A DNA Sequence Containing the Control Sites for the *uxaB* Gene of *Escherichia coli*

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(Received 7 August 1985; revised 14 October 1985)

The nucleotide sequence of a 286 bp fragment containing the *uxaB* control region of *Escherichia coli* has been determined. The transcriptional start of the *uxaB* gene has been located and the promoter signals identified. Various fragments of the *uxaB* promoter-proximal region were fused *in vitro* with the *lacZ* gene. Results obtained with these fusions indicate that the operator-promoter sites are located on a 110 bp restriction fragment. The determination of the amino acid sequence of the NH₂-terminus of the *uxaB* gene product revealed that the *uxaB* gene is not initiated with the AUG codon but with the unusual GTG codon. CRP, the cyclic AMP receptor protein, does not bind to the *uxaB* control region DNA even though expression of the *uxaB* gene is sensitive to catabolite repression.

INTRODUCTION

Escherichia coli K12 can grow on the aldohexuronates D-glucuronate and D-galacturonate, which are metabolized via the Ashwell pathway (Ashwell, 1962). The first steps in the catabolism of galacturonate involve, in order, products of the structural genes *exuT*, *uxaC*, *uxaB* and *uxaA*. These genes form the *exu* regulon. Their expression is subject to negative control by the ExuR repressor, but UxuR, another repressor of the hexuronate system, has no effect on their expression (Portalier *et al.*, 1980; Ritzenthaler *et al.*, 1983). Their expression is also sensitive to catabolite repression by glucose (Hugouvieux-Cotte-Pattat, 1981). The *uxaB* gene is located at min 52 on the *E. coli* genome (Bachmann & Low, 1980) and codes for altronate oxidoreductase, the synthesis of which is induced by galacturonate, tagaturonate and fructuronate (Robert-Baudouy *et al.*, 1974).

The *exuR* and *uxuR* regulatory genes, and the *uxaB* gene, have been individually cloned into the plasmid vector pBR322 or its derivatives (Ritzenthaler *et al.*, 1981; Ritzenthaler & Mata-Gilsinger, 1982; Blanco *et al.*, 1983). The regulatory region of *uxaB* was isolated on a 2.5 kb *EcoRI*₁–*EcoRI*₂ fragment (Fig. 1), and it has been shown that *uxaB* is transcribed from the *EcoRI*₁ towards the *EcoRI*₂ site (Blanco *et al.*, 1983). The purpose of this work was to determine the nucleotide sequence of the *uxaB* control region and to locate on this sequence the promoter and the operator. The latter were more precisely located by the construction and expression of *uxaB*–*lacZ* fusions formed *in vitro* on plasmid vectors.

METHODS

Bacterial strains, plasmids and culture media. All the strains were *E. coli* K12 derivatives: HB101, Pro[–] Leu[–] Str^R HsdM[–] HsdR[–] EndoI[–] RecA[–] LacY[–] (Boyer & Roulland-Dussoix, 1969); MC1061, *araD139* Δ (*ara-leu*)7697 Δ *lacX74* GalU[–] HsdR[–] Str^R (Minton, 1984); 71-18, Δ (*lac-pro*) F'*lacI*^a *lacZ* Δ M15 Pro⁺ SupE[–] (Dente *et al.*, 1983); 1475, ExuR[–] ArgG[–] HisA[–] (Portalier *et al.*, 1980).

The *lac* fusion plasmids pNM480, pMN481 and pMN482 (Minton, 1984) were used for the detection of *uxaB*

Abbreviation: SS-DNA, single-stranded DNA.

translational initiation signals. Plasmids pEB3 (*uxaB*⁺), pEB7 (*uxaBo*⁺) (Blanco *et al.*, 1983), pRU101 (*uxuR*⁺) and pRE101 (*exuR*⁺) (Ritzenthaler *et al.*, 1983), are pACYC184 (*ter*⁺, *cat*⁺) derivatives (Chang & Cohen, 1978); plasmids pEMBL8 and pEMBL9 (Dente *et al.*, 1983) were used for cloning and sequencing *uxaB* fragments.

