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CHARACTERIZATION OF THE OPERATOR SITES OF THE *EXU* REGULON IN *ESCHERICHIA COLI* K-12 BY OPERATOR-CONSTITUTIVE MUTATIONS AND REPRESSOR TITRATION

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ABSTRACT

In *Escherichia coli*, the *exu* regulon of the hexuronate system involves the three *exuT*, *uxaCA* and *uxaB* operons and is under the negative control of the *exuR* regulatory gene product. The technique developed by CASADABAN, CHOU and COHEN was employed to construct two plasmids containing operon fusions in which the lactose genes were fused to the *uxaCA* and *exuT* operons. These fusions were transferred into the chromosome by a reciprocal recombination event, and the resulting strains were used for isolation of mutants defective in repression. Two types of operator-constitutive mutants were obtained: one specific for the *uxaCA* operon expression and the other affecting the *exuT* gene expression. This genetic evidence confirms that these two operons which are divergently transcribed each possess their own operator site.—The derepressed expression of the two *exuT-lac* and *uxaCA-lac* operons and the *uxaB* gene was also examined upon introduction of plasmids bearing various operators of the *exu* regulon. The results of testing *exuR* repressor titration by multiple copies of the *exu* operators allowed us to show a gradation in the affinity degrees for the three *exu* operators: *uxaBo* has the strongest affinity for the *exuR* repressor and *uxaCo* the weakest, although that of *exuTo* seems to be just slightly greater. This gradation may play a role in the control of the *exu* regulon expression.

IN *Escherichia coli* K-12, the aldohexuronates, glucuronate and galacturonate, are degraded according to the ASHWELL (1962) pathway (Figure 1). They enter the cell by the same specific transport system (protein I) and are dissimilated in two parallel pathways using the same first step (enzyme II). The *exu* regulon involves the *uxaB* operon (altronate oxidoreductase) located at 52 min and the *exu* region located at 68 min (BACHMANN 1983). This region includes the *exuT* gene (aldohexuronate transport protein), the *uxaCA* operon (uronate isomerase and altronate hydrolyase) and the *exuR* regulatory gene which codes for a repressor (PORTALIER, ROBERT-BAUDOUY and STOEBER 1980). The induction exerted by galacturonate and tagaturonate is restricted to the four *exu* regulon enzymes, whereas glucuronate and mannonic amide are able to induce the synthesis of all of the enzymes belonging to the hexuronate system (ROBERT-BAUDOUY, PORTALIER and STOEBER 1974). Tagaturon-

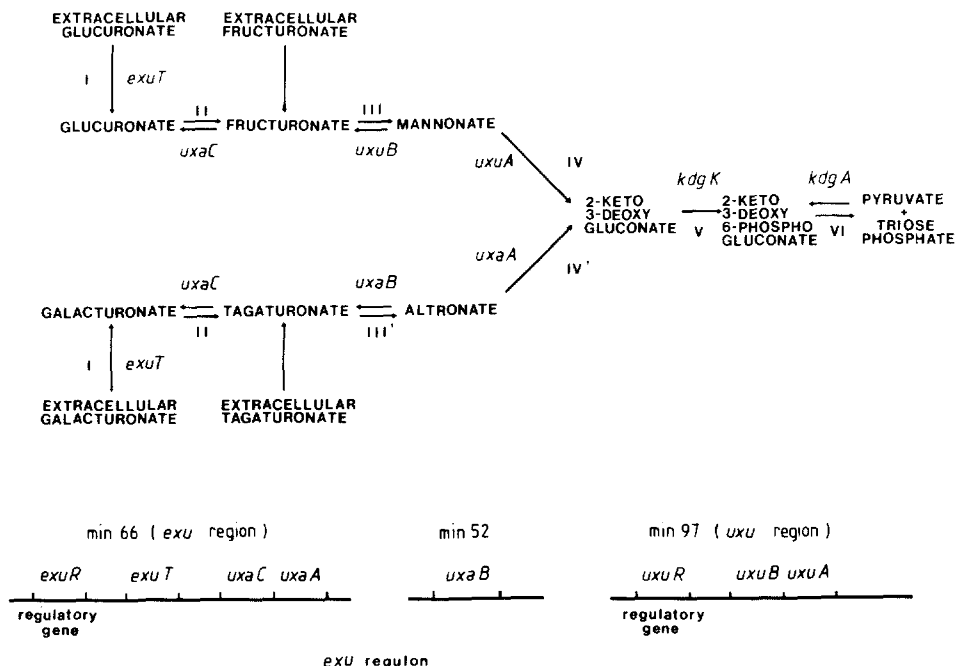

FIGURE 1.—Degradative pathway of hexuronates in *E. coli* K-12. The different steps are catalyzed by the following enzymes: I, aldohexuronate transport system; II, uronate isomerase (EC 5.3.1.12); III, mannonate oxidoreductase (EC 1.1.1.57); IV, mannonate hydrolyase (EC 4.2.1.8); III′, altronate oxidoreductase (EC 1.1.1.58); IV′, altronate hydrolyase (EC 4.2.1.7); V, 2-keto-3-deoxygluconate kinase (EC 2.7.1.45); VI, 2-keto-3-deoxy-6-phosphogluconate aldolase (EC 4.1.2.14). The symbols under each roman numeral are the structural genes of the corresponding enzymes. At the bottom of the figure, is given the location on the *E. coli* chromosome of some of these structural genes and of the corresponding regulatory genes.

