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Regulation of *Escherichia coli* K-12 Hexuronate System Genes: *exu* Regulon

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Two types of *Escherichia coli* K-12 regulatory mutants, partially or totally negative for the induction of the five catabolic enzymes (uronic isomerase, *uxaC;* altronate oxidized nicotinamide adenine dinucleotide: *uxaB;* mannonate hydrolyase, *uxuA*) and the transport system (*exuT*) of the hexuronate-inducible pathway, were isolated and analyzed enzymatically. Hexuronate-catabolizing revertants of the negative mutants showed a constitutive synthesis for some or all of these enzymes. Negative and constitutive mutations were localized in the same genetic locus, called *exuR*, and the following order for the markers situated between the min 65 and 68 was determined: *argG-exuR-exuT-uxaC-uxaA-tolC*. The enzymatic characterization of the pleiotropic negative and constitutive mutants of the *exuR* gene suggests that the *exuR* regulatory gene product exerts a specific and total control on the three *exuT*, *uxaB*, and *uxaC-uxaA* operons of the galacturonate pathway and a partial control on the *uxuA-uxuB* operon of the glucuronate pathway. The analysis of diploid strains containing both the wild type and a negative or constitutive allele of the *exuR* gene, as well as the analysis of thermosensitive mutants of the *exuR* gene, was in agreement with a negative regulatory mechanism for the control of the hexuronate system.

In *Escherichia coli* K-12, aldohexuronates (D-galacturonate and D-glucuronate) are metabolized into 2-keto-3-deoxy-D-gluconate via the action of the aldohexuronate permease (18) and five inducible enzymes, uronic isomerase, altronate, and mannonate hydrolyase (Fig. 1 of reference 2), which constitute the hexuronate system. Kinetic studies of the induction of the hexuronate system have been previously described (18, 28). Two patterns of induction were identified in wild-type strains. The first one was observed in the presence of galacturonate or tagaturonate and was restricted to the three galacturonate degradative enzymes (uronic isomerase, altronate NAD^+:oxidoreductase, and altronate hydrolyase) and the aldohexuronate permease. The second one was triggered by glucuronate or fructuronate and extended to the whole hexuronate system. The effect of D-mannonic amide, a gratuitous inducer (9) which is able to induce the whole system (28), suggests that the structural genes coding for these proteins are coordinately regulated.

Using a set of single mutants of the hexuronate system, it was established that the true inducers were tagaturonate in the first mode of induction and fructuronate in the second (28). The kinetic analysis of the hexuronate system induction showed that the syntheses of uronic isomerase and altronate hydrolyase on the one hand, and mannonate NAD^+:oxidoreductase and the hydrolyase on the other hand, were coordinately induced, suggesting therefore that structural genes coding for both enzyme groups belong to two distinct operons. The genetic identification of two distinct clusters supports this suggestion. The six structural genes of the five enzymes and the transport system were localized in the *E. coli* chromosome as shown in Fig. 1. The genes coding for uronic isomerase (*uxaC*) (19) and altronate hydrolyase (*uxaA*) (20) are contiguous and located at min 66 (4). The structural gene of the hexuronate transport sytem (*exuT*) maps close by the above two genes and is cotransducible with them (18). The gene coding for the altronate NAD^+:oxidoreductase (*uxaB*) is located at min 52 (19). The structural genes of the mannonate NAD^+:oxidoreductase and hydrolyase, *uxuB* and *uxuA*, respectively, are both situated at min 97 (26).

The following study describes pleiotropic-negative mutants which are partially or totally deficient for the synthesis of the hexuronate enzymes and the hexuronate transport system. In the same way, some of their galacturonate- or glucuronate-catabolizing revertants showing a constitutive synthesis for some or all of enzymes in the system were characterized. The location of these negative and constitutive mutations on the *E. coli* chromosome was determined. The

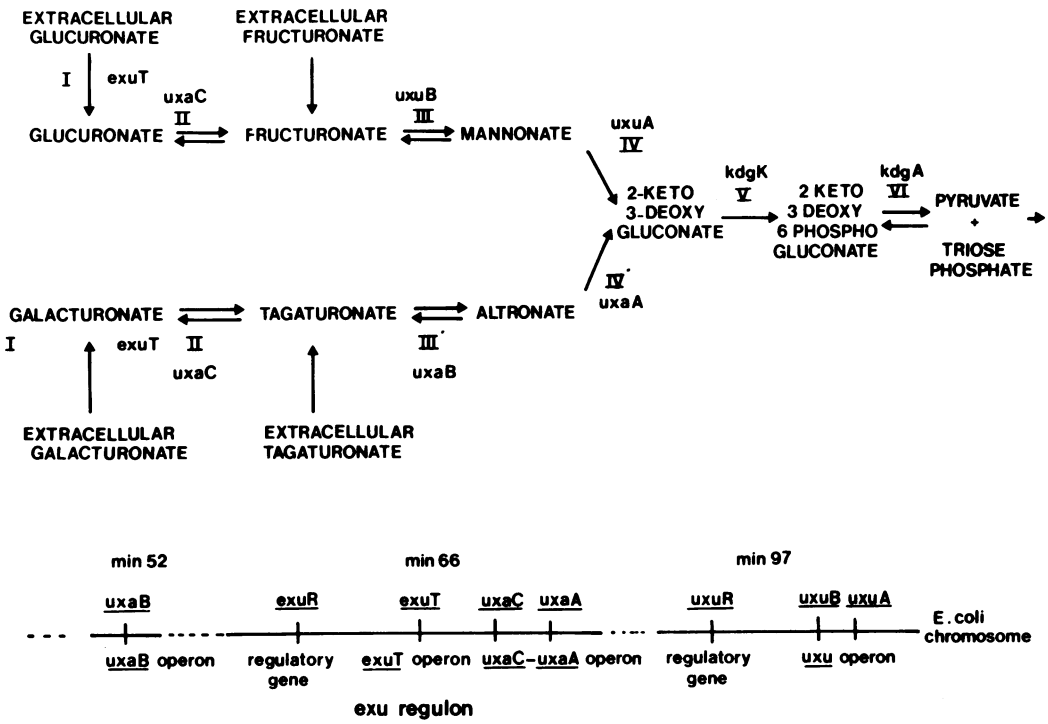

FIG. 1. *Degradative pathway of hexuronates in E. coli K-12. The different steps are catalyzed by the following enzymes: I, aldohexuronate transport system; II, uronic isomerase (EC 5.3.1.12); III, mannonic oxidoreductase (EC 1.1.1.57); IV, mannonic hydrolyase (EC. 4.2.1.8); III', altronic oxidoreductase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

analysis of diploids which contained both the wild-type allele and different mutated alleles helped to define the regulatory mechanism which controls the expression of the hexuronate system.

These results demonstrate the existence of a hexuronate "regulon" (13).

(This work formed part of a thesis submitted by J. R.-B. to the University of Lyon, Lyon, France, 1900.)