Media for growth were identical to those described by Miller (1972). The synthetic medium was M63 (pH 7.2) and contained glycerol (5 g l⁻¹). When needed, ampicillin and chloramphenicol were used at final concentrations of 25 µg ml⁻¹.

Chemicals. D-Galacturonate and *p*-aminobenzyl β-D-thiogalactosamine agarose were purchased from Sigma, and chloramphenicol and ampicillin from Serva. Purified CRP protein was a gift of B. Blazy. RNA polymerase was obtained from BRL. Fructuronate was synthesized in our laboratory.

Enzyme induction, preparation and assay. The conditions for induction, extraction and assay of altronate oxidoreductase were as outlined previously (Robert-Baudouy *et al.*, 1974; Portalier & Stoeber, 1972). β-Galactosidase was assayed by the method of Miller (1972) in exponentially growing cells. All the specific activities reported are the means of at least three separate assays.

Isolation and analysis of plasmid DNA. This was done as described by Blanco *et al.* (1983) and Maniatis *et al.* (1982).

3'-labelling of DNA fragments. *EcoRI*-*HindIII* fragments purified from pEMBL-E plasmids were 3'-end-labelled with DNA polymerase I Klenow fragment.

Construction of pEMBL-E vectors. The *EcoRI*₁-*EcoRI*₂ fragment of pEB7 bearing the regulatory region of the *uxaB* gene was purified and introduced into the *EcoRI* site of pEMBL8 in each of the two possible orientations, yielding pEMBL-E1 and pEMBL-E2 (Fig. 1). In pEMBL-E1, the *EcoRI*₂ site is located near the *lacZ* sequence of pEMBL8, whereas in pEMBL-E2 it is *EcoRI*₁ that is adjacent to *lacZ*. The *EcoRI*₁-*EcoRI*₂ fragment possesses a unique *NruI* site and no *SmaI* site (Blanco *et al.*, 1983); pEMBL-E1 and -E2 were digested with *NruI* and *SmaI* and ligated *in vitro* with T4 DNA ligase. The resulting plasmids bearing *NruI*-*SmaI* deletions were named pEMBL-E3 and pEMBL-E4 (Fig. 1).

The purified *EcoRI*₁-*EcoRI*₂ fragment was digested with *Sau3A*, and the fragments generated were inserted into the *BamHI* site of pEMBL8. The pEMBL8 hybrids containing *uxaB Sau3A* fragments were introduced into strain 71-18 by transformation. Transformant colonies were screened for constitutive altronate oxidoreductase synthesis, since multiple copies of the *uxaB* operator cause derepression of the *uxaB* chromosomal gene due to titration out of the *ExuR* repressor. In clones showing derepression of *uxaB*, the recombinant plasmids contained a unique 200 bp *Sau3A* fragment. These plasmids were named pEMBL-E5. The 200 bp *Sau3A* fragment was transferred from pEMBL8 to pEMBL9 using the *EcoRI* and *HindIII* sites flanking the pEMBL linker. The resulting plasmid was named pEMBL-E6.

The *NruI*-*HincII* (*SalI* site of pEMBL polylinker) fragment of pEMBL-E5 was deleted, yielding pEMBL-E7; similarly pEMBL-E8 and pEMBL-E9 respectively resulted from the deletion of the *HincII* fragment of pEMBL-E5 and pEMBL-E4.

Construction of *uxaB-lac* fusions. *EcoRI*-*HindIII* fragments of pEMBL-E were transferred to the vectors pNM480, -481 and -482, since pNM plasmids possess the same polylinker cloning sites as pEMBL. The resulting pNM vectors were named pNM-Ex according to the fragment Ex inserted. Fusions were made in the three reading frames using pNM480, -481 and -482. β-Galactosidase synthesis was obtained only with one of the three pNM vectors.

