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Regulation of Expression of the *uxu* Operon and of the *uxuR* Regulatory Gene in *Escherichia coli* K12

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Gene fusions between the *lac* structural genes and various genes of the hexuronate system of *Escherichia coli* K12 were isolated by the technique of Casadaban. Mud(Ap^r *lac*) and λ*plac*–Mu insertion mutants were constructed in which the *lac* genes were fused to the regulatory region of the *uxu* operon. In all the *uxu–lac* fusion strains, β-galactosidase expression was shown to be inducible by the natural inducers of the *uxu* operon (glucuronate and fructuronate) and sensitive to catabolite repression by glucose. In addition we isolated a Mud(Ap^r *lac*) fusion where the *lac* genes were fused to the *uxuR* regulatory gene. In this fusion the synthesis of β-galactosidase reflects the regulation of the *uxuR* gene. In the presence of a wild-type *uxuR* allele, partial repression of β-galactosidase expression was found; the repression was removed when inducer was added. This result indicates that while the *uxuR* gene is subject to autogenous control, the *uxuR* repressor may have only a low affinity for its own operator.

INTRODUCTION

Glucuronate can serve as a carbon source for growth of *Escherichia coli* K12. It is degraded by bacteria after adaptation, according to the pathway first discovered by Ashwell (1962); the pathway is illustrated in the accompanying paper (Ritzenthaler & Mata-Gilsinger, 1983). Glucuronate enters the cell by a specific transport system I (THU). It is isomerized to fructuronate by enzyme II, reduced by enzyme III to mannonate, and then is further dehydrated by enzyme IV into 2-keto-3-deoxy-D-gluconate. This last compound, a common intermediate of hexuronate degradation, is phosphorylated by enzyme V and split by the specific aldolase VI into pyruvate and triose phosphate. Galacturonate is degraded in a parallel pathway, using the same transport system (I) and the same first step (enzyme II). The *uxu* operon includes the two genes specifically involved in glucuronate degradation, *uxuA* and *uxuB* (structural genes for enzyme III and IV; Robert-Baudouy *et al.*, 1974), at min 97. The *uxu* operon is principally regulated by the product of the *uxuR* gene (min 97) and partially by that of the *exuR* gene (min 68) (Robert-Baudouy *et al.*, 1981; Portalier *et al.*, 1980).

The expression of many structural genes of the hexuronate system (*uxaB*, *uxaCA* and *exuT*) has been studied by analysis of their fusion with *lac* genes (Hugouvieux-Cotte-Pattat & Robert-Baudouy, 1981). Moreover, the isolation of *exuR–lac* gene fusions showed that the *exuR* gene is autoregulated (Hugouvieux-Cotte-Pattat & Robert-Baudouy, 1982).

In this paper we report the construction of fusions of the *lac* genes with the *uxu* operon, by use of the *in vivo* methods devised by Casadaban (1976*a*) and Casadaban & Cohen (1979). This approach confirms the results of physiological analysis (Robert-Baudouy *et al.*, 1974). Glucuronate induces the *uxu* operon, but only after its transformation into fructuronate; it is not an inducer of this operon in *uxaC* mutants. In contrast, fructuronate appears to be a true inducer; it induces in *uxuB* mutants without being metabolized further. Mannonic amide is a gratuitous inducer of the *uxu* operon.

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Table 1. *Strains of Escherichia coli K12 and phage*

Strain	Genotype*	Source or reference
E. coli		
MC4100	F⁻ *araD139 ΔlacU169 rpsL thi*	Casadaban (1976*a*)
MAL103	F⁻ *Δpro–lacXIII rpsL thi* (Mud(Ap^r *lac*) Muc*ts*)	Casadaban & Cohen (1979)
MCR1	as MC4100 but *exuR1*	Hugouvieux-Cotte-Pattat & Robert-Baudouy (1981)
MCR1-1	as MC4100 but *exuR1-1*	as MCR1
MI2	as MC4100 but *uxaC*::Muc*ts*	as MCR1
NU1 to NU7	as MC4100 but *uxu*::Mud(Ap^r *lac*)	this work
MU1 to MU17	as MC4100 but *uxu*::Muc*ts62*	this work
MUF1 to MUF6	as MU11 but *uxu*::–ΔMu–::λp123(209)	this work
NC13	as MC4100 but *uxuR*::Mud(Ap^r *lac*)	this work
P4X	Hfr *metB thi*	this laboratory
RJ54	F⁻ *ΔuxuAB–uxuR thr leu arg rpsL thi*	Robert-Baudouy & Portalier (1974)
Phage		
Muc*ts62*	*cts62*	This laboratory
Mud(Ap^r *lac*)	*cts* d1(Ap^r *lac*)	Casadaban & Cohen (1979)
λp1(209)	*lacA?YZO'–ΔW209–trp'AB'*::(+Mu')	Casadaban (1976*a*)
λp123(209)	*lacA?YZO'–ΔW209–trp'ABCDE?'*::(–Mu')	Casadaban (1976*a*)