MATERIALS AND METHODS

Chemicals. Intermediate substrates of the hexuronate pathway were synthesized in our laboratory: D-tagaturonic acid (7), D-fructuronic acid (2), and D-mannonic amide (28). D-Glucuronate and D-galacturonate were purchased from Sigma Chemical Co.; *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was from Aldrich Chemical Co. [*U*-^{14}C]glucuronate potassium salt was purchased for the Radiochemical Centre, Amersham (England). All other substrates were analytical grade.

Nomenclature. The genetic nomenclature was according to Bachmann et al. (4). A new symbol, *exuR*, was used for the structural gene of the repressor. Strains HJ1 to 8 were pleiotropic mutants which did not grow on galacturonate as the sole carbon source, and the symbol for this phenotype was Gar$^-$. The corresponding mutations were named *exuR1* to *exuR8*. RC1 to 14 were pleiotropic mutants which did not grow on either galacturonate or glucuronate as a sole carbon source, and they had both Gar$^-$ and Gur$^-$ phenotypes. The associated mutations were designated by *exuR10* to *23*.

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

Media. M63 mineral medium (31) was supplemented as described elsewhere (20). Oxoid solid media contained either glucose (5 mg/ml) or glycerol (5 mg/ml) or glucuronate or galacturonate (2.5 mg/ml). Aldohexuronate Difco MacConkey media contained the sugars at 15 mg/ml.

Enzyme induction and extraction. The conditions for induction and extraction were described previously (28). The methods of determination of specific activity and differential rate of synthesis (17) are to be found in the same reference (28).

Enzyme assays. Aldonic oxidoreductases and hydrolyases were assayed according to previously published methods (22, 23, 27, 29); uronic isomerase was measured by a coupling method described previously

TABLE 1. *Bacterial strains*[a]

Strains	Sex	Genotype	Origin or deviation
P4X	Hfr	*metB1*	E. Wollman
A314	Hfr	*metB1 kdgA2*	(24)
KL16-99	Hfr	*recA*	(12)
MH1	Hfr	*metB1 uxaC1*	(19)
HJ1 to 8	Hfr	*metB1 exuR1* to *8*	Spontaneous revertant from A314, *kdgA*$^+$ transductant
HJ1ra1 to 8	Hfr	*metB1 exuR1-1* to *1-8*	Spontaneous revertant from HJ1 to 8 on galactouronate at 42°C
HJ1ru1 to 8	Hfr	*metB1 exuR1-9* to *1-16*	Spontaneous revertant from HJ1 to 8 on glucuronate at 42°C
RC1 to 14	Hfr	*metB1 exuR10-23*	NTG[b] mutants of P4X
RC1ra1 to 14	Hfr	*metB1 exuR10-1* to *10-14*	Spontaneous revertants from RC1 on galacturonate at 42°C
RC1rt1	Hfr	*metB1 exuR10-15*	Spontaneous revertant from RC1 on tagaturonate at 42°C
RC1ru1	Hfr	*metB1 exuR10-16*	Spontaneous revertant from RC1 on glucuronate at 42°C
PAT 317	F$^-$	*proA thr leu argH hisA thyA rpsL*	Hoffnung
AT2699	F$^-$	*argG metC thyA hisA rpsL*	A. L. Taylor
EW1b	F$^-$	*argG tolC hisA rpsL*	E. Whitney (33)
PB1	F$^-$	*argG uxaC1 metC hisA rpsL*	(19)
PB3	F$^-$	*tolC uxaC1 hisA rpsL*	(19)
JR38	F$^-$	*argG uxaA1 metC metB rpsL*	(20)
MR1	F$^-$	*argG exuR1 metC rpsL*	Thy$^+$ *rpsL* recombinant of the HJ1 × AT2699 cross
828	F′	*leu lac hisA recA mol xyl metB thyA argG rpsL*/F(*thyA*$^+$ *argG*$^+$)	B. Low, KLF22/KL110
812	F$^-$	*argG exuR1 thyA hisA rpsL*	MetC$^+$ *rpsL* recombinant of HJ1 × AT2699 cross
814	F$^-$	*argG exuR1 thyA recA rpsL*	His$^+$ *rpsL* recombinant of K16-99 × 812 cross
844	F′	*hisA recA exuR1 argG rpsL*/F(*exuR*$^+$ *argG*$^+$)	ArgG$^+$ *rpsL* recombinant of 828 × 814 cross
820	F$^-$	*uxaA1 metC thyA rpsL recA*	His$^+$ *rpsL* recombinant of KL16-99 × RJ27 cross
RJ27	F$^-$	*uxaA1 metC thyA rpsL hisA*	(20)
811	F$^-$	*argG uxaC1 metC thyA recA rpsL*	His$^+$ *rpsL* recombinant of KL16-99 × PBT1 cross (19)
816	F$^-$	*argG exuR1-2 thyA hisA rpsL*	TolC$^+$ *rpsL* recombinant of HJ1ra2 × EW1b *thyA* cross
817	F$^-$	*argG exuR1-2 thyA rpsL recA*	His$^+$ *rpsL* recombinant of KL16-99 × 816 cross
849	F′	*argG exuR1-2 thyA rpsL recA*/F(*thyA*$^+$ *argG*$^+$ *exuR*$^+$)	Diploid of 828 × 817 cross
1312	F$^-$	*argG exuR10 thyA hisA rpsL*	TolC$^+$ *rpsL* recombinant of RC1 × EW1b cross
1382	F$^-$	*argG exuR10 recA hisA rpsL*	RecA *thy*$^+$ recombinant of KL16-99 × 1312 cross
1422	F′	*argG exuR10 recA hisA rpsL*/F(*arg*$^+$ *exuR*$^+$)	828 × 1382 cross
1311	F$^-$	*argG exuR10-1 hisA thyA rpsL*	TolC$^+$ *rpsL* recombinant of RC1ru1 × EW1b *thyA* cross
1366	F$^-$	*argG exuR10-1 hisA recA rpsL*	RecA Thy$^+$ recombinant of KL16-99 × 1311 cross
1423	F′	*argG exuR10-1 hisA recA rpsL*/F(*argG*$^+$ *exuR*$^+$)	KLF22 × 1366 cross

[a] The allele numbers of usual genetic markers are these of the Coli Genetic Stock Center, Yale University, New Haven, Conn.

[b] NTG, *N*-Methyl-*N*′-nitro-*N*-nitrosoguanidine.

(19); the hexuronate transport system was assayed as described (18).

Plate assays for aldonic oxidoreductase activities. In situ plate assays were performed as described in (21). For a specific detection of altronate NAD$^+$: oxidoreductase activity, we used the inhibitory effect of parachloromercuribenzoate (10 mM) on the mannonate NAD$^+$:oxidoreductase.

Genetic methods. (i) Mutagenesis. Negative mutants either were isolated after mutagenic treatment of Hfr P4X with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (1) or were spontaneous mutants isolated from strain A314 (*kdgA*) as detailed previously (20). Strain A314 (lacking aldolase, enzyme VI, see Fig. 1), grown on glycerol + aldohexuronate, accumulated toxic 2-keto-3-deoxy-6-phosphate-D-gluconate and died. Sec-

ondary mutants of this strain blocked early in the hexuronate pathway did not accumulate the poisonous compound and could grow under the above conditions.

