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Regulation of β-Glucuronidase Synthesis in *Escherichia coli* K-12: Pleiotropic Constitutive Mutations Affecting *uxu* and *uidA* Expression

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Among the β-glucuronidase (UID)-constitutive mutants obtained by growth on methyl-β-D-galacturonide, some strains are also derepressed for the two enzymes of the *uxu* operon: mannonate oxidoreductase (MOR) and mannonate hydrolyase (HLM). By conjugation and transduction experiments, two distinct constitutive mutations were separated in each pleiotropic mutant strain. One of them was specific for *uidA* gene expression and was characterized as affecting either *uidO* or *uidR* sites. The second type of mutation was mapped close to the *uxu* operon and was found to be responsible for the pleiotropic effect revealed in the primary mutants: after separation such a mutation still fully derepresses MOR and HLM synthesis but weakly derepresses UID synthesis. The pleiotropic effect of this mutation was maintained even though the activity of the structural genes was altered. This rules out the occurrence of an internal derepressing interaction between these enzymes. In merodiploid strains, *uxu*-linked constitutive mutations were recessive to the wild-type allele, suggesting that these mutations could affect a regulatory gene. The *uxuR* gene is probably a specific regulatory gene for a very close operon, *uxu*. Moreover, it has a weak effect on *uidA* expression. Thus, UID synthesis would be negatively controlled through the activity of two repressor molecules that are synthesized by two distinct regulatory genes, *uidR* and *uxuR*. These two repressing factors are antagonized, respectively, by phenyl-thio-β-D-glucuronide and mannonic amide and could cooperate in a unique repression/induction control over *uidA* expression. Constitutive mutations affecting the control sites of *uidA* gene probably characterize two distinct attachment sites in the operator locus for each of the repressor molecules.

By growing *Escherichia coli* K-12 on methyl-β-D-galacturonide (MeGalU), a noninducing substrate of β-glucuronidase (UID), two types of constitutive mutants for UID synthesis were obtained. The mutants of the first type were specific for UID synthesis and carried only a mutation(s) located close to *uidA*, the structural gene of UID. The mutation(s) affected two loci: *uidO*, the presumed operator site of *uidA*; and *uidR*, a specific regulatory gene for *uidA* expression. This work is reported in the accompanying article (14).

The second type of MeGalU-positive mutant is studied in the present work. They have been identified by specific assays on solid media as strains simultaneously derepressed for UID and for mannonate oxidoreductase (MOR) (14). We first investigated whether these constitutive mutations exhibited a pleiotropic effect on the synthesis of the hexuronate pathway (see Fig. 1 of reference 14); physiological analysis, genetic mapping, and dominance studies were then carried out to establish their identity and significance.

MATERIALS AND METHODS

Materials, symbols, and general methods are described in the accompanying article (14). The bacterial strains were all derived from *E. coli* K-12 and are listed in Table 1. The color tests on solid media have already been described for UID (12) and MOR (17).

Assay for KDG kinase activity. 2-Keto-3-deoxygluconate (KDG) kinase was identified in toluene-treated cells (30 min at 37 C) taken from cultures grown on glycerol, according to Pouysségur and Stoeber (20). Induced or constitutive kinase activity was inferred by comparing the assayed strains with suitable reference strains.

Hexuronate enzyme assays. Presumed derepressed pleiotropic mutants, or their derivatives, were grown in glycerol liquid medium. The cells

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TABLE 1. *Principal bacterial strains*[a]

Strains	Sex	Genotype	Origin or derivation
PA309	F⁻	*thr-1,leu-6,thi-1,his-1,argH1, trp-1,str-9,lacY1,malA1,xyl-7, mtl-2,gal-6,ara-13*	E. Wollman (via J. Puig)
PA601	F⁻	Like PA309 but *trp⁺,purE43,proA*	E. Wollman (via J. Puig)
K63	F⁻	*his,fadD88,man-4,gal,str*	P. Overath (J. Pouyssegur)
KL132	F⁻	*pyrB3,thi-1,pro-27,thr-1,leu-6, his-1,thyA25,xyl-7,lacY1,gal-6, malA1,ara-13,str-9,recA1*	K. B. Low
F112/JC1553	F′	F *met⁺/metB1,leu-6,argG6, his-1,str-104,gal-6,xyl-7, mtl-2,lacY1* or *Z4,malA,recAI*	K. B. Low (B. Bachmann)
F101/AB2463	F′	F *thr⁺,leu⁺/thr-1,leu-6, proA2,his-4,argE3,thi-1,str-31, xyl-5,mtl-1,ara-14,galK2,lacY1, recA13*	K. B. Low (B. Bachmann)
F121/KL132	F′	F *pyr⁺,thr⁺*/KL132	K. B. Low (B. Bachmann)
JB1	Hfr	*metB1,uxuA1*	(25)
CM1	Hfr	*metB1,uxuB1*	(25)
CM8	Hfr	*metB1,uxuB8*	(23)
AU128	Hfr	*metB1,uxaC*	(11)
GMG22	F⁻	Like PA601, Δ*uxu,uxuR10*	(12; This paper)
GM290	F⁻	*argE3,aroD6,str-700,mtl-1,lac, gal,uidA1*	(12)
GMS343	F⁻	Like GM290 but *uid⁺,man-4*	(12)
GMS529	F⁻	Like GMS343 but *thy*	Trimethoprim resistance (28)
GMS407	F⁻	Like GMS343 but *aro⁺,str⁺,uidA1*	(12)
GM146	F⁻	*aroD6,thi-1,argE3,his-4,galK2, lacY1,xyl-5,mtl-1,str-700*	Gur⁺ derivative from AB3302 (12)
GMS799	Hfr	*argE3*	Transduction of P4X by P1 grown on GM146, selection for Met⁺
GMS830	Hfr	*metB1,man-4*	Transduction of GM291 by P1 grown on K63, selection for Uid⁺
GMS838	Hfr	*argE3,man-4*	Transduction of GMS830 by P1 grown on GM146, selection for Met⁺
GMS788	Hfr	*argH1,uxaC*	Transduction of AU128 by P1 grown on PA601, selection for Met⁺
GMS848	Hfr	*uxuA1,man-4*	*arg⁺ met⁺* from a cross between GMS838 F⁻ phenocopy and Hfr JB1
GMS878	Hfr	*uxuR1,man-4*	*uxu⁺* from GMS848 by P1 transduction from GM230
GMS565	F⁻	Like KL132 except *his⁺,rec⁺*	From a cross between KL132 and Hfr KL16
GMS566	F⁻	Like KL132 except *thy⁺,rec⁺*	From a cross between KL132 and Hfr KL16
GMS578	F⁻	Like KL132 except *pro⁺,thy⁺, rec⁺,uxuR2*	From a cross between GMS566 and Hfr GK2
GMS812	F⁻	Like KL132 except *leu⁺,rec⁺,uxuR3*	From a cross between GMS565 and Hfr GMX10
GMS784	F⁻	Like GMS566 except *thr⁺,leu⁺, pro⁺,uxaA1*	From a cross between GMS566 and Hfr JB1
GMS361	F⁻	Like GM290 except *uidA⁺,his⁺,uidO1, uxuR1,xyl*	From a cross between GM146 and Hfr GM230
GMS368	F⁻	Like GMS343 except *aro⁺,uidO1*	From a cross between GMS343 and Hfr GM230
GMS513	F⁻	Like GMS361 except *aro⁺,uidA1*	From GMS361 by P1 transduction from GMS407; this strain is able to grow on MeGalU after only addition of *uidA⁺*