* The nomenclature is that described by Bachmann & Low (1980).

Catabolite repression exerted by glucose on the expression of the *lac* genes in the fusion strains demonstrated that the genes of the *uxu* operon were very sensitive to the glucose effect and that this repression was partially removed by addition of cAMP. Moreover, analysis of a particular Mud(Ap^r *lac*) insertion strain indicated that the strain contained an *uxuR–lac* fusion and permitted us to study *in vivo* the regulation of this regulatory gene. Previous indications that the *uxuR* gene was autogenously regulated were based on analysis of a *uxuR–lac* fusion constructed *in vitro* and carried by a multicopy plasmid (Ritzenthaler & Mata-Gilsinger, 1982). Our *in vivo* fusion showed that this autoregulation takes place *in vivo*.

METHODS

Bacterial and bacteriophage strains. All bacterial strains used were derivatives of *E. coli* K12. Bacterial and phage strains are listed in Table 1.

Media and growth conditions. The media used were described previously (Miller, 1972). Cells were usually grown in complete medium (L) or in synthetic medium (M63) supplemented with glycerol (5 mg ml^{-1}), glucose (5 mg ml^{-1}), lactose (5 mg ml^{-1}), glucuronate or galacturonate (2·5 mg ml^{-1}). Aldohexuronate/MacConkey media contained these compounds at 15 mg ml^{-1}. 5-Bromo-4-chloro-3-indolyl-β-D-galactoside was added to minimal medium at 40 μg ml^{-1}.

Enzyme assays. β-Galactosidase was assayed in exponentially growing cells by the method of Miller (1972). Mannonate oxidoreductase and mannonate hydrolyase were measured by previously published methods (Portalier & Stoeber, 1972; Robert-Baudouy & Stoeber, 1973).

Enzymes were assayed in toluene-treated cells (Miller, 1972; Robert-Baudouy *et al.*, 1974). All the specific activities reported are the average of at least three different assays.

Plate assays for aldonic oxidoreductase activities (enzymes III and III'). *In situ* plate assays were performed, after toluene treatment, on clones grown overnight on glycerol minimal agar medium supplemented or not with inducer (usually galacturonate or glucuronate), as described by Portalier & Stoeber (1972). Only clones derepressed (constitutive) or induced for one aldonic oxidoreductase activity became blue.

Chemicals. Intermediate substrates of the hexuronate pathway (tagaturonate, fructuronate) and mannonic amide (gratuitous inducer) were synthesized in our laboratory (Robert-Baudouy & Stoeber, 1973).

Transduction and matings. Transductions with the generalized transducing phage P1 and non-interrupted mating experiments were carried out according to Miller (1972).

Preparation of phage lysates. Muc*ts62* and Mud(Ap^r *lac*) were prepared by thermoinduction of lysogenic strains, MC4100 (Muc*ts62*) and MAL103, respectively. λ lysates were prepared by lytic growth (Miller, 1972) on strain MC4100. Once lysis had occurred, chloroform was added and the lysate was shaken and centrifuged to remove cell debris.

Assays for Mu lysogeny. (i) *By phage release.* Colonies were replicated into a lawn of strain MC4100 on L-agar plates. After overnight incubation at 42 °C, lysogens showed a halo of lysis around the colony because they release phages that kill nearby Mu-sensitive cells.

(ii) *By cross-streaking.* Colonies picked with a sterile toothpick were cross-streaked against a lysate of Mu*cts62*. After overnight incubation at 42 °C, immune or resistant colonies were unaffected whereas sensitive colonies were lysed at their intersection with the phage lysate.