All mutants grew as white colonies on MacConkey agar plates containing aldohexuronates at 15 mg/ml, whereas wild-type colonies were red. Spontaneous mutants selected by the second method were able to grow on an Oxoid minimal medium supplemented with glycerol plus hexuronates (glucuronate, galacturonate, or both).

(ii) Genetic methods. Bacterial matings, bacteriophage P1 *kc* transductions, and preparation of diploid strains were performed according to Miller (15).

(iii) Reversions. Spontaneous revertants from hexuronate non-catabolizing strains were obtained at a rate of about 10^{-5} on solid minimal medium supplemented with glucuronate, galacturonate, or tagaturonate at various temperatures.

Construction of diploid strains containing wild-type allele and various mutated alleles of the *exuR* regulatory gene. Hfr strains HJ1, RC1, HJ1ra2, and RC1ru1 were mated with F^- strain EW1b, and TolC$^+$ *rpsL* (streptomycin resistant) recombinants carrying the *exuR1* (strain 812), *exuR10* (strain 1312), *exuR1-2* (strain 816), or *exuR10-16* (strain 1311) alleles were selected. The *recA* marker was then introduced in TolC$^+$ *rpsL* recombinants by mating with Hfr KL16-99 and selection of His$^+$ *rpsL* or Thy$^+$ *rpsL* recombinants; the corresponding *recA* derivatives were named 814, 1382, 816, and 1366. The KLF22 episome from diploid strain KLF22/KL110 (strain 828) was transferred in *recA* strains and maintained through selection for *argG* and *thyA* markers in diploid strains 844, 1422, 849, and 1423. The merodiploid capacity to transfer an episome was tested on *recA* F^- strains with a single mutation in the structural gene *uxaA* (strain 820) or *uxaC* (strain 811). It was thus verified that the episome was not deleted or integrated.

RESULTS

Isolation and characterization of different regulatory gene mutants (*exuR* locus). (i) *exuR* (Gar$^-$) type mutants and their revertants. A collection of independent spontaneous mutants of the *exuR* (Gar$^-$) type were isolated from strain A314 (*kdgA*) (24) which were able to grow at 37°C on minimal agar medium supplemented with glycerol and galacturonate. These mutants were still unable to grow on glycerol in the presence of glucuronate at 37°C. Presumably, the toxic accumulation of 2-keto-3-deoxy-phosphate-D-gluconate did not occur when galacturonate was the precursor. To study the effect of these mutations (called *exuR* [Gar$^-$]), the *kdgA*$^+$ allele was tranduced to all spontaneous double mutants (*kdgA-exuR* [Gar$^-$]) with phage P1 to obtain the *exuR* (Gar$^-$) mutations alone (strains called HJ).

Eight independent mutants (HJ1 to 8) were more thoroughly analyzed for their growth patterns on hexuronates at different temperatures and inducibility or constitutivity of the aldonate oxidoreductases (Table 2). All mutants were characterized by a deficiency in altronate NAD$^+$:oxidoreductase at 37 and 42°C which was related to their lack of growth on galacturonate at both temperatures. HJ strains showed other phenotypic properties which allowed their classification into four groups, as follows.

Mutants of class I were unable to grow on galacturonate and induce altronate NAD$^+$:oxidoreductase at any temperature. They maintained a wild-type phenotype for growth on glucuronate and for mannonate NAD$^+$:oxidoreductase induction.

Mutants of class II were similar to class I strains but could develop on galacturonate and induce altronate NAD$^+$:oxidoreductase at 30°C.

Mutants of class III, like class II strains, could induce altronate NAD$^+$:oxidoreductase at 30°C but did not grow on galacturonate at this temperature; in addition, mannonate NAD$^+$:oxidoreductase induction was reduced at 42°C as well as growth on glucuronate.

Mutants belonging to class IV were identical to those of class I as far as growth on galacturonate (lack of growth at all temperatures) and synthesis of altronate NAD$^+$:oxidoreductase (no induction at all temperatures) were concerned; however, class IV mutants had a defect in inducibility of mannonate NAD$^+$:oxidoreductase at 42°C which entailed the lack of growth on glucuronate at this temperature.

Revertants from mutants HJ1 to 8 (*exuR1* to *8*) were isolated as clones able to grow at 42°C on galacturonate or glucuronate as the sole carbon source. They were designated as HJra or HJru strains, respectively, and were obtained with a frequency of 10^{-6} to 10^{-7}. Their phenotypic characteristics are given in Table 2. From each HJ mutant, it was possible to obtain an HJra revertant; these revertants usually showed a constitutive synthesis for altronate oxidoreductase at 30 or 42°C and occasionally for mannonate NAD$^+$:oxidoreductase at 42°C. All HJra revertants grew on glucuronate at 42°C. Several HJru revertants were still unable to grow on galacturonate and induce alternate NAD$^+$:oxidoreductase at low or high temperature; some of them showed a constitutive synthesis of mannonate or altronate oxidoreductase.

(ii) Enzyme analysis. Enzyme levels of the hexuronate system were quantitatively assayed in the presence or absence of various inducers in HJ1 and HJ4 pleiotropic mutants in comparison to the wild-type strain P4X and three of their HJra-type revertants. Strains HJ1 and HJ4 were chosen as representative clones of classes I and III (Table 2). Results are shown in Table 3.

TABLE 2. *Phenotypic properties of various HJ (exuR1 to 8) mutants and their revertants*[a]

Class	Strain	Growth				Constitutivity[b]				Inducibility[c]			
		On galacturonate		On glucuronate		Altronic oxidoreductase		Mannonic oxidoreductase		Altronic oxidoreductase[d]		Mannonic oxidoreductase[e]	
		30°C	37 and 42°C	30 and 37°C	42°C	30°C	42°C	30°C	42°C	30°C	37 and 42°C	30 and 37°C	42°C
	P4X (wild type)	+	+	+	+	−	−	−	−	+	+	+	+
I	HJ1, HJ6	−	−	+	+	−	−	−	−	−	−	+	+
	HJ1ra1, HJ6ra1	−	+	+	+	−	+	−	(+)	(+)		+	+
	HJ1ra2, HJ6ra2	+	+	+	+	+	++	−	−			+	+
	HJ1ra3	+	+	+	+	+	++	−	+			+	−
II	HJ2	(+)	−	+	+	−	−	−	−	+	−	+	(+)
	HJ2ra1	+	+	+	+	+	++	−	−			(+)	+
III	HJ3, HJ4	−	−	+	−	−	−	−	−	+	−	+	(+)
	HJ3ra1, HJ4ra1	+	+	+	+	+	++	−	−			+	+
	HJ3ra2, HJ4ra2	+	+	+	+	−	−	−	−	+	(+)	+	++
	HJ4ra3	+	+	+	+	+	++	(+)	++				
	HJ3ru1	−	−	+	+	−	−	−	−	ND	−	ND	+
	HJ3ru2, HJ4ru1	+	+	+	+	−	(+)	−	+	ND	+	ND	
	HJ3ru3, HJ4ru2	+	+	+	+	++	+	−	+			ND	
IV	HJ5, HJ7, HJ8	−	−	+	−	−	−	−	−	−	−	+	(+)
	HJ5ra1	+	+	+	+	+	−	−	−		+	(+)	+
	HJ7ra1	+	+	+	+	+	−	−	+		+	(+)	
	HJ5ra2, HJ7ra2, HJ8ra1	+	+	+	+	+	++	−	−			(+)	+
	HJ5ru1, HJ7ru1, HJ8ru1	−	−	+	+	−	−	−	−	ND	−	ND	+
	HJ8ru2	−	−	+	+	+	−	−	+		−	ND	
	HJ5ru2	+	−	+	+	−	−	−	+	ND	−	ND	
	HJ7ru2	+	−	+	+	+	+	−	+			ND	
	HJ5ru3, HJ8ru3	+	+	+	+	+	+	−	−			ND	ND
	HJ7ru3	+	+	+	+	+	+	−	+			ND	

