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Regulation of Hexuronate System Genes in *Escherichia coli* K-12: Multiple Regulation of the *uxu* Operon by *exuR* and *uxuR* Gene Products

JANINE ROBERT-BAUDOUY,* RAYMOND PORTALIER, AND FRANÇOIS STOEBER

Laboratoire de Microbiologie de l'Institut National des Sciences Appliquées de Lyon, 69621 Villeurbanne, France

New regulatory mutants of *Escherichia coli* K-1 carrying alterations of the *uxuR* gene were isolated and characterized. In the presence of superrepressed or derepressed *uxuR* mutations, mannonic hydrolyase (*uxuA*) and oxidoreductase relationship analyses suggested that the *uxuR* gene product acted as a repressor in the control of *uxuA-uxuB* operon expression. *uxuR* mutations were localized near min 97, and the following gene order was established: (*argH*)-*uxuR*-*uxuB*-*uxuA*-(*thr*). Properties of *exuR* (point and deletion) mutants showed that both *exuR* and *uxuR* regulatory gene products were involved in the control of the *uxuA uxuB* operon. Analysis of *exuR uxuR* double-derepressed mutants suggested that *exuR* and *uxuR* repressors act cooperatively to repress the *uxu* operon.

In *Escherichia coli* K-12, D-fructuronate is transformed into 2-keto-3-deoxy-D-gluconate by the action of D-mannonic NAD^+:oxidoreductase (21) and D-mannonic hydrolyase (28) (Fig. 1). The structural genes of mannonic hydrolyase (*uxuA*) (26) and oxidoreductase (*uxuB*) are clustered and situated near min 97 (25) on the *E. coli* genetic map (3). In the wild-type strain, both enzymes are inducible by glucuronate or fructuronate; fructuronate has been shown to be their common internal inducer (25). As the syntheses of both enzymes are strictly coordinated, it has been proposed the *uxuA* and *uxuB* genes constitute the *uxu* operon (27).

Results given in an earlier paper (19) showed that the *exuR* regulatory gene product exerts a negative control over the expression of the galacturonate pathway operons (*exuT*, *uxaC-uxuA*, and *uxaB*; Fig. 1) as well as over the *uxuA-uxuB* operon. In the presence of *exuR* mutations, called "superrepressed" mutations, leading to an uninducible expression of part of the *exu* region (*exuT*, *uxaC-uxaA*, and *uxaB* operons), the induction of the *uxuA-uxuB* operon never exceeded 50% of the wild-type value.

On the other hand, in the presence of *exuR* mutations, called "derepressed" mutations, leading to a constitutive expression of the *exuT*, *uxaC-uxaA*, and *uxaB* operons, in the absence of inducer, the constitutive expression of the *uxuA-uxuB* operon varied between 10 and 30% of its fully induced level, although it remains fully inducible.

In this study, we present the properties of new superrepressed (uninducible) or derepressed (constitutive) mutants for the *uxu* operon. The corresponding mutations are located in a second regulatory gene, *uxuR*, which negatively controls the *uxu* operon.

The analysis of *exuR uxuR* double constitutive mutants revealed a cooperative effect of the two repressors for the control of the *uxuA-uxuB* operon.

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MATERIALS AND METHODS

Nomenclature. The genetic nomenclature is as described by Bachmann et al. (3). The following abbreviations are used: *uxuR*, for the structural gene of the *uxuR* repressor; Gur^+ (Gur^-) and Gar^+ (Gar^-), for the ability (inability) to catabolize glucuronate and galacturonate, respectively; $UxuR^-$ ($UxuR^+$), for constitutive (inducible) expression of the *uxuA-uxuB* operon; "*uxu* operon," for the "*uxuA-uxuB* operon;" and "*exu* regulon," for the various operons (*exuT*, *uxaC-uxaA*, *uxaB*, and *uxuA-uxuB*) controlled by the *exuR* gene product (19).

Chemicals. Intermediate substrates of the hexuronate pathway were synthesized in our laboratory: D-tagaturonic acid (5), D-fructuronic acid (2), and D-mannonic amide (27); D-glucuronate and D-galacturonate were purchased from Sigma Chemical Co.; and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) was from Aldrich Chemical Co. All other substrates were of analytical grade.

Strains. All bacterial strains used were *E. coli* K-12 derivatives; they are listed in Table 1.

