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Identification and Characterization of Genes (*xapA*, *xapB*, and *xapR*) Involved in Xanthosine Catabolism in *Escherichia coli*

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We have characterized four genes from the 52-min region on the *Escherichia coli* linkage map. Three of these genes are directly involved in the metabolism of xanthosine, whereas the function of the fourth gene is unknown. One of the genes (*xapA*) encodes xanthosine phosphorylase. The second gene, named *xapB*, encodes a polypeptide that shows strong similarity to the nucleoside transport protein NupG. The genes *xapA* and *xapB* are located clockwise of a gene identified as *xapR*, which encodes a positive regulator belonging to the LysR family and is required for the expression of *xapA* and *xapB*. The genes *xapA* and *xapB* form an operon, and their expression was strictly dependent on the presence of both the XapR protein and the inducer xanthosine. Expression of the *xapR* gene is constitutive and not autoregulated, unlike the case for many other LysR family proteins. In minicells, the XapB polypeptide was found primarily in the membrane fraction, indicating that XapB is a transport protein like NupG and is involved in the transport of xanthosine.

Four different nucleoside phosphorylases have been found in *Escherichia coli*. Thymidine phosphorylase (encoded by *deoA*) and uridine phosphorylase (encoded by *udp*) both are specific for pyrimidine nucleosides (27, 38), while purine nucleoside phosphorylase (encoded by *deoD*) and xanthosine phosphorylase (encoded by *xapA*) are specific for purine nucleosides (12, 16, 17). The nucleoside phosphorylases catalyze the phosphorolytic breakdown of the N-glycosidic bond in the nucleoside molecule, with the formation of the corresponding free bases and pentose-1-phosphate. The bases can be utilized as precursors in the synthesis of nucleotides catalyzed by the enzymes in the purine and pyrimidine salvage pathways and used as a nitrogen source (31, 32). The pentose-1-phosphate formed serves as a carbon source. A consequence of the action of the nucleoside phosphorylases is that *E. coli* can grow on nucleosides as the sole carbon and energy source. Purine nucleoside phosphorylase plays a crucial part in the breakdown of all the purine nucleosides except xanthosine (22). However, in 1980, a second purine nucleoside phosphorylase (XapA), which could cleave xanthosine, was found (8, 12). This xanthosine phosphorylase activity is found only when *E. coli* is grown in the presence of xanthosine (12). Xanthosine appears to be the true inducer and is the only nucleoside that can induce the expression of the *xapA* gene. XapA can degrade all purine nucleosides except adenosine and deoxyadenosine. This substrate specificity resembles the specificity of mammalian purine nucleoside phosphorylase more than that of the other *E. coli* purine nucleoside phosphorylase (14, 28). No other enzymes involved in nucleoside catabolism appear to be induced by xanthosine (12). The isolation of regulatory mutants (*xapR* mutants) with high levels of XapA could indicate that the *xapA* gene expression is dependent on a functional *xapR* gene product which probably acts as an transcriptional activator when xanthosine is present (8, 20). The regulation of the expression of *deoD* and *xapA* is different. Expression of the *deoD* gene is regulated negatively by the two repressors DeoR and CytR and is activated by the cyclic AMP (cAMP) receptor protein-cAMP complex; however, even under completely repressed conditions, a low basal level of purine nucleoside phosphorylase is maintained (for a review, see reference 11). In contrast to this, the xanthosine phosphorylase activity can be measured only when cells are grown in the presence of xanthosine, and the xanthosine phosphorylase activity is not influenced by DeoR or CytR. Xanthosine phosphorylase has been purified and partly characterized (3, 12, 24).

