on MOPS minimal medium to mid-log growth phase and total RNA was prepared by using RNA*later* (Ambion) and purified using an RNeasy MinElute (QIAGEN) column (31). Taqman probes and primers were designed by using Primer Express software provided with the ABI Prism 7000 sequence detector (Applied Biosystems). Transcript levels were normalized to 16S rRNA as described previously (9), and the values were expressed relative to cells grown on glucose.

Cloning of *uxuR* and purification of UxuR. UxuR was prepared by using the His tag modification system from QIAGEN. PCR primers were designed for amplification of the *uxuR* gene: 5′-CAAGCTTTGATGAAAATGCACC-3′ and 5′-CGGTACCAAATCTGCCACCTCT-3′. The amplified DNA fragment was digested with KpnI and HindIII and ligated into pQE30 to create pCU107. UxuR was overproduced and purified on a nickel-nitrilotriacetate column, as described previously (18).

Mobility shift binding assay. The DNA probe used for the gel retardation assays was PCR amplified with primers 5′-TCATGGTTGTTGCTGCAAA-3′ and 5′-TTTCATAGTGTGCAGCAGCG-3′, which produced a 113-bp fragment containing the putative UxuR binding site upstream of *gntP*. The DNA fragment was end labeled using [δ-^{32}P]ATP and T4 polynucleotide kinase. The binding mixture contained 1 μg of salmon sperm DNA, 0.66 mM dithiothreitol, the indicated amount of UxuR protein, 8 nmol of probe, and binding buffer (50 mM Tris, pH 7.5, 100 mM KCl, 10% glycerol, and 1 mM EDTA) in a total volume of 17.5 μl. The binding mixture was incubated at room temperature for 30 min, loaded onto a 5% nondenaturing agarose gel prepared in Tris-borate-EDTA buffer, and electrophoresed for 2 h. Dried gels were subjected to autoradiography. A small amount of pure fructuronate was provided by Hans J. Nelis from the University of Gent. Other sugars were purchased from Sigma-Aldrich Co.

To construct mutant fragments of the operator, PCR primers were designed to introduce two nucleotide replacements in the UxuR binding site. Primers for pCU108 were 5′-GATATGTTATGTAAATTAATCAACCATTGTTGCGATG-3′ and 5′-CATCGCAACAATGGTTGATTAATTTACATAACATATC-3′ and for pCU109 were 5′-GATATGTTATGTAAATTGGTCGGCCATTGTTGCGATG-3′ and 5′-CATCGCAACAATGGCCGACCAATTTACATAACATATC-3′, using pCU110 as a template. The mutant binding sites were confirmed by sequencing.

Biosynthesis of fructuronate. Fructuronate was biosynthesized in cell extracts of *E. coli* ABN14 (pABN13) which overproduces glucuronate isomerase and is deficient for fructuronate oxidoreductase (Fig. 1). The mutant strain was grown in LB medium supplemented with 0.5 mM IPTG (isopropyl-β-D-thiogalactopyranoside), harvested at mid-log phase, and lysed by sonication. The cell extract was then centrifuged and the supernatant incubated in a 20 mM solution of glucuronate at 37°C for 30 min. The resulting mixture of glucuronate and fructuronate was heat inactivated and filtered (0.45-μm pore size). To prepare fructuronate from this mixture, MOPS minimal medium was added to grow *E. coli* ABN12 (*gntP* mutant), which selectively consumed the glucuronate. Likewise, fructuronate was removed from the mixture by growth of *E. coli* ABN13 (*uxaC* mutant), leaving only glucuronate. The cells were centrifuged and the supernatants, which were significantly enriched for fructuronate or glucuronate, respectively, were collected and filter sterilized for use in growth experiments. Biochemical confirmation of fructuronate in the enriched sample was prevented by the cell extract components.

RESULTS

Transcriptional analysis of the UxuR regulon. To identify the conditions in which *gntP* is induced, expression from a single-copy chromosomal *gntP-lacZ* operon fusion was measured (Table 2). In wild-type cells, *gntP* was induced fivefold by glucuronate but not by other sugar acids. Northern hybridization analysis confirmed induction of a 1.5-kb *gntP* transcript (10) by glucuronate (Fig. 2A). The *uxuA* and *uxuB* transcripts were also induced by growth on glucuronate (Fig. 2B). The presence of two transcripts that hybridized to the *uxuA* and *uxuB* probes suggests that the primary 2.9-kb transcript is processed. Glucuronate induction of *gntP* and *uxuB*, as well as *eda*, which encodes the Entner-Doudoroff aldolase, was confirmed by QPCR (Table 3). These results suggest that transcription of *gntP*, *uxuA*, and *uxuB* is coordinated. Thus, *gntP* behaves like other known glucuronate-induced genes.