Mutagenesis. Induced mutants were obtained

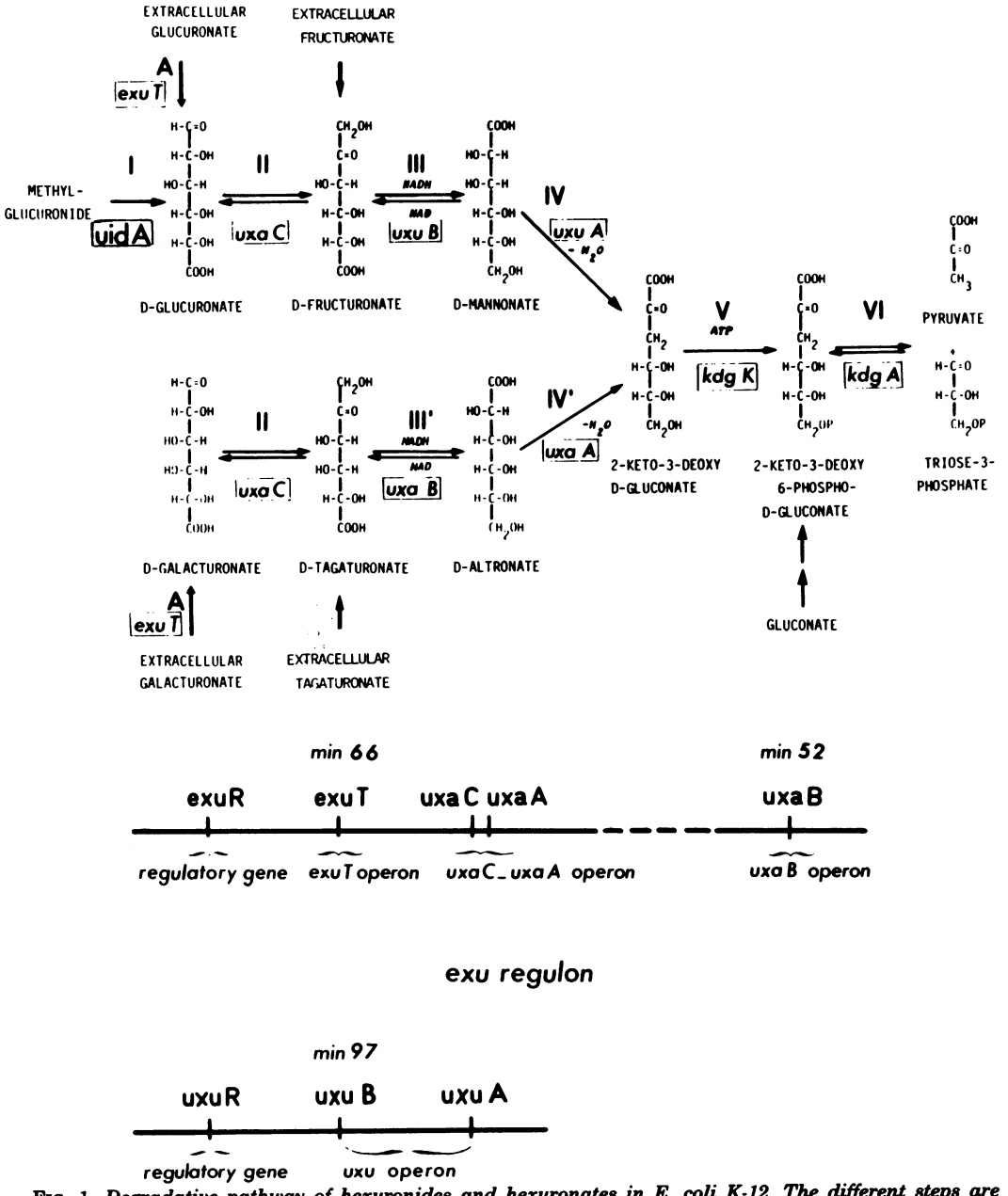

FIG. 1. *Degradative pathway of hexuronides and hexuronates in E. coli K-12. The different steps are catalyzed by the following enzymes: I, β-glucuronidase (EC 3.2.1.31); II, uronic isomerase (EC 5.3.1.12); III, mannonic oxidoreductase (EC 1.1.1.57); IV, mannonic hydrolyase (EC 4.2.1.8); III', altronic oxidoreductase (EC 1.1.1.58); IV', altronic hydrolyase (EC 4.2.1.7); V, 2-keto-3-deoxygluconic kinase (EC 2.7.1.45); VI, 2-keto-3-deoxy-6-phosphogluconic aldolase (EC 4.2.1.14). A, Aldohexuronic transport system (THU). The symbols under each roman numeral (or A) are the structural genes of the corresponding enzymes. At the bottom of the figure, the distribution of these genes in different regulons or operons (or both) is given together with the corresponding regulatory genes and the chromosomal location.*

after NTG treatment of strain Hfr P4X (3 mg/ml for 20 min with a survival rate of 50%) (1).

Spontaneous mutants were isolated from strain A314 (*kdgA*) as previously described (17–19, 22, 23). Growth of strain A314 (lacking aldolase; enzyme VI in Fig. 1) on glycerol plus glucuronate or fructuronate results in the accumulation of toxic 2-keto-3-deoxy-6-*p*-gluconate and then death. Secondary mutants of