S1 nuclease mapping. RNA was purified by hot phenol extraction (Salser *et al.*, 1967) from strain 1475 (*ExuR*⁻) containing the pEB3 (*uxaB*⁺) plasmid. Cells were grown on minimal medium M63 containing glycerol (0.4%, w/v).

Single-stranded (SS) DNA probes, complementary to the *uxaB* sequences cloned into pEMBL-E1 and pEMBL-E7, were prepared according to Burke (1984). SS-DNA template was annealed with the universal sequencing primer and the primer was extended with the DNA polymerase I Klenow fragment. After cleavage of the primer at a unique 5' site, the reaction products were denatured, and separated by electrophoresis on acrylamide gels.

RNA (100 µg) and SS-DNA probe (100 000 c.p.m.) were co-precipitated. The dried pellet of nucleic acids was resuspended in 10 µl hybridization buffer (0.4 M-NaCl, 10 mM-PIPES pH 6.5, 80%, v/v, formamide), incubated for 10 min at 80 °C and cooled slowly to 40 °C overnight. After dilution with 9 vols S1 nuclease buffer (0.25 M-NaCl, 0.03 M-sodium acetate pH 4.6, 1 mM-ZnSO₄), unhybridized DNA was digested with S1 nuclease (50 U; BRL) for 30 min at 37 °C. The ³²P-labelled SS-DNA products were sized on denaturing sequencing gels (6%, v/v, acrylamide, 8 M-urea) alongside a typical DNA sequence.

Purification of *uxaB-lacZ*-encoded protein. Hybrid β-galactosidase encoded by a *uxaB-lacZ* fusion was purified by affinity chromatography, from a culture of strain 1475 containing plasmid pEBG1 (Blanco *et al.*, 1983). Cells were disrupted in a French pressure cell and the mixture was centrifuged at 12000 r.p.m. for 15 min. The supernatant was made 36% saturated with ammonium sulphate, and centrifuged at 12000 r.p.m. for 15 min; the resulting pellet was dissolved in buffer (10 mM-Tris pH 7, 10 mM-magnesium acetate, 0.1 M-NaCl, 10 mM-2-mercaptoethanol), and applied to a 10 ml column of *p*-aminobenzyl β-D-thiogalactosamine agarose. The eluted

Fig. 1. Structure of the pEMBL-Ex plasmids. See Methods for details of their construction. Only the restriction fragments of the *uxaB* control region inserted in the plasmids pEMBL are shown (thin lines). These fragments were introduced into pEMBL opened at one or two of its polylinker sites (hatched areas). E, Sm, B, Sa, Hinc, P and H represent respectively *EcoRI*, *SmaI*, *BamHI*, *SaII*, *HincII*, *PstI* and *HindIII* polylinker sites of pEMBL8. SD, ribosome-binding site (Shine-Dalgarno sequence); +1, transcriptional start point of the *uxaB* gene.

hybrid β -galactosidase was repurified on the same column. The purified enzyme was 90% pure as judged by SDS-polyacrylamide gel electrophoresis. Its NH_2 -terminal amino acid sequence was determined by J. Bonicel (Service Commun Sequenceur LCB-CBBH, CNRS, Marseille, France).

RESULTS

Nucleotide sequence of the *uxaB* regulatory region

The 2.5 kb *EcoRI* fragment containing the *uxaB* regulatory region was purified from pEB7 and cloned into pEMBL-8, in the two orientations, yielding pEMBL-E1 and -E2 (Fig. 1). Various restriction fragments of the *EcoRI*₁–*EcoRI*₂ segment bearing the entire or part of the *uxaB* regulatory region were cloned into pEMBL-8 and pEMBL-9, yielding pEMBL-E3 to -E9 (Fig. 1). The SS-DNA of these plasmids was used as template for the determination of the nucleotide sequence of the *uxaB* control region by the dideoxy chain terminator method of Sanger *et al.* (1977). Except for the sequence extending from the *Sau3A*₁ to the *EcoRI*₁ sites, the nucleotide sequence shown in Fig. 2 was determined several times in both strands according to the various overlaps obtained.

