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Cloning and Endonuclease Restriction Analysis of *uidA* and *uidR* Genes in *Escherichia coli* K-12: Determination of Transcription Direction for the *uidA* Gene

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The two genes of the *Escherichia coli* K-12 *uid* region (the structural gene *uidA* and the regulatory gene *uidR*) were isolated on a ColE1-*uid* hybrid plasmid from the bank of Clarke and Carbon. We made a restriction map of this region and correlated it with the genetic map by subcloning the *uid* restriction fragments into plasmids pBR322, pBR325, and pACYC177. In these plasmids, amplification of the products of the *uidA* and *uidR* genes occurred. The enzyme coded for by *uidA* was identified by polyacrylamide gel electrophoresis of crude extracts from strains containing the *uidA* plasmid. A 1-megadalton *Eco*RI-*Bam*HI segment contained the *uidAo* operator, and the direction of transcription of the *uidA* gene was determined. The restriction analysis also suggested that the order of the loci in this region is *manA*, *uidA*, *uidAo*, *uidR*.

β-D-Glucuronidase is the first enzyme of the hexuronide-hexuronate pathway in *Escherichia coli* K-12 (1; F. Stoeber, D. Sc. thesis, University of Paris, Paris, France, 1961). Methyl-β-D-glucuronate induces the synthesis of this enzyme (20). The *uidA* structural gene of β-D-glucuronidase and the *uidR* regulatory gene, which controls *uidA* expression, are located at 36 min on the *E. coli* linkage map, between the *manA* and *add* genes (14, 21). Genetic evidence suggests that β-D-glucuronidase synthesis is negatively controlled by the product of *uidR* (22). The regulatory gene *uxuR* also exerts a weak negative control on *uidA* expression (23).

In this work, as a first step in studying the multiple regulation of hexuronide metabolism in vitro, we cloned the *E. coli uid* chromosomal region into multicopy plasmid vectors. We present a restriction map of this DNA segment and its correlation with the genetic map. Some strains in which the *uidA* gene has been cloned produce large amounts of β-D-glucuronidase. The *uidAo* operator site was located on a small endonuclease-generated fragment, and the transcription direction of *uidA* was determined.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this investigation are listed in Table 1. All of the bacterial strains were *E. coli* K-12 derivatives.

Culture media. The media used for growth were those described by Novel and Novel (21). The minimal medium used was M63 (pH 7.2) (27) or M9 (pH 7.2) (19). Solid media contained glucose (5 g/liter), glycerol (5 g/liter), glucuronate (2.5 g/liter), or methyl-β-D-glucuronide (2 g/liter). When needed, ampicillin, tetracycline, chloramphenicol, or kanamycin was added at a final concentration of 25 μg/ml. Colicin E1 was prepared from strain JF390 by the method of Spudich et al. (28).

Chemicals and enzymes. Methyl-β-D-glucuronide (Stoeber, D. Sc. thesis) was synthesized in our laboratory. D-Glucuronate was purchased from Sigma Chemical Co., St. Louis, Mo.; *p*-nitrophenyl-β-glucuronide was from Calbiochem, La Jolla, Calif. *Eco*RI, *Bam*HI, *Hin*dIII, and T4 DNA ligase were obtained from Miles Laboratories, Inc., Elkhart, Ind., and *Bgl*II, *Sma*I, *Sal*I, and *Pst*I were from Boehringer Mannheim Corp., New York, N.Y. Chloramphenicol was from Sigma; tetracycline, ampicillin, and kanamycin were obtained from Serva Feinbiochemica, Heidelberg, Germany. Purified *E. coli* K-12 β-D-glucuronidase was obtained from Boehringer Mannheim.

Enzyme induction and extract preparation. The conditions used for induction and extract preparation of β-D-glucuronidase have been described by Novel et al. (20).

Enzyme assays. β-D-Glucuronidase was assayed as described by Novel and Novel (22). In situ plate assays for β-D-glucuronidase activity were performed on clones grown overnight on glycerol agar medium, as described by Novel and Novel (22). Only clones derepressed for β-D-glucuronidase activity became yellow under these conditions. Assays for β-D-glucuronidase in polyacrylamide gels were performed by covering the gel surface with a solution of 10 mM *p*-nitrophenyl-β-D-glucuronide. After 15 min of incubation at 37°C, the protein bands with β-D-glucuronidase activity became yellow. β-Lactamase activity was measured as described by Sykes and Nordström (30).