TABLE 1. *Bacterial strains*

Strain	Sex	Genotype/phenotype	Origin or derivation
P4X	Hfr	*metB1*	E. Wollman
A314	Hfr	*metB1 kdgA*	(23)
RJ48recA	F⁻	*proA leu thr uxuA1 rpsL recA*	His⁺ RpsL recombinant from a cross between RJ48 (26) and KL166
1758	Hfr	*metB1 uxuR1*	Spontaneous mutant from A314, transduced for *kdgA*⁺
1759	Hfr	*metB1 uxuR1-1*	Spontaneous revertant from 1758 on fructuronate
CM8	Hfr	*metB1 uxuB8*	(25)
HR1 to 3	Hfr	*metB1 uxaB1* to *3*	(17)
1077	Hfr	*metB1 uxaB1 uxuR11*	HR1 revertant on galacturonate at 42°C
1081	Hfr	*metB1 uxaB2 uxuR12*	HR2 revertant on galacturonate at 42°C
1084	Hfr	*metB1 uxaB3 uxuR13*	HR3 revertant on galacturonate at 42°C
KL166	Hfr	*recA* Rif^r	M. Hofnung
HJ1	Hfr	*metB1 exuR1*	(27)
RC1	Hfr	*metB1 exuR10*	(27)
HJ1 ra2	Hfr	*metB1 exuR1-2*	Spontaneous revertant from HJ1 (19)
RC1 ra42	Hfr	*metB1 exuR10-42*	Spontaneous revertant from RC1 (19)
1414	F⁻	*leu thr uxuA1 uxuR14 argH rpsL exuR1-2*	Thy⁺ UxaB⁺ recombinant from a cross between HJ1ra2 and 1176
1455	F⁻	*leu thr uxuA1 uxuR14 argH rpsL exuR10-42*	Thy⁺ UxaB⁺ RpsL recombinant from a cross between RC1ra42 and 1176
RJ27	F⁻	*leu thr uxuA1 argH rpsL thyA hisA proA*	(26)
PAT317	F⁻	*leu thr argH rpsL thyA hisA proA*	M. Hofnung
1828	F⁻	*leu uxuR1 rpsL thyA hisA proA*	Thr⁺ RpsL recombinant from a cross between 1758 and PAT317
1828bis	F⁻	*leu uxuR1 rpsL recA hisA proA*	Thy⁺ RpsL recombinant from a cross between KL166 and 1828
897	F′	KLF134 (*metB*⁺-*leu*⁺)/JC1553 *metB1 his-1 leu-6 recA mtl xyl malA gal lac rpsL* λ^r *sup-48*	B. Low via J. P. Lecocq
1828ter	F′	KLF134(*metB*⁺-*leu*⁺)/1828bis	Cross between 897 and 1828bis
1176	F⁻	*leu thr uxuA1 uxuR14 argH rpsL uxaB3 thyA*	Revertant on galacturonate at 42°C from an His⁺ RpsL UxaB3 recombinant from a cross between RJ27 and HR3
1375	F⁻	*leu thr uxuA1 uxuR14 argH rpsL recA uxaB3*	Thy⁺ RpsL recombinant from a cross between KL166 and 1176
KLF121/422	F′	KLF121(*pyrB*⁺-*thr*⁺)/*pro thr pyrB rpsL recA*	M. Hofnung
1383	F′	KLF121(*pyrB*⁺-*thr*⁺)/1375	Cross between KLF121/422 and 1375

this strain blocked early in the glucuronate pathway do not accumulate the poisonous compound and can grow under the above conditions.

Isolation and construction of strains carrying *uxuR* regulatory mutations. (i) Isolation of *uxuR* regulatory mutants. Pleiotropic negative mutants specifically altered for both *uxuA* and *uxuB* gene expression were screened among clones unable to grow on glucuronate but able to grow on galacturonate and giving a negative reponse in the in situ plate assay for mannonic oxidoreductase (20; see below).

Among 27 independent NTG-induced mutants showing such a pleiotropic phenotype, 9 were negative for mannonic hydrolyase activity and constitutively synthesized β-glucuronidase.

From 96 independent spontaneous clones derived from strain A314, 12 mutants devoid of both mannonic oxidoreductase and hydrolyase were analyzed in more detail.

All Gur⁺ revertants isolated from negative NTG-induced mutants showed an inducible phenotype for mannonic oxidoreductase and hydrolyase, so NTG-induced mutants and revertants were not studied any further.

Most spontaneous mutants did not revert to growth on glucuronate or fructuronate and showed constitutive synthesis of β-glucuronidase; they were identified as deletion mutants in the *uxu* region and were similar to previously described mutants (25).

Among seven pleiotropic negative spontaneous mutants able to revert for growth on glucuronate and fructuronate, five gave inducible revertants; the last two strains, one of which was strain 1758, reverted either to an inducible or a constitutive phenotype for mannonic oxidoreductase and hydrolyase as well as β-glucuronidase syntheses.

(ii) Selection of mannonic oxidoreductase constitutive mutants from altronic oxidoreductase-deficient strains. HR strains carried mutations in the altronic oxidoreductase structural gene (*uxaB*) and as a consequence were unable to transform galacturonate beyond tagaturonate (17) (Fig. 1). Tagaturonate, which was not an inducer of mannonic oxidoreductase, was a secondary substrate of this enzyme (21).

To isolate mutants able to synthesize constitutively mannonic oxidoreductase, 10^9 cells of each HR mutant were spread on a plate containing minimal agar sup-

plemented with galacturonate as the sole carbon source. Mannonic oxidoreductase-constitutive revertants were identified among wild-type revertants by the previously described in situ plate assay (20).