Fig. 2. Nucleotide sequence of a 286 bp fragment of the *uxaB* control region. The proposed -35 and -10 regions of the promoter are boxed. The start point of *uxaB* transcript (position +1) and the ribosome-binding site (SD) are indicated, and the NH₂-terminal amino acid sequence of altronate oxidoreductase is shown. Relevant restriction sites are arrowed.

Identification of the transcriptional start of the *uxaB* gene

The 5' end of *uxaB* messenger RNA was located by S1 nuclease mapping using uniformly labelled SS-DNA probes containing the relevant strand of the DNA fragments. The SS-DNA molecules derived from products of S1 mapping were detected and sized on sequencing gels, in comparison to sequencing ladders derived from pEMBL-E vectors. The *EcoRI*₁-*NruI*, *NruI*-*EcoRI*₂ and *Sau3A*₁-*Sau3A*₂ SS-DNA fragments were used as probes for S1 mapping. The *NruI*-*EcoRI*₂ fragment hybridized with mRNA was not cleaved by S1 treatment, showing that the transcription start point is upstream of the *NruI* site. The sizes of the RNA : DNA hybrid molecules obtained with *EcoRI*₁-*NruI* and *Sau3A*₁-*Sau3A*₂ were respectively 61 ± 1 bp and 144 ± 1 bp, indicating that the start point of *uxaB* transcription is at position 110 ± 1 on the nucleotide sequence.

Putative -10 and -35 regions of the *uxaB* promoter can be identified. The presumptive -10 region, TGTCGT (nucleotides 100 to 105), is 18 nucleotides distant from the -35 region, TCGGCG.

Expression and regulation of *uxaB*-*lacZ* fusions

All fragments cloned on pEMBL vectors and sequenced were transferred into the fusion probe vectors pNM480, -481 and -482. These vectors carry the *lac* operon in which the

Table 1. Regulation of *uxaB-lacZ* gene expression

β -Galactosidase activity is expressed in milliunits [$\text{nmol product min}^{-1} (\text{mg dry weight})^{-1}$], and is normalized to a constant level of β -lactamase activity. All the assays were at 30 °C. The values reported are means of at least three separate assays, results from which varied by <5% about the mean. No β -galactosidase activity was detectable in the presence of the hybrid plasmids pNM-E2, -E4, -E6 or -E9, or that of the parental plasmids pNM-480, -481 and -482.

Growth medium*	Plasmid in <i>trans</i>	β -Galactosidase specific activity* in the presence of:				
		pNM-E1	pNM-E3	pNM-E5	pNM-E7	pNM-E8
Glycerol	none	1410	1980	1490	4010	980
Glucose	none	160	190	150	390	70
Glucose + cAMP (10 mM)	none	1580	2240	1840	4580	1010
Glycerol	pACYC184	1420	2020	1530	4050	1080
	(control)					
Glycerol	pRE101	20	20	10	30	10
	(<i>exuR</i> ⁺)					
Glycerol + tagaturonate (5 mM)	pRE101	290			790	
	(<i>exuR</i> ⁺)					
Glycerol	pRU101	1450	1910	1490	3820	
	(<i>uxuR</i> ⁺)					

* M63 medium with the additions shown. Glycerol and glucose were used at 5 g l⁻¹.

regulatory region and the first eight codons of *lacZ* have been removed and replaced by the same polylinker cloning sites as in pEMBL. They allow the expression of hybrid β -galactosidase when a DNA fragment containing transcriptional and translational signals is introduced in the correct orientation in front of the *lac* genes. The gene fusion can be made in all three translational readings frames using the three vectors pNM480, -481 and -482. When the inserted fragment contains a promoter but no translational signals, only the *lacY* gene is expressed.