TABLE 1—*Continued*

Strains	Sex	Genotype	Origin or derivation
F112/GMS784	F'	F112/GMS784	This study
GM230	Hfr	*uidO1,uxuR1*	MeGalU$^+$ mutant of Hfr H (14)
GMH34	Hfr	*uidR14,uxuR5*	MeGalU$^+$ mutant of Hfr H (14)
GMH39	Hfr	*uidR9*	MeGalU$^+$ mutant of Hfr H (14)
GMH47	Hfr	*uidR15,uxuR6*	MeGalU$^+$ mutant of Hfr H (14)
GMK2	Hfr	*uidR2,uxuR2*	MeGalU$^+$ mutant of KL16 (14)
GMX5	Hfr	*uidR10,uidO3*	MeGalU$^+$ mutant of P4X (14)
GMX10	Hfr	*uidR12,uxuR3*	MeGalU$^+$ mutant of P4X (14)
GMX13	Hfr	*uidR4*	MeGalU$^+$ mutant of P4X (14)
GMX21	Hfr	*uidR13,uxuR4*	MeGalU$^+$ mutant of P4X (14)
GMX29	Hfr	*uidR3*	MeGalU$^+$ mutant of P4X (14)
GMX31	Hfr	*uidR6*	MeGalU$^+$ mutant of P4X (14)
GMX49	Hfr	*uidR16,uxuR8,uxuA1*	MeGalU$^+$ mutant of JB1 (14)
GMX58	Hfr	*uidR17,uxuR7,uxuA1*	MeGalU$^+$ mutant of JB1 (14)
GMX55	Hfr	*uidR18,uxuR11,uxuB8*	MeGalU$^+$ mutant of CM8 (14)

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

were collected in the exponential phase, suspended in 10 mM imidazole buffer (pH 7.4), and disrupted in the presence of 10 mM dithioerythritol in a Raytheon (10-kHz) oscillator for 20 min. Cell extracts were assayed for the following enzymes: uronate isomerase (25), MOR and altronate oxidoreductase (18, 19), mannonate hydrolyase (HLM) and altronate hydrolyase (J. Jimeno-Abendano, Dr.Ing thesis, Lyon, Lyon, France, 1968; 26). Hydrolyase assays were carried out at 37 C; the other assays were at 30 C.

Enzymatic units. One unit of UID has been defined as the amount of enzyme hydrolyzing 1 μmol of *p*-nitrophenyl-β-D-glucuronide (pNPGlcU) per min under the assay conditions (405 nm, 30 C) (12). In the present article, we define 1 unit of MOR as the amount of enzyme required for 1 mmol of reduced nicotinamide adenine dinucleotide to reduce fructuronate per min under the assay conditions (340 nm, 30 C) (19).

Heat and PCMB treatment of enzymatic extracts. The enzymatic extracts were diluted fivefold in a phosphate buffer (10 mM, pH 7.0) previously incubated at the inactivation temperature. After incubation, the samples were diluted fivefold in a cold phosphate buffer and assayed. *p*-Chloromercuribenzoate (PCMB) was added to the usual altronate oxidoreductase assay medium (18) and preincubated at 30 C before addition of the enzymatic extract.

Construction of isogenic derivative strains. The *uidR* or *uidO* mutation was transferred to strain GMS830 or GMS838 (P4X), after growth of P1 on mutant strains, with selection for Uxu$^+$; the *uxuR1* mutation was added to *uidR* mutants by transduction from a Man$^+$*uidR* derivative of GMS848, using the same selection. Such methods were not possible with *uxu* mutants; the *uxuR* mutation was therefore transferred by uninterrupted conjugation between Hfr P4X (*uxuR*) and the female phenocopy of a *man*$^+$*uidR* derivative of strain GMS838, using Arg$^+$Met$^+$ selection.

Construction of constitutive strains carrying *uidA1* mutation. (i) **Strain GMS602.** The *uidA1* mutation was transferred by phage P1 grown on strain GM291 (*uidA1*) to strain GMS501 (*uxuR1,man*), with selection for Man$^+$ recombinants. Strain GMS501 had been constructed previously by crossing GM230 (*uxuR1*, HfrH) with an F-minus strain, K63, and selecting for Gal$^+$,Str recombinants.

(ii) **Strain GMS513.** Strain GMS361 (*uidO1, uxuR1,aroD*) (Table 1) was transduced by phage P1 (grown on GMS407 [*uidA1,man*]), with selection for Aro$^+$ recombinants. Four of 211 Aro$^+$ clones received *uidA1*. To determine whether they had retained *uidO1*, each clone was then transduced by P1 phage (HfrH, wild type), and the culture was spread on a solid medium containing MeGalU as the sole carbon source. Only one of the Aro$^+$ strains was able to yield MeGalU$^+$ transductants; this means that the P1 had brought *uidA*$^+$ without deleting *uidO1* since the latter is necessary, in cooperation with *uxuR1*, to again establish the MeGalU$^+$ phenotype. Finally, the transduction was repeated with P1 (GMS343 [*man*]), and a normal cotransduction value was obtained between the MeGalU$^+$ phenotype and the added *man* character.

Chemicals. PCMB was purchased from Calbiochem Co.; DTE was from Merck, Darmstadt. Phenyl-thio-β-glucuronide (TPU) was a kind gift from F. Stoeber (Dr.Sc. thesis, Univ. of Paris, Paris, France, 1961).

RESULTS

Analysis of derepressed enzymes in primary mutants. Enzymatic assays confirmed the high derepression of UID and MOR (already observed on solid medium). HLM was also strongly derepressed (Table 2), but uronate isomerase, altronate hydrolyase, and KDG ki-

TABLE 2. *Constitutive enzymatic activities in MeGalU-positive double mutants and derivatives*