(iii) Construction of strain 1176 carrying *uxuA1* and *uxuR* mutations. Hfr HR3, which carried the *uxaB3* mutation in the altronic oxidoreductase structural gene (17) was crossed with F⁻ RJ27 carrying the *uxuA1* mutation in the mannonic hydrolyase structural gene (26). His⁺ RpsL recombinants were selected and analyzed. An *uxuA1 uxaB3* clone was identified among the His⁺ recombinants through its double Gur⁻-Gar⁻ phenotype at 42°C. Strain 1176 was isolated from the *uxuA1 uxaB3* double mutant by reversion on galacturonate at 42°C by using the method described above and had the following genotype: *leu thr uxuA1 uxuR argH rpsL thyA uxaB3.*

Strain 1375, a *recA* derivative of strain 1176, was obtained after mating Hfr KL166 with F⁻ 1176 and selection of ThyA⁺ RpsL recombinants.

(iv) Construction of strains 1414 and 1455 carrying mutations *uxuR14* and *exuR1-2*, and *exuR-42*, respectively. Strain 1176 (*uxuR14 uxuA1 uxaB3*) was crossed with Hfr HJra2 carrying the derepressed *exuR1-2* mutation and with Hfr RC1ra42 carrying the derepressed *exuR10-42* allele (19). In each case Thy⁺ RpsL recombinants were selected and analyzed for the coinheritance of both *uxaB*⁺ and derepressed *exuR* alleles. The altronic oxidoreductase-constitutive phenotype was ascertained by the in situ plate assay for aldonic oxidoreductases (see below).

Two strains carrying two sets of derepressed mutated alleles were obtained: strain 1414 carried the *uxuR14* and *exuR1-2* mutations, and strain 1455 carried the *uxuR* and *exuR10-42* mutations.

(v) Construction of diploid strains containing the wild-type allele and various mutated alleles of the *uxuR* regulatory gene. KLF134 (*metB*⁺ *leu*⁺) episome from diploid strain 897 was transferred into strain 1828bis (*leu uxuR1 rpsL recA hisA proA*) and maintained in diploid strain 1828ter through selection for *thr* marker. KLF121 (*pyrB*⁺ *thr*⁺) episome from diploid strain KLF121/422 was introduced into strain 1375 (*leu thr uxuA1 uxuR14 argH rpsL recA uxaB3*) through selection for *thr* marker.

The presence of episome KLF134 or KLF121 in strain 1828ter or 1383 was tested by the capacity to transfer the Thr⁺ Gur⁺ phenotype to strain RJ48 *recA* (*proA leu thr uxuA1 rpsL recA*). It was thus verified that the episome was not deleted for *uxu thr* markers or integrated.

Deletion mutants obtained after induction of HfrH58. For curing H58 λ lysogens by thermal treatment, the method developed by Shimada et al. (29) was used, as modified by Mata et al. (10).

Current genetic methods. Conjugation, transduction with phage P1, and construction of diploid strains were performed by the methods of Miller (11).

Media. M63 mineral medium (30) was supplemented as described previously (18). Oxoid solid media contained glucose (5 mg/ml), glycerol (5 mg/ml), glucuronate, tagaturonate, or galacturonate (2.5 mg/ml); aldohexuronate MacConkey media (Difco Laboratories) contained 15 mg of glucuronate or galacturonate per ml.

Reversions. Spontaneous revertants were obtained at various temperatures by plating 10^8 to 10^{10} cells on solid minimal medium supplemented with glucuronate, fructuronate, or tagaturonate.

Enzyme induction and extraction. The conditions for enzyme induction and extraction were outlined previously (27). Specific activities and differential rates of synthesis (12) were specified previously (27).

Enzyme assays and units. Aldonic oxidoreductases and hydrolyases were assayed by previously published methods (21, 22, 24, 28); hexuronic isomerase was measured by a coupling method described previously (17); hexuronate transport system was performed as described previously (13). One unit of β-glucuronidase has been defined as the amount of enzyme hydrolyzing 1 μmol of *p*-nitrophenyl-β-D-glucuronide per min at 30°C (by absorbance at 405 nm) (14).

Specific assay of altronic NAD⁺:oxidoreductase in the presence of mannonic NAD⁺:oxidoreductase. Both altronic and mannonic oxidoreductase activities were qualitatively analyzed by the in situ plate assays described by Portalier and Stoeber (20). As both enzymes could transform altronate into tagaturonate, specific detection of altronic oxidoreductase activity in the presence of mannonic oxidoreductase required the addition of 1 mM *para*-chloromercuribenzoate sodium salt in the plate assay to inhibit mannonic oxidoreductase activity (20).

RESULTS

Isolation and characterization of *uxuR* regulatory mutants. (i) *uxuR* derivatives of strain A314. Independent clones were originally isolated at 30 or 42°C from strain A314 as spontaneous mutants able to grow on glycerol in the presence of glucuronate but specifically unable to express both mannonic oxidoreductase and hydrolyase. Strain 1758 was chosen as a representative clone of this class of pleiotropic negative (superrepressed) mutants; it was subsequently transduced to *kdgA*⁺.

Mutant 1758 did not grow on glucuronate or fructuronate but grew normally on galacturonate or tagaturonate.

In this mutant, mannonic oxidoreductase and hydrolyase could not be induced by their regular inducers, glucuronate and fructuronate, whereas the three other enzymes of the hexuronate system coded for by the *exu* regulon (19), uronic isomerase, altronic oxidoreductase, and hydrolyase, were still induced by galacturonate, tagaturonate, glucuronate, or fructuronate (Table 2). In the presence of glucuronate or fructuronate these last three enzymes were even superinduced, but it should be noticed that fructuronate, the true inducer derived from glucuronate, was not catabolized in strain 1758. These results showed that the glucuronate and fructuronate transport systems were present in this strain.