Transcriptional and translational signals were examined in the three reading frames by introducing pNM derivatives into strain MC1061 (Δlac). β -Galactosidase activity was detected in strain MC1061 containing pNM-E1, pNM-E3, pNM-E5, pNM-E7 and pNM-E8, showing the presence of transcriptional and translational signals in the corresponding DNA fragments. As expected in each case, these signals were only detected with one of the three pNM vectors. All the experiments with the various inserts gave the same result in relation to the translational phase of the *uxaB* gene. To seek promoter activity in fragments -E2, -E4, -E6 and -E9, pNM vectors bearing appropriate inserts were introduced into HB101 (LacY⁻). In no case did these plasmids restore a Lac⁺ phenotype to strain HB101, demonstrating the absence of a promoter activity in the insert fragments.

These results indicated that transcriptional and translational signals are both included in the 110 bp *Sau3A*₁-*Nru*I fragment. To prove that these signals belonged to *uxaB* gene, the regulation of β -galactosidase synthesis was analysed in strain MC1061 containing plasmids pACYC184, pRE101 (*exuR*⁺) or pRU101 (*uxuR*⁺) in *trans* to the fusion plasmid. β -Galactosidase synthesis was specifically repressed upon introduction of pRE101 (*exuR*⁺); transformation with the control plasmid pACYC184 or with pRU101 (*uxuR*⁺) had no effect on the expression of the *uxaB-lacZ* gene. The repression exerted by the ExuR regulatory molecule could be reversed upon addition of tagaturonate as inducer (Table 1).

Hugouvieux-Cotte-Pattat (1981) showed that *uxaB* expression is sensitive to catabolite repression by glucose, which is reversed by cAMP. The *uxaB-lac* fusions carried by the pNM vectors were also sensitive to this catabolite repression. β -Galactosidase synthesis was strongly decreased in cells grown on glucose; when cAMP was added, the β -galactosidase activity was identical to that observed in glycerol-grown cells (Table 1). The 110 bp *Sau3A*₁-*Nru*I fragment in pNM-E7 was sufficient to confer sensitivity to glucose repression (Table 1). All the regulatory properties of the expression of the *uxaB-lacZ* fusions were identical to those observed for the wild-type *uxaB* gene.

The repression of β -galactosidase synthesis exerted by the *exuR* product in strains containing

Table 2. *Expression of *uxaB* in strain 71-18 containing the plasmids pEMBL-E1 to -E9*

Specific activities are given in milliunits [nmol product min⁻¹ (mg dry weight)⁻¹]. Cells were grown at 37 °C to an appropriate cell density in glycerol M63 medium with or without inducer and assayed for enzyme activity after treatment in a French press. The induced activities measured in the presence of the plasmids pEMBL-E1 to -E9 were identical to those obtained with the parental plasmid pEMBL8 or 9. The values reported are means of at least three separate assays, results from which varied by <5% about the mean.

Plasmid	Inducer (galacturonate, 5 mM)	Altronate oxidoreductase specific activity
pEMBL8 or 9	—	20
	+	1010
pEMBL-E1	—	490
pEMBL-E2	—	220
pEMBL-E3	—	390
pEMBL-E4	—	30
pEMBL-E5	—	410
pEMBL-E6	—	380
pEMBL-E7	—	370
pEMBL-E8	—	450
pEMBL-E9	—	20

pNM-E7 showed that the 110 bp *Sau3A*₁–*Nru*I segment carries the operator site of *uxaB*. This result was confirmed by the ability of this fragment to titrate out the chromosomal ExuR repressor, leading to constitutive expression of the *uxaB* gene (Table 2). All the other fragments containing the *Sau3A*₁–*Nru*I segment had the same effect. The level of derepression observed in the presence of pNM-E7 was greater than that obtained in the presence of pEB7, a pACYC184 derivative containing the *Eco*RI₁–*Eco*RI₂ fragment (Blanco *et al.*, 1983). This difference may be explained by the fact that pEMBL and pNM vectors are both pBR322 derivatives affected for copy number control (Dente *et al.*, 1983; Minton, 1984) as found for pAT153 (Twigg & Sherratt, 1980), where the copy number is increased 1.5- to 3-fold.