Isolation and manipulation of plasmid DNA. Plasmid DNA was amplified in growing cultures by adding 150

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype	Reference
Bacterial strains		
1830	*uidA manA arg lac gal mtl xyl*	This laboratory
JF390	*Para Coli* Ca57 (Colc)	11
JA200	Δ*trpE5 thr leu recA lacY*	9
HB101	*pro leu rpsL hsdM hsdR endoI recA lacY*	6
2523	*uidR recA rpsL fadD88 gal*	This laboratory
Sφ200	*metB rpsL purB* Δ(*add-uid-man*)	14
Plasmids		
pBR322	*bla*$^+$ *tet*$^+$	5
pBR325	*bla*$^+$ *tet*$^+$ *cat*$^+$	4
pACYC177	*bla*$^+$ *aac*$^+$	8
pMC874	*aac*$^+$	7

μg of chloramphenicol per ml (10). Extraction and purification of plasmid DNA were achieved by the alkaline extraction procedure described by Birnboim and Doly (3) for rapid analysis of restriction endonuclease digestion patterns. The cleared-lysate technique of Guerry et al. (12) was also used. Further purification of the DNA was done by dye-buoyant centrifugation in CsCl gradients containing ethidium bromide (24). Restriction endonuclease digestion and DNA ligation were carried out as described previously (25). *E. coli* cells were prepared for transformation with plasmid DNA by the method of Mandel and Higa (16), as modified by Wensink et al. (33).

Preparation of crude cell extracts. Cells were grown to the early stationary phase in M9 medium containing antibiotics and glycerol, washed with 20 mM phosphate buffer (pH 7.0), concentrated 20 times in the same buffer, and disrupted with a Raytheon oscillator (10 kHz) for 15 min at 4°C.

Agarose and polyacrylamide gel electrophoresis. The conditions used for electrophoresis of DNA on agarose gels were identical to those described by Ritzenthaler et al. (26). For polyacrylamide gel electrophoresis under nondenaturing conditions, samples were made 10% glycerol and 5% β-mercaptoethanol and electrophoresed overnight at 4°C on 7.5% polyacrylamide gels by the methods of Laemmli (15) and Studier (29).

Construction of pCB plasmids. The pCB plasmids used (see Fig. 2) were constructed by the following method. A plasmid vector (pBR322, pBR325, or pACYC177) and plasmid U1 were digested with the restriction enzymes and ligated in vitro with T4 DNA ligase. The resulting ligated mixture was used to transform strain HB101, and we selected colonies which were resistant to one antibiotic and sensitive to the antibiotic whose resistance gene was inactivated. Hybrid plasmid DNA was extracted from these strains and digested with the same enzymes used previously for cloning, and the cloned U1 fragment was identified after electrophoresis on an agarose gel. The 3.9- and 8.1-megadalton (Md) *Eco*RI-*Hin*dIII fragments of plasmid U1 were inserted into the *cat* gene of pBR325, yielding pCB1 and pCB2, respectively. The *Bam*HI-2–*Pst*I-4 fragment was cloned in plasmid pACYC177. This plasmid, which was ampicillin sensitive and kanamycin resistant, was designated pCB6; pCB7 and pCB8 were ampicillin-sensitive hybrids of pBR322 containing the *Eco*RI–*Pst*I-3 and *Eco*RI–*Pst*I-4 fragments of plasmid U1, respectively. Plasmid pCB9 was derived from pCB8 by deletion of the *Sma*I-2–*Pst*I-4 segment of pCB8.

The 11- and 1-Md *Bam*HI fragments of plasmid U1 were subcloned into the tetracycline resistance gene of pBR322, yielding pCB3 and pCB5, respectively. The orientation of the *Bam*HI fragment in pBR322 was assessed by measuring the size of the fragments obtained after cleavage with a restriction endonuclease having only a single site in the cloned fragment and a single site in the plasmid DNA. pCB4 was a U1-pBR322 hybrid plasmid in which the 1-Md *Bam*HI-1–*Bam*HI-2 fragment lay in the opposite orientation relative to the U1 sequences.

Plasmid pCB10 was derived from plasmid pCB5, from which the *Eco*RI–*Bam*HI-2 segment was deleted.