Catabolite repression of *gntP*. Sequence analysis indicated a CRP binding site in the *gntP* regulatory region. This location, at position −42 with respect to the previously mapped transcription start site (10), is indicative of class II activation (3). A *gntP-lacZ* operon fusion was repressed 7- and 3.5-fold in the presence of glucose or gluconate, respectively, by comparison to LB medium alone (Table 2). Also, addition of glucose to cells growing on LB-glucuronate resulted in 30-fold catabolite repression of *gntP* transcription compared to glucuronate alone (Table 2). QPCR confirmed that the *gntP* transcript is eightfold higher on minimal fructuronate medium compared

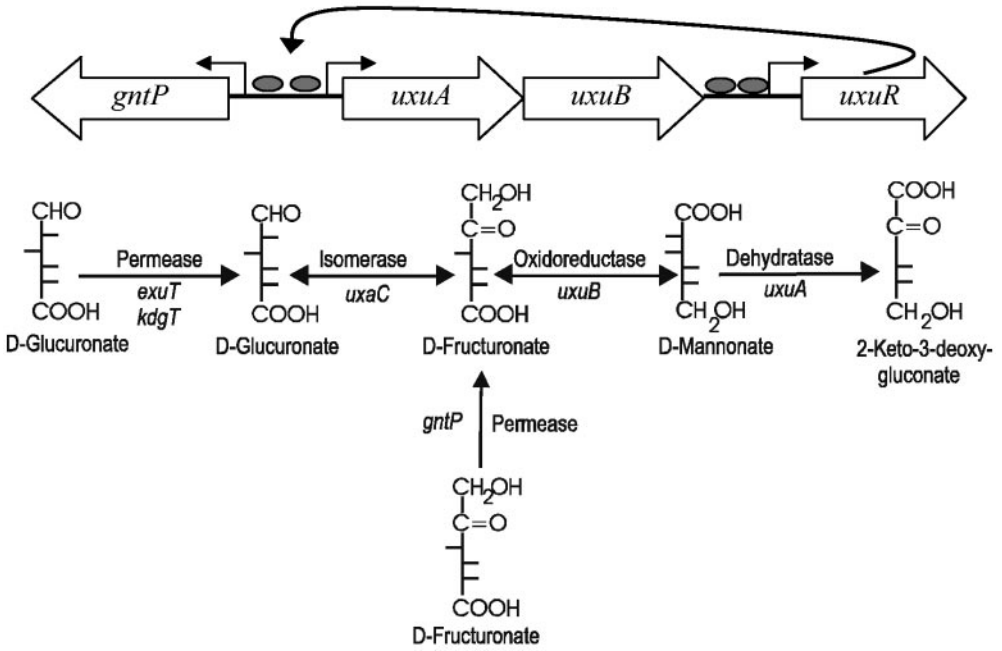

FIG. 1. Gene map of *gntP* region (top). Grey ovals indicate putative UxuR binding sites (27). Bottom, proposed pathway for fructuronate metabolism in *E. coli*.

with the mixture of fructuronate plus glucose (Table 3). Likewise, the *uxuB* transcript was more than twofold higher on fructuronate than on fructuronate plus glucose (Table 3). Exogenous cAMP relieved catabolite repression of glucuronate-dependent induction of *gntP* transcription caused by glucose, presumably by allowing formation of cAMP-CRP complex and activation by binding to the CRP site (Table 2). Exogenous cAMP also caused a twofold induction over that by glucuronate alone (Table 2).

To further examine cAMP-dependent catabolite repression, a *gntP-lacZ* fusion was constructed in *cya* and *crp* mutants. In the wild-type background expression of the *gntP-lacZ* fusion was approximately 100-fold higher than in either the *crp* or *cya* mutants, indicating that cAMP and CRP are required for activation (Table 2). As expected, addition of exogenous cAMP to the *cya* mutant, but not the *crp* mutant, relieved catabolite repression. These results demonstrate that transcription of *gntP* requires activation by the cAMP-CRP complex.