Isolation of mutants induced with Mud(Ap^r *lac) phage.* A fresh saturated culture of strain MC4100 was infected with a Mud(Ap^r *lac*) lysate for 20 min at 30 °C (Casadaban & Cohen, 1979). The multiplicity of infection was 1 by calculation from plaque-forming units of the lysate. To allow the expression of ampicillin resistance, the mixture was diluted with 10 volumes of L-broth and incubated with shaking for 30 min at 30 °C. Ampicillin-resistant transductants were selected by plating on MacConkey/glucuronate agar plates containing ampicillin (25 μg ml^{-1}). White colonies on this medium (ampicillin resistant and Glucuronate$^-$) which grew on lactose minimal medium only when this was supplemented with glucuronate were retained as potential fusion mutants.

Isolation of mutants induced with Mucts62 phage. Drops of a Mu*cts* lysate were spotted on to a lawn of strain MC4100 on L-agar plates supplemented with $CaCl_2$ (1 mM) and $MgSO_4$ (2·5 mM) and incubated overnight at 30 °C (Bukhari & Ljungquist, 1977). Survivors of Mu*cts* infection were grown in L-broth to exponential phase (about 10^8 cells ml^{-1}) and a suitable dilution was spread on MacConkey/glucuronate agar plates. Mutants that appeared as white colonies on this medium were tested for Mu lysogeny by phage release.

The procedure used to obtain Mu*cts62* lysogens yielded single- as well as multiple-site Mu insertions. Bacteriophage P1 transductions were carried out to determine which strains were single Mu*cts62* insertions. Lysogens were transduced to Glucuronate$^+$ by a P1 lysate made on strain MC4100. Only lysogens carrying a single prophage in a gene involved in glucuronate degradation would be cured of the Mu*cts62* phage. The other lysogens were either multiple Mu*cts62* insertions or had a mutation in the glucuronate pathway and an unlinked Mu*cts62* insertion.

Selection for gene fusion with Mucts62-induced mutants and λplac phages. Lysogens for λpl(209) and λp123(209) of each Mu*cts62* insertion mutant were isolated by the method of Casadaban (1976*a*). An overnight L-broth culture of each λ,Mu dilysogenic strain was centrifuged and resuspended in 10^{-3} M-$MgSO_4$. About 10^9 cells were spread on to a lactose/glucuronate minimal plate and incubated at 42 °C overnight, then at 37 °C for 3 d. Colonies that appeared were patched on to lactose minimal plates and lactose/glucuronate minimal plates. Colonies that grew better on this second medium than on the first were retained.

Construction of a plasmid carrying uxuR$^+$. To clone the *uxu* region of a wild-type *E. coli* strain, we used *in vivo* cloning on the RP4::mini-Mu plasmid pULB113 [phenotype: Tra$^+$ Ap Km Tc(Mu3A)] (Van Gijsegem & Toussaint, 1982). R′ plasmids able to complement RJ54, a Δ*uxuR-uxuB-uxuA* strain (Robert-Baudouy & Portalier, 1974), were selected on glucuronate minimal medium. The donor strain (P4X) was counterselected with streptomycin (200 μg ml^{-1}). The presence of the plasmid was checked by testing for resistance to the three antibiotics kanamycin (20 μg ml^{-1}), tetracycline (20 μg ml^{-1}) and ampicillin (20 μg ml^{-1}). The presence of the regulatory gene *uxuR* on the plasmid was tested by a normal inducible expression of the products of the *uxuA* and *uxuB* genes. Such plasmids bearing the whole *uxu* region *uxuR*, *uxuA* and *uxuB* were isolated and called R′*uxu*.

RESULTS

Isolation of Mucts62 or Mud(Ap^r *lac) insertion mutants*

Strain MC4100 was mutagenized with Mu*cts62* or Mud(Ap^r *lac*) phages as described in Methods. Glucuronate non-fermenting Mu insertion mutants were screened as white colonies on MacConkey/glucuronate plates; they appeared at a frequency of about 10^{-3} for Mud(Ap^r *lac*) and 5×10^{-3} for Mu*cts*. Mutants were purified three times on the same plates and then analysed to determine the site of their Mu insertion. Their growth phenotype on galacturonate minimal medium showed whether the mutated gene coded for an enzyme of the common degradative pathway or for one specific to glucuronate degradation. Only strains mutated in a gene involved specifically in glucuronate degradation were retained as potential Mu insertions in the *uxu* operon.