Insertion of the 1-Md *Bam*HI fragment of U1 into pMC874. Strains bearing insertions of the 1-Md *Bam*HI fragment of U1 in the unique *Bam*HI site of pMC874 were obtained by transformation of *E. coli* strain HB101 with a ligated mixture of *Bam*HI-cleaved pCB5 and pMC874. Kanamycin-resistant transformants showing a constitutive expression of *uidA* were kept. The orientation of U1 in pMC874 was assessed by measuring the size of the fragments obtained after cleavage with *Eco*RI. The 1-Md *Bam*HI fragment of U1 was ligated in both orientations relative to the pMC874 sequences in the various plasmids.

RESULTS

Identification of ColE1 hybrid plasmids carrying the 36-min region of the *E. coli* chromosome. Selection was based on the following observation. As in the case of the *lac* operator (2, 13, 17), the multiple copies of a plasmid containing a binding site for the *uid* repressor titrated out the *uid* repressor present in the cell and thereby derepressed the *uid* operon, resulting in a constitutive synthesis of β-D-glucuronidase. This approach was used to isolate ColE1 hybrid plasmids that caused constitutive β-D-glucuronidase synthesis; the Clarke-Carbon clone bank of strain JA200 carrying *E. coli* DNA (9) was screened for the presence of β-D-glucuronidase activity in the absence of inducer by using the in

situ plate assay described above. Of 500 clones tested, 4 showed weak constitutive synthesis of β-D-glucuronidase. Three of the four selected plasmids had a molecular weight of 12×10^6, and the fourth had a molecular weight of 9×10^6. One of the 12-Md plasmids was designated U1. To confirm that U1 contained the *uidA* gene, this plasmid was purified and introduced by transformation into strain 1830 carrying the *uidA* mutation (Table 1). Since after transformation this mutant was able to catabolize methyl-β-D-glucuronide, the *uidA* structural gene was present on plasmid U1.

The *manA* and *add* genes code for phosphomannose isomerase and adenosine deaminase, respectively. These genes are located on both sides of the *uidA* gene, and transduction experiments have revealed that each of these markers cotransduces with the *uidA* gene with a frequency of 89% (14). To check for the presence of these two markers, strain Sϕ200, which bore a deletion covering the *manA-uidA-add* region (Table 1), was transformed by plasmid U1. Strain Sϕ200 recovered the ability to use D-mannose and methyl-β-D-glucuronide as carbon sources, but the *add* gene function was not restored. Thus, U1 carried *uidA* and *manA*.

β-D-Glucuronidase activity was measured in strain JA200 containing plasmid U1 (Table 2). In this strain, the induced and noninduced enzyme levels were about 200 and 16%, respectively, of the fully induced level of this strain without the plasmid. These low values showed that the product of the *uidA* cloned gene was not highly amplified and also suggested the presence of the *uidR* regulatory gene on plasmid U1.

Restriction map of the ColE1-*uid* plasmid. As a first step toward studying the gene organization in the *uid* region, we constructed a restriction map of plasmid U1. The plasmid DNA was purified, and the restriction map was determined by analyzing the fragment patterns obtained on agarose gels after single and double digestions. The single *Sma*I site or the two *Pst*I cleavage sites on ColE1 plasmid DNA were used as reference points for the locations of the other

TABLE 2. Activity of β-D-glucuronidase in strain JA200

Strain	Inducer concn[a] (mM)	Sp act of β-D-glucuronidase (%)[b]
JA200	0	0
	10	100
JA200(U1)	0	16
	10	183

[a] The inducer was methyl-β-D-glucuronide.

[b] Percentage of the induced value in the isogenic haploid strain.