Effect of a *uxuR* mutation on *gntP* expression. To investigate the role of UxuR on the expression of *gntP*, the *gntP-lacZ* chromosomal fusion was transduced into an *uxuR* mutant to create *E. coli* CU111. The *gntP-lacZ* fusion in *E. coli* CU111 was derepressed 10-fold in the absence of glucuronate, in comparison to the wild type (Table 2). Expression of the *gntP-lacZ* fusion was repressed approximately twofold in the presence of glucuronate compared with the fully induced level in the wild type grown on glucuronate. These results confirm that UxuR is the negative regulator of *gntP* and suggest that glucuronate, in addition to glucose and gluconate, may be a catabolite repressing sugar. However, there is no evidence that glucuronate is preferred over fructuronate, as growth of *E. coli* on the mixture does not result in diauxie (data not shown).

Binding of UxuR to the *gntP* operator. Since mutation of *uxuR* derepressed *gntP* expression, we measured binding of UxuR to the *gntP* regulatory region. Other researchers have searched all known genes involved in hexuronate catabolism to derive a putative UxuR operator consensus sequence, AAAT TGGTNNACCAATTT (27). The *gntP* UxuR operator lies within the promoter region, centered at −22 with respect to the transcriptional start site (10). The UxuR operator of *gntP*

TABLE 2. Effects of *cya*, *crp*, and *uxuR* mutations on *gntP*::*lacZ* expression[a]

E. coli strain	Relevant genotype	β-Galactosidase activity (Miller units)							
		None	Glc	Gnt	Galnt	Gln		Gln+Glc	
						None	+cAMP	None	+cAMP
CU110	wt	176	25	50	171.5	870	1,693	27	1,470
CU111	*uxuR*	1,700	ND	ND	ND	394	ND	ND	ND
CU112	*cya*	19	ND	ND	ND	13	1,985	0	1,565
CU113	*crp*	8	ND	ND	ND	8	8	0	0

[a] Growth conditions were in LB medium plus the relevant carbon source (Glc, glucose; Gnt, gluconate; Galnt, galacturonate; Gln, glucuronate; none, no added cAMP). wt, wild type; ND, results have not been determined.

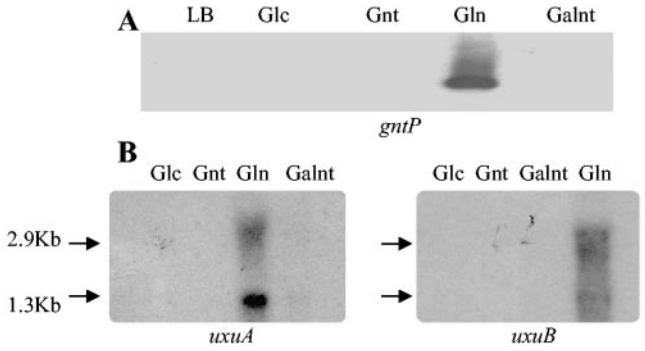

FIG. 2. A. Northern blot analysis of *gntP* transcription with a *gntP*-specific probe. RNA samples from *E. coli* W1485 grown in LB medium with indicated carbon sources (0.2%): LB, none; Glc, glucose; Gnt, gluconate; Gln, glucuronate; Galnt, galacturonate. B. Northern blot of the *E. coli uxuA* and *uxuB* transcripts with gene-specific probes. RNA samples were isolated from *E. coli* W1485 cells grown in LB medium with the indicated carbon sources (0.2%).

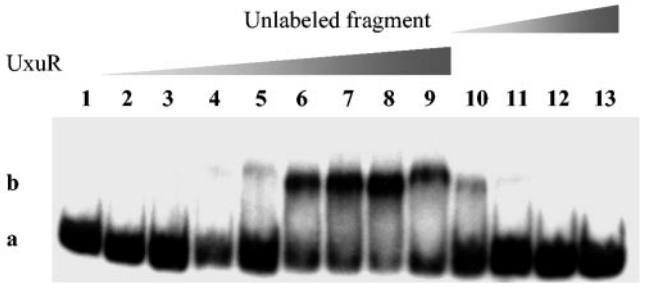

FIG. 3. Gel retardation of a ^{32}P-labeled *gntP* promoter probe by purified UxuR. All lanes contained 8 nmol of a radioactively labeled DNA fragment containing the UxuR operator. Lane 1, no protein. Lanes 2 to 9 contain increasing amounts of UxuR: 0.9, 1.8, 3.6, 7.2, 14.4, 28.8, 57.6, and 115.2 nmol, respectively. Lanes 10 to 13 represent competition experiments using unlabeled *gntP* fragments: lane 10, 25×; lane 11, 100×; lane 12, 200×; and lane 13, 300× unlabeled fragment (by weight). The shifted band (b) and free DNA (a) are indicated on the left.