The *xapR* and *xapA* genes have been located at 52 min on the *E. coli* chromosome, almost exactly 180° from the *deoD* gene, which maps at 100 min (8). The *xapR* and *xapA* genes cotransduce (P1 phage) with a frequency of approximately 90% (8, 21). DNA sequencing of the 51- to 52-min region has revealed an open reading frame (ORF), ORF294, which is homologous to the LysR family of transcriptional activators (6). Since *xapR* is the only regulatory gene mapped to this region, ORF294 was proposed to be the *xapR* gene (6). We have isolated a part of the 52-min region from the Kohara library and determined the DNA sequence. We show that ORF294 does encode the XapR protein and identify two other ORFs that form an operon clockwise of *xapR*. One ORF shows homology to the mammalian purine nucleoside phosphorylase gene and is the *xapA* gene, and between *xapA* and *xapR* is another gene, *xapB*, which encodes a membrane-associated protein that probably is a nucleoside transport protein. The XapR protein is a member of the LysR family, which comprises more than 50 proteins (13, 37). All of these proteins are approximately 300 amino acids in length. The N-terminal sequences appear to be involved in DNA binding and contain highly conserved helix-turn-helix motifs. The C-terminal sequences, in contrast, appear to be only slightly conserved and probably are involved in inducer binding. Most LysR proteins act as both transcriptional activators and repressors. Often they repress their own transcription while they activate the transcription of a neighboring gene located immediately upstream but divergently transcribed.

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MATERIALS AND METHODS

DNA techniques. The methods used for transformation, isolation of DNA fragments, and plasmid isolation were those described by Sambrook et al. (35).