[a] +, Growth or presence of the enzyme; ++, presence of high activity of enzyme; −, no growth or absence of enzyme activity or constitutivity; (+), slight growth or low enzyme activity; ND, not done.
[b] Enzyme activity in cells grown in the absence of inducer (plate assays).
[c] Inducibility could not be identified on the basis of plate assays when strains were constitutive.
[d] In the presence of galacturonate (plate assays).
[e] In the presence of glucuronate (plate assays).

Strain HJ1 showed a low but significant constitutivity of the *uxaC-uxaA* and *exuT* (18) operons were nevertheless weakly inducible by tagaturonate, glucuronate, and fructuronate. The *uxaB* operon did not show any constitutive synthesis and was not induced by galacturonate or tagaturonate, but was weakly induced by glucuronate or fructuronate. The low activity of uronic isomerase in the presence of glucuronate seemed to be sufficient to permit HJ1 growth on this compound at all temperatures. The absence of altronate NAD^+:oxidoreductase induction by galacturonate or tagaturonate in strain HJ1 explained its inabililty to grow on galacturonate (Table 2).

In mutant HJ4, the *uxaC-uxaA* operon was not expressed at the normal level in the absence of inducer and could not be induced. In that strain the *uxaB* operon was weakly induced by fructuronate. The complete absence of altronate NAD^+:oxidoreductase and hydrolyase induction in the presence of tagaturonate (a true inducer) explained the lack of HJ4 growth on galacturonate. The temperature-sensitive growth pattern of HJ4 on glucuronate (Table 2) could be the consequence of the temperature-sensitive synthesis of the uronic isomerase.

In both HJ1 and HJ4 strains, the level of induction of the *uxuA-uxuB* operon by fructuronate was lower than that of the wild-type strain; this induction was temperature sensitive in strain HJ4. The isomerase activity of mutant

TABLE 3. *Enzymatic activities in mutants HJ1 and HJ4 and their revertants*

Strain	Inducer (5 mM)	Temp of growth (°C)	Differential rate of synthesis (mU/mg)[a]				
			Uronic isomerase (*uxaC*)	Altronic hydrolyase (*uxaA*)	Altronic oxidoreductase (*uxaB*)	Mannonic oxidoreductase (*uxuB*)	Mannonic hydrolyase (*uxuA*)
P4X	None	30	10 (1)	10 (1)	70 (<1)	80 (1)	<1 (<1)
	None	37	10 (1)	9 (1)	220 (1)	350 (3)	5 (3)
	None	42	15 (2)	5 (<1)	70 (<1)	10 (1)	1 (<1)
HJ1	None	37	35 (5)	30 (5)	12 (<1)	46 (<1)	<1 (<1)
	Galacturonate	37	25 (5)	20 (5)	<1 (<1)		
	Tagaturonate	37	110 (15)	71 (11)	72 (<1)		
	Glucuronate	37	80 (15)	60 (15)	900 (9)	8,000 (58)	98 (60)
	Fructuronate	37	63 (14)	80 (21)	1,080 (9)	6,160 (54)	80 (51)
HJ1ra2	None	37	800 (112)	600 (94)	17,000 (94)	500 (4)	10 (6)
	Galacturonate	37	250 (49)	330 (75)	4,000 (31)		
	Tagaturonate	37	130 (18)	62 (10)	6,560 (36)		
	Glucuronate	37	360 (68)	215 (53)	9,500 (96)	15,000 (109)	150 (91)
	Fructuronate	37	900 (205)	500 (128)	20,000 (163)	5266 (46)	68 (43)
HJ1ra1	None	30	540 (79)	560 (89)	220 (3)	10 (<1)	<1 (1)
	Galacturonate	30	320 (97)	350 (114)	80 (9)		
	Glucuronate	30	190 (73)	192 (80)	230 (4)	5,600 (72)	50 (63)
	None	42	420 (58)	360 (56)	7,000 (80)	1,300 (13)	10 (8)
	Galacturonate	42	250 (68)	250 (132)	6,200 (82)		
	Glucuronate	42	250 (56)	190 (105)	6,000 (69)	9,000 (90)	100 (85)
HJ4	None	37	1 (<1)	<1 (<1)	50 (<1)	46 (<1)	<1 (<1)
	Tagaturonate	37	24 (3)	<1 (<1)	66 (<1)		
	Fructuronate	30	22 (5)	<1 (<1)	573 (13)	6,430 (84)	70 (88)
	Fructuronate	42	<1 (<1)	<1 (<1)	517 (9)	5,875 (59)	65 (57)
	Glucuronate	42	<1 (<1)	<1 (<1)	50 (<1)	400 (4)	<1 (<1)
HJ4ra3	None	30	700 (103)	608 (97)	14,138 (184)	738 (9)	6 (8)
	None	42	930 (129)	778 (122)	26,300 (302)	2,525 (25)	18 (15)

[a] Numbers in parentheses represent: in the absence of inducer, percent best induction obtained in the wild-type strain P4X at the corresponding temperature (data not shown); in the presence of inducer, percent induction obtained in wild-type strain P4X with the same inducer at the corresponding temperature (data not shown).

HJ1, growing on glucuronate at 37°C, was sufficient to transform glucuronate into the true inducer, fructuronate, which allowed the induction of the *uxuA-uxuB* operon; this was presumably not the case for strain HJ4 at 42°C.

The HJra-type revertants did not recover a wild-type phenotype but showed an altered pattern of synthesis for the hexuronate system enzymes.

After growth on glycerol at 37°C, HJ1ra2, a non-temperature-sensitive revertant of HJ1, showed a high constitutive level of the galacturonate degradative pathway enzymes (*uxaC-uxaA* and *uxaB* operons) (Table 3). These results were extended by qualitative measurement (Table 2). The specific enzymes of the glucuronate pathway, mannonate NAD^+:oxidoreductase and hydrolyase (*uxuA-uxuB* operon), were present constitutively at very low levels (twice the basal level of the wild type in these conditions). The *uxuA-uxuB* operon of HJ1ra2 was normally inducible by glucuronate at 37°C.