ate enters the cell by way of a separate transport system distinct from that encoded by *exuT*.

The two adjacent operons *exuT* and *uxaCA* were shown to be transcribed in opposite directions (HUGOUVIEUX-COTTE-PATTAT and ROBERT-BAUDOUY 1982b). However, they have different regulatory regions, and the position of their respective operator sites relative to the endonucleases sites was determined (MATA-GILSINGER and RITZENTHALER 1983). Here, we describe the isolation and characterization of operator-constitutive mutants of the *uxaCA* and *exuT* operons. The derepressed expression of the four chromosomal *exu* regulon genes (*exuT, uxaCA, uxaB*) caused by introduction of multiple copies of *exu* operators is also examined, and a hierarchy in the repressor affinity is deduced.

MATERIALS AND METHODS

Bacterial strains, phages and plasmids: The bacterial strains, phages and plasmids used in this investigation are listed in Table 1. The bacterial strains were *E. coli* K-12 derivatives.

Culture media: Media for growth were identical with those described by MILLER (1972). Synthetic

TABLE 1

Bacterial strains, bacteriophages and plasmids used in this study

Designation	Relevant genotype	Source
Bacterial strains[a]		
HB101	*leu pro hsdM hsdR recA rpsL lacY*	BOYER
EW1b	*hisA argG tolC rpsL*	WHITNEY
MC4100	*araD139 ΔlacU169 rpsL*	CASADABAN
2161	As MC4100 but *kdgA2*	This laboratory
2510	As MC4100 but *recA1*	This laboratory
3215	As MC4100 but *exuT9*	This laboratory
3216	As MC4100 but *uxaA1*	This laboratory
2500	As MC4100 but *uxaCA-lacZY kdgA2*	This study
3141	As MC4100 but *exuT-lacZY*	This study
Plasmids[b]		
pMC903	bla^+ aac^+ $lacZ^+$ $lacY^+$	CASADABAN
pEB3	bla^+ $uxaB^+$ *uxaBo*	BLANCO
pEB7	tet^+ *uxaBo*	BLANCO
pRU9	bla^+ *uxu*	RITZENTHALER
pRE	bla^+ or tet^+ exu^+	RITZENTHALER
pRK4	tet^+ $kdgT^+$	MANDRAND-BERTHELOT
Bacteriophages		
λ*pexu4*	CI857 $exuT^+$	MATA
λ*pexu6*	CI857 $exuR^+$ $exuT^+$ $uxaCA^+$	MATA

[a] The O^c derivatives of strains 2500 and 3141 are not listed in this table.
[b] Plasmids pREG3 and pREG4 that are shown in Figure 2 are not represented in this table. The precise genotype of plasmids pRE used in this study was described in a previous paper (RITZENTHALER, MATA-GILSINGER and STOEBER 1981). All of them have a fragment of the *exu* region inserted in either the *tet* or the *bla* gene of pBR322.

medium was M63 (pH 7.2) (SISTROM 1958) or M9 (pH 7.2) (MILLER 1972) and contained either glucose, glycerol (5 g/liter) or glucuronate, galacturonate, tagaturonate, fructuronate (2.5 g/liter); aldohexuronate MacConkey media (Difco Laboratories) contained glucuronate or galacturonate, 15 mg/ml. When needed, ampicillin, chloramphenicol and kanamycin were used at final concentrations of 25 μg/ml, and tetracycline, 15 μg/ml, was added.

Chemicals and enzymes: Intermediate substrates of the hexuronate pathway (tagaturonate, fructuronate) were synthesized in our laboratory (ROBERT-BAUDOUY, PORTALIER and STOEBER 1974). D-glucuronate and D-galacturonate were purchased from Sigma Chemical Company. Restriction enzymes were purchased from Boehringer Mannheim and chloramphenicol, tetracycline, ampicillin and kanamycin from Serva Feinbiochemica. [U-^{14}C]glucuronate, potassium salt, was purchased from Radiochemical Centre, Amersham, England.

Genetic methods: Transduction with phage P1 or phage λ and conjugation were carried out according to the method of MILLER (1972).