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genotype or description	Reference, source, construction, or selection
Bacteria		
RB400	HfrH Δ(*gal-deoR*) *thi tyrT*$^{+}$ *deoD*::(λ*c*I857S7)	7
BD1854	*minA minB thi his rpsL lac mtl man mal xyl tonA*	34
DC520	MZ-1 Δ*lac pro*::Tn*10 his ilv galK*Δ*8*	D. Court
GD746	JC7623 *xapR*::*kan*	Kan^{r} transformants with linear pCP4
GD749	JC7623 Δ(*xapABR*)::*kan*	Kan^{r} transformants with linear pGD222
GD790	JC7623 Δ*xapB*::*kan*	Kan^{r} transformants with linear pGD228
GD792	JC7623 *xapA*::*kan*	Kan^{r} transformants with linear pGD230
JC7623	F^{-} *recB21 recC22 sbcB15 thr leu proA2 hisG argE thi ara lacY galK xyl mtl rpsL tsx supE*	25
HO449	F^{-} *supF relA spoT rpsL lamB metB udp gsk-3 pncA hemA deoD zjj*::Tn*10*	B. Hove-Jensen
SØ3076	F^{-} *thr1 leuB6 rpsL galK lacY1 cytR*	Laboratory collection (C600 origin)
SØ6416	SØ3076 *deoD zjj*::Tn*10*	SØ3076+P1v(HO449), Tet^{r}
SØ6435	SØ3076 *deoD*	SØ6416, selection of Tet^{s}
SØ6436	SØ6435 Δ*lac pro*::Tn*10*	SØ6435+P1v(DC520), Tet^{r}
SØ6444	SØ6436 *xapR*::*kan*	SØ6436+P1v(GD746), Kan^{r}
SØ6447	SØ6436 Δ(*xapABR*)::*kan*	SØ6436+P1v(GD749), Kan^{r}
SØ6685	SØ6436 *xapA*::*kan*	SØ6436+P1v(GD792), Kan^{r}
SØ6686	SØ6436 Δ*xapB*::*kan*	SØ6436+P1v(GD790), Kan^{r}
Plasmids		
pBR322	Vector	5
pCC1	7,745-bp *Hin*dIII fragment from Kohara phage λ417 in pBR322/*Hin*dIII	This work
pCP4	1,264-bp *Bam*HI fragment from pUC4k in pCC1/*Bgl*II	This work
pCP10	481-bp *Nco*I-*Afl*II fragment (Klenow) from pCC1 in pGD56/*Sma*I	This work
pCP19	Filling in of the *Bgl*II site in pCP10 (Klenow) and religation	This work
pCS14	Deletion of 1,060-bp *Eco*NI-*Bgl*II fragment from pGD216	This work
pCS59	Deletion of 1,029-bp *Cla*I fragment from pGD216	This work
pCS60	Deletion of 895-bp *Mam*I-*Ecl*136II fragment from pGD216	This work
pCS63	1,537-bp *Nco*I-EcoNI fragment (Klenow) from pGD216 in pSU18/*Sma*I	This work
pCS64	Filling in of the *Bgl*II site in pGD216 (Klenow) and religation	This work
pGD56	*galK* promoter cloning vector DNA sequence (accession no. X67018)	
pGD111	3,094-bp *Eco*RI-SacI fragment from pGD408 into pOU250/*Eco*RI/*Sac*I	This work
pGD112	4,733-bp EcoRI-*Spl*I fragment from pGD409 into pOU250/*Eco*RI/*Spl*I	This work
pGD114	2,441-bp *Bam*HI-*Ecl*136II fragment from pGD410 into pOU250/*Bam*HI/*Ecl*136II	This work
pGD115	3,958-bp *Eco*RI-*Spl*I fragment from pGD411 into pOU250/*Eco*RI/*Spl*I	This work
pGD116	1,812-bp *Eco*RI-*Sac*I fragment from pGD412 into pOU250/*Eco*RI/*Sac*I	This work
pGD117	Deletion of 1,138-bp *Eco*RI-*Bam*HI fragment from pGD111	This work
pGD118	Deletion of 1,812 bp *Eco*RI-*Ecl*136II fragment from pGD116	This work
pGD216	Deletion of 3,723-bp *Eco*NI fragment from pCC1	This work
pGD222	Deletion of 1,631-bp *Mlu*I-*Ecl*136II fragment from pCP4	This work
pGD228	Replacement of 947-bp *Sgr*AI-*Nco*I fragment from pGD216 with 1,252-bp *Hin*cII fragment from pUC4K	This work
pGD225	Deletion of 2,484-bp *Sgr*AI-*Eco*NI and 539-bp *Hin*dIII-SphI fragments from pGD216	This work
pGD230	1,252-bp *Hin*cII fragment from pUC4K in pCC1/*Ecl*136II	This work
pGD408	1,265-bp *Hae*III fragment from pGD216 in pRAK82/*Sma*I	This work
pGD409	1,949-bp *Eco*RI-*Nru*I fragment from pGD216 in pRAK82/*Eco*RI-*Sma*I	This work
pGD410	484-bp *Bam*HI-*Bgl*II fragment from pCP10 in pRAK80/*Bam*HI	This work
pGD411	1,167-bp *Ecl*136II-*Sal*I fragment from pGD216 in pRAK80/*Sma*I-*Sal*I	This work
pGD412	2,046-bp *Sgr*AI (Klenow)-*Eco*RI fragment from pGD216 in pRAK81/*Eco*RI-*Sma*I	This work
pOU250	Mini-R1 plasmid carrying a promoterless *lacZ* gene	P. Valentin-Hansen
pSU18	pACYC184-derived vector	2
pRAK80 to -82	Vectors for translational fusions to *lacZ* in 3 reading frames	19
pTZ19R and -U	Vectors	40
pUC4K	pUC plasmid carrying a kanamycin cassette	40
pUC18 and -19	Vectors	40

Bacterial strains. The bacterial strains used are listed in Table 1. SØ6436 was constructed from SØ3076 in several steps. First, a *deoD* mutation was introduced to prevent the purine nucleoside phosphorylase from metabolizing inosine in the XapA assay. Next, a deletion of the *lac* operon was introduced. In SØ6436 it is possible to study both *galK* and *lacZ* fusions. GD746, GD749, GD790, and GD792 were constructed by recombining the *kan* insertions of pCP4, pGD222, pGD228, and pGD230, respectively, into JC7623 (*recBC*) by transforming JC7623 with *Apa*LI-linearized plasmid DNA. Recombinants growing on Luria broth plus kanamycin (30 μg/ml) but not on Luria broth plus ampicillin (50 μg/ml) were selected and further tested on minimal medium. P1v phages grown on GD746, GD749, GD790, and GD792 were used to transduce SØ6436 as described by Miller (30), resulting in SØ6444, SØ6447, SØ6686, and SØ6685, respectively (the final constructions are outlined in Fig. 3). The kanamycin insertions were verified by Southern analysis (data not shown) with a PCR-generated probe covering nucleotides 834 to 1168 and radiolabelled with [α-^{32}P]dCTP by using the Ready-to-go labelling kit from Pharmacia.