has the sequence ACAATGGTTGACCAATTT, which matches the right-half site exactly and six of eight positions in the left-half site of the consensus binding site. To determine interactions between UxuR and the *gntP* operator and to identify the inducer of the regulon, gel mobility shift assays were performed. A DNA probe containing the *gntP* operator was end labeled with ^{32}P and incubated with increasing amounts of UxuR protein. A single band with reduced mobility, corresponding to binding of UxuR, was observed (Fig. 3). Addition of a 200-fold excess of the unlabeled DNA fragment completely abolished the shift.

To determine the inducer of the UxuR regulon, various pure sugars were tested for their ability to inhibit formation of the UxuR-DNA complex (Fig. 4). Pure fructuronate, provided by Hans J. Nelis, was the only sugar that inhibited formation of the complex. To prove that UxuR binds specifically to the putative consensus UxuR operator sequence, we constructed mutants carrying two base pair substitutions in either the left- or right-half sites; both mutations drastically reduced binding (Fig. 5). These results indicate that UxuR binding to the UxuR operator is sequence specific and that binding is inhibited by fructuronate.

Induction of the UxuR regulon by fructuronate. Since fructuronate inhibits UxuR binding to the *gntP* operator, we tested whether fructuronate was able to induce genes of the UxuR regulon. QPCR showed that *gntP* and *uxuB* were induced 88- and 400-fold, respectively, by fructuronate and 120- and 140-fold, respectively, by glucuronate compared to glucose (Table 3). In addition, fructuronate induced *eda* approximately four-fold, but *edd*, which is not in the Ashwell pathway, was not induced (Table 3). Thus, the results indicate that growth on fructuronate induces GntP and the enzymes involved in fructuronate catabolism. Since glucuronate does not inhibit binding of UxuR to the *gntP* operator, the results confirm that fructuronate formed from glucuronate is responsible for induction of the UxuR regulon during growth on glucuronate.

Mutation of *gntP* prevents growth on fructuronate. The wild-type strain grew to a final density of 1.61 (A_{600} units) on the mixture of glucuronate and fructuronate, which was biosynthesized as described in Materials and Methods (Table 4). Diminished cell yield of the wild type on the glucuronate and fructuronate preparations provided evidence that the biosynthesis strategy worked to selectively remove the countercomponent of the mixture. The ratio of cell yields (A_{600} 1.10 versus 0.49) on the enriched preparations indicated that the original mixture contained glucuronate and fructuronate in approximately a 2:1 ratio. Since the cell yield on the enriched preparations is roughly additive, it appears that *E. coli* uses the two sugars with similar efficiency. The *uxaC* mutant had final cell yields on the mixture (A_{600}, 0.57) and on fructuronate (A_{600}, 0.51) equivalent to that of the wild-type strain grown on fructuronate alone (Table 4). The *uxaC* mutant was unable to grow on glucuronate and grew on fructuronate with a generation time similar to that of the wild type (Table 5). The *gntP* mutant grew on the mixture and on glucuronate to final densities of A_{600} 1.20 and 1.11, respectively, which is equivalent to that of the wild-type strain grown on glucuronate alone (Table 4). Furthermore, the *gntP* mutant grew on glucuronate at the same

TABLE 3. Transcript levels of key pathway genes measured by QPCR[a]

Substrate	*gntP*		*uxuB*		*edd*		*eda*	
	Δ Fold	SD	Δ Fold	SD	Δ Fold	SD	Δ Fold	SD
Gluconate	2.2	0.16	0.79	0.070	53	7.4	1.7	0.040
Glucuronate	120	6.4	140	7.2	0.87	0.090	1.5	0.15
Frn	88	2.0	400	43	0.63	0.10	4.4	0.52
Frn + glucose	11	0.92	180	27	3.5	0.35	9.5	1.1

[a] Δ Fold, relative to cells grown on minimal glucose medium. Frn, fructuronate; SD, standard deviation.