Enzyme induction and preparation: The conditions for induction and extraction of the enzymes of the hexuronate system were as outlined previously (ROBERT-BAUDOUY, PORTALIER and STOEBER 1974).

Enzyme assays: The enzyme β-galactosidase was assayed by the method of MILLER (1972) in exponentially growing cells. D-altronate:NAD oxidoreductase, altronate hydrolyase and uronic isomerase were measured according to previously published methods (PORTALIER and STOEBER 1972b; ROBERT-BAUDOUY, JIMENO-ABENDANO and STOEBER 1975; PORTALIER, ROBERT-BAUDOUY and NEMOZ 1974). *In situ* plate assays for D-altronate:NAD oxidoreductase activity were performed

as described by PORTALIER and STOEBER (1972a). Glucuronate uptake experiments were carried out as described previously (NEMOZ, ROBERT-BAUDOUY and STOEBER 1976).

Isolation and analysis of plasmid DNA: Procedures for the isolation of plasmid DNA (BIRNBOIM and DOLY 1979; GUERRY, LE BLANC and FALKOW 1973; RADLOFF, BAUER and VINOGRAD 1967), construction, cloning, restriction endonuclease cleavage and gel analysis of recombinant plasmids (COHEN *et al.* 1973; MANIATIS, JEFFREY and VAN DE SANDE 1975; SHARP, SUGDEN and SAMBROOK 1975; RITZENTHALER, MATA-GILSINGER and STOEBER 1980), and transformation of *E. coli* with plasmid DNA (MANDEL and HIGA 1970) have already been described.

Construction of plasmids pREG3 and pREG4

Plasmid pMC903 (CASADABAN, CHOU and COHEN 1980) is a derivative of the pACYC177 ampicillin- and kanamycin-resistant plasmid vector. At its unique *Bam*HI site is inserted a *Bam*HI fragment containing the W205 *trp-lac* fused operon. The two operon fusions, *uxaCA-lac* and *exuT-lac*, were constructed *in vitro* by inserting in the correct orientation the *Bam*HI-*lac* fragment of plasmid pMC903 into the *Bgl*II restriction site of the *uxaA* gene and into the *Bam*HI site of the *exuT* gene, respectively (Figure 2).

Plasmid pREG3: Plasmid pRE16 (RITZENTHALER, MATA-GILSINGER and STOEBER 1981) is a derivative of pBR322 which has inserted a *Pst*I DNA fragment containing the *uxaCA* operon in the *bla* gene. This plasmid had a unique *Bgl*II restriction site located in the *uxaA* gene. Plasmids pMC903 and pRE16 were digested with *Bam*HI and *Bgl*II endonucleases, respectively, mixed after thermoinactivation of the restriction enzymes, ligated with T4 DNA ligase and used to transform *lac* deletion strain 2510 (Table 1). Tetracycline-resistant and Lactose$^+$ transformants were detected on lactose-MacConkey plates with tetracycline. Their plasmid DNA was extracted and analyzed by endonuclease digestions. pREG3 is a derivative of plasmid pRE16 containing the *Bam*HI-*lac* fragment of pMC903 inserted into its *Bgl*II site in the orientation shown in Figure 2.

Plasmid pREG4: Plasmid pRE6 (RITZENTHALER, MATA-GILSINGER and STOEBER 1981) is a derivative of pBR322 in which a part of the *tet* gene was substituted by a chromosomal DNA segment bearing the *exuT* gene. The sole *Bam*HI restriction site of pRE6 is situated in the *exuT* gene. Plasmids pMC903 and pRE6 were mixed, digested with *Bam*HI and ligated with T4 DNA ligase. After transformation of strain 2510, ampicillin-resistant and Lactose$^+$ colonies were detected on lactose-MacConkey plates with ampicillin and tested for kanamycin resistance. Only the kanamycin-sensitive clones were retained since they contained the plasmid pRE6 with the *Bam*HI-*lac* fragment inserted in the *exuT* gene. Plasmid pREG4 was isolated from one of these transformants, and its structure is shown in Figure 2.

An approximate determination of plasmid copy number per cell has been made in various plasmid-bearing strains used in Tables 4 and 5: a given amount of exponentially growing cells yielded the same quantity of plasmid DNA (± 20%) in each case after plasmid DNA extraction by the method of BIRNBOIM and DOLY (1979). All of the plasmids are derivatives of pBR322 (except pEB7) with small *exu* inserts and are approximately of similar size (3.7 to 5 megadaltons) except pRE9 (6.90 megadaltons).