Enzymic analysis of polar mutants obtained by Mu insertion

Seven mutants with Mud(Ap^r *lac*) insertions and 17 with Mu*cts62* insertions resulting in a Glucuronate$^-$ phenotype were assayed for enzymes III and IV with glucuronate as inducer. These Mu insertion mutants fell into three classes based upon their enzyme levels.

Table 2. *Enzymic analysis of strain NC13*

Strains were grown at 30 °C on glycerol minimal medium to exponential phase (about 5×10^8 cells ml^{-1}) and enzymes were assayed after toluene treatment. Glucuronate was added as inducer at 5×10^{-3} M. The specific activities of enzymes III and IV are expressed in μmol product per min per mg bacterial dry weight. β-Galactosidase activity is expressed in units per mg protein. One unit of β-galactosidase activity is the amount of enzyme that will hydrolyse 1 nmol *O*-nitrophenyl-β-D-galactoside per min. —, Not determined.

		Specific activity		
Strain	Inducer	Enzyme III (*uxuA* gene product)	Enzyme IV (*uxuB* gene product)	β-Galactosidase
MC4100	None	17	40	—
	Glucuronate	159	760	—
NC13	None	233	1035	167
	Glucuronate	—	—	143
NC13(R'*uxu*)	None	25	85	87
	Glucuronate	192	930	146

Class I strains (15 independent clones) showed no detectable levels of enzymes III and IV (data not shown). They are probably Mu insertions in *uxuA* (the first gene of the *uxuAB* operon) which show a strong polar effect on the *uxuB* gene.

Class II strains (8 independent clones) synthesized enzyme IV but no enzyme III (data not shown). They are probably Mu insertions located in the *uxuB* gene.

A class III strain, the Mud(Ap^r *lac*) insertion mutant NC13, showed a high constitutive level of enzymes III and IV (Table 2), but very low growth on glucuronate. The fact that this clone had a Glucuronate$^-$ phenotype was not unexpected. It had already been observed that mutants with constitutive expression of the *uxu* operon (by inactivation of the *uxuR* gene or of the *uxuO* operator) are unable to metabolize glucuronate (Robert-Baudouy *et al.*, 1981). This strain probably had a Mu insertion in the *uxuR* gene.

Isolation of mutants with lac gene fusions by using Mud(Ap^r lac)

The seven mutants obtained after Mud(Ap^r *lac*) mutagenesis were grown on lactose minimal plates with and without glucuronate to determine if the mutated gene was fused to the *lac* genes. Three mutants were Lactose$^-$ with or without glucuronate; they probably resulted from integrations of the phage that orientated the *lac* genes incorrectly with respect to the promoter of the mutated gene. Three other mutants (NU2, NU3 and NU7) showed very low growth on lactose alone and good growth in lactose plus glucuronate. Mutant NC13 grew very weakly on lactose with or without glucuronate. The Mud(Ap^r *lac*) mutants NU2, NU3, NU7 and NC13, which had a Lactose$^+$ phenotype, were selected as potential fusion mutants.

In all these mutants the following characters could be cotransduced by phage P1 (about 70%): the Glucuronate$^-$ phenotype, ampicillin resistance (the selected character), growth on lactose (only in presence of glucuronate for NU2, NU3 and NU7), Mu phage immunity (shown by cross-streaking of the strain against Muc*ts*) and, for NC13, the constitutive expression of the *uxu* operon. The cotransduction of these phenotypes indicated that the Glucuronate$^-$ phenotype was the result of Mud(Ap^r *lac*) phage insertion. The phenotype with respect to growth on lactose resulted from fusion of the *lac* operon with a hexuronate-inducible gene in all the NU strains and in NC13 with a constitutively expressed gene. These strains were then analysed in more detail to determine the site of their Mu insertions.

Analysis of the fusion strain NC13

This strain was made into a partial diploid by introduction of R'*uxu* an RP4::mini-Mu derivative which transferred the entire *uxu* region including a wild-type *uxuR*$^+$ allele. The introduction of this R'*uxu* into the mutants NU2, NU3 and NU7 restored the Glucuronate$^+$ phenotype, indicating that the Glucuronate$^-$ mutation was caused by inactivation of the *uxu* operon.