Original mutant and derivatives	Allele			UID		Sp act (U/mg)			
	uxuR	*uidR*	*uidO*	Toluene-treated cells	Cell extracts	MOR	HLM	ISO[a]	HLA[b]
GM230	*1*	+	*1*	6.60	6.50	6.20	26.0	9.0	3.0
GMS591[c]	*1*	+	+	0.27	0.27	4.70	36.0	20.0	6.5
GMS501[d]	*1*	+	+	0.10					
GMS397[e]	+	+	*1*	0.10					
GMS510[d,e]	*1*	+	*1*	6.73					
GMS873[f]	+	+	*1*	0.33					
GMS891[g]	*1*	+	*1*	8.00					
GMK2	*2*	*2*	+	6.80	7.20	1.17	21.0	7.0	3.0
GMS589[c]	*2*	+	+	0.20	0.20	4.84	49.0	18.0	9.0
GMS471[e]	+	*2*	+	2.42					
GMS874[f]	+	*2*	+	5.65					
GMX10	*3*	*12*	+	10.30	6.4	0.71	7.0	8.0	3.0
GMS638[c]	*3*	+	+	0.27					
GMS832[h]	*3*	+	+		0.03	0.36	5.5		
GMS802[i]	+	*12*	+	0.25					
GMX21	*4*	*13*	+	12.20	4.13	2.16	20.0	7.0	3.5
GMS631[c]	+	*13*	+	0.39					
GMS867[h]	*4*	+	+		0.02	1.97	18.0		
GMS643[j]	+	*13*	+	1.00					
GMH34	*5*	*14*	+	6.50	6.98	5.23	26.5	8.0	11.0
GMS808[c]	*5*	+	+		0.24	4.74	31.0	18.0	6.0
GMH47	*6*	*15*	+	3.00	3.89	2.29	14.0	<1.0	3.0
GMX58	*7*	*17*	+	13.97	9.52	4.72	7.0	3.0	15.0
GMS807[i]	*7*	+	+	0.27					
GMS863[h]	*7*	+	+		0.02	1.71	2.5		
GMS806[i]	+	*17*	+	8.90					
GMX49	*8*	*16*	+	13.85	8.60	2.15	6.0	<1	15.5
GMS805[i]	*8*	+	+	0.25					
GMS804[i]	+	*16*	+	3.58					
GMX55	*11*	*7*	+	12.71					
GMS897[j]	*11*	+	+	0.08					
GMS710[h]	+	*7*	+	1.59					

[a] ISO, Uronate isomerase (EC 5.3.1.12).
[b] HLA, Altronate hydrolyase (EC 4.2.1.7).
[c] From Uxu$^+$ by P1 transduction from JB1 (*uxuA1*).
[d] Gal$^+$, Str^r recombinant from short Hfr conjugation with K63 F$^-$.
[e] Transduction of K63 by P1 grown on GMK2, selection for Man$^+$.
[f] Transduction of GMS838 by P1 grown on GMK2, selection for Man$^+$.
[g] Transduction of GMS848 (*uxuA,man*) by P1 grown on GM230, selection for Uxu$^+$; next transduction by the same lysate, selection for Man$^+$.
[h] Pyr$^+$ selection from Hfr conjugation with GM565.
[i] Met$^+$,Arg$^+$ recombinant from Hfr conjugation with female phenocopy of GMS838 (*argE3*).
[j] Transduction of GMS343 by P1 grown on GMX55, selection for Man$^+$.

nase were not derepressed. A weak derepression of oxidoreductase activity towards tagaturonate was also observed. Several lines of evidence showed that this activity was not due to the altronate oxidoreductase but to the capacity of MOR to use tagaturonate to a slight extent (19). First, at 53 C and in the presence of dithioerythritol (2 mM), oxidoreductase activity was similarly suppressed against fructuronate and tagaturonate in extracts of two different pleio-

tropic mutants (GMS863 and GMS867: residual activities after 10 min of incubation were, respectively, 28 to 29% and 47 to 47.5% of the initial activity). Moreover, whereas a purified altronate oxidoreductase extract (18) was not significantly inactivated (11 to 18% of the initial activity) in the presence of 0.1 mM PCMB, this pseudoaltronate oxidoreductase activity was strongly inactivated (40 to 95%) in four different mutant extracts (GMH34, GMX49, GMX58, and GM230).

Occurrence of different pNPGlcU$^+$ phenotypes. To map mutations giving rise to the pNPGlcU$^+$ phenotype (12, 14), crosses were performed between Hfr mutants and F$^-$ polyauxotroph strains. Two different Hfr's were used (GM230 [HfrH] and GMK2 [HfrKL16]) and yielded four types of recombinants: (i) a strong pNPGlcU$^+$ and pleiotropic donor type (with both Hfr's); (ii) a weak pNPGlcU$^+$, but still pleiotropic, type (with both Hfr's); (iii) a strong pNPGlcU$^+$, but not pleiotropic, type (with GMK2); and (iv) a weak pNPGlcU$^+$ and nonpleiotropic type (with GM230).

With these two Hfr's of opposite polarity, the first type was always transferred as a distal marker: with *his*$^+$ (17%) for GM230 and with *thr*$^+$,*leu*$^+$ (30%) for GMK2 (Fig. 1). The second type was transmitted as a proximal marker by HfrH (61% of the Thr$^+$,Leu$^+$ and 43% of the Pro$^+$ recombinants in short crosses) and as a distal marker by KL16, with the same markers.

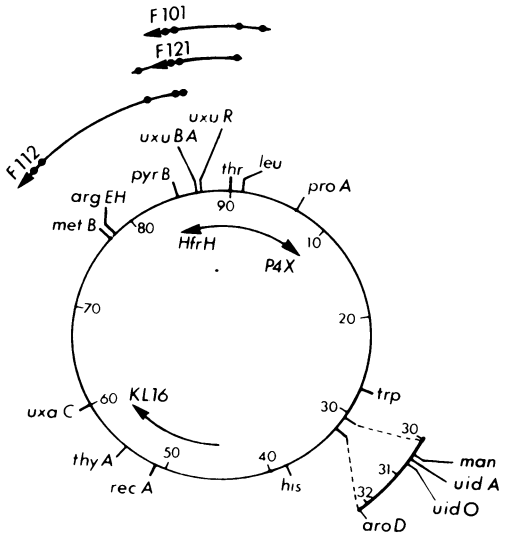

FIG. 1. *Genetic map of E. coli K-12 showing relevant markers, the Hfr point of origin of HfrH, P4X, and KL16, and the point of origin of F-primes (9) spanning the uxuR region.*

As for the nonpleiotropic third and fourth types, they were transmitted with the *his-trp* region: the third type by GMK2 (19% of cotransmission with *trp*$^+$ in a cross with PA309) and the fourth type by GM230 (30% with *his*$^+$ in a cross with PA601).

These results suggested that the various phenotypes observed arose from segregation in the crosses of two mutations carried by the primary mutants. One of them, located in the *his-trp* region of the chromosome, was not pleiotropic, whereas the other, in the *thr-leu* region, was.

Accurate mapping of the constitutive mutations linked to the *his-trp* region. All of the mutant strains proved to be able to transfer, through a single transductional event, a nonpleiotropic constitutive character to wild-type recipient strains. This character was strongly cotransduced with *man* and weakly with *aroD* (Table 3), like the *uidA* (12), *uidO* (14), and *uidR* (14) genes. In transduced derivatives, UID activity varied from one mutant to another but was constant for the different clones from the same primary mutant (Table 2). The specific activity was always weaker than the original value of the double mutant. In some derivatives, this activity even proved to be extremely low (1.5 to 8% of the primary mutant activity in GM230, GMX10, and GMX21).

Accurate mapping of the constitutive mutations linked to the *thr-leu* region. In this region, *uxuA* and *uxuB* were mapped at min 86 and characterized as the structural genes of HLM (24) and MOR (23), respectively. Because of the shift of the markers in this region, according to Taylor and Trotter (30), these mutations must, in fact, lie at min 87.