TABLE 2. *Activities of the hexuronate system enzymes in uxuR regulatory mutants 1758 (uxuR1) and 1759 (uxuR1-1)*

Strain	Inducer (5 mM)	Differential rate of synthesis (mU/mg)[a]					
		Uronic isomerase (*uxaC*)	Altronic hydrolyase (*uxaA*)	Altronic oxidoreductase (*uxaB*)	Mannonic oxidoreductase (*uxuB*)	Mannonic hydrolyase (*uxuA*)	β-Glucuronidase (*uidA*)
1758	None	30	<1	28	<1	<1	783
	Fructuronate	(191)[b] 785	(168) 410	(217) 16,760	(<1) <1	(<1) <1	617
	Tagaturonate	(95) 646	(87) 550	(100) 7,739	—[c]	—	—
	Glucuronate	(185) 482	(127) 307	(132) 8,584	(1) 127	(<1) <1	—
	Galacturonate	(113) 373	(103) 310	(124) 11,228	—	—	—
1759	None	(12) 84	(<1) <1	(6) 488	(32) 2,509	(32) 25	27

[a] All assays were carried out at 30°C.

[b] Numbers within parentheses represent: in the absence of inducer, percentage of the best induction obtained in wild-type strain P4X at the corresponding temperature; in the presence of inducer, percentage of the induction obtained in the wild-type strain with the same inducer at the corresponding temperature.

[c] —, Not determined.

Only β-glucuronidase (Fig. 1) was weakly constitutively synthesized in mutant 1758.

Revertants able to grow at 42°C on glucuronate or fructuronate were isolated from strain 1758; most of them recovered an inducible wild-type pattern of synthesis for the hexuronate system enzymes, but 10% of the revertants which grew poorly on glucuronate displayed a constitutive pattern for mannonic oxidoreductase and hydrolyase syntheses. Revertant 1759 was a representative strain of the latter class (Table 2).

Pleiotropic mutations associated with strains 1758 and 1759 were named *uxuR1* and *uxuR1-1*, respectively.

(ii) *uxuR* derivatives of altronic oxidoreductase-deficient strains. Strains HR1, HR2, and HR3 carried mutations (*uxaB1*, *uxaB2*, and *uxaB3*, respectively) in the altronic oxidoreductase structural gene (17).

Spontaneous secondary mutants showing a constitutive pattern of mannonic oxidoreductase and hydrolyase syntheses were isolated at 42°C with a rate of 10^{-7}/cell by plating each HR strain on minimal medium supplemented with galacturonate (see above); 90% of the galacturonate revertants showed such a constitutive phenotype.

Revertants 1077 and 1081, isolated from mutants HR1 (*uxaB1*) and HR2 (*uxaB2*) and carrying mutations *uxuR11 and uxuR12*, respectively, were quantitatively analyzed (Table 3). The constitutive phenotype was restricted to the *uxuA-uxuB* operon, which was not fully derepressed in the absence of inducer. In the presence of D-mannonic amide, a gratuitous inducer of the hexuronate system (27), the expression of the *uxuA-uxuB* operon was increased but did not reach the wild-type fully induced level. As parental strains, revertants were still altronic oxidoreductase deficient; uronic isomerase and altronic hydrolyase were normally inducible in these revertants.

Mapping studies. (i) Genetic localization of the *uxuR* gene. The pleiotropic negative *uxuR1* mutation was mapped by a noninterrupted cross between Hfr 1758 (*uxuR1*) and F⁻ PAT317. The analysis of the Pro^+, $(Thr\text{-}Leu)^+$, Arg^+, and His^+ recombinants for the Gur^- phenotype showed that the Gur^- character was strongly linked to the *thr-leu* locus and suggested that the *uxuR1* mutation was localized between the *thr-leu* and *argH* markers. No segregation was observed between β-glucuronidase-constitutive and Gur^- phenotypes in the various recombinants (data not shown).

A more precise localization of the *uxuR1* mutation could not be defined, as no cotransduction could be demonstrated between the *uxuR* locus and known markers of the *thr-leu* region, except for the *uxuA* and *uxuB* genes (see below).

(ii) Ordering of the *uxuA* and *uxuR* genes with the *argH* and *thr-leu* markers. Segregation of *uxuA* and *uxuR* mutations was followed among recombinants obtained after a noninterrupted cross between Hfr P4X (wild type) and F⁻ 1176 carrying the *uxuR* and *uxuA1* alleles. The *uxuA1* mutation has been previously localized near min 97 in the structural gene of the mannonic hydrolyase, between the *thr-leu* and *argH* markers (26). Strain 1176 showed a Gur^- phenotype (inability to grow on glucuronate or fructuronate), as well as a mannonic oxidoreductase-constitutive $UxuR^-$ phenotype.