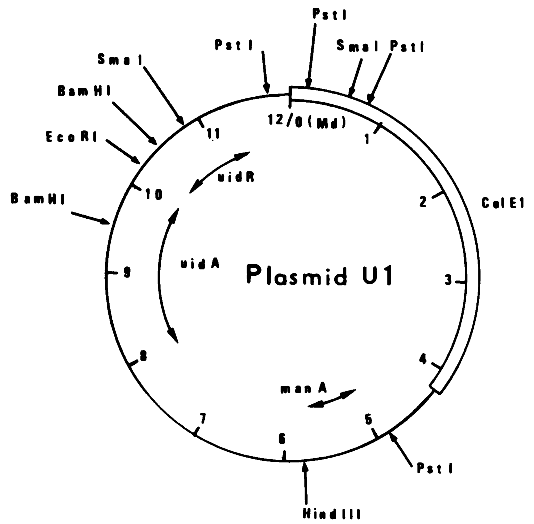

FIG. 1. Restriction and genetic map of plasmid U1. The ColE1 vector is indicated by the double line, and *E. coli* chromosomal DNA is represented by the single line. The locations of the *uid* genes were determined as described in the text. Map units are in megadaltons.

sites. As Fig. 1 shows, the cloned region contained two *Pst*I sites, two *Bam*HI sites, one *Hin*dIII site, one *Eco*RI site, and one *Sma*I cleavage site but no *Bgl*II or *Sal*I sites. Plasmid U1 had a molecular weight of 12×10^6; since the ColE1 plasmid alone had a molecular weight of 4.2×10^6, the molecular weight of the inserted chromosomal fragment was estimated to be 7.8 $\times 10^6$.

Construction and characterization of the pCB plasmids. To assign individual restriction endonuclease fragments to each gene of the cloned region, we subcloned the different endonuclease-generated fragments of plasmid U1 into plasmids pBR322, pBR325, and pACYC177. The resulting plasmids were designated pCB1 to pCB10 and are shown in Fig. 2. The genotypes of the pCB plasmids were determined as follows. Each recombinant plasmid was introduced by transformation into strains HB101, Sϕ200, 1830, and 2523. All of these strains except HB101 carried mutations or deletions covering the 36-min region of the *E. coli* chromosome. The Sϕ200 transformants were analyzed for growth on minimal agar containing mannose as the sole carbon source, whereas the constitutive expression of the *uidA* gene was determined in strain HB101, Sϕ200, and 2523 transformants (Table 3).

Location of the *manA* gene. When pCB2 was introduced into strain 1830, the ability to catabolize mannose was restored. This result demonstrated that pCB2 carried an intact *manA* gene.

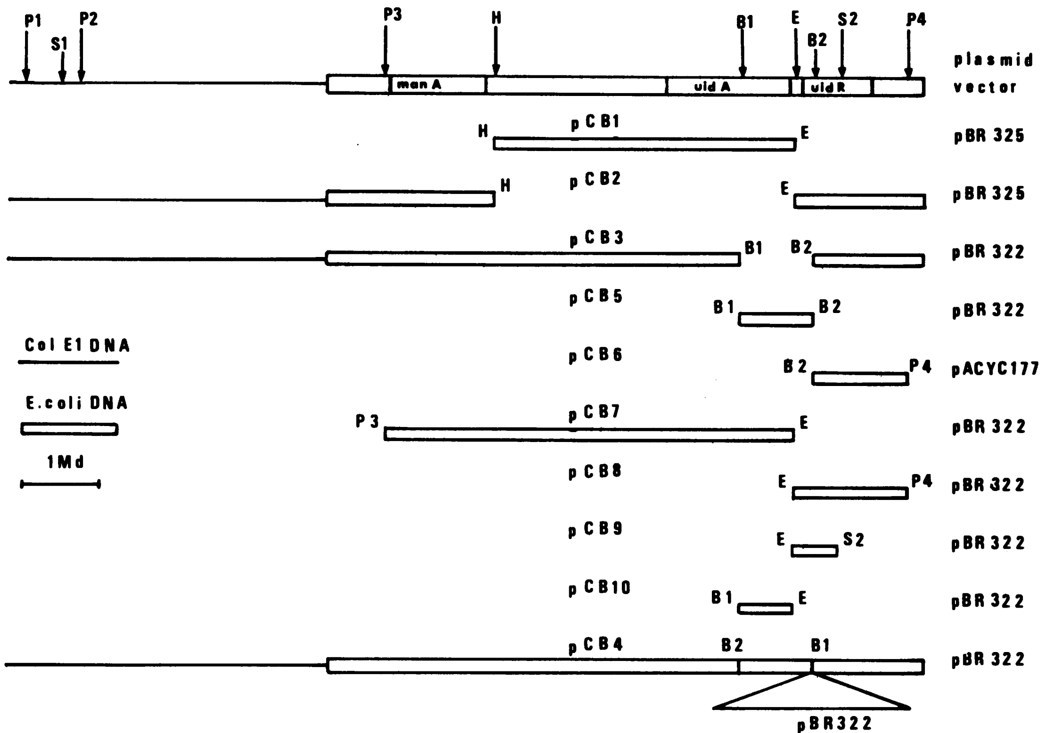

FIG. 2. Physical maps of recombinant plasmids carrying portions of the *uid* region. At the top is a map of plasmid U1, indicating the appropriate restriction sites. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sma*I; P, *Pst*I.