No cotransduction was obtained between the lesion producing the pNPGlcU$^+$ phenotype and gene *pyrB* (min 85) (0/161 PyrB$^+$ obtained in transduction of GMS442 [pNPGlcU, *pyrB*] by P1 grown on HfrH). On the other hand, the lesion was located quite close to *uxuA*: upon P1 transduction from GM230 to JB1 (*uxuA1*), 85% of the Uxu$^+$ recombinants inherited the pNPGlcU$^+$ phenotype (Table 4).

To determine the order of the markers in this region, we relied on the following observation. First, the Uxu$^-$ phenotype completely prevents growth on glucuronate and, therefore, on β-glucuronide, which is the preceding substrate in the hexuronide-hexuronate pathway. Secondly, the transfer of the pleiotropic pNPGlcU$^+$ character significantly inhibits growth on both substrates. Thus, in a mating between strain JB1 (P4X, *uxuA1*) and an F$^-$ derivative of GM230, when we selected the MeGlcU$^+$ clones, we favored the appearance of a crossing-over between these two very close markers:

TABLE 3. *Location of the uidA-linked constitutive mutations carried by some MeGalU⁺ mutants*

uid mutation	P1 donor	Recipient strain	Selected phenotype	No.	Percent unselected markers				
					Man⁺	Aro⁺	Uid⁺	pNPGlcU⁺	pNPGlcU⁻
O2	GMS368 (+)[a]	GM290 (*uidA1*)	Aro⁺	202	2.5		4	4[b]	0
			Uid⁺	103	88	1		88[b]	
									0
	GM290 (*uidA1*)	GMS368 (+)	Man⁺	98		<1	90	90[b]	0
	GMS343	GMS513 (*uidA1*) (+)	Uid⁺	508	86	0.8		0	100
			MeGalU⁺	20	90	<5	100	100[c]	0
R2	GMK2 (+)	GMS407 (*uidA1*)	Man⁺	59			85	85	0
			Uid⁺	95	86			97	3
	GMK2	GMS343	Aro⁺	124	3.2			6.4	
			Man⁺	70		<1		85	
R12	GMX10 (+)	GMS343	Man⁺	32		<3		91	
			Aro⁺	95	<1			<1	
R13	GMX21 (+)	GMS343	Man⁺	31		<3		97	
R14	GMH34 (+)	GMS529	Man⁺	60		<2		85	
			Aro⁺	96	2			2	
R17	GMX58 (+)	GMS529	Man⁺	24		<4		87	
R16	GMX49 (+)	GMS529	Man⁺	24		<4		75	
R7	GMX55 (+)	GMS529	Man⁺	32		<3		78	
			Aro⁺	32	7			7	

[a] (+) indicates the pNPGlcU⁺ phenotype.
[b] Only Uid⁺ recombinants can express the pNPGlcU⁺ phenotype.
[c] All of the MeGalU⁺ recombinant clones necessarily have the pNPGlcU⁺ phenotype.

pNPGlcU⁻ of the donor and Uxu⁺ of the recipient. A four-point test (Table 5) revealed the absence of the Thr⁻,Pyr⁺ class among the MeGlcU⁺ recombinants and gave the following order as being the most probable: *pyrB* (min 85), *uxuA* (min 87.5), pNPGlcU⁺ (min 87.5), and *thr* (min 90) (Fig. 2).

All of the MeGalU⁺ pleiotropic mutants carried a constitutive mutation at this locus (70 to 95% cotransducible with *uxuA* [Table 4]). When the strains carried, in addition, an *uxuA1* or an *uxuB* mutation, the mapping was performed by conjugation (Table 4).

Analysis of derivatives carrying only the constitutive mutation linked to *uxuA*. In the course of conjugation or transduction experiments, no disjunction appeared between the constitutive expression of the synthesis of UID, MOR, and HLM. In all of the derivatives, the constitutivity of MOR and of HLM remained at the same level as that of the primary mutants. In contrast, UID synthesis always appeared to

TABLE 4. *Mapping of the uxuA-linked pleiotropic constitutive mutations (uxuR)*

Type of mapping	Mutant donor	Selected phenotype	No. of recombinants	Percent pNPGlcU recombinants
P1 transduction from JB1 *(uxuA1)*	GM230	Uxu$^+$	134	85
	GMK2	Uxu$^+$	144	88
	GMX10	Uxu$^+$	32	95
	GMX21	Uxu$^+$	32	70
	GMH34	Uxu$^+$	31	90
	GMH47	Uxu$^+$	31	87
Hfr conjugation with F$^-$ (GMS565 or GMS566)	GMX49 (*uxuA1*)	Thr$^+$	16	25
		Pyr$^+$	14	50
	GMX58 (*uxuA1*)	Leu$^+$	67	40
		Xyl$^+$	67	42
	GMX55 (*uxuB8*)	Pyr$^+$	48	73

TABLE 5. *Tentative ordering of the uxuR2-constitutive mutation in the uxuA region*

Donor Hfr strain	Recipient strain	Selected phenotype	No.	Percent unselected phenotype			
				Thr$^+$	pNPGlcU$^-$	Uxu$^-$	Pyr$^+$
JB1	GMS578	Thr$^+$	144		63	62	42
uxuA1	+						
metB1	+	Pyr$^+$	347	80	90	88	
+	*thr*						
−	pNPGlcU$^+$	Gur^{+a}	59	78	100[a]	<2[a]	29
+	*pyrB*						

[a] See text and Fig. 2.

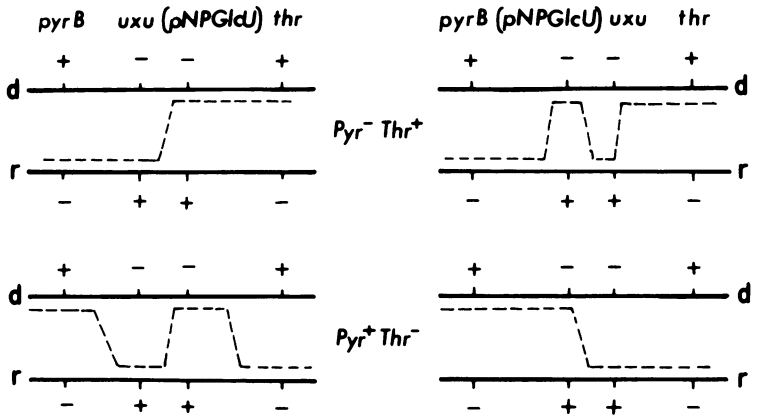

FIG. 2. *Tentative ordering of the constitutive locus in the uxuA region among Gur$^+$ recombinants (data from Table 5). The broken lines represent the crossing-over necessary for formation of Gur$^+$ recombinants (pNPGlcU$^-$, Uxu$^+$). d, Donor; r, recipient.*

be weak (Table 2): 1 to 4% of the constitutive activity of the primary mutants. This observation explains why such derivatives lost their ability to grow on MeGalU.