HJ1ra1, a temperature-sensitive revertant for growth on galacturonate (Table 2), also showed a temperature-sensitive pattern of enzyme synthesis (Table 3). The *uxaB* operon, which could not be induced at 30°C, became clearly constitutive at 42°C; this is to be compared with the temperature-sensitive growth of HJ1ra1 on galacturonate. The *uxaC-uxaA* operon showed a constitutive expression at all temperatures. Lastly, the *uxuA-uxuB* operon, nonconstitutive at 30°C, was weakly constitutive at 42°C. There was no difference in the induction level of the wild-type strain P4X at 30 and 42°C for the different operons of the hexuronate system (data not shown).

(iii) *exuR* (Gar^-, Gur^-) type mutants and

their revertants. The mutants (RC strains) unable to develop (or growing very slowly) on both hexuronates (galacturonate or glucuronate) were identified on MacConkey hexuronate medium after mutagenic treatment and growth at 37°C. The phenotypes of 14 independent isolates of RC type, carrying an *exuR* (Gar^-, Gur^-) mutation (out of a collection of 250 clones), are shown in Table 4.

Mannonate and altronate oxidoreductases were not inducible in most RC mutants. In some strains, however, altronate NAD^+:oxidoreductase remained inducible at low temperature (RC10, RC11) or at any temperature (RC12, RC13). The inducibility of mannonate oxidoreductase was only detectable in a limited number of mutants and was temperature sensitive in some (RC7, RC9) and not in others. RC9 was clearly a temperature-sensitive strain for growth and enzyme synthesis.

The properties of revertants isolated from RC1 at 37 or 42°C on galacturonate (RC1ra), glucuronate (RC1ru), or tagaturonate (RC1rt) are presented in Table 4; analogous revertants from other RC strains gave similar results. If the ability to grow on galacturonate always implied the ability to grow on glucuronate, the reverse was not systematically observed. All RC1 revertants seemed more or less constitutive for mannonate NAD^+:oxidoreductase whether they

TABLE 4. *Phenotypic properties of various RC (exuR10 to 23) mutants and RC1 revertants selected on various substrates*[a]

Strain	Growth				Constitutivity[b]				Inducibility[c]			
	On galacturonate		On glucuronate		Altronic oxidoreductase		Mannonic oxidoreductase		Altronic oxidoreductase[d]		Mannonic oxidoreductase[e]	
	30°C	42°C	30°C	42°C	30°C	37 and 42°C	30°C	37 and 42°C	30°C	42°C	30°C	42°C
P4X	+	+	+	+	−	−	−	−	+	+	+	+
RC1, RC2, RC3, RC4, RC6, RC8, RC14	−	−	−	−	−	−	−	−	−	−	−	−
RC5	−	−	−	(+)	−	−	−	−	+	−	+	+
RC7	−	−	−	−	−	−	−	−	+	−	+	−
RC9	+	−	+	−	−	−	−	−	(+)	−	(+)	−
RC10, RC11	(+)	−	−	−	−	−	−	−	+	−	−	−
RC12, RC13	−	−	−	−	−	−	−	−	+	+	−	−
RC1 revertants												
RC1ra1, RC1rta1	+	+	+	+	++	++	+	++				
RC1ra2	−	+	+	+	−	(+)	(+)	++	(+)			
RC1ra42	(+)	+	+	+	(+)	+	(+)	+				
RC1ru1	+	+	+	+	+	+	(+)	+				
RC1ru2,ru6	−	+	(+)	+	−	+	(+)	+				
RC1ru27	+	−	(+)	+	+	−	(+)	+				
RC1ru8	−	−	(+)	+	+	+	(+)	+				
RC1ru10	−	−	(+)	+	−	+	(+)	+				
RC1ru13	−	−	(+)	+	+	−	(+)	+				
RC1ru17	−	−	(+)	+	−	−	(+)	+				
RC1ru49	(+)	+	(+)	+	−	+	(+)	+				
RC1ru35	+	+	+	+	−	+	+	+				
RC1ru24	+	+	+	+	+	−	(+)	(+)				
RC1ru14	+	+	(+)	+	+	+	+	−				
RC1ru31	−	+	(+)	+	+	+	−	+				
RC1ru26	+	+	−	+	+	+	+	+				
RC1ru44	−	+	(+)	+	+	+	+	+				

[a] +, Growth or presence of the enzyme; ++, presence of heavy quantity of enzyme; −, no growth or absence of inducibility or constitutivity; (+), slight growth or low enzyme activity.

[b] Enzyme activity in cells grown in the absence of inducer (plate assays).

[c] Inducibility could not be estimated on the basis of plate assays when strains were constitutive in the same conditions.

[d] In the presence of galacturonate (plate assays).

[e] In the presence of glucuronate (plate assays).

were isolated on glucuronate or galacturonate. This was not always observed for altronate oxidoreductase.

(iv) Enzyme analysis. Enzyme analysis of mutant RC1 is summarized in Table 5. In this strain, *uxaC-uxaA* and *uxaB* operons could not be induced even by tagaturonate or fructuronate. Induction of the *uxuA-uxuB* operon by fructuronate was about half of the wild-type level; in the absence of uronic isomerase, glucuronate was not transformed in fructuronate and consequently did not induce this operon. It should be noted that this strain, which did not grow on glucuronate, utilized fructuronate normally as a sole carbon source. The *exuT* operon was not inducible in mutant RC1 (data not shown; see reference 18). In *exuT* mutants (which are also unable to grow on both hexuronates), however, *uxaC-uxaA* and *uxaB* operons were induced by tagaturonate or fructuronate (18). The enzyme analysis of mutants RC10 and RC14 gave comparable results (data not shown).

Whatever the substrate used for the selection was, revertants RC1ru1, RC1rt1, and RC1ra1 isolated from mutant RC1 at 37°C showed a constitutive synthesis of *uxaC-uxaA* and *uxaB* operons (Table 5). Constitutivity of the *uxaB* operon was highest in strain RC1ru1 and higher than that of the *uxaC-uxaA* operon in all revertants. Since uronic isomerase was constitutively synthesized in revertants, the *uxuA-uxuB* operon could be induced by glucuronate. Glucuronate or fructuronate induction, however, still remained limited, as has been observed in mutant RC1 in the presence of fructuronate. The *uxuA-uxuB* operon was weakly but clearly constitutive in strain RC1rt1. Revertant RC1ra42 was selected for growth on galacturonate at 42°C and showed an interesting temperature-sensitive constitutive pattern of synthesis for the whole hexuronate system. Constitutivity of the *uxaC-uxaA* operon was higher than that of the *uxuA-uxuB* operon at 30 and 42°C. The temperature effect was maximum for the *uxaB* operon. Strain RC1ra42 properties could be compared with those of strain HJ1ra1, a temperature-sensitive revertant derived from strain HJ1 (Table 3).