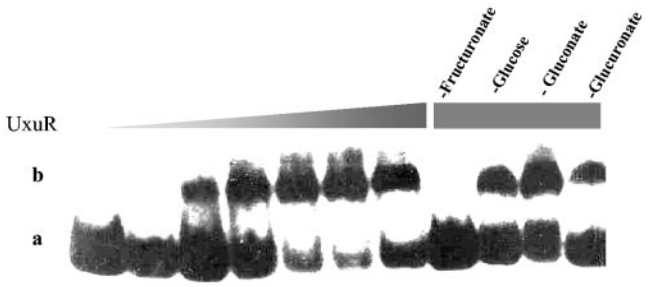

FIG. 4. Binding of UxuR to the *gntP* operator in the presence of various sugars. All lanes contained 8 nmol of radioactively labeled DNA probe. Lane 1 contains no protein and lanes 2 to 7 contain increasing amounts of UxuR protein: 0.9, 1.8, 3.6, 7.2, 14.4, and 28.8 nmol, respectively. Lanes 8 to 11 contain 28.8 nmol of UxuR protein together with 100 mM D-fructuronate (lane 8), D-glucose (lane 9), D-gluconate (lane 10), or D-glucuronate (lane 11). The shifted band (b) and free DNA (a) are indicated on the left.

rate as the wild type but was unable to grow on fructuronate (Table 5). Growth of the *gntP* mutant was not affected on glucose or galacturonate. The results confirm the hypothesis that a *gntP* mutant cannot grow on fructuronate, presumably due to a defect in fructuronate transport.

DISCUSSION

The primary findings of the present study are that *gntP* is a member of the UxuR regulon, induced by fructuronate, and that GntP is required for growth on fructuronate. Since GntP is in the major facilitator superfamily of sugar transporters (28) and is a proven sugar acid transporter (10), we conclude that GntP is the transporter for fructuronate. The scheme for fructuronate transport and catabolism is shown in Fig. 1.

The *gntP* gene is adjacent to and divergently transcribed from the *uxuAB* operon (2, 10, 24). The results shown in Tables 2 and 3 and Fig. 2 demonstrate that transcription of *gntP* is induced by glucuronate and fructuronate. Genes in the UxuR regulon are known to be induced by glucuronate via its conversion to fructuronate and are under the negative control of UxuR (17, 26). Derepression of *gntP* in an *uxuR* mutant strongly suggests that it is a new member of the UxuR regulon

TABLE 4. Final growth yield of wild-type and mutant strains grown on fructuronate, glucuronate, and fructuronate plus glucuronate[a]

Substrate	WT		*uxaC*		*gntP*	
	A_{600}	SD	A_{600}	SD	A_{600}	SD
Gln	1.10	0.045	0.10	0.042	1.11	0.06
Frn	0.49	0.024	0.51	0.030	0.09	0.02
Frn + Gln	1.61	0.023	0.57	0.089	1.20	0.04

[a] Carbon sources biosynthesized and enriched as described in Materials and Methods. Frn, fructuronate; Gln, glucuronate; WT, wild type; SD, standard deviation.

(Table 2). UxuR is known to bind to a consensus operator site that is conserved in several species (27). We identified a putative UxuR binding site overlapping the *gntP* promoter and confirmed that this site is bound in vitro (Fig. 3 and 5). Furthermore, we demonstrated that binding of the UxuR operator in the *gntP* regulatory region is specifically inhibited by fructuronate (Fig. 4). Taken together, these results prove that *gntP* is a member of the UxuR regulon.

The data in Tables 2 and 3 indicate that *gntP* and, to a lesser extent, *uxuB* are catabolite repressed by glucose. As expected, cAMP-CRP is required to activate *gntP* transcription. These results indicate that *E. coli* prefers glucose over fructuronate and glucuronate. We have observed diauxic growth on a mixture of glucose and glucuronate but no diauxie for growth on the mixture of fructuronate and glucuronate (data not shown).

GntP is an inner membrane protein with 12 membrane-spanning domains (10). GntP was previously categorized as a high-affinity gluconate transporter, but growth on gluconate does not require GntP (data not shown) and *gntP* is not induced by gluconate (10). Despite the fact that *gntP* is induced by glucuronate, it does not appear to be the glucuronate transporter, since glucuronate does not compete with gluconate for uptake by GntP in *E. coli* (10). On the basis of these observations, we postulated that GntP is actually a fructuronate transporter. This hypothesis was confirmed by the finding that a *gntP* mutant is unable to grow on fructuronate as a sole carbon and energy source (Tables 4 and 5).