RESULTS

Transfer of the uxaCA-lac operon fusion from plasmid pREG3 to the chromosome: The construction of plasmid pREG3 in which the lactose operon structural genes were situated downstream of the *uxaCA* promoter has been described. In this plasmid, the lactose operon was surrounded by the two parts of the *uxaA* gene, so that the *uxaA-lac* fusion could be transferred to the chromosome by a reciprocal recombination event. The integration of this fusion results in an inactivation of the *uxaA* gene. The recombination of the fusion into the chromosome can easily be selected by use of strain 2161(Δ*lac*, *kdgA*). This strain is unable to grow on glycerol plus aldohexuronate, due to the accumulation of toxic 2-keto-3-deoxy-6-phosphogluconate resulting in the cell death

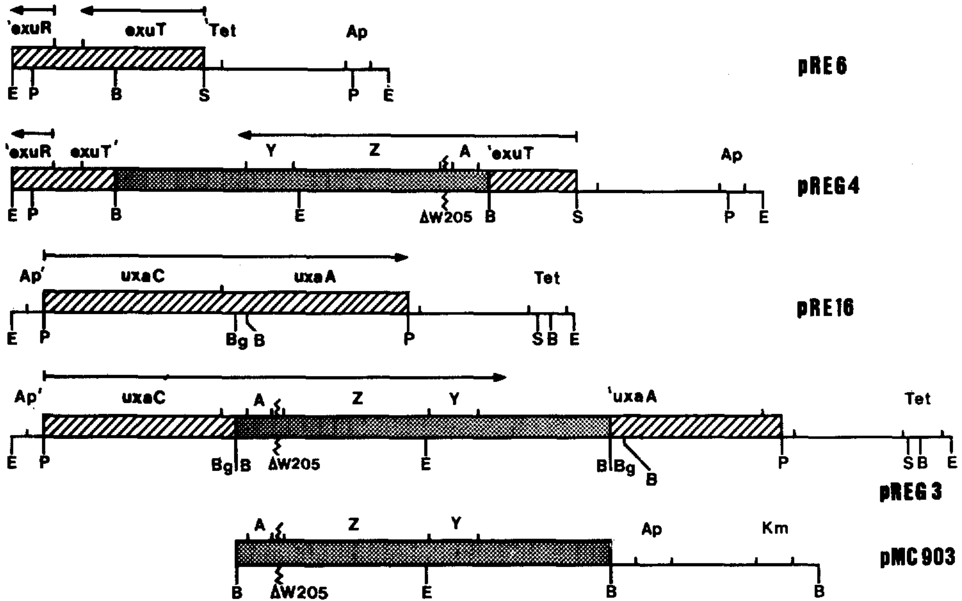

FIGURE 2.—Structure of the *exu-lac* operon fusion plasmids pREG3 and pREG4. Their construction is described in the text. These maps show the plasmids arbitrarily opened at an *Eco*RI or *Bam*HI site. Plasmid DNA is represented by a thin line, *exu* chromosomal DNA by a double hatched line and *lac* chromosomal DNA by a double stippled line. The scale of *lac* DNA is reduced twofold with respect to pRE6 and pRE16. Arrows on the plasmids indicate the direction of transcription; primes indicate that the gene is interrupted on the side the prime is written. Abbreviations: B, *Bam*HI; E, *Eco*RI; P, *Pst*I; Bg, *Bgl*II; S, *Sal*I; Ap, *bla* gene; Tet, *tet* gene; Km, *aac* gene; Z, *lacZ* gene; Y, *lacY* gene.

(POUYSSEGUR 1972). Secondary mutations that occur earlier in the hexuronate pathway prevent the cell from the accumulation of the poisonous compound and allow a growth in the presence of this sugar.

The tetracycline-resistant plasmid pREG3 was introduced into strain 2161 containing the plasmid pRU9 (*uxu* fragment inserted into the *tet* gene by tetracycline resistance selection). These two plasmids possess the same replicon and are incompatible. The double transformants of 2161 were plated on glycerol plus galacturonate in the presence of ampicillin. This antibiotic allowed the maintenance in the cell of plasmid pRU9 whose role was to favor elimination of plasmid pREG3 after integration of the *lac* fusion onto the chromosome. The mutants able to grow on this medium were isolated and then analyzed to distinguish the *uxaA* mutation due to the *uxaA-lac* fusion recombination on the chromosome from spontaneous mutations in the structural genes of the hexuronate system. Among these mutants, those that were not poisoned on glycerol plus glucuronate carried a mutated gene coding for an enzyme of the common degradative pathway and were discarded. After this screening the remaining clones were expected to be *uxaA* or *uxaB* mutants. These mutants can be distinguished by *in situ* plate assays for altronate oxidoreductase activity: in the presence of galacturonate as inducer, in *uxaA* mu-