As 9% of the $(Thr\text{-}Leu)^+$ Gur^+ recombinants were $UxuR^-$, whereas no $UxuR^-$ clones could be identified among Arg^+ Gur^+ recombinants, the following gene order was suggested: *argH-uxuR-uxuA-thr*. This order is in agreement with the

TABLE 3. *Enzymatic activities in uxaB strains constitutive for the uxuA-uxuB operon*

Strain	Genotype	Inducer (5 mM)	Differential rate of synthesis (mU/mg)[a]			
			Uronic isomerase (*uxaC*)	Altronic hydrolase (*uxaA*)	Mannonic oxidoreductase (*uxuB*)	Mannonic hydrolyase (*uxuA*)
P4X	*metB1*	None	(1)[b] 10	(1) 9	(2, 5) 350	(3) 5
		D-Mannonic amide	(100) 540	(100) 400	(100) 10,500	(100) 150
1077	*metB1 uxaB1 uxuR11*	None	(2) 12	(1) 10	(43) 5,900	(40) 66
		D-Mannonic amide	(89) 480	(95) 380	(80) 8,500	(71) 106
1081	*metB1 uxaB2 uxuR12*	None	(1) 8	(<1) 6	(23) 41,000	(29) 48
		D-Mannonic amide	(92) 496	(105) 420	(62) 6,500	(51) 76

[a] All assays were carried out at 37°C.

[b] Numbers within parentheses represent: in the absence of inducer, percentage of the best induction obtained in wild-type strain P4X at 37°C; in the presence of inducer, percentage of the induction obtained in the wild-type strain with the same inducer at 37°C.

gradient of transmission of the *uxuA*$^+$ (74%) and *uxuR*$^+$ (67%) alleles in (Thr-Leu)$^+$ recombinants. The weak segregation between Gur$^+$ and UxuR$^+$ phenotypes also suggested that the *uxuR* and *uxuA* genes are close together.

(iii) Cotransduction of *uxuA* and *uxuR* markers. The relative proximity of the *uxuA* and *uxuR* genes was confirmed by transduction studies using phage P1 where the donor strain 1084 carried the *uxuR13* constitutive allele and the recipient strain RJ27, the *uxuA1* mutation. 50% of the Gur$^+$ recombinants inherited both the *uxuA*$^+$ and the *uxuR13* alleles.

(iv) Ordering of the *uxuR*, *uxuB*, and *uxuA* genes. For ordering the *uxuR*, *uxuB*, and *uxuA* genes, transduction mapping with phage P1 was carried out. The donor strain, CM8, contained the *uxuB8* mutation in the mannonic oxidoreductase structural gene; this mutant was temperature sensitive and displayed a Gur$^+$ phenotype at 30°C but a Gur$^-$ phenotype at 42°C (25). The recipient strain, 1176, carried the *uxuR* constitutive allele and the *uxuA1* mutation; it was characterized by a Gur$^-$ phenotype at 30 and 42°C.

A total of 350 Gur$^+$ transductants were selected at 30°C. Since the *uxuA* and *uxuB* genes were very closely linked (25), only 3% of these transductants showed a Gur$^+$ phenotype at 42°C (*uxuB*$^+$ *uxuA*$^+$ genotype); all of them retained the constitutive UxuR$^-$ phenotype. Moreover, the few Gur$^+$ transductants selected at 42°C were also constitutive.

These results strongly suggested the following gene order: *argH-uxuR-uxuB-uxuA-thr*. (If the order were *uxuR-uxuA-uxuB*, about 50% of the Gur$^+$ transductants at 42°C would have inherited the inducible UxuR$^+$ phenotype.) The same gene order has been established by independent studies using subcloning of different fragments of the *uxu* region into multicopy plasmid vectors (Ritzenthaler et al., submitted for publication).

Analysis of dominance relationships between the different *uxuR* alleles. Activities of the hexuronate system enzymes were estimated in merodiploid strains containing combinations of the wild type and a superrepressed (*uxuR1*) or a constitutive (*uxuR14*, *uxuR1-1*, *uxuR11*, or *uxuR12*) mutant allele of the *uxuR* gene. Results obtained with the *uxuR1* and *uxuR14* alleles are given in Table 4; similar results were achieved with other mutations (data not shown). In diploid strain 1828ter the superrepressed *uxuR1* allele exerted a *trans* dominant effect over the wild-type *uxuR*$^+$ allele, as mannonic oxidoreductase and hydrolyase were not induced by D-mannonic amide. In the same diploid strain, the expression of the three enzymes, uronic isomerase, altronic oxidoreductase, and hydrolyase, strictly controlled by the *exuR* gene product, remained normally inducible, as was the case in the haploid strain 1828bis. Inducibility of these enzymes showed that the D-mannonic amide uptake is not controlled by the *uxuR* gene.

The haploid strain 1375 contained the derepressed *uxuR* allele associated with the *uxuA1* mutation. Enzymatic analysis of the haploid control strain 1375 and the diploid strain 1383 containing both the *uxuR*$^+$ and *uxuR* alleles (Table 4) showed that the *uxuR*$^+$ allele was transdominant to the *uxuR* allele.

Effects of *exuR* deletions on the *uxu* operon expression. Partial deletions of the *exuR* regulatory gene (10) were associated with a low constitutive expression of the *uxuA-uxuB* operon (3 to 9% of the fully induced level). Such a derepression effect was equal to 3 to 9 times the wild-type (HfrH) basal level value (data not shown).