Peptide sequence of the NH₂-terminus of altronate oxidoreductase

An identical reading frame was found for all the *uxaB*–*lacZ* fusions, but no ATG initiator codon could be detected in this reading frame. Fusions performed at the *Nru*I site led to the expression of a hybrid β -galactosidase, suggesting that an initiator codon is located upstream of this site. A putative initiator codon GTG at position 160 of the nucleotide sequence is preceded by a good ribosome-binding site AAGG (Shine & Dalgarno, 1974). To confirm the role of GTG as the initiator codon for the *uxaB* gene, the amino acid sequence of the NH₂-terminus of altronate oxidoreductase was determined. Hybrid β -galactosidase encoded by the *uxaB*–*lacZ* fusion of plasmid pEBG1 was purified to near homogeneity and subjected to Edman degradation. The NH₂-terminal sequence determined was NH₂-Met-Lys-Thr-Leu, which corresponds to the NH₂-terminal sequence of altronate oxidoreductase fused to β -galactosidase; it is in complete agreement with the codons from nucleotides 150 to 161. This result shows unambiguously the use of the GTG codon as initiator codon for the *uxaB* gene.

*The *uxaB* regulatory region has no CRP-binding site*

In most known catabolite-activated operons, principally those involved in the catabolism of growth substrates, the activation is mediated by the catabolite activator protein CRP. In the presence of its effector, cAMP, CRP stimulates the initiation of transcription at the promoter of these genes. Since the *uxaB* and *uxaB*–*lac* fusions were sensitive to glucose catabolite repression which was reversed by cAMP, we searched for putative CRP-binding sites in the *uxaB* sequence. The binding of CRP–cAMP complex to *uxaB* sequences was tested by the gel electrophoretic method described by Garner & Revzin (1981), Fried & Crothers (1983) and Kolb *et al.* (1983). The binding of proteins to DNA can be easily detected since it alters the electrophoretic mobility of the DNA fragment. The 3'-end-labelled *Sau3A*₁–*Sau3A*₂ fragment

Fig. 3. Test for specific CRP binding to the *uxuAB* and *uxaB* promoter regions. Each assay contained 200 μ M-cAMP and from 0 to 400 nM-CRP. (a) Migration of the *uxaB* *Sau*3A₁–*Sau*3A₂ fragment and of DNA fragments containing the *uxuAB* control region; (b) migration of the *uxaB* *Sau*3A₁–*Sau*3A₂ fragment alone. Each restriction fragment was 3'-end-labelled.

was incubated in the presence of various concentrations of CRP, with 200 μ M-cAMP, and the protein–DNA mixture was submitted to electrophoresis in 8% polyacrylamide gels. No binding of CRP to the *uxaB* fragment was found, whereas in the same conditions, an 180 bp DNA fragment containing the *uxuAB* CRP-binding site was displaced in a well-defined band (Fig. 3).

DISCUSSION

In vitro fusions of *uxaB* with the *lacZ* gene have shown that the entire *uxaB* control region resides in a 110 bp *Sau*3A₁–*Nru*I DNA fragment, which contains the *uxaB* promoter, the *uxaB* operator and the DNA signals necessary for catabolite repression. The nucleotide sequence of the *uxaB* control region was determined and we tried to locate the various regulatory signals on this sequence. The transcription start was identified at position 110 ± 1 on the nucleotide sequence. As regards the mRNA start, the examination of the sequence reveals the presence of –10 and –35 sequences which have poor correspondence with the consensus sequences that have been established for *E. coli* promoters (Rosenberg & Court, 1979; Hawley & McClure, 1983). The Pribnow box is imperfect, and the spacing between these two elements (18 bp) is more than the optimal 17 bp (Hawley & McClure, 1983; Aoyama *et al.*, 1983). Such features suggest that this putative promoter would show only weak activity. To correlate the high *in vivo* expression of the *uxaB* gene or the *uxaB*–*lac* fusions with this weak promoter sequence we propose an activation mechanism. Physiological and genetic studies have never shown the action of a specific activator for *uxaB* expression, so we think that such an activator must have a pleiotropic effect, like the CRP–cAMP complex.