The UxuR regulon is best described as a sugar acid catabolism module whose role, first and foremost, is for growth on

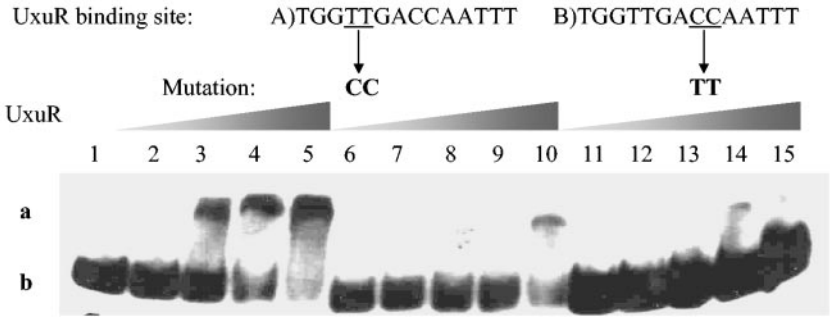

FIG. 5. Effect of UxuR binding site mutations on formation of the repressor-operator complex. The wild-type UxuR binding site is shown at the top with the mutated base pairs within the binding sites underlined and the alterations shown in boldface below the wild-type binding site. Lane 1 contains the wild-type DNA probe and no protein. Lanes 2 to 5 contain the wild-type binding site and increasing amounts of UxuR: 0.9, 1.8, 3.6, and 7.2 nmol, respectively. Lanes 6 to 10 contain the DNA probe with a mutation in the left-half site (A) and increasing amounts of UxuR (same concentrations as lanes 2 to 5). Lanes 11 to 15 contain the DNA probe with a mutation in the right-half site (B) with increasing amounts of UxuR (same concentrations as lanes 2 to 5). The shifted band (b) and free DNA (a) are indicated on the left.

TABLE 5. Generation times of wild-type and mutant strains grown on sugar acids[a]

Substrate	WT		*uxaC*		*gntP*	
	G (min)	SD	G (min)	SD	G (min)	SD
Glucose	79	5	78	0.10	81	4.9
Glucuronate	81	8.9	NG		81	0.2
Galacturonate	105	4.5	NG		106	4.3
Fructuronate	96	1.1	105	25	NG	

[a] G (min), generation time in minutes. NG, no growth; SD, standard deviation; WT, wild type.

fructuronate. Fructuronate is the true inducer of the UxuR genes, including *gntP*, a member of the UxuR regulon that encodes the fructuronate transporter. Thus, catabolism of fructuronate requires only the UxuR regulon and the KdgR regulon, which lies downstream in the catabolic scheme (Fig. 1).

Although glucuronate also induces the UxuR regulon, it does so via its conversion to fructuronate (26). Transport of glucuronate is not mediated by a transporter in the UxuR regulon, but rather by ExuT, which is a member of the ExuR regulon (17). Glucuronate catabolism requires first the ExuR regulon, which controls transport of glucuronate and galacturonate and their conversion to fructuronate and tagaturonate, respectively, and then the UxuR and KdgR regulons. Thus, hexuronate catabolism in *E. coli* involves three distinct functional modules encoded by the ExuR, UxuR, and KdgR regulons. Within these modules, the pathway enzymes have overlapping functions as well as multiple target substrates. Repressors belonging to the three regulons also act cooperatively in inducing hexuronate metabolism. Previous studies showed that synthesis of enzymes encoded by *uxuAB* is principally regulated by UxuR and partially regulated by ExuR (26). Our finding that *gntP* is induced by both fructuronate and glucuronate is consistent with the interplay that exists within the hexuronate catabolic modules. Glucuronate, because it proceeds through a fructuronate intermediate, must induce the UxuR regulon and must also induce the ExuR regulon, which encodes the glucuronate transporter, ExuT, and the first step in its catabolism, UxaC. The UxuR regulon, on the other hand, can be thought of as a module that is principally involved in catabolism of fructuronate, which is transported by GntP.

Sweeney et al. suggested that a *gntP* mutation diminished the fitness of a human commensal *E.* coli strain for colonization of the mouse intestine (30). This finding suggested the possibility that fructuronate is important for *E. coli* colonization. Recently we completed a systematic nutritional characterization of *E. coli* MG1655 colonization with respect to its carbon nutrition requirements (4). In contrast to the earlier result, we found that a *gntP* mutant of *E. coli* MG1655 was perfectly fit for colonization of the mouse intestine. To our knowledge, fructuronate has not been found in the mouse intestine (19). In fact, there are no published reports that describe the natural occurrence of fructuronate. Thus, the ecological importance of GntP as a fructuronate transporter in *E. coli* remains to be established.

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