Enzymes III and IV were constitutively expressed in NC13, so it was reasonable to assume the absence of any functional *uxuR* repressor due to a Mud(Ap^r *lac*) insertion in the *uxuR* gene. The presence of the R′*uxu* in NC13 suppressed the constitutivity of enzymes III and IV and restored normal inducibility (Table 2). Therefore the constitutive allele was recessive to the wild-type *uxuR* allele, which is characteristic of a mutation inactivating a negative regulatory gene. Moreover, the *uidA* gene coding for β-glucuronidase (min 36) is also subject to partial negative control by the *uxuR* gene product (Novel & Novel, 1976). In NC13 β-glucuronidase was found to be synthesized constitutively, consistent with inactivation of the *uxuR* gene (data not shown). We conclude that the Mu-induced mutation of NC13 is located in the region carried by the R′*uxu* and almost certainly represents an insertion inactivating the *uxuR* gene.

The *lacZ* product (β-galactosidase) was assayed in the *uxuR–lac* fusion strain, under different growth conditions and after introduction of the wild-type *uxuR* gene. The presence of the R′*uxu* lowered the synthesis of β-galactosidase to about 50% without inducer; it caused no significant reduction in the presence of glucuronate (Table 2). We conclude that NC13 carries a *uxuR–lac* fusion properly oriented and that the autoregulation of the *uxuR* gene can be demonstrated *in vivo*.

Isolation of additional uxu–lac fusion strains by using Mucts62 and λp(lacMu) phage

Among the 17 mutants which had inserted Mu*cts62* phage in the *uxu* operon, only the 10 monolysogenic mutants were retained. These 10 mutants were lysogenized with λp1(209) and λp123(209) phages, and two independent lysogens for each λ were selected. In the 40 resulting dilysogenic strains, Mu*cts* phage was heat-induced to obtain *lac* fusion deletions. These fusions were selected on lactose minimal medium supplemented with glucuronate, under conditions of derepression of the *uxu* operon. All dilysogenic strains, except MU11(λp123(209)), yielded clones which were still Lac$^+$ in the absence of inducer, indicating that the *lac* genes were not under the control of the *uxu* operon. In contrast, the two MU11(λp123(209)) lysogens gave, at a frequency of 10^{-7}, Lac$^+$ clones; of these, 30% could ferment lactose only in the presence of glucuronate. In these clones the deletion fuses the *lac* genes to the *uxu* regulatory region.

Six fusion strains (MUF1 to 6), derived from MU11(λp123(209)), which were Lac$^+$ only in the presence of glucuronate, were chosen for further characterization.

Orientation of the Mucts62 prophage in strain MU11

The orientation of the Mu insertion in MU11 could be deduced from the observation that only λp123(209) lysogens of this strain gave rise to *uxu* operon fusions. The two λ phages used, λp123(209) and λp1(209), lack the aa′ attachment site and, in the absence of the chromosomal *lac* genes, lysogenize stably in a Mu lysogen strain by integration via Mu homology. In MU11, integration put the *lac* genes in the *uxu* operon. λp1(209) phage carries the *c* end of phage Mu whereas λp123(209) carries its *S* end, and so they can integrate the *lac* genes in opposite directions (Casadaban, 1976*a*). Only the lysogens having the *lac* genes integrated in the same direction as the *uxu* operon could give operon fusions. In the case of MU11, λp123(209) put the *lac* genes in the correct orientation. The Mu orientation in MU11 is deduced as being (+) because the *c* gene on the phage Mu is counter-clockwise with respect to its *S* gene on the *E. coli* chromosome map, as usually represented (Howe & Bade, 1975).

Inducibility of the fused genes

The results of β-galactosidase assays for fusion strains NC13, NU2, NU3, NU7, MUF1, MUF6 and MUF8, with different inducers of the hexuronate system, are given in Table 3. All the fusion strains showed a significant basal level (without inducer). In no fusion strain was β-galactosidase activity induced by isopropyl-β-D-thiogalactopyranoside (1 mM) the *lac* inducer in a wild-type strain (data not shown). In all strains (except NC13) a high induction ratio was obtained with fructuronate (Table 3). The same ratio was found with glucuronate, but glucuronate was capable of being transformed into fructuronate in these strains. No induction of β-galactosidase by glucuronate was found when the NU3 or NU7 fusions were transduced by phage P1 in an *uxaC* mutant, while β-galactosidase remained induced by fructuronate.

Table 3. *Induction of β-galactosidase in uxuR–lac and in uxu–lac fusion strains*

β-Galactosidase specific activity was determined in exponentially growing cells in glycerol (or glucose) minimal medium, with or without an inducer. The numbers in parentheses indicate activity when cAMP (5 mM) was added 2 h before the assay.