The expression of *uxaB* and *uxaB*–*lac* fusions is sensitive to catabolite repression by glucose, which is reversed by cAMP. The structure of the *uxaB* promoter is consistent with activation by the CRP–cAMP complex. In fact, examination of the sequences around the transcription start point of CRP-regulated promoters reveal a particularly poor correspondence with the consensus sequences determined for *E. coli* promoters: in most cases the –35 sequence is barely apparent, the Pribnow box is often imperfect, and the spacing between these two elements is more than the optimal 17 bp (De Crombrughe *et al.*, 1984). Unfortunately no CRP-binding site was detected in the *uxaB* sequence by alteration of electrophoretic mobility. This result is not surprising since the only putative CRP-binding site, presenting some homology with the consensus sequence AA-TGTGA-N₇-CACA (De Crombrughe *et al.*, 1984), is located at position 149. The highly conserved sequence TGTGA is present, but the location of this putative site downstream of the promoter makes it unlikely that this sequence has a physiological role. We can exclude the possibility of a CRP-binding site being located upstream of the sequenced fragment because the expression of the *uxaB*–*lac* fusion obtained with the *Sau*3A₁–*Nru*I fragment is sensitive to catabolite repression.

The catabolite repression exerted by glucose on the *uxaB-lac* fusions is possibly not mediated by the CRP-cAMP complex. The inducer exclusion phenomenon (Kornberg *et al.*, 1980) could affect *uxaB* gene expression in the wild-type strain, but the *uxaB-lac* genes carried by multicopy vectors are expressed constitutively, due to repressor titration, thus expression cannot be abolished by an inducer exclusion mechanism. Catabolite repression is not exclusively regulated by the intracellular level of cAMP (Ullman & Danchin, 1980), it affects *lac* genes in a Δ *cya* strain (Dessein *et al.*, 1978). So it is possible that the catabolite repression of *uxaB-lac* fusions is mediated by another effector, possibly CMF (Ullman *et al.*, 1976). The catabolite repression mechanism exerted on *uxaB* expression, and the existence of an activator for *uxaB* transcription, need further investigation.

The use of GUG as the initiator codon might decrease the rate of initiation of translation of the *uxaB* mRNA, as is the case for *lacI* (Steege, 1977). Nevertheless, the features observed in the DNA sequence of *uxaB* show that the use of the weak GUG initiator codon is compensated by a good Shine-Dalgarno sequence, by an optimal spacing separating this sequence from the GUG codon, and by the presence of the AAAA sequence following this codon (Kozak, 1983; Stormo *et al.*, 1982; Gheysen *et al.*, 1982). Thus the use of the weak GUG initiator codon for *uxaB* is compensated by the organization and the nature of the DNA sequence around the initiator codon. The presence of a weak promoter and GUG codon would be expected to lead to low expression of the *uxaB* gene. In fact, in induced cells (wild-type or containing *uxaB*⁺ plasmids) the *uxaB* product is synthesized in large amounts, allowing its easy purification (Blanco *et al.*, 1983; Portalier & Stoeber, 1972).

The operator of the *uxaB* gene was located to within the *Sau*3A₁-*Nru*I fragment. The DNA sequence of this fragment presents some homologies with that of the *exuT-uxaCA* regulatory region (unpublished observations). We think that the homologous regions are probably the sequence recognized by the ExuR repressor.

This work was supported by grants from the Centre National de la Recherche Scientifique (Laboratoire Propre du CNRS no. UM 380024).

We are indebted to B. Blazy for providing purified CRP protein and to D. Boxer for reading the manuscript. We thank G. Luthaud for typing the manuscript and C. Van Herreweghe for help in the preparation of the illustrations.

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