tants, the *uxaB* gene is expressed, resulting in the synthesis of the altronate oxidoreductase, whereas, in *uxaB* mutants, this enzyme is absent. In the *uxaA* mutants, the inactivation of the gene is due to a spontaneous mutation or to the insertion of the *uxaA-lac* fusion. Some of the *uxaA* mutants were sensitive to tetracycline, proving that pREG3 has been eliminated, and also showed low growth on lactose and good growth when galacturonate was added to the lactose. One of these mutants, numbered 2500, was studied in more detail. First, this strain was cured of pRU9 by many generations of growth without antibiotic selection. The *uxaCA* operon expression was then examined: in the presence of inducer, the *uxaC* gene was expressed at a level corresponding to that of a wild-type isogenic strain, whereas the altronate hydrolase coded for by *uxaA* was not synthesized. The β-galactosidase synthesis was inducible by mannonic amide (Table 2). The cotransduction frequency by phage P1 of the presumed *uxaA* mutation of strain 2500 with *uxaC* was 98%. These results indicate that in strain 2500 the *uxaA* gene is inactivated by insertion of the lactose operon.

Transfer of exuT-lac operon fusion from plasmid pREG4 to the chromosome: An analogous strategy was employed to obtain an *exuT-lac* fusion on the chromosome. In plasmid pREG4, the lactose operon is inserted into the *exuT* gene in the correct orientation, and the ampicillin gene is still functional. Plasmid pRK4 (Table 1) carries the *tet* gene and the *kdgT* gene responsible for 2-keto-3-deoxygluconate uptake (POUYSSEGUR and LAGARDE 1973). The *kdgT* transport system also exhibits a low affinity for glucuronate. Introduction of the multicopy plasmid pRK4 into a wild-type strain causes a high level of transport activity (M. A. MANDRAND-BERTHELOT, P. RITZENTHALER, and M. MATA-GILSINGER, unpublished results). The role of pRK4 was to favor the disappearance of pREG4 after insertion of the *exuT-lac* fusion into the chromosome and to render the resulting *exuT* mutant capable of incorporating glucuronate.

Plasmids pREG4 and pRK4 were introduced into strain 2161 by ampicillin and tetracycline resistance selection, and mutants able to grow on glycerol plus galacturonate and tetracycline were selected. Only clones still poisoned on glycerol plus glucuronate and on glycerol plus tagaturonate corresponding to *exuT* mutants were retained. Most of them were sensitive to ampicillin, proving that pREG4 had been eliminated. In one of these mutants, named 3141, the point mutation *kdgA2* was suppressed by reversion on fructuronate, and plasmid pRK4 was removed. The putative mutation *exuT* of 3141 was localized by transduction to Galacturonate$^+$ with bacteriophage λp*exu4* carrying the entire *exuT* gene (Table 1). The cotransduction frequency by phage P1 of the Galacturonate$^-$ Glucuronate$^-$ phenotype with the locus *tolC* (66 min) of strain EW1b was 4%. In strain 3141, the β-galactosidase synthesis was inducible by tagaturonate or mannonic amide (Table 3). All of these data indicate that the *exuT-lac* fusion had been integrated into the chromosome, resulting in the inactivation of the *exuT* gene.

Isolation and characterization of operator-constitutive mutations in uxaCo and exuTo: Strains 2500 and 3141 carrying the *uxaCA-lac* and *exuT-lac* fusions, respectively, were used for selecting spontaneous mutants with derepressed expression of the *lac* genes by plating on minimal agar containing lactose as the sole

TABLE 2

Activities of the exu *regulon enzymes in* uxaCo *mutants*

Strain	Inducer (mannonic amide) (5 mM)	Specific activities[a]			
		Uronate isomerase (*uxaC*)	β-Galactosidase (*uxaA-lac*)	Altronate oxidoreductase (*uxaB*)	THU (%)[b] (*exuT*)
2500	−	10	110	30	15
	+	480	1780	3450	100
2500-01	−	450	1780	25	18
	+	420	1750	3540	95
2500-02	−	90	420	30	16
	+	460	1550	3320	95
2500-05	−	480	1680	20	15
	+		1740	3220	90
2500-06	−	520	1730	20	18
	+		1540	3480	95

Mutants 2500-01 and 02 were Lac^+ revertants of strain 2500 selected in the absence of plasmid. Mutants 2500-05 and 06 were Lac^+ revertants selected in the presence of plasmid pRE2 carrying the *exuR* regulatory gene; after the Lac^+ reversion, pRE2 was removed to allow a normal induction of the *exu* enzymes. Cells were grown at 37° to an appropriate cell density in glycerol M9 minimal medium and assayed for enzymes activities after treatment in a French press.

[a] Specific activities are given in milliunits (nanomoles of product per min) per milligram of dry weight.

[b] THU is the aldohexuronate transport protein coded for by *exuT*. The activities of THU are expressed in percentage of the value induced in strain 2500.