Preparation of double mutants from the two segregated primary mutants. To eliminate the possibility of a "strain effect" modifying UID synthesis in recipient strains (which could have explained the low activities observed in dissociated mutations) isogenic derivatives were constructed. Even in such isogenic strains, partial activities depending on each of the two original mutations remained low compared with the high activity observed in pri-

mary mutants (e.g., GM230 and GMX10 in Table 2).

To avoid the possibility of another mutation remaining masked in the primary mutant, preparation of the double mutant was performed starting with the two separated mutations. Both GM230 mutations were independently transferred by transduction to a wild-type chromosome. The new strains thus prepared exhibited the full constitutive characteristics of the primary mutant. As expected, these strains had recovered the ability to grow on MeGalU.

Similarly, the mutation linked to *uxuA* in strain GM230 was transferred to several simple *uidR* mutants (14). A strong derepression resulted that was particularly striking in the case of weak *uidR* mutations (Table 6).

Expression of the pleiotropic constitutive character in strains carrying a negative mutation in the structural gene of the enzymes concerned. The pleiotropic effect of mutations linked to *uxuA* could have been due to internal induction of a set of genes (for example *uxuA* and *uxuB*) by the metabolic function of the other gene (*uidA*) or vice versa. This was ruled out by the following results. MeGalU$^+$ mutants belonging to the pleiotropic class (by their genetic mapping and their properties) were obtained from strains negative for either one of the *uxu* enzymes; in such strains, UID synthesis remains constitutive (GMX49, GMX55, and GMX58 in Table 2). Conversely, a *uidA* mutation was introduced in pleiotropic mutants without preventing the constitutive expression of MOR and HLM synthesis (cf. the constitutive activities of GMS601 [*uidA*], which are 2.66 for MOR and 35 for HLM, with those of GMS591 [Uid$^+$], which are 4.71 and 36, respectively).

Moreover, during previous *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis experiments (12), we obtained a GMG22 mutant that was simultaneously Uxu$^-$- and UID-constitutive, albeit weakly. No crossing experiments led to the separation of these two characters, which both lie in the *thr-leu* region. Such a mutant could carry a deletion or a multisite mutation in the *uxu* region since it was not capable of reverting to the Uxu$^+$ wild-type phenotype nor of yielding Uxu$^+$ recombinants in conjugation with a *uxuA* strain (JB1, Table 7). This defect could affect simultaneously the *uxuA* locus and the locus responsible for the constitutive synthesis described above for MeGalU$^+$ pleiotropic mutants.

Expression of *uxuA*-linked mutations in merodiploids. Three F-prime strains from B. Low were used (9) (Fig. 1); they proved able to cover the *uxuA* region and also to exchange the pNPGlcU$^+$ character in an F$^-$Rec$^+$ strain. They were F121 and F101, HfrH derivatives, and F112. Shortening of the latter's very long episome (ca. 10.5 min) was prevented by continuous selection on a Uxu$^+$,PyrB$^+$ medium.

Crosses were performed, Thr$^+$, Leu$^+$, or PyrB$^+$ was selected accordingly, and MOR-, UID-, and HLM-constitutive synthesis was analyzed (Table 8). For all of the mutations, expression of constitutive synthesis for each of the three enzymes was severely repressed by

TABLE 6. *Effect on UID-constitutive synthesis of the introduction of the uxuR1 mutation in uidR mutant strains*

Strain	*uidR* allele	*uxuR* allele	Other mutations	UID sp act (U/mg of dry wt)		
				Expt 1	Expt 2	Expt 3
GMX29	*3*	+		0.48	0.31	0.49
GMS880[a]	*3*	*1*		8.43	8.21	6.76
GMX31	*6*	+		1.14	0.91	
GMS881[a]	*6*	*1*		7.76	6.38	7.80
GMX27	*11*	+		3.16	2.70	
GMS984[a]	*11*	*1*		7.25	5.83	
GMK1	*1*	+		4.22		
GMS990[a]	*1*	*1*		7.70	7.57	
GMX13	*4*	+		5.62	4.29	5.58
GMS892[a]	*4*	*1*		6.75	9.02	
GMX11	*19*	+		3.84	5.32	
GMS994[a]	*19*	*1*		13.38		
GMX5	*10*	+	*uidO3*	14.11	11.75	12.37
GMS934[a]	*10*	*1*	*uidO3*	11.07	14.85	
GMS744	*21*	+	*uidR101*	2.17	2.34	
GMS894	*21*	*1*	*uidR101*	4.43	8.91	

[a] Transduction of GMS878 by P1 grown on *uidR* strain, selection for Man$^+$.

TABLE 7. *Recombination between uxu mutations in conjugation with Hfr P4X JB1 (uxuA1)*[a]

Cross: Hfr × F⁻ (Uxu⁻)	No. of recombinants/ml of conjugation medium			
	Thr⁺,Leu⁺	Thr⁺,Leu⁺,Uxu⁺	Arg⁺,Uxu⁺	PyrB⁺,Uxu⁺
P4X × GMG22 (*uxuR10*)	1.3×10^6	3×10^5	6.7×10^5	
JB1 × GMG22 (*uxuR10*)	1.3×10^6	<10	<10	
P4X × S812 (*uxuR3*)	2.0×10^6	ND[b]		1.1×10^5
JB1 × S812 (*uxuR3*)	2.0×10^6	2.7×10^4		2.0×10^3
P4X × GMG67 (*uxu*)	9.0×10^5	ND	2.9×10^4	
JB1 × GMG67 (*uxu*)	7.0×10^5	<10	<10	

[a] Noninterrupted crosses between Hfr and F⁻ strains were done, and recombinants selected on prototrophic media containing glucose or glucuronate (Uxu⁺) were numbered and reported for 1 ml of the cross medium.
[b] ND, Not done.

TABLE 8. *Expression of UID synthesis in merodiploid strains carrying uxuR mutations*

Strains	Episome: *uxuR* allele	Chromosome: *uxuR* allele	Phenotype selected	Sp act (U/mg of dry wt) in cells grown on glycerol		
				UID	MOR	HLM
GMS864		*1*		0.082	3.97	29.0
F121/GMS864	+	*1*	Pyr⁺	0.003	0.05	3.5
F101/GMS864	+	*1*	Leu⁺		0.13	4.6
GMS581		*2*		0.092	4.84	53.0
F112/GMS581[a]	+	*2*	Pyr⁺	0.005	0.24	5.0
F112/GMS581[b]	+	*2*	Pyr⁺	0.017	0.33	5.8
GMS867		*4*		0.018	1.97	21.0
F101/GMS867	+	*4*	Thr⁺,Leu⁺	<0.002	0.05	3.0
GMS831		*5*		0.042	1.84	10.0
F112/GMS831[a]	+	*5*	Pyr⁺	0.008	0.53	7.8
F112/GMS831[b]	+	*5*	Pyr⁺	0.008	0.75	7.8
GMS863		*7 uxuA1*		0.019	1.71	2.4
F101/GMS863[a]	+	*7 uxuA1*	Thr⁺,Leu⁺	<0.002	0.03	2.7
F101/GMS863[b]	+	*7 uxuA1*	Thr⁺,Leu⁺	<0.002	0.02	2.7
GMS634		*8 uxuA1*		0.020	0.46	4.0
F112/GMS634[a]	+	*8 uxuA1*	Pyr⁺	<0.005	0.04	4.0
F112/GMS634[b]	+	*8 uxuA1*	Pyr⁺	<0.005	0.02	4.0
GMS832		*3 uxuB*		0.035	0.36	5.5
F112/GMS832[a]	+	*3 uxuB*	Pyr⁺	0.004	0.08	5.6
F112/GMS832[b]	+	*3 uxuB*	Pyr⁺	0.003	0.07	5.3
GMS773		*10* Δ*uxu*		0.029	0.006	2.0
F101/GMS773	+	*10* Δ*uxu*	Thr⁺,Leu⁺	0.002	0.005	1.8

[a] Clone 1.
[b] Clone 2.

the corresponding wild-type allele of the episome, e.g., 81 to 96% repression of UID synthesis.