Plasmids pCB3 and pCB7 had the same effect as pCB2, whereas pCB1 and pCB8 did not. A comparison of the physical maps of these recombinant plasmids showed clearly that the structural gene of the phosphomannose isomerase was located on the *Hin*dIII–*Pst*I-3 fragment.

Location of the *uidA* gene. Plasmid U1 was cleaved into two fragments by *Eco*RI-*Hin*dIII double digestion. When plasmid pCB1 carrying the smaller *Eco*RI-*Hin*dIII endonuclease fragment of plasmid U1 was introduced into strain 1830 or Sϕ200, β-D-glucuronidase activity was detected, whereas the larger *Eco*RI-*Hin*dIII fragment introduced into strain Sϕ200 did not

TABLE 3. Complementation patterns of pCB plasmids in various mutant strains[a]

Plasmid	Growth on mannose of strain Sϕ200 [Δ(*manA-uidA-uidR*)]	β-D-Glucuronidase activity in:[b]		
		Strain 2523 (*uidR*)	Strain HB101	Strain Sϕ200 [Δ(*manA-uidA-uidR*)]
pBR322	−	++	−	−
U1	+		+	+
pCB1	−	++	+	++
pCB2	+	−	−	−
pCB3	+	++	−	−
pCB4	−	++	−	−
pCB5	−	++	+	−
pCB6	−	++	−	−
pCB7	+	++	+	++
pCB8	−	−	−	−
pCB9	−	++	−	−
pCB10	−	++	+	−

[a] Symbols: −, absence of growth or enzyme activity; +, growth or low enzyme activity; ++, presence of large quantity of enzyme.

[b] The presence of β-D-glucuronidase activity was tested with an in situ plate assay, as described in the text (cells were grown in the absence of inducer).

cause expression of the *uidA* gene. Thus, the *uidA* gene was carried on the smaller *Hin*dIII-*Eco*RI fragment of pCB1. This result was confirmed by the properties of plasmid pCB7 (*Pst*I-3–*Eco*RI segment), which behaved like pCB1. The *uidA* mutation of strain 1830 or Sϕ200 was not complemented by pCB2, pCB5, or pCB10 (Table 3). A comparison of the structures and phenotypes of these plasmids with those of pCB1 indicated that *uidA* was not completely encoded by the *Hin*dIII–*Bam*HI-1 or *Bam*HI-1–*Eco*RI segment; therefore, the *Bam*HI-1 restriction site lay within the *uidA* gene. The phenotype of pCB4 confirmed this result. This plasmid consisted of the entire U1 plasmid with pBR322 inserted in the *Bam*HI-2 site. In addition, the *Bam*HI-1–*Bam*HI-2 fragment of U1 was in the opposite orientation. This inversion caused inactivation of the *uidA* gene.

Location of *uidR*. To determine the precise location of the *uidR* gene, plasmids pCB2 and PCB8 were introduced into strain 2523, which produced an inactive *uidR* repressor. In the resulting transformants, the constitutive synthesis of β-D-glucuronidase was abolished, showing that *uidR* was included in the *Eco*RI–*Pst*I-4 fragment. Moreover, in the strain 2523(pCB5), 2523(pCB6), and 2523(pCB9) transformants, the constitutive expression of β-D-glucuronidase persisted. Therefore, the *uidR* gene overlapped the *Bam*HI-2 and *Sma*I-2 sites. These results were confirmed by the behavior of plasmids pCB3 and pCB4, which had the same effect in strain 2523 as pCB5, pCB6, and pCB9. The inversion of the *Bam*HI-1–*Bam*HI-2 fragment in plasmid pCB4 or the deletion of this fragment in plasmid pCB3 inactivated the *uidR* gene.