Strain	Growth conditions: Carbon source	Inducer	β-Galactosidase activity [units (mg protein)$^{-1}$]
NC13	Glycerol	None	167
		Glucuronate	143
		Fructuronate	170
		Galacturonate	158
		Tagaturonate	141
	Glucose	None	114
NU3	Glycerol	None	4
		Glucuronate	130
		Fructuronate	118
		Galacturonate	6
		Tagaturonate	8
		Mannonic amide	107
	Glucose	None	2 (2)
		Glucuronate	10 (22)
		Fructuronate	9 (24)
NU2	Glycerol	None	21
		Glucuronate	250
NU7	Glycerol	None	5
		Glucuronate	24
MUF1	Glycerol	None	4
		Glucuronate	17
MUF6	Glycerol	None	15
		Glucuronate	294
MUF3	Glycerol	None	6
		Glucuronate	170
NU3(*uxaC*)	Glycerol	None	5
		Glucuronate	7
		Fructuronate	102
NU7(*uxaC*)	Glycerol	None	5
		Glucuronate	4
		Fructuronate	20

Mannonic amide efficiently induced β-galactosidase in NU2, NU3 and NU7. Galacturonate and tagaturonate were not inducers of β-galactosidase synthesis in these fusion strains. None of the compounds tested affected β-galactosidase synthesis in NC13 (Table 3).

Catabolite repression of the fused genes

The β-galactosidase activity of the fusion strains NU3 and NC13 was assayed in minimal medium containing glucose (Table 3). NU3 glucose-grown cells showed a β-galactosidase level reduced to 8% of that found for glycerol-grown cells with the same inducer. When cAMP was added to the glucose culture, the amount of β-galactosidase increased about threefold (Table 3). The synthesis of β-galactosidase in NC13 was only slightly inhibited by glucose (20% inhibition).

Expression of the lac fusions in the presence of regulatory gene mutations

The *uxu* operon is under the negative control of the *exuR* and the *uxuR* regulatory genes (Robert-Baudouy *et al.*, 1981). Two different mutated alleles of the *exuR* gene, *exuR1* (super-repressed and *exuR1-1* (derepressed), were introduced into strain MC4100, yielding strains MCR1 and MCR1-1 (Table 1). Strains MCR1 and MCR1-1 were first lysogenized with Mu*cts62* to prevent zygotic induction after introduction of Mud(Ap^r *lac*) by transduction. The *lac* fusions

Table 4. *Expression of β-galactosidase in the fusion strains in the presence of different alleles of the exuR gene*

β-Galactosidase specific activity was determined in exponentially growing cells in glycerol minimal medium without inducer, for the fusion strains derived from MC4100 (NC13, NU2 and NU3) and for the corresponding fusions transduced into MCR1 and MCR1-1, which respectively bear the *exuR1* (super-repressed) and *exuR1-1* (derepressed) allele of the *exuR* regulatory gene.

Strain	β-Galactosidase activity [units (mg protein)$^{-1}$]
NC13	167
NC13(*exuR1*)	151
NC13(*exuR1-1*)	190
NU2	21
NU2(*exuR1*)	15
NU2(*exuR1-1*)	45
NU3	4
NU3(*exuR1*)	5
NU3(*exuR1-1*)	15

NU2, NU3 and NC13 were then transduced into the lysogens by selecting for ampicillin resistance (about 90% cotransduction was seen between Ap^R and the fusion). The *exuR1* mutation had little effect on the fused genes (Table 4). When the *exuR1-1* allele was present, the *lac* genes of NU2 were expressed at twice the rate, those of NU3 at four times the rate, whereas those of NC13 only showed a 15% increase.

An attempt to introduce derepressed and super-repressed alleles of *uxuR* into our fusion strains failed, possibly because of the tight linkage between *uxuR* and *uxuA* or *uxuB*. These mutations might have had a greater effect on β-galactosidase synthesis than the *exuR* mutations.

Calculation of the number of repressor molecules per cell

The level of expression of the *uxuR* gene can be estimated from the level of expression of the *lac* genes in the *uxuR–lac* fusion strain. An induced wild-type *lacZ* gene produces 10^4 to 3×10^4 β-galactosidase monomers per cell (Miller, 1972). Comparison of the level of β-galactosidase expression in the *uxuR–lac* fusion strain (Table 2) with that for a fully induced *lac* operon showed that the *uxuR* promoter was about 1% as active as the *lac* promoter. If we assume that the *uxuR* and *lac* messenger RNA molecules are equally stable and are translated equally efficiently, this would indicate a steady-state level of about a few hundred monomers of the *uxuR* gene product per cell. On this hypothesis the number of *uxuR* repressor molecules in the cell would be about a hundred or less if this molecule is an oligomer. In the presence of an inducer of the hexuronate pathway, this number can double.