Hereafter, *uxuAB*-linked constitutive mutations will be called *uxuR* (see Discussion).

Expression of *uidA*-linked constitutive mutations in merodiploids. F-prime strain 500 (14) was crossed with F⁻ *recA* strains each of which carried *uidA*-linked mutations. In every merodiploid derivative an important repression of UID synthesis occurred, except in the one carrying the GM230 mutation (Table 9, line 1); moreover, this mutation was shown to be *cis* dominant over the wild-type allele (Table 9, line 2).

These two kinds of constitutive mutations

have already been described with the simple MeGalU$^+$ mutants and been ascribed to defects in *uidR* gene and the *uidO* locus (14).

When the chromosome carries the two mutations of the pleiotropic primary mutant, the dominant behavior is maintained (Table 9, lines 3 and 7).

UID inducibility in *uidR*, *uidO*, and *uxuR* derivative strains. Derivatives carrying only the segregated *uidR* mutation behave as simple *uidR* mutants (14): UID synthesis can be induced by MeGlcU (only under nonblocked conditions) and, to a lesser extent, by glucuronate; it can also be freely induced by mannonic amide (Table 10, lines 1 to 4).

Derivatives carrying only the *uxuR* mutation can also be induced by MeGlcU or by glucuronate (Table 11). This inducibility is decreased (but not entirely suppressed) in the presence of a *uxaC* mutation (isomerase negative) (13, 15). Mannonic amide is incapable of bringing about a derepression of UID synthesis but can act in conjunction with MeGlcU to restore a good induction of UID in *uxuR,uxaC* strains (Table 11).

Recently we (M. A. Mandrand-Berthelot, G.

TABLE 9. *Expression of UID synthesis in some merodiploid derivative strains carrying uidO- or uidR- constitutive mutations*

Primary mutant	Haploid relevant genotype	Sp act (U/mg) on glycerol		Diploid relevant genotype	Sp act (U/mg) on glycerol	
		F$^-$	Segregated		Clone 1	Clone 2
GM230	*uidO1*	0.04	0.09	F *uidO$^+$/uidO1*	0.13	0.09
	uidO1,uidA	0.001		F *uidA$^+$ uidO$^+$/uidA,uidO1*	0.001	0.001
	uidO1,uxuR1	3.25	2.76	F *uidO$^+$/uidO1,uxuR1*	1.21	2.25
GMX5[a]	*uidO3*	0.10	0.06	F *uidO$^-$/uidO3*	0.06	0.06
GMK2	*uidR2*	2.42	2.61	F *uidR$^+$/uidR2*	<0.05[b]	<0.05[b]
GMX10	*uidR12*	0.15	0.15	F *uidR$^+$/uidR12*	0.01	<0.01
	uidR12,uxuR3	9.57	10.00	F *uidR$^+$/uidR12,uxuR3*	0.06	0.06
GMX21	*uidR13*	0.26		F *uidR$^+$/uidR13*	0.01	<0.05
GMX58	*uidR17*	2.26		F *uidR$^+$/uidR17*	0.03	0.03
GMX55	*uidR18*	1.59		F *uidR$^+$/uidR18*	0.02	0.03
GMX49	*uidR16*	2.23		F *uidR$^+$/uidR16*	0.02	0.03
GMH34	*uidR14*	0.23		F *uidR$^+$/uidR14*	0.01	0.005

[a] See reference 14.
[b] Cells were grown on mannose.

TABLE 10. *Induction of UID synthesis in uidO, uidR(R^c) and uidR(R^s) derivatives*

Mutant strain	*uid* allele	UID sp act (U/mg) in cells grown on glycerol plus:					
		Nothing	MeGlcU[a]	GlcU[a]	TPU[a]	ManN[a]	ManN + MeGlcU[a]
GMS802[b]	*R12*	0.30	4.06	1.90		5.96	
		0.32			0.29	7.28	
GMS875[c]	*R13*	0.29	3.90				
		0.27				6.21	
GMS397[d]	*O1*	0.09	5.15	2.90			
GMS873[c]	*O1*	0.21	3.47			4.01	
GMS999[e]	*O1,uxaC*	0.14		0.52	0.17	3.51	
GMS906[f]	*O3*	0.29			8.33	0.47	
		0.25			7.34	0.63	
GMS979[f]	*R101*	0.001	0.03		0.01	0.10	0.10
GMS744[f]	*R101, R21*	2.34	2.25	1.83			
		2.17				3.52	
GMS889[f]	*R101,uxuR1*	0.23			0.26	0.27	

[a] Concentration: MeGlcU, 10 mM; GlcU, 10 mM; ManN, 3mM; TPU, 0.1 mM.
[b] See Table 2, footnote *i*.
[c] See Table 2, footnote *f*.
[d] See Table 2, footnote *e*.
[e] Arg$^+$,Met$^+$ recombinant from a cross between GMS788 *(uxaC)* and an F$^-$ phenocopy of GMS873.
[f] See reference 14.

TABLE 11. *Induction of UID synthesis in uxuR,uxaC strains under gratuitous conditions*

Strain	*uxuR* allele	UID sp act (U/mg) in cells grown on glycerol plus:[a]					
		Nothing	MeGlcU	GlcU	TPU	ManN	MeGlcU (or GlcU) + ManN
AU128	+			0.89			
GMS791[b]	*1*	0.15	0.69	0.46			
		0.11		0.59		0.10	(4.04)
		0.10	0.57	0.39	4.41		
GMS789[b]	*2*	0.16	0.71	0.47			
		0.15	0.47	0.54		0.10	4.07
GMS825[b]	*3*	0.15	0.35			0.06	3.49
		0.09	0.33	0.68	3.55		
GMS826[b]	*4*	0.20	1.08			0.15	
			0.52			0.08	3.45
GMS827[b]	*5*	0.22	1.61	0.51			
		0.17	0.88	1.18		0.19	4.94
GMS823[b]	*9*	0.15	1.33	0.80		0.39	4.26
		0.27		0.54		0.24	
GMS996[c]	*10*	0.02	0.06			0.03	1.64
		0.01	0.02	0.13	0.54		

[a] For the concentrations used, see footnote *a* of Table 10.
[b] Arg$^+$,Met$^+$ selection from Hfr mutant conjugation with an F$^-$ phenocopy of GMS788.
[c] His$^+$,Met$^+$ selection in cross between AU128 and GMG22.