Physiological evidence for the presence of the *uid* genes on the pCB plasmids. To confirm the results of the complementation experiments, β-D-glucuronidase activity was measured in strains carrying the various pCB plasmids. β-D-Glucuronidase was synthesized at a high rate in strain Sϕ200(pCB1) (Table 4); this rate was about 15 times the induced rate of a haploid strain. In strain HB101(pCB1), the enzyme level was enhanced by the addition of an inducer since unlike Sϕ200, HB101 has a functional chromosomal *uidR* gene. When plasmids pCB2, pCB3, pCB4, pCB5, pCB8, and pCB9 were introduced into Sϕ200, no activity was detected. These results clearly suggested that β-D-glucuronidase was overproduced in strains that harbored a multicopy *uidA* plasmid.

To determine whether *uidR* cloned on multicopy plasmids was able to reduce *uidA* gene expression further, enzyme activity was measured in various strains transformed with the plasmids described above. β-D-Glucuronidase synthesis remained constitutive in strains 2523(pCB3), 2523(pCB4), and 2523(pCB6), and strain HB101 carrying the same plasmids showed a wild-type phenotype. Therefore, no functional *uidR* gene was carried by plasmid pCB3, pCB4, or pCB6. In contrast, the constitutive expression of *uidA* in strains 2523(pCB2) and 2523(pCB8) completely disappeared. In the presence of methyl-β-D-glucuronide, *uidA* expression in strains HB101 and 2523 bearing pCB2 was significantly lower than *uidA* expression in a wild-type haploid strain. No induction of β-D-glucuronidase could be obtained in strain HB101(pCB8) or 2523(pCB8), as discussed below. These observations were consistent with the expectation that the *uidR* repressor is overproduced in strains bearing *uidR* on multicopy plasmids.

Operator location and transcription direction of the *uidA* gene. Despite the absence of an entire *uidA* gene in pCB5, weak constitutive expression of β-D-glucuronidase was observed in wild-type strain HB101 containing pCB5 (Table 3), suggesting the presence of a sequence allowing derepression of the chromosomal *uidA* gene. According to the negative regulation model described by Novel and Novel (23), this sequence should be the operator of the *uidA* gene and should be located on the *Bam*HI-1–*Bam*HI-2 segment. The *Bam*HI-1–*Eco*RI fragment of pCB10 had the same effect when it was introduced into strain HB101 (Table 3). Surprisingly, pCB4, which also contained the *Bam*HI-1–*Bam*HI-2 segment, did not cause constitutive expression of the *uidA* gene in strain HB101, but in this case the transformants showed a lower β-lactamase activity than observed in strain HB101(pCB5) (data not shown). This is discussed below.

The location of the *uidAo* operator on the *Bam*HI-1–*Eco*RI segment and the location of the *uidA* gene on the *Hin*dIII-*Eco*RI fragment suggested that the transcription direction of the *uidA* gene was from *Eco*RI to *Bam*HI-1. To confirm the transcriptional direction of *uidA*, we inserted the small *Bam*HI fragment of plasmid U1 into the *lac* fusion plasmid pMC874. This plasmid, which was constructed by Casadaban et al. (7), contains a portion of the *lac* operon (*lacZY*) without the regulatory region and the first eight codons of the amino-terminal end of the *lacZ* gene (β-galactosidase). A unique *Bam*HI cleavage site was incorporated instead of these eight codons. Insertion of DNA fragments containing appropriate regulatory signals into this plasmid leads to the formation of an operon fusion in which *lacY* is transcribed from the promoter sequence of the inserted fragment.

The *lacY* mutation prevented strain HB101 (Table 1) from growing on lactose minimal medium. When pMC874 inserted a promoter se-

TABLE 4. β-D-Glucuronidase activity in *E. coli* strains containing different pCB plasmids

Plasmid	Functional *uid* gene on plasmid	Inducer concn[a] (mM)	Sp act of β-D-glucuronidase (%)[b]		
			Strain 2523 (*uidR*)	Strain HB101	Strain Sϕ200 [Δ(*add-uidA-manA*)]
pBR322	None	0	72	2	0
		10	100	100	0
pCB1	*uidA*	0		157	1,530
		10		1,628	1,400
pCB2	*uidR*	0	1	1	0
		10	5	65	0
pCB3	None	0	80	1	0
		10	110	96	0
pCB4	*uidAo*	0	80	8	0
		10	125	110	0
pCB5	*uidAo*	0	70	28	0
		10	105	120	0
pCB6	None	0	75		
		10.	100		
pCB8	*uidR*	0	0	0	
		10	0	0	