TABLE 3

Activities of exu *regulon enzymes in* exuTo *mutants*

Strain	Inducer (mannonic amide) (5 mM)	Specific activities[a]			
		β-Galactosidase (*exuT-lac*)	Uronate isomerase (*uxaC*)	Altronate hydrolyase (*uxaA*)	Altronate oxidoreductase (*uxaB*)
3141	−	87	10	ND	20
	+	1790	520	38	3850
3142	−	1420	20	ND	20
	+	1380	560	33	3830
3143	−	1780	15	ND	20
	+	1490	590	30	3940
3148	−	1480	10	ND	20
	+	1510	590	32	3490
3149	−	1620	15	ND	25
	+	1580	500	39	3980

Mutants 3142 and 3143 were Lac^+ revertants of strain 3141 selected in the absence of plasmid. Mutants 3148 and 3149 were Lac^+ revertants of strain 3141 selected in the presence of plasmid pRE2, after the Lac^+ reversion pRE2 was removed. ND = activity not detectable.

[a] Specific activities are given in milliunits (nanomoles of product per min) per milligram of dry weight.

carbon source. Most of the Lac^+ revertants were *exuR* mutants, since they constitutively synthesized the *uxaB* gene product. The remaining *lac* constitutive mutants were expected to be operator constitutive (O^c). To counterselect

the class of *exuR* mutants and to isolate O^c mutants having a strongly reduced repressor affinity, Lac^+ revertants were also selected starting from strains 2500 and 3141 but in the presence of plasmid pRE2 carrying the *exuR* regulatory gene.

Eight independent mutants derived from the *uxaCA-lac* fusion strain were used as donors in the transduction of strain 3215 (*exuT*, Δ*lac*) to $Glucuronate^+$ with phage P1; all of the $Glucuronate^+$ $Galacturonate^-$ recombinants expressed the *lac* genes constitutively. In the same way, ten independent mutants derived from strain 3141 were analyzed by phage P1 transduction. With strain 3216 (*uxaA*, Δ*lac*) as recipient, all of the $Tagaturonate^+$ $Galacturonate^-$ recombinants exhibited constitutive synthesis of β-galactosidase. The enzyme activities of representative strains of each type of mutants are shown in Tables 2 and 3. Hence, all of the putative O^c mutations lie in the *exu* region. The mutants were rendered diploid by lysogenization with bacteriophage λ*pexu*6 which transduces the whole *exu* region including the *exuR* regulatory gene (MATA, Delstanche and ROBERT-BAUDOUY 1978). In each diploid strain, the constitutive expression of the *lacZ* gene was retained, whereas the *uxaA* gene provided by λp*exu*6 was expressed only in the presence of inducer (data not shown). These results demonstrate the *cis*-dominant character of the mutations.

The expression of the *exu* regulon and *lacZ* genes was analyzed in the O^c derivatives of strain 2500 and 3141. In the Lac^+ revertants of strain 2500, under uninduced conditions, the *uxaC* gene was constitutively expressed, as was the *lacZ* fusion, but *exuT* and *uxaB* were still repressed, proving that the *uxaCA* operon, contrary to the *uxaB* and *exuT* genes, was no longer regulated by the *exuR* repressor (Table 2). Among the Lac^+ revertants of strain 2500, two kinds of O^c mutants were revealed: the first type was selected in the presence of the one chromosomal copy of *exuR* and exhibited a low level of constitutivity except for one mutant (2500-01); the second type was obtained in the presence of a high copy number of *exuR* and showed constitutive enzyme levels identical with the fully derepressed rates of strain 2500 (2500-05 and 2500-06). In the *exuTo* mutants derived from strain 3141, the β-galactosidase synthesis was constitutive, whereas the altronate hydrolyase, the altronate oxidoreductase and the uronate isomerase syntheses were inducible at a level identical with that of strain 3141 (Table 3). It is noteworthy that only *exuT-lac* mutants with fully derepressed synthesis of β-galactosidase were obtained by the two types of selection (in the presence of one copy or multicopies of *exuR*).

The isolation of operator-constitutive mutations for one operon that did not affect the expression of the other operon confirmed that the *uxaCA* and *exuT* genes each possess separate control regions.

Hierarchy of the exu regulon operons in the exuR repressor binding: Strains 2500 and 3141 were used to measure the derepression of the three *exu* operons (*exuT-lac*, uxaA-lac, uxaB) caused by introduction of multicopy plasmids bearing the *uxaBo*, *uxaCo* and/or *exuTo* operators (Figure 3). An estimate of the copy number of the various plasmids has been made in each strain as described before: all of the plasmids were supposed to be in an approximately equal copy