Novel and M. Novel, manuscript in preparation) have shown that TPU, which is not metabolized by *E. coli* cells (F. Stoeber, Dr.Sc. thesis, Univ. of Paris, Paris, France, 1961), was able, in conjunction with mannonic amide, to derepress a *uxaC,uidR*$^+$,*uxuR*$^+$ strain. It can be seen in Table 11 that TPU alone strongly derepresses UID synthesis in the *uxuR* derivatives. In contrast, TPU was incapable of inducing *uidR* strains (14; Table 10). In *uidR*(*R*^{s}), *uxuR* doubly mutated strains (14), TPU had lost the inducing property attached to the *uxuR* mutation (Table 10).

Two dominant constitutive mutations have been characterized which can be distinguished by their behavior in the presence of inducer molecules: *uidO1* (from GM230) was induced by mannonic acid and not by TPU; *uidO3* (from GMX5) (14) was induced by TPU and not by mannonic acid.

DISCUSSION

Mutants obtained by growth on MeGalU always carry at least one mutation located in the region of the UID structural gene, *uidA*, which results in the constitutive synthesis of this enzyme. Study of the behavior of these mutations in merodiploids and the characterization of superrepressed mutants (type I^s) enabled us to define two distinct loci: *uidO* and *uidR* (14; this article).

Some MeGalU$^+$ mutants carry, in addition, another mutation very far from *uidA*, in the neighborhood of the *uxu* operon. Several observations established the regulatory nature of this new type of mutation. The expression of the *uxu* operon (MOR and HLM enzymes) is strongly derepressed, and a weak but constant derepression of UID synthesis occurs. No other group of genes of the hexuronate pathway is affected. The presence of the corresponding wild-type allele in merodiploids simultaneously abolishes the derepression of the three enzymes: MOR, HLM, and UID. Defects either in the *uidA* gene or *uxu* operon do not abolish the constitutive synthesis of the remaining enzymes.

Consequently, these pleiotropic constitutive

TABLE 12. *MOR activity in induced and uninduced cultures of some uidR strains*[a]

Strains	*uidR* allele	MOR sp act (U/mg)		Derepression ratio
		+ Nothing	+ ManN	
GMX31	*6*	0.076	1.53	20.0
GMX37	*28*	0.075	1.85	25.0
GMX34	*27*	0.067	1.28	19.0
GMK1	*1*	0.083	1.79	22.0
GMG81	*101*	0.131	2.41	19.0
P4X	+	0.087	1.90	22.0

[a] Cells grown on glycerol with or without ManN 3 mM were treated with toluene for 30 min at 37 C and then assayed for MOR specific activity.

mutations do seem to affect a regulatory gene, which we have called *uxuR* because it strongly affects the very close *uxu* operon; it also effects a weak control on the expression of *uidA*.

In certain cases the *uxuR* mutations are associated with an altered expression of the *uxu* operon: in the GMX10 (*uxuR3*) mutant a constitutive but weak MOR and HLM activity is observed, but this activity is no longer inducible by glucuronate.

As for *uidA* expression, the control exerted by *uxuR* does appear to be negative. A probable deletion in the *uxuAB-uxuR* region (strain GMG22, *uxuR10* mutation) produces the constitutive synthesis of UID.

The control exerted by *uidR* is also probably negative. In this gene, superrepressed mutations have been characterized that are dominant, as can be expected from a strictly negative system (1). It can be concluded from the dominance studies that each regulatory gene probably synthesizes a diffusible product: *uxuR*, the molecule Ruxu; and *uidR*, the molecule Ruid. Furthermore, UID synthesis apparently depends on the functioning of these two regulatory genes.

Before discussing how these two molecules act in concert to repress *uidA* expression, let us summarize our main results.

First, the two expression processes are quantitatively different. Derepression of UID synthesis due to *uidR* mutations varies widely, depending on the mutant, but can be very strong (14). It is weak, however, in all of the *uxuR* mutants.

Second, induction of UID synthesis in wild-type strains requires the simultaneous presence of two effector molecules: a β-glucuronide and fructuronate (11). We have shown that strong induction occurs after addition of two gratuitous inducer molecules: TPU (a thio-β-D-glucuronide) and ManN (mannonic acid, an analogue of fructuronate). When alone, neither compound can induce UID (M. A. Mandrand-Berthelot et al., manuscript in preparation).

Third, induction of MeGalU$^+$ mutants no longer requires those two inducers. Indeed, *uidR* mutants (14; Table 10), but not *uxuR* mutants, are induced by ManN alone. Thus, the ManN molecule is able, by itself, to relieve the repression produced by the Ruxu molecule on *uidA* expression. On the other hand, TPU can, by itself, induce *uxuR* mutants (Table 11) but not *uidR* mutants (Table 10). Therefore, TPU must antagonize (directly) the repression caused by *uidR*. It should be noted here that neither MeGlcU, under gratuitous conditions (in the presence of a *uxaC* mutation), nor GlcU gives rise in *uxuR* strains to the strong expected induction and that induction is restored by the addition of ManN (Table 11, last column). The requirement for those two inducers to derepress *uidA* may, in this case, only be apparent, since ManN may still interfere in glucuronate metabolism, even in strains derepressed for its use, by stimulating MeGlcU penetration. It has been demonstrated (G. Nemoz, 3rd cycle thesis, Univ. of Lyon, Lyon, France, 1975) that ManN induces gene *exuT* for permease, this gene being under the control of a regulatory gene, *exuR* (29). On the other hand, the uptake of MeGlcU, which is still not well-known, must be partially controlled by the activity of those two genes and therefore is sensitive to ManN (M. A. Mandrand et al., manuscript in preparation).

Finally, it is only the association in the same strain of two separate derepression processes that is able to bring about the full expression of *uidA*: (i) by the association of two negative mutations, *uxuR* and *uidR*; (ii) by the action of TPU on a *uxuR* or *uidO3* mutation; (iii) by the action of mannonic amide on a *uidR* or *uidO1* mutation (Fig. 3).

Two hypotheses can be put forward to explain the interaction between these two derepression processes at the *uidA* level. Either both molecules have a mutual affinity for each other and associate freely in the cytoplasm to form a single repressor molecule or Ruxu and Ruid molecules attach independently to the deoxyribonucleic acid (DNA) in the *uidA* region.

The first may be ruled out since according to this hypothesis the single molecule (Ruid Ruxu) would very likely repress in the same way the expression of *uxuAB* and of *uidA*. This is not the case: (i) derepression of the *uxuAB* operon only requires the action of ManN (25), whereas *uidA* requires, both in wild-type strains and *uxuR* mutants, another inducer molecule; (ii) *uxuR*-constitutive mutants are strongly derepressed for MOR and HLM synthesis but only slightly for UID synthesis; (iii) *uidR*-constitutive mutants are not derepressed for MOR synthesis (Table 12); (iv) no mutant fully derepressed for both *uxu* and *uidA* has been found to date.