[a] The inducer was methyl-β-D-glucuronide.
[b] Percentage of the induced value in the isogenic haploid strain carrying plasmid pBR322.

quence causing *lac* gene transcription, it conferred the Lac$^+$ phenotype to strain HB101 since *lacY* was expressed from the plasmid. When the 1-Md *Bam*HI fragment of plasmid U1 was introduced into pMC874 in both orientations, only one orientation of this fragment was able to restore the growth of HB101 transformants on lactose. This orientation corresponded to a direction of transcription from the *Bam*HI-2 site to the *Bam*HI-1 site. In Lac$^+$ and Lac$^-$ transformants a weak constitutive synthesis of β-D-glucuronidase was observed, since the chromosomal *uidR* repressor was titrated by the numerous copies of the *uidAo* operator borne by the multicopy plasmid.

To confirm that *lacY* expression was under the control of the *uidA* promoter in this *lac* fusion plasmid, pCB8 carrying a wild-type *uidR* gene was introduced into Lac$^+$ HB101 transformants. These clones were not able to grow on lactose, whereas introduction of plasmid pCB8M (plasmid pCB8 carrying an inactive *uidR* gene [unpublished data]) in the above-described HB101 transformants did not inhibit growth on this sugar. The effect of the *uidR* gene on the expression of the *lac* fusion plasmids confirmed the presence of the *uidA* operator on the *Bam*HI-1–*Bam*HI-2 fragment of plasmid U1.

From all of these experiments we concluded that *uidA* was transcribed from the *Bam*HI-2 site to the *Bam*HI-1 site (i.e., counterclockwise on the standard *E. coli* map) and that the order of the loci in this region was *uidR*, *uidA* promoter-operator, *uidA*.

Identification of the *uidA* gene product. A second line of evidence demonstrating the presence of the *uidA* gene on the vectors was obtained by analyzing the proteins synthesized by cells containing pCB plasmids. Under nondenaturing conditions, acrylamide gel electrophoresis of the proteins in crude extracts of strains Sϕ200(pBR322), Sϕ200(pCB2), and HB101 (pBR322) failed to show a peptide band in the normal position for β-glucuronidase (Fig. 3, lanes a, c, and f), and no enzymatic activity was detected by the dye test. However, strains Sϕ200(pCB1) and Sϕ200(U1) produced a β-D-glucuronidase protein; this protein was detectable by the dye test in both cases but was visualizable by Coomassie blue coloration only in the case of pCB1 (Fig. 3, lanes b and d). β-D-glucuronidase was not synthesized in sufficient amounts by strains Sϕ200(U1) to yield a visible blue band on the gel. The same behavior was observed with strain HB101(pCB5); an enzymatic activity band corresponding to the *uidA* gene product was observed, but this band did not appear by Coomassie blue coloration (Fig. 3, lane g). Since multicopy plasmid pCB5 carried the *uidAo* operator, the titration of the repressor caused weak constitutive synthesis of β-D-glucuronidase coded for by the chromosomal *uidA* gene. Plasmid pCB5 had no effect on β-D-glucuronidase expression in strain Sϕ200 because this strain had no *uidA* gene (Fig. 3, lane e). A peptide band of β-D-glucuronidase was observed with strain 2523(pBR322) but not with strain 2523(pCB8) (Fig. 3, lanes h and j). This result showed that the *uidA* gene was repressed by the *uidR* gene product produced by pCB8.

DISCUSSION

A ColE1-*uid* hybrid plasmid carrying part of the 36-min region of *E. coli* was isolated from the Clarke-Carbon *E. coli* gene bank. The gene