TABLE 4. *Expression of superrepressed or derepressed alleles of the uxuR gene in the merodiploid strains*

Strain	Genotype	Inducer (5 mM)	Differential rate of synthesis (mU/mg)				
			Uronic isomerase (*uxaC*)	Altronic hydrolyase (*uxaA*)	Altronic oxidoreductase (*uxaB*)	Mannonic oxidoreductase (*uxuB*)	Mannonic hydrolyase (*uxuA*)
PAT317	*leu thr argH thyA hisA proA* (wild type)	None	(6)[a] 25	(2) 7	(2) 89	(2) 130	(<1) <1
		D-Mannonic amide	(100) 428	(100) 270	(100) 1720	(100) 5037	(100) 20
1828bis	*proA leu uxuR1 argH recA*	None	(7) 30	(2) 6	(1) 40	(<1) 16	(<1) <1
		D-Mannonic amide	(124) 530	(96) 260	(105) 1800	(<1) <1	(<1) <1
1828ter	F(*metB*$^+$-*thr*$^+$)/ 1828bis	None	(5) 22	(2) 7	(<1) 34	(<1) 15	(<1) <1
		D-Mannonic amide	(159) 680	(100) 270	(99) 1700	(<1) <1	(<1) <1
1375	*leu thr uxuA1 uxuR14 argH recA uxaB3*	None	(5) 20	(1) 3	–[b]	(85) 4448	–
		D-Mannonic amide	(91) 390	(101) 273	–	(155) 7815	–
1383	F(*pyrB*$^+$-*thr*$^+$)/ 1375	None	(5) 23	(<1) <1	–	(6) 393	(<1) <1

[a] Numbers within parentheses represent: in the absence of inducer, percentage of the best induction; in the presence of inducer, percentage of the induction obtained with the same inducer in the wild-type strain at 37°C.
[b] –, Not detectable.

On the other hand, when an *exuR*$^+$ transducing phage was introduced into *exuR* deletion mutants, the low constitutive *uxuA-uxuB* expression disappeared.

Control of the *uxu* operon by the *exuR* and *uxuR* genes. The uninduced level of expression of the *uxu* operon (estimated through mannonic oxidoreductase activity) was analyzed in strains containing various combinations of *exuR* and *uxuR* alleles (Table 5).

The presence of only one derepressed *exuR* or *uxuR* allele (*exuR1-2*, *exuR10-42* [thermosensitive], or *uxuR*) in *E. coli* (strains HJ1ra2, RC1ra42, or 1176, respectively) resulted in a partially constitutive expression of the *uxu* operon. In these strains, the derepressive effects of *exuR* alleles were lower than that of the *uxuR* mutation.

When derepressed (constitutive) alleles of both *exuR* and *uxuR* genes were simultaneously present (strains 1414 and 1455), the constitutive differential rate of synthesis of mannonic oxidoreductase was always much higher than the sum of the individual constitutive rates measured in the presence of only one derepressed allele. It was also higher than the induced level in wild-type strains at the same temperature. Moreover, strains 1414 and 1455 were not superinducible by fructuronate (this true inducer was not metabolized in the *uxuA1* strains and acts as a gratuitous inducer, data not shown).

DISCUSSION

Results given in this paper as well as those presented in an independent article (19) show that part of the hexuronate system genes, namely, the *uxuA-uxuB* operon coding for mannonic hydrolyase and oxidoreductase is under the dual control the regulatory genes *uxuR* and *exuR*, respectively.

We demonstrated above that when the *exuR* gene was deleted or inactivated by λ insertion, the *uxuA-uxuB* operon was partially expressed. These results showed that the *exuR* gene product is involved in the control of the *uxuA-uxuB* operon and suggest the existence of another regulatory gene for this operon.

This second regulatory gene, called *uxuR*, has been identified in this study and is situated at min 97 between the *argH* and *uxuB* markers. It partially affects the expression of the *uxu* operon, but has no detectable effect on the expression of the *exuT*, *uxaC-uxaA*, and *uxaB* operons. The different *uxuR* alleles we have characterized here are similar to previously described alleles of the I regulatory gene in the *lac* operon (32), and their properties suggest that the *uxuR* gene product exerts, at least, a specific repressor function on the *uxuA-uxuB* operon.

As with *lacI* superrepressed alleles (31), the *uxuR1* superrepressed allele in strain 1758 totally blocked mannonic oxidoreductase and hy-

TABLE 5. *Mannonic oxidoreductase activity in exuR-uxuR double regulatory mutants*

Strain	Relevant genotype	Growth temp. (°C)	Mannonic oxidoreductase constitutive differential rate of synthesis (mU/mg)	
			Experimental[a]	Theoretical[b]
PAT317	*exuR*$^+$	30	(1) 40	
	uxuR$^+$	37	(3) 130	
		42	(1) 30	
HJ1ra2	*exuR1-2*	37	(4) 500	
	uxuR$^+$	30	(6) 500	
RC1ra42	*exuR10-42*	42	(21) 2,144	
	uxuR$^+$			
1176	*exuR*$^+$	30	(87) 3,068	
	uxuR14	37	(87) 4,580	
		42	(87) 3,160	
1414	*exuR1-2 uxuR14*	37	(168) 8,810	5,080
1455	*exuR10-42 uxuR14*	30	(260) 9,140	3,568
		42	(231) 8,360	5,304

[a] Numbers in parentheses represent percentage of the best induction in the appropriate wild-type strain (PAT317 or P4X).