Plasmids. DNA carrying ORF294 and 4 kbp downstream of ORF294 was isolated from λ417 of the Kohara library (23). A 7,744-bp *Hin*dIII fragment was

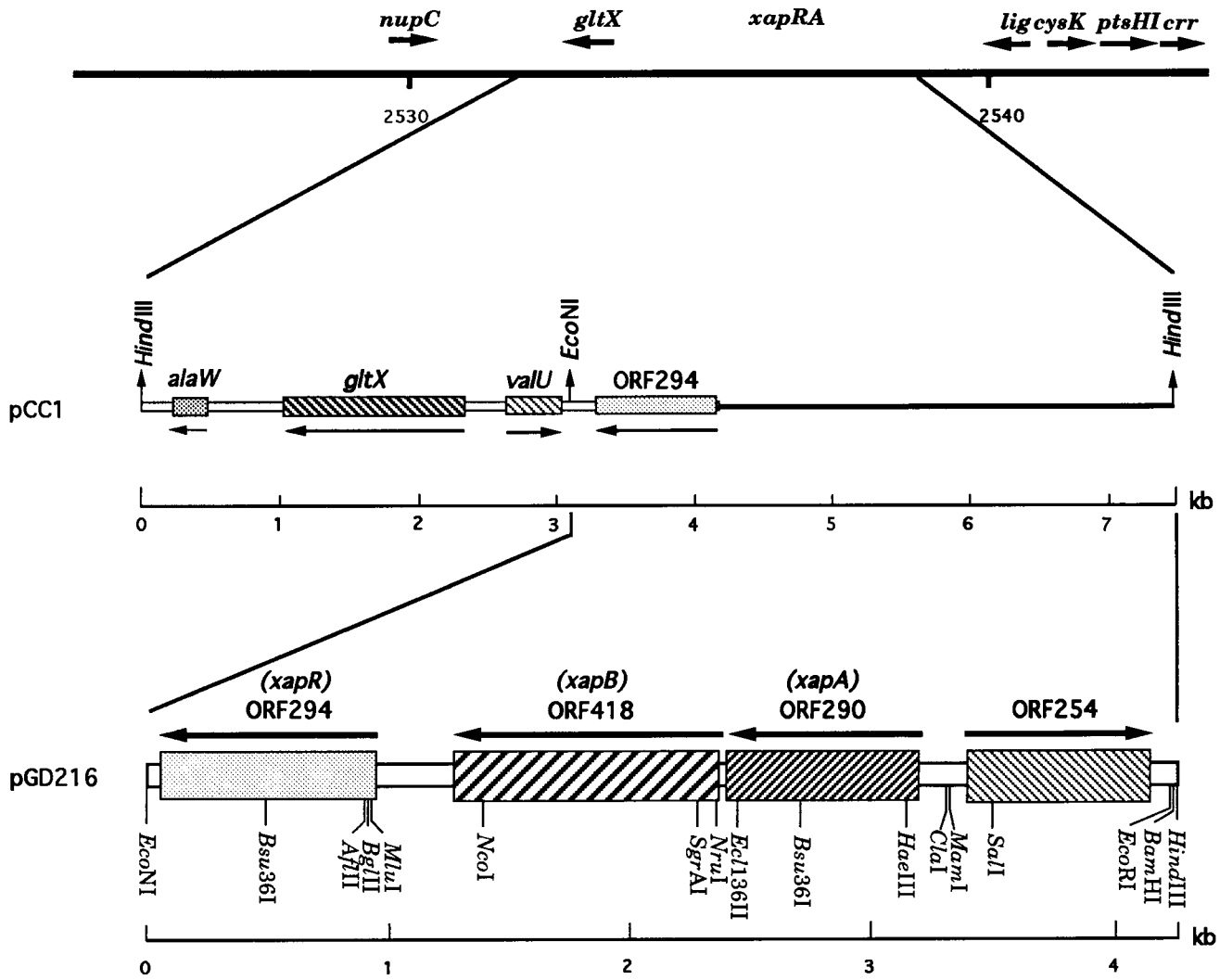

FIG. 1. Map of the 52-min region of the *E. coli* chromosome. The numbers indicate the positions on the Kohara map. The ORF shown in pCC1 is taken from reference 6. The arrows indicate the orientations of the reading frames. The restriction sites shown are those used for subcloning as described in Table 1.

cloned into the *Hin*dIII site of pBR322, resulting in pCC1 (Fig. 1). From this plasmid an *Eco*NI fragment of 3,723 bp carrying the DNA downstream of the *xapR* gene was deleted, and the plasmid was religated. The resulting plasmid (pGD216) has been used for the construction of a number of subclones, as outlined in Table 1 (see also Fig. 1). The low-copy-number translational *lacZ* fusions were constructed in two steps (Table 1). First, the fusions were constructed in the multicopy plasmids pRAK80 to pRAK82 (19), and then the fusions were transferred to the low-copy-number mini-R1 plasmid pOU250.

DNA sequencing. Fragments of DNA were isolated from the plasmid pGD216 and cloned in pUC18, pUC19, pTZ19R, or pTZ19U (40). All plasmids used for double-stranded DNA sequencing were purified by the CTAB-DNA precipitation method (10). Sequencing was performed as described by Sanger et al. (36), using α-^{35}S-dATP (New England Nuclear) as a radioactive marker and the Sequenase kit (7-deaza-dGTP, version 2.0) from U.S. Biochemical Corp.

Growth of cells and preparation of cell extracts. Cells were grown in AB glucose minimal medium (9) supplemented with the appropriate required amino acids, with Casamino Acids, and either with xanthosine (1 mg/ml) or not. Plasmid-containing strains were supplemented with 25 (pOU250 derivatives) or 100 (pBR322 derivatives) μg of ampicillin per ml, while pSU18 derivatives