FIG. 3. Analysis of the proteins synthesized by uninduced cells containing plasmids pBR322, U1, pCB1, pCB2, pCB5, and pCB8. Crude cell extracts were run on 7.5% polyacrylamide gels under nondenaturing conditions to allow detection of β-D-glucuronidase activity. After protein separation by electrophoresis, the presence of β-D-glucuronidase was tested by the in situ dye test; then, the protein bands were visualized by Coomassie blue coloration. Slot k was loaded with a partially purified preparation of β-D-glucuronidase. Slot a, Strain Sϕ200(pBR322); slot b, strain Sϕ200(U1); slot c, strain Sϕ200(pCB2); slot d, strain Sϕ200(pCB1); slot e, strain Sϕ200(pCB5); slot f, strain HB101(pBR322); slot g, strain HB101(pCB5); slot h, strain 2523(pBR322); slot i, strain 2523(pCB1); slot j, strain 2523(pCB8). The arrow indicates the position of β-D-glucuronidase activity on the gel.

order *manA-uidA-uidAo-uidR* was established by subcloning different restriction fragments from this plasmid into multicopy plasmid vectors. The locations of the different genes in the 36-min region with respect to the restriction sites enabled us to determine maximal and minimal values for the molecular weights of the proteins encoded by the endonuclease fragments and to estimate the distances between the genes. *manA* and *uidA* are encoded by the 5.3-Md *Pst*I-3–*Eco*RI fragment (Fig. 2). The product of the *manA* gene, which is located on the *Hin*dIII–*Pst*I-3 fragment, has a maximum molecular weight of 78,000. Less than 1.0 Md separates *uidA* and *uidR* since *Bam*HI-1 and *Bam*HI-2 lie within *uidA* and *uidR*, respectively. *uidR* is probably very close to *uidA*, as *exuR* is close to the *exuT* operon (18) and *uxuR* is close to the *uxuA-uxuB* operon (25).

We demonstrated that *uidR* overlaps *Sma*I-2 and *Bam*HI-2 sites (separated by 0.3 Md) and is entirely within the *Eco*RI–*Pst*I-4 fragment (1.5 Md). This result suggests that the *uidR* repressor (monomer) molecular weight is more than 18,000 and less than 80,000. Strains carrying plasmid pCB1, which has the *uidA* gene, produce elevated levels of β-glucuronidase. When plasmids containing the *uidR* gene are introduced into the wild-type strain, the chromosomal expression of *uidA* in the presence of an inducer is far lower than that of the isogenic haploid strain. This suggests that strains containing *uidR* inserted in plasmids overproduce the *uidR* repressor. The normal amount of this regulatory protein in a cell is extremely low; thus, the use of these plasmids should facilitate purification of this molecule. We observed that strains harboring plasmid pCB8 could not be induced, whereas strains bearing plasmid pCB2 could. This may be explained by greater amplification of the small pCB8 plasmid than the larger pCB2 plasmid. This hypothesis seemed to be confirmed by the lower level of plasmid pCB2-encoded β-lactamase compared with the level of plasmid pCB5, which obviously is the same size as pCB8. Previous work has demonstrated that the level of this enzyme is directly proportional to the plasmid copy number (31, 32). Therefore, it seems likely that strains with plasmid pCB8 produce more repressor than strains with plasmid pCB2.

Evidence for the *uidAo* location is given by the behavior of plasmids pCB10 and pCB5. As observed with the *lac* operator (13, 17) and with the *uxaCo* (26) and *uxuAo* operators (25), a multicopy plasmid containing the operator of the *uidA* gene introduced in a cell is able to titrate the repressor synthesized by the chromosomal *uidR* gene and thus cause constitutive expression of the chromosomal β-D-glucuronidase gene. Transformants bearing plasmid pCB10 or pCB5 show a weak constitutive synthesis of the *uidA* gene product. Therefore, the operator is on the *Eco*RI–*Bam*HI-1 fragment. In contrast, pCB4, which also contains the regulatory sequences, has no effect, probably because the low plasmid copy number per cell is insufficient to bind enough of the *uidR* repressor to overcome repression. This hypothesis seems to be confirmed by the higher levels of plasmid pCB5- and pCB10-encoded β-lactamases compared with the level with plasmid pCB4. Finally, fusions that bring *lacY* gene expression under the control of transcriptional and translational signals within the *uidA* gene were used to confirm the direction of transcription of this gene.

Cloning of the operator region of the *uidA* gene will be very helpful for selecting operator-constitutive mutations and for determining the nucleotide sequence of this regulatory region in wild-type and mutant strains. This study should lead to a better definition of the controlling elements for *uidA* gene expression since the operator locus is probably made up of two distinct attachment sites for the *uxuR* and *uidR* repressors (23).

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