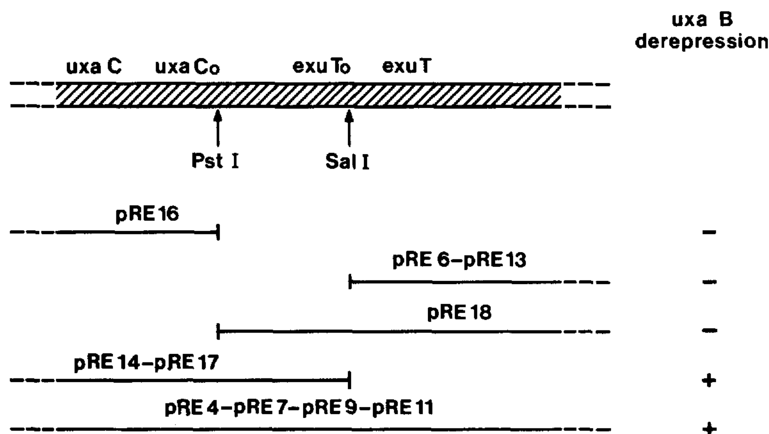

FIGURE 3.—Repressor titration by plasmids bearing *exu* operators. The repressor titration is followed by the derepression of the altronate oxidoreductase synthesis (*uxaB* gene product). At the top of the figure, the *exu* chromosomal region is represented by a double hatched line. The thick lines indicate the parts of the *exu* region present on the plasmids pRE.

number in the cell. Under these conditions, the derepression ratio of β-galactosidase and altronate oxidoreductase synthesis depends on the affinity of the *exuR* repressor molecule for the *exu* operator present on the plasmid. The results of testing repressor titration are shown in Tables 4 and 5.

Plasmid pRE16 bearing *uxaCo* caused no significant alteration in repressibility of the *exu-lac* genes, whereas plasmid pRE18 (*exuTo*) weakly derepressed the expression of the two fused genes. These two plasmids did not affect the *uxaB* gene expression in strain HB101. In contrast, when *exuTo* and *uxaCo* were both present on a plasmid (pRE14), significant constitutive synthesis of β-galactosidase (Table 4) and of altronate oxidoreductase (Table 5; Figure 3) was observed. Greater derepression of the *exu* operons was obtained upon addition of multiple copies of the *uxaB* operator (plasmid pEB7 or pEB3). The level of β-galactosidase (corresponding to that of altronate hydrolyase or aldohexuronate transport protein) reached as much as 50% of the fully induced level in the presence of multiple copies of *uxaBo*, whereas under the same conditions, the derepressed level of altronate oxidoreductase represented less than 20% of the fully induced level.

DISCUSSION

Fusions of the *lac* genes to either one of the two divergently transcribed *exuT* and *uxaCA* operons were isolated on plasmid vectors and transferred onto the chromosome. Selecting growth on lactose media permitted the isolation of spontaneous regulatory mutants. This procedure was used by BARKER, KUHN and CAMPBELL (1981) for selecting operator mutations in the *bio* operon. Some of the Lac$^+$ mutants presented the characteristics expected of operator-constitutive mutations: the mutations allowed constitutive expression of the *exu* gene fused to the *lac* operon, they were *cis*-acting and were located in the *exu* region.

TABLE 4

Repressor titration by plasmids bearing exu *operators in the* exuT-lac *and* uxaC-lac *fusion strains*

Strain	Plasmid	*exu* operator on the plasmid	Specific activity of β-galactosidase	Extent of derepression (%)[a]
2500 (*uxaA-lacZ*)	None	None	110	6
	pRE16	*uxaCo*	175	10
	pRE18	*exuTo*	380	21
	pRE14	*uxaCo-exuTo*	802	45
	pEB7	*uxaBo*	1120	63
	pEB3	*uxaBo*	1090	61
3141 (*exuT-lacZ*)	None	None	87	5
	pRE16	*uxaCo*	113	6
	pRE18	*exuTo*	233	13
	pRE14	*uxaCo-exuTo*	555	31
	pEB7	*uxaBo*	903	50
	pEB3	*uxaBo*	950	53

[a] The extent of derepression is calculated as the ratio of the derepressed level in the presence of *exu* o^+ plasmids to the induced level. For strains 2500 and 3141 grown on glycerol synthetic medium plus mannonic amide, the specific activities of β-galactosidase were 1780 and 1790, respectively (Tables 2 and 3).

TABLE 5

Constitutive expression of uxaB *gene caused by* exu *operator plasmids in strain HB101*

Plasmid	*exu* operator on the plasmid	Specific activity of altronate oxidoreductase (*uxaB*)	Extent of derepression (%)[a]
None	None	25	1
pRE6	None	30	1
pRE16	*uxaCo*	30	1
pRE18	*exuTo*	20	1
pRE4	*uxaCo-exuTo*	400	18
pRE9	*uxaCo exuTo*	300	13
pRE11	*uxaCo exuTo*	390	18
pRE12	*uxaCo exuTo*	370	17
pRE14	*uxaCo exuTo*	400	18
pRE17	*uxaCo exuTo*	420	19
pEB7	*uxaBo*	390	18

[a] The extent of derepression is calculated as the ratio of the derepressed enzyme level to the fully induced level. The specific activity of altronate oxidoreductase in strain HB101 in the presence of inducer is 2200 milliunits/mg and serves as reference in the calculation of the depression ratio.