Mapping of different *exuR* mutations. (i) Noninterrupted crosses. Mapping of the *exuR1* mutation was performed by crossing the mutant Hfr HJ1 with the F^- AT2699. Results (data not shown) placed the *exuR1* mutation (traced by the Gar^- phenotype) between the *argG* and *metC* markers.

Eight independent Hfr mutants, HJ1 to 8

TABLE 5. *Enzymatic activities in mutant RC1 and different RC1 revertants isolated on different substrates*

Strain	Inducer (5 mM)	Temp of growth (°C)	Differential rate of synthesis (mU/mg)[a]				
			Uronic isomerase (*uxaC*)	Altronic hydrolyase (*uxaA*)	Altronic oxidoreductase (*uxaB*)	Mannonic oxidoreductase (*uxuB*)	Mannonic hydrolyase (*uxuA*)
RC1	None	37	8 (1)	8 (<1)	98 (<1)	263 (2)	<1 (<1)
	Galacturonate	37	12 (2)	3 (<1)	146 (1)		
	Tagaturonate	37	28 (4)	3 (<1)	60 (<1)		
	Glucuronate	37	<1 (<1)	3 (<1)	376 (4)	900 (7)	13 (8)
	Fructuronate	37	10 (2)	2 (<1)	427 (3)	6,570 (58)	50 (32)
RC1ru1	None	37	1,112 (156)	452 (138)	45,663 (253)	1,020 (7)	<1 (<1)
	Galacturonate	37	404 (78)	328 (75)	12,080 (94)		
	Tagaturonate	37	596 (84)	516 (81)	22,250 (124)		
	Glucuronate	37	420 (80)	370 (91)	16,220 (165)	9,415 (68)	75 (46)
	Fructuronate	37	430 (98)	390 (100)	12,220 (100)	4,729 (42)	70 (44)
RC1rt1	None	37	496 (70)	424 (66)	38,554 (214)	2,760 (20)	48 (29)
	Galacturonate	37	618 (120)	400 (90)	29,857 (231)		
	Glucuronate	37	674 (128)	450 (111)	28,840 (292)	10,438 (76)	95 (58)
RC1ra1	None	37	510 (72)	354 (55)	17,514 (97)	225 (2)	4 (2)
	Tagaturonate	37	460 (65)	548 (86)	31,545 (175)		
	Fructuronate	37	494 (112)	270 (69)	23,423 (191)	7,800 (67)	83 (53)
RC1ra42	None	30	130 (18)	92 (14)	685 (9)	500 (6)	6 (8)
	None	42	430 (59)	348 (54)	20,756 (239)	2,144 (21)	27 (23)

[a] In the absence of inducer, numbers in parentheses represent percent best induction obtained in wild-type strain P4X at the corresponding temperature. In the presence of inducer, they represent percent induction obtained in wild-type with the same inducer at the corresponding temperature.

(*exuR1* to *8*; phenotype, Gar$^-$), were crossed with the F$^-$ strain JR38 carrying the *uxaA1* mutation (autronic hydrolyase deficient; phenotype, Gar$^-$); this mutation was previously mapped at min 66 between *argG* and *metC*. In each cross, the Gar$^+$ ArgG$^+$ *rpsL* and Arg$^+$ *rpsL* recombinants were selected. The Arg$^+$ *rpsL* recombinants permitted the correction of the variations of the absolute number of the Gar$^+$ recombinants in the different crosses. After this normalization, 10^2 to 10^3 times more Gar$^+$ recombinants were obtained with the wild-type Hfr P4X than with the Hfr HJ1 to 8. From these results it appears that the different *exuR* mutations were closely linked to the *uxaA1* mutation.

Using the same methodology, it was possible to show that independent mutations *exuR1* to *8* and *exuR10* to *23* were very strongly linked together and situated between the *argG* and *metC* markers.

(ii) Cotransduction studies of *exuR*. The *exuR* locus was more precisely mapped by determining cotransduction frequencies of different alleles of this gene (*exuR1*, *exuR10*, *exuR-2*, *exuR10-16*) with *tolC* (33), *uxaA1* (20), *uxaC1* (19), *exuT* (18), and *argG* (4), which were previously localized between min 65 and 68 (Table 6).

The different *exuR* mutations were weakly cotransducible (about 2%) with the extreme markers *tolC* and *argG*. Cotransduction frequencies of *uxaA*, *uxaC*, *exuT*, and *exuR* with *tolC* decreased from 22 to 2%, suggesting the following gene order: *tolC-uxaA-uxaC-exuT-exuR*.

Cotransduction values of the *uxaA*, *uxaC*, *exuT*, and *exuR* markers with *argG* never exceeded 1 to 2% and so could not be used for gene ordering. When mutant HJ1 (*exuR1*; phenotype, Gar$^-$) was used as a donor and strain PB3 (*uxaC1 tolC*; phenotype Gar$^-$ and Gur$^-$) as a recipient, 69% of the Uxu$^+$ TolC$^+$ transductants showed a Gar$^-$ phenotype (Table 6). These results confirmed that the *exuR* gene was situated out of the *tolC-uxaC* segment.

Unpublished results (P. Ritzenthaler et al., personal communication) and analysis of different deletions obtained from the λcI857 (inserted in the *exuR* gene) excision (14) confirmed the gene order given above.

Analysis of dominance relationships between the different *exuR* alleles. Activities of the hexuronate system enzymes were estimated in merodiploid strains containing different combinations of the wild-type and a negative or constitutive mutated allele of the *exuR* gene (Tables 7 and 8).

In the presence of the true inducer, tagaturonate or fructuronate, genes *uxaC*, *uxaA*, *uxaB*, and *exuT* were not expressed in diploid strains containing *exuR 1* or *exuR 10* negative mutations (strains 844, 1422) as observed in the haploid controls (strains 814, 1382) (Table 7).

The partial repressive effect of the *exuR1* and *exuR10* mutations on the induction of *uxuA* and *uxuB* genes was also maintained in merodiploids (Table 7, strains 844 and 1422).

These results showed that all the effects of the *exuR1* and *exuR10* mutations were dominant over the wild-type *exuR*$^+$ allele.

Analysis of diploid strains containing the constitutive *exuR1-2* (strain 849) or *exuR10-16*