[b] Calculated sum from the differential rates of synthesis measured in single *exuR* and *uxuR* derepressed mutants.

drolyase syntheses, was transdominant to the wild-type inducible allele *uxuR*$^+$, and reverted to the constitutive *uxuR1-1* allele.

As with *lacI* derepressed alleles (8), derepressed alleles of *uxuR* (*uxuR*, *uxuR1-1*, *uxuR11*, and *uxuR12*) allowed a constitutive expression of the *uxuA-uxuB* operon and were recessive to the wild-type allele. Such constitutive expression of the *uxuA-uxuB* operon never exceeded 40% of its fully induced level but remained hyperinducible; this result suggests that in derepressed mutants a repressive action of the *exuR* gene product was still operative. As in the *lac* operon, one may assume that the repressor from the superrepressed allele of *uxuR* probably lost affinity for the inducer, and that the repressor from the constitutive alleles of *uxuR* probably lost afinity for the operator of the *uxu* operon. In most *uxuR* constitutive mutants, the β-glucuronidase (structural gene, *uidA*) synthesis was constitutive; this result was in agreement with independent observations by Novel and Novel (15), who showed that *uidA* and *uxuA-uxuB* operons were constitutively expressed (but repressed in diploid strains in the presence of the wild-type allele) in mutants selected for growth on methyl-β-D-galacturonide.

An independent observation confirmed the negative model of regulation; new mutants have been isolated after Mu insertion in the *uxuR* gene which show only a constitutive expression of the *uxuA-uxuB* operon (Hugouvieux-Cotte-Pattat and Robert-Baudouy, unpublished data).

The properties of mutants carrying only one mutated allele of *exuR* or *uxuR* gene strongly suggest that both *exuR* and *uxuR* repressors are involved in the control of the *uxu* operon. In the wild-type strain, both repressors are inactivated in the presence of the true common inducer, fructuronate (27).

As the *uxu* operon could be induced in strains carrying a superrepressed *exuR* allele but was not inducible in mutants harboring a superrepressed *uxuR* allele, the *uxuR* control should be more stringent, at least in the *uxuR* mutant, than the *exuR* one. However, as soon as one lock has been lost in strains carrying a derepressed *exuR* or *uxuR* allele, the second wild-type regulatory allele, *uxuR*$^+$ or *exuR*$^+$, was insufficient to completely inhibit the *uxuA-uxuB* operon. It thus seems that each repressor more or less partially represses the *uxu* operon, but that their simultaneous action results in a complete repression of this operon. Moreover, the analysis of double mutants carrying two derepressed alleles of *exuR* and *uxuR* genes (Table 5) unambigously shows that both repressors do not act independently but cooperate in some way for repressing the *uxu* operon. Such a cooperative process is illustrated by the following observation: the values of the mannonate oxidoreductase rates of synthesis measured in double derepressed mutants are higher than the arithmetical sums of those values estimated in each simple regulatory mutant. One may compare this situation with the known cases of cumulative inhibition and cooperative inhibition of an allosteric enzyme affected by two inhibitors (31). In cumulative inhibition, in which the two inhibitors act independently and their effects are simply additive, the product of both residual activities of the enzyme measured in the presence of one inhibitor equals the residual activity estimated in the presence of both inhibitors. When this last activity is lower than the mentioned product, the inhibition is said to be cooperative (or synergistic). An analogous result was observed in a case of cumulative repression (16).

In our case, the product of the residual activities (rate of synthesis in the single derepressed strain/rate of synthesis in double derepressed strain) in the *exuR* mutant RC1ra42 and in the *uxuR* mutant 1176 at 30°C (Table 5) is $0.055 \times 0.336 \simeq 0.018$; this product is clearly higher than the residual activity in the uninduced wild-type strain PAT317 at 30°C (rate of synthesis in uninduced wild-type strain/rate of synthesis in double derepressed strain): 0.004. At 42°C this comparison is yet more conclusive: $0.256 \times 0.378 \simeq 0.097 > 0.004$.

Bacterial systems with a known double negative regulation are not numerous. One may mention the case of the *gal* operon in *E. coli*, which is controlled by the two regulatory genes *galR*

and *capR* (4, 7, 9). The *deo* operons of *E. coli* (catabolism of nucleosides) are also simultaneously controlled by two regulatory genes, *cytR* and *deoR* (6); the *uidA* operon (catabolism of glucuronides) is regulated by both the *uidR* and *uxuR* genes (15). In these two last cases biderepressed mutants were described in which the derepression effect is higher than the sum the derepression effect for each monoderepressed mutant. Although this observation suggested a mechanism of cooperation between the two repressors, this cooperation was nevertheless not further substantiated.

To conclude, it must be emphasized that the molecular basis of the complex regulatory mechanism operating in the dual control of the *uxuA-uxuB* operon cannot be elucidated only through in vivo physiological and genetical studies but will require in vitro analyses with purified operators and repressors.

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