For the *uxaCA-lac* fusion, two types of O^c mutations were obtained: one set with a relatively low constitutive level of β-galactosidase and the other set with a fully derepressed synthesis of this enzyme. The first class corresponds to the mutated *uxaC* operator which retains some residual affinity for the *exuR* repressor, as has already been observed in other systems such as *lac* (SMITH and SADLER 1971; JOBE, SADLER and BOURGEOIS 1974) or *recA* operator (GINSBURG *et al.* 1982). The isolation of specific operator-constitutive mutants of the

uxaCA operon and of the *exuT* operon suggests that these two operons have no common control region; therefore, they form two distinct operons. This genetic evidence is in accordance with the location of the *exuT* and *uxaC* operators relative to the endonuclease sites, as reported in a previous paper (MATA-GILSINGER and RITZENTHALER 1983).

Knowing the precise location of the *exu* operators and having previously constructed hybrid plasmids bearing them (RITZENTHALER, MATA-GILSINGER and STOEBER 1981; BLANCO, MATA-GILSINGER and RITZENTHALER 1983), we were able to measure the *exuR* repressor titration by each *exu* operator. The *exuR*-encoded repressor has the strongest affinity for the *uxaBo* operator: multiple copies of *uxaBo* caused a greater derepression effect on the three *exu* operons than did *exuTo* or *uxaCo*, whereas, at the other extreme, the ratio of the derepressed enzyme activity in the presence of the *exu* operators to the induced activity was always lower for the *uxaB* gene when compared with that of *exuT* or *uxaCA* operons; *uxaCo* seems to have the lowest repressor affinity, although that of *exuTo* appears to be just slightly greater since multiple copies of *uxaCo* or *exuTo* did not affect or affected only weakly the expression of *uxaCA* and *exuT*, respectively. This could be explained by the fact that the cell responds to *exuR* titration by synthesizing more repressor molecules in a first phase, since the *exuR* gene is known to regulate its own expression negatively (HUGOUVIEUX-COTTE-PATTAT and ROBERT-BAUDOUY 1982a); a derepressed expression of the *exu* genes could only be detected when, in a second phase, the *exu* operator copies titrate out more repressor molecules than can be synthesized. It is noteworthy that the presence of both *exuT* and *uxaCA* control regions results in derepression, whereas either alone is ineffective. Identical results were obtained for the *lac* operator (BETZ and SADLER 1981): plasmid pBR325 carrying one *lac* operator derepressed the chromosomal *lac* operon of HB101 much less than did the same plasmid carrying more tandem *lac* operators.

These results indicate that the *exuR* repressor displays different affinities for the three *exu* operons, as has already been observed for the *arg* regulon (JACOBY and GORINI 1969), the *hut* operons of *Salmonella typhimurium* (HAGEN and MAGASANIK 1976), the *trp* operons (BOGOSIAN, BERTRAND and SOMERVILLE 1981), the λ operators (MEYER, MAUER and PTASHNE 1980) or the *lexA* and *recA* operons (BRENT and PTASHNE 1981). This hierarchy in repressor-operator interactions permits a noncoordinate control of the expression of the unclustered *exu* genes by a single *exuR* repressor. We can imagine that the difference in the *exu* operator affinity serves to set the basal levels of the different *exu* enzymes as was reported for the SOS operons (HUISMAN, D'ARI and CASAREGOLA 1982). It was previously demonstrated by NEMOZ, ROBERT-BAUDOUY and STOEBER (1976) that the *exuT*-encoded aldohexuronate transport system had a relatively high basal level. The *uxaCo* site, which has the weakest repressor affinity, may also be responsible for the high basal level of the uronate isomerase enzyme. Therefore, the galacturonate could enter the cell and be degraded to tagaturonate which is known to be the true inducer, even if the extracellular concentration of galacturonate is low. A sufficient amount of

repressor remains to repress the *uxaB* gene, but *exuT* and *uxaCA*, which required a higher level of repressor protein for protection of their operators, can be expressed, allowing first an accumulation of tagaturonate and then a derepression of the *uxaB* gene expression. This hypothesis suggests that the enzymes upstream of the true inducer in the hexuronate pathway, such as those coded for by *exuT* and *uxaC*, are synthesized at a relatively high basal level, whereas the basal level of the enzymes downstream of the inducer, such as that encoded by *uxaB*, has to be lower. Nevertheless, the differences in operator affinity could also affect the inducibility of the *exu* regulon genes.

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