TABLE 6. *Cotransduction of exuR with genetic markers of the min 66 region*

Donor (P1)	Donor genotype	Recipient	Recipient genotype	Selected marker	No. analyzed	Inheritance of unselected markers		Linkage
						Class	Percent	
HJ1	*exuR1*	EW1b	*tolC argG*	*tolC*$^+$	104	Gar$^-$[a]	3	*exuR1-tolC*
HJ1	*exuR1*	PB3	*tolC uxaC1*	*tolC*$^+$	623	Gar$^-$[a]	2.8	*exuR1-tolC*
RC1	*exuR10*	EW1b	*tolC argG*	*tolC*$^+$	312	Gur$^-$	1.3	*exuR10-tolC*
HJ1ra2	*exuR1-2*	EW1b	*tolC argG*	*tolC*$^+$	306	ExuR^c[b]	3	*exuR1-2-tolC*
RC1ru1	*exuR10-16*	EW1b	*tolC argG*	*tolC*$^+$	156	ExuR^c[b]	1	*exuR10-16-tolC*
HJ1	*exuR1*	EW1b	*tolC argG*	*argG*$^+$	200	Gar$^-$	21.8	*exuR1-argG*
RC1	*exuR10*	EW1b	*tolC argG*	*argG*$^+$	156	Gur$^-$	3.3	*exuR10-argG*
HJ1ra2	*exuR1-2*	EW1b	*tolC argG*	*argG*$^+$	170	ExuR^c[b]	<1	*exuR1-2-argG*
RC1ru1	*exuR10-16*	EW1b	*tolC argG*	*argG*$^+$	312	ExuR^c[b]	1	*exuR10-16argG*
HJ1	*exuR1*	PB3	*uxaC1 tolC*	Gur$^+$	364	Gar$^-$	66	*uxaC1-exuR1*
HJ1ra2	*exuR1-2*	MH1	*uxaC1*	Gur$^+$	100	ExuR^c[b]	88.5	*uxaC1-exuR1-2*
RC1rt1	*exuR10-15*	PB1	*uxaC1*	Gur$^+$	59	ExuR^c[b]	90	*uxaC1-exuR10-15*
HJ1	*exuR1*	PB3	*uxaC1 tolC*	Gur$^+$ *tolC*$^+$[c]	145	Gar$^-$	69	

[a] Gur$^+$.

[b] ExuR^c, Constitutive altronate oxidoreductase activity.

[c] TolC$^+$ recombinants were ColE1 sensitive and deoxycholate resistant.

TABLE 7. *Expression of different superrepressed alleles of the exuR regulatory gene in merodiploid strains*[a]

Strain	Relevant genotype for the *exuR* region	Inducer (5 mM)	Differential rate of synthesis (mU/mg)[b]					
			Uronic isomerase (*uxaC*)	Altronic hydroxylase (*uxaA*)	Altronic oxidoreductase (*uxaB*)	Mannonic oxidoreductase (*uxuB*)	Mannonic hydroxylase (*uxuA*)	Hexuronate transport system (*exuT*)
PAT317	F⁻ wild type *exuR*⁺	None	5	4	60	130	<1	35
		Tagaturonate	440 (100)	290 (100)	3,900 (100)	—[c]	—	
		Fructuronate	433	283	3,349	(100) 5,247	65 (100)	
828	F′*exuR*⁺/*exuR*⁺ wild type	None	17	10	<1	<1	<1	
		Tagaturonate	722 (100)	620 (100)	1,536 (100)	—	—	
		Fructuronate	422	280	1,340	4,263	38	
814	F⁻*exuR1 recA*	None	34 (8)	16 (6)	15 (<1)	63 (1)	<1 (<1)	
		Tagaturonate	43 (10)	16 (6)	24 (<1)	—	—	
		Fructuronate	90 (20)	28 (10)	206 (6)	1,179 (22)	11 (17)	
844	F′*exuR*⁺/*exuR1*	None	9 (1)	1 (<1)	50 (3)	32 (<1)	<1 (<1)	
		Tagaturonate	67 (9)	25 (4)	<1 (<1)	—	—	
		Fructuronate	35 (8)	8 (3)	177 (13)	1,065 (20)	8 (12)	
1382	F⁻*exuR10 recA*	None	10 (2)	<1 (<1)	20 (<1)	81 (2)	<1 (<1)	5.3
		Tagaturonate	19 (4)	<1 (<1)	13 (<1)	—	—	2.6
		Fructuronate	13 (3)	<1 (<1)	41 (1)	698 (13)	6 (9)	
1422	F′*exuR*⁺/*exuR10*	None	15 (2)	<1 (<1)	<1 (<1)	<1 (<1)	<1 (<1)	2.5
		Tagaturonate	56 (8)	<1 (<1)	<1 (<1)	—	—	3.5
		Fructuronate	24 (6)	<1 (<1)	165 (12)	885(17)	8 (12)	

[a] All assays were made at 37°C.

[b] Numbers in parentheses represent: in the absence of inducer, percent best induction; in the presence of inducer, percent induction obtained with the same inducer in the more adequately wild-type strain (haploid or diploid).

[c] —, Not determined.

TABLE 8. *Expression of different derepressed alleles of exuR regulatory gene in merodiploid strains*[a]

Strain	Relevant genotype for the *exuR* region	Differential rate of synthesis (mU/mg)				
		Uronic isomerase (*uxaC*)	Altronic hydrolyase (*uxaA*)	Altronic oxidoreductase (*uxaB*)	Mannonic oxidoreductase (*uxuB*)	Mannonic hydrolyase (*uxuA*)
817	F⁻*exuR1-2 recA*	480 (109)	180 (62)	2,857 (73)	228 (4)	<1 (<1)
849	F′*exuR*⁺/*exuR1-2*	94 (13)	53 (9)	16 (1)	49 (1)	<1 (<1)
1366	F⁻*exuR10-16 recA*	540 (122)	310 (107)	8,334 (213)	231 (4)	<1 (<1)
1423	F′*exuR*⁺/*exuR10-16*	33 (5)	14 (2)	14 (1)	20 (<1)	<1 (<1)

[a] All assays were made in the absence of inducer and at 37°C. For explanation of numbers on parentheses, see Table 7, footnote *b*.

(strain 1423) alleles (Table 8) showed that the derepressive effects of these mutations on the *exuR* regulon and *uxuA-uxuB* operon (seen in haploid controls 817 and 1366) were completely recessive in the presence of the wild-type *exuR*⁺ allele.

DISCUSSION

We have isolated and characterized two types of *E. coli* K-12 regulatory mutants partially or totally blocked ("superrepressed") in the induction of the enzymes and the transport system of the hexuronate pathway. Revertants of these mutants have been obtained in which the synthesis of some or all of these enzymes became constitutive (thermosensitive or not). All these mutations were localized at min 66 in the locus *exuR* and were cotransducible with some markers of the hexuronate pathway: *exuT*, *uxaC*, and *uxaA*, as well as *tolC* and *argG* characters. The folowing order of these markers was established (Table 6): *argG-exuR-exuT-uxaC-uxaA-tolC*. The mutations in the *exuR* gene were always pleiotropic and affected all the operons of the hexuronate system, so this gene may be considered to have a regulatory role on these operons. The different mutant alleles of the *exuR* gene profoundly affected the expression of the *exuT* (18), *uxaC-uxaA*, and *uxaB* operons; on the other hand, with the same alleles

of *exuR* the effect on the expression of the *uxuA-uxuB* operon was less pronounced. Moreover, partial deletions of the *exuR* gene or its inactivation by insertion of phage λ (14), which involve complete absence or inactivation of the *exuR* gene product, always make the *exuT* (18), *uxaC-uxaA*, and *uxaB* operons fully constitutive and the *uxuA-uxuB* operon only weakly constitutive (Robert-Baudouy et al., unpublished data). It follows that the independent *exuT*, *uxaC-uxaA*, and *uxaB* operons belong to a "regulon" (13) controlled by the common and, as far as we know, unique regulatory gene *exuR*. This gene, however, only partially controls the *uxuA-uxuB* operon, which may be simultaneously regulated by a second and independent regulatory gene. The isolation and characterization of other regulatory mutants which only affect the *uxuA-uxuB* operon (Robert-Baudouy et al., unpublished data) support this hypothesis.