DISCUSSION

To further studies of regulation in the *exu* regulon, we have isolated strains in which the *lac* genes are fused to various genes involved in glucuronate degradation (*uxu* operon) and to the *uxuR* regulatory gene.

The pattern of β-galactosidase induction in the fusion strains was as expected from previous studies of the *uxu* operon. Glucuronate, fructuronate and mannonic amide were good inducers of β-galactosidase synthesis in *uxu–lac* fusion strains; galacturonate and tagaturonate had no inducing activity (Table 3). Fructuronate induced β-galactosidase synthesis in a *uxuB* mutant, indicating that fructuronate is a true inducer of *uxu* operon, since this sugar is not further metabolized in *uxuB* mutants. In contrast, glucuronate was not an inducer by itself but only after transformation into fructuronate by enzyme II (the *uxaC* gene product). As expected, in *uxaC* mutants the expression of *uxu–lac* operon was not induced by glucuronate.

The induction ratio for enzymes III and IV is about 30 in non-fusion strains (Robert-Baudouy *et al.*, 1974). In the *uxu–lac* fusions, β-galactosidase induction ratios were between 5 and 30,

depending on the strain tested. A range of activity in *uxu–lac* fusion strains has been observed in other systems (Casadaban, 1976*b*; Débarbouillé & Schwartz, 1979; Komeda & Iino, 1979) and presumably depends upon the point of Mud(Ap^r *lac*) insertion within the gene or the extent of the deletion obtained after Mu*cts62* excision.

The induced expression of the fused *uxu* operon was very sensitive to catabolite repression exerted by glucose; cAMP partially reversed the glucose effect (Table 3). The expression of the *uxuR* regulatory gene appeared to be only slightly catabolite repressed (Table 3).

Analysis of the effect on the fusion strains of derepressed or super-repressed alleles of *exuR*, the other regulatory gene of the hexuronate system, confirmed that the *uxu* operon was also controlled by the *exuR* gene product (Table 4). On the other hand, the *exuR* repressor seems to have no action, or a very weak effect, on the expression of the *uxuR* regulatory gene.

The *lacZ* product (β-galactosidase) was assayed in the *uxuR–lac* fusion strains, in different growth conditions, and after introduction of the wild-type *uxuR*$^+$ gene. In the *uxuR–lac/uxuR*$^+$ merodiploids, *lac* expression was found to decrease by a half in comparison with the corresponding *uxuR–lac* haploid fusion strain. This repression was partially removed after addition of an inducer of the hexuronate system. These results imply that the expression of *uxuR*, reflected by β-galactosidase synthesis in the fusion strains, is repressed by the *uxuR* gene product but expressed after addition of inducer. The *uxuR* gene is thus autoregulated; its product controls its own synthesis. This autoregulatory effect was also found by analysis of an *uxuR–lac* fusion constructed *in vitro* (Ritzenthaler & Mata-Gilsinger, 1982). As shown here, the *uxuR* repressor regulates its own synthesis *in vivo*. Autogenous repression was also shown in the case of *exuR*, the other regulatory gene of the hexuronate system (Hugouvieux-Cotte-Pattat & Robert-Baudouy, 1982). While in the case of *exuR*, this repression reaches five-fold, the *uxuR* protein represses its own synthesis only by a half. The autoregulation of *exuR* (which controls all the genes of the hexuronate system) probably plays a major role in the regulation of hexuronate degradation. In contrast, the *uxuR* repressor controls its own synthesis weakly; this may be sufficient to avoid too great a variation in the concentration of repressor under various growth conditions.

Autoregulation has also been found in the case of many other regulatory genes: *araC* (Casadaban, 1976*b*), *hut* repressor (Smith & Magasanik, 1971), *trpR* (Gunsalus & Yanofsky, 1980), *lexA* (Brent & Ptashne, 1980), *tyrR* (Camakaris & Pittard, 1982), and is probably a common way of maintaining the appropriate concentration of regulatory proteins in the cells.

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