The second hypothesis appears to be more likely. Each repressor molecule would be bound to DNA in the *uidA* region, with a separate repressive activity. Each repressor would be antagonized by a specific inducer, but full induction could only be reached by the addition of the two inducer molecules. Independent attachment of Ruid and Ruxu to DNA is substantiated by the finding of two classes of dominant

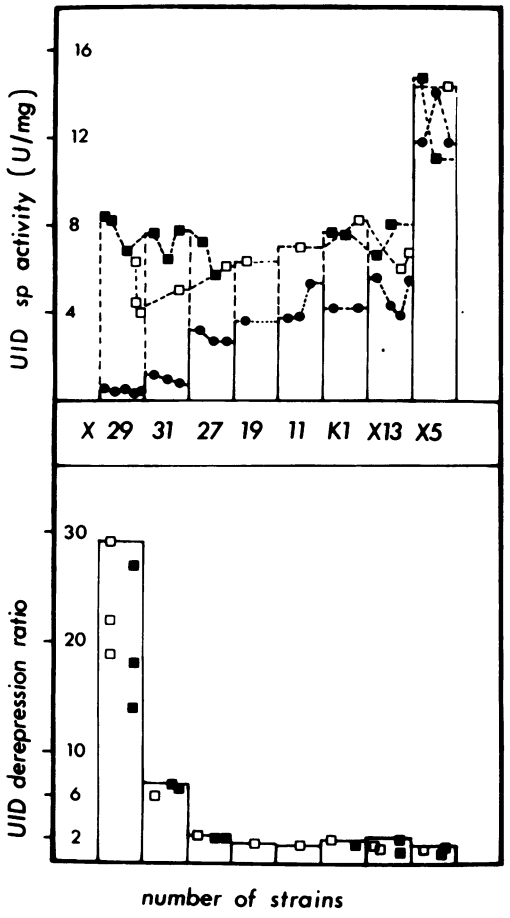

FIG. 3. *Effect of mannonic amide or of transfer of the uxuR1 mutation on the derepression of some uidR mutants. Top, Data obtained with some uidR mutants (classes I, II, and III) were taken from Table 6 of this paper and Table 6 of reference 14. Each sign refers to an independent experiment. uidR⁻ cells were grown on glycerol (●) or glycerol plus ManN (■); uidR,uxuR1 strains were grown on glycerol (□). Bottom, Derepression was measured, using data from each experiment, as the ratio: specific activity in uidR strains induced with ManN (■) or in uidR,uxuR1 strains grown on glycerol (□)/specific activity in uidR strains grown on glycerol.*

constitutive mutations. These mutations, presumed to be O^c, respond differently to TPU and ManN: *uidO1* is insensitive to TPU, as are the *uidR* mutations, and *uidO3* is insensitive to ManN, as are the *uxuR* mutations (Table 10). The former mutation seems to affect the DNA binding site of the Ruid molecule, and the latter seems to affect that of the Ruxu molecule.

This second hypothesis does not exclude the possibility of the two repressor molecules binding after their attachment to DNA. This could explain the synergistic pattern of their actions: (i) the conjunction of two weak constitutive mutations (of operator or regulator type) associated in the same strain produces a complete UID derepression; (ii) weakly derepressed mutations are not at all inducible by the specific inducer of their own gene, contrary to what happens in O^c mutants with the *gal* operon towards D-fucose (10) or in weak I^- mutants, described by Shineberg, towards isopropyl-β-D-thiogalactopyranoside (27).

In fact, in the literature, the distinction between the synergistic and additive processes is not clear. A cumulative effect has been described for the regulation of the *gal* operon: expression of *gal* enzymes is controlled by two molecules produced, respectively, by genes *galR* and *capR* (2, 3, 6, 8, 10). Action of gene *capR* is independent of the specific induction of *gal*; it occurs in a *galR*-superrepressed mutant as well as in $galR^+$ wild-type strains, whether induced or noninduced (6). According to the results of Mackie and Wilson (10), additivity has only been detected for weakly induced cells (growth of the superrepressed mutant on glucose) and not for well-induced cells (growth of the wild-type strain on glycerol). In this latter case, repression by the two repressor molecules does not appear to be cumulative in the sense given by the authors.

Independent control systems do not necessarily result in an additive repression level. Thus, complete repression of the *lac* operon under the double independent control of *trpR* and *lacI* in strains having the *lac-trp* fusion (21) is not the addition of the two repression processes (total repression/*lac* repression/*trp* repression = 165/4 to 5/5 to 6).

Recently, Hua and Markovitz (7) demonstrated, in the *gal* operon region, the existence of a second operator site which responds specifically to *capR* control. This new site does lie, as expected by the authors (8), in the operator-promoter region of the *gal* operon.

Another example of multiple regulation in the catabolism of nucleosides has recently been reported (5). Two regulatory genes, *cytR* and *deoR*, control the expression of the four enzymes of the *deo* operon. Each of the products of these genes is antagonized by its specific inducer molecule. Derepression of *deo* genes by cytidine (or *cytR* mutation) seems to be added to the derepression by deoxyribose-5-phosphate (or *deoR* mutation). It represents about 30% of the latter. In a *cytR,deoR* mutant the activities of the *deo* enzymes are higher than the sum of the activities in a *cytR* mutant plus those in a *deoR* mutant (5).

The gene *uidA* regulation seems to be caused by the interdependence of the two repressor molecules during the repression/induction process. Weakly derepressed operator-constitutive mutations are insensitive to their own inducing molecule. A superrepressed *uidR* mutant is insensitive to the action of ManN and to the transfer of a *uxuR* mutation. Isolation of deletions in this region should enable the clarification of the exact relationship between these two repressor molecules and the control sites of gene *uidA*. Furthermore, since *uidA* expression is strongly affected by catabolic repression (G. Novel, manuscript in preparation), it would be interesting to investigate the location of the sites responsible for the positive control by catabolite receptor protein-cyclic adenosine 3′,5′-monophosphate.

The significance of the dual control of *uidA* expression is not fully understood. UID synthesis is not induced, through $uxuR^+$ repression of *uidA*, unless glucuronate (produced by UID from β-glucuronide) can be isomerized into fructuronate (see Fig. 1 of accompanying paper [14]). The presence of fructuronate in *E. coli* K-12 appears as a prerequisite for the induction of UID (to produce internal glucuronate) and for the induction of glucuronate enzymes (to further metabolism). Such a hypothesis implies that the basal level of isomerase should be high enough to produce the amount of fructuronate required to fully induce isomerase synthesis. Another explanation might be that glucuronate itself would be able to induce isomerase. It has been shown that the uronic isomerase and altronate hydrolyase are coordinately induced (25) and that their structural genes, *uxaC* and *uxaA*, respectively, are very close on the chromosome (15). If these genes belong to the same operon, the hydrolyase should be induced by glucuronate; this is not the case (25). However, the occurrence of this operon still has not been demonstrated fully (29).

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