The following observations argue that the *exuR* gene synthesizes a repressor which negatively controls the expression of the *exu* regulon, unless it is inactivated by binding an inducer.

(i) The pleiotropic noninducible (superrepressed) mutations of *exuR* are fully similar to the superrepressed i^s mutations of the *lacI* regulatory gene of the *lac* operon (34), a system with a typically negative regulation. As these i^s mutations, the superrepressed mutations of *exuR* always revert into a constitutive phenotype.

(ii) In merodiploid strains, however, the different superrepressed alleles of *exuR*, as the i^s alleles for the *lac* operon (34), are dominant to the wild-type inducible allele $exuR^+$. The different constitutive alleles of *exuR*, as the constitutive i^- alleles for the *lac* operon, (9), are recessive to the wild-type inducible allele $exuR^+$. As in the *lac* operon, one may assume that the repressor from the superrepressed alleles of *exuR* probably loses affinity for the inducer, and that the repressor from the constitutive alleles of *exuR* probably loses the affinity for the operators of the different operons of the system.

(iii) The repressor nature of the *exuR* gene product is very consistent with the properties of the thermosensitive constitutive revertants of the HJ1ra1 and RC1ra42 type. These two strains, constitutive at any temperature for the *exuT* (18) and *uxaC-uxaA* operons, remain uninduced at 30°C for the *uxaB* operon. At 42°C this last operon also becomes constitutive. These mutants, therefore, as far as the *uxaB* operon is concerned, behave like thermosensitive regulatory mutants of the negatively controlled *lac* operon (8, 30) and of other negatively controlled systems (6, 10, 25, 32). We suggest that in these two mutants the mutated *exuR* gene synthesizes a modified and thermolabile repressor. The mutation of the repressor is such that it has reduced or no affinity at 30°C for the operators of the *exuT* and *uxaC-uxaA* operons, but remains able to bind the operator of the *uxaB* operon, which thus has the strongest affinity for the repressor; moreover, at 30°C the repressor retains the initial superrepressed mutation, thus hindering the induction of the *uxaB* operon. At 42°C it is denatured and fully inactive. The thermosensitivity of this repressor affects even the other operons of the *exu* regulon and the *uxuA-uxuB* operon in the RC1ra42 mutant, where the constitutivity of all operons increases with the temperature.

(iv) The phenotype of the mutants where *exuR* is deleted or has been inactivated by inserting the phage λ (14) gives very convincing support for the role of the *exuR* gene product as a repressor. In this case (as at 42°C with the thermolabile products of the HJ1ra1 and RC1ra42 constitutive revertants), the product of the *exuR* gene is abolished. Only the complete disappearance of a negatively acting repressor can entail the total constitutive phenotype we observed here; the supression of a positive controlling activator would have resulted in a noninducible phenotype.

Very similar results have been found in other negatively controlled systems where the regulator gene was deleted (25). As in the case of the *lac* repressor, all these results are compatible with the product of the *exuR* gene being a repressor with two binding sites, one for binding the inducer (abolished in our superrepressed mutations) and one for binding the operators (abolished in our constitutive mutations).

This notion is strengthened by the peculiar properties of the conditional constitutive mutants HJ1ra1 and RC1ra42. These revertants of a superrepressed mutant are, at 30°C, simultaneously constitutive for *exuT* and *uxaC-uxaA* and still superrepressed for *uxaB*. This shows that the mutant retained the initial superrepressed mutation besides the new constitutive mutation. As in a similar case with the i^s i^- double mutants of the regulatory gene of the *lac* operon, it suggests that the corresponding binding sites for inducer and operator in our system are lying on the same molecule; otherwise the superrepressed phenotype would remain dominant (5).

Lastly, one of the studied mutants, HJ1, is of a new and original type which, so far as we know, has never been described for inducible operons. This mutant is entirely superrepressed for the *uxaB* operon, but weakly constitutive for the *exuT* (18) and *uxaC-uxaA* operons; since these last two operons are nevertheless noninducible,

they may also be considered as superrepressed. In this partially constitutive and partially supperrepressed mutant, which is obviously due to a single mutation (obtained with a relatively high mutation rate), one may reasonably assume that a unique mutational event in the *exuR* gene simultaneously partially inactivated the binding site of the repressor for some operators and entirely inactivated the binding site for the inducer. It is comparable to regulatory mutants of class III of the repressible *arg* regulon (11), which are partially derepressed but fully insensitive to the action of the corepressor.

The present experimental results do not allow us to decide whether the *exuR* repressor only possesses one or two distinct binding sites for the true, different-acting inducers, tagaturonate and fructuronate (the first one derepresses only the *exu* regulon; the second one additionally represses the *uxuA-uxuB* operon). Nor is it presently known whether this repressor has one recognition site for all the operators of the system (which would thus have different affinities for this unique site), or several sites (for instance, one for the operators of the *exu* regulon and one for the operators of the double-regulated *uxuA-uxuB* operon). Only the in vitro study of the purified *exuR* gene product will allow us to answer these different questions and definitively prove the repressor nature of the *exuR* gene product.

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ERRATA

Cytochrome c_3 from the Sulfate-Reducing Anaerobe *Desulfovibrio africanus* Benghazi: Purification and Properties

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Volume 140, no. 3, p. 898, Table 4: The number of Phe residues from *D. africanus* Benghazi should read "4" rather than "8," and the difference should read "1.5" rather than "5.5."

Regulation of *Escherichia coli* K-12 Hexuronate System Genes: *exu* Regulon

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Volume 143, no. 3 p. 1095, column 1, lines 5–7: Should read "... uronic isomerase, altronate NAD^+:oxidoreductase, altronate hydrolyase, mannonate NAD^+:oxidoreductase, and mannonate hydrolyase (Fig. 1) (2), which constitute...."

Page 1096, column 1, line 10: "1900" should read "CNRS:A08780."

Page 1098, column 1, line 30: "816" should read "817."

Page 1099, column 1, lines 2 and 3: Should read "... and *exuT* (18). These operons were...."

Page 1101, Table 4, column 4, last line: "(+)" should read "+."

Page 1103, column 1, line 24: "*exuR-2*" should read "*exuR1-2*."

Page 1103, column 1, line 33: Should read "*tolC* were 22, 13, 5, and 2%, respectively, suggesting...."

Page 1103, Table 6, column 5: "$tolC^+$" should read "$tolC^{+c}$."

Page 1103, Table 6, column 8: "21.8" should read "2.8."

Page 1105, column 1, lines 9–10 and 20: "unpublished data" should read "J. Bacteriol., in press."

Glyoxylate Cycle in *Mucor racemosus*

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Volume 143, no. 1, p. 419, legend to Fig. 7, line 3: "5 M" should read "5 μM," and "50 M" should read "50 μM."