were grown in the presence of 100 μg of chloramphenicol per ml. The cells were harvested in the exponential phase by centrifugation (12,000 × *g*, 8 min), washed with AB medium, and resuspended in 100 mM Tris-HCl–2 mM EDTA (pH 7.4) to a cell density of approximately 2×10^9 bacteria per ml. After determination of the final optical density at 436 nm (OD_{436}), the suspension was sonicated for 60 s and centrifuged (3,000 × *g*, 10 min). The supernatant was used for enzyme assays.

Enzyme assays. Xanthosine phosphorylase activity was measured at 37°C (assay A) as described by Hammer-Jespersen et al. (12). β-Galactosidase activity was assayed at 28°C as described by Miller (30). Protein determination was carried out with the bicinchoninic acid protein assay kit from Pierce with bovine serum albumin as the standard. One enzyme unit is defined as the amount of enzyme which catalyzes the conversion of 1 nmol of substrate per min. Specific activity is either units per OD_{436} unit or units per milligram of protein. One milligram of protein per milliliter corresponds to 15 OD_{436} units per milliliter.

Labelling of plasmid-encoded proteins. Transformants of the minicell-forming strain BD1854 (Table 1), carrying various plasmids, were grown overnight in 100 ml of AB glucose minimal medium supplemented with xanthosine. The minicell fractions were separated from normal cells by two consecutive sucrose gradients as described by Reeve (34). Protein synthesis in minicells was performed as described by Westh-Hansen et al. (42).

Fractionation of the proteins synthesized was performed as described by Weiner et al. (41). Polyacrylamide gel electrophoresis was performed on sodium dodecyl sulfate (SDS)–12.5% polyacrylamide gels as described by Laemmli (26) and Ames (1). The samples were shaken for 1 h at 37°C immediately before they were applied to the gel. The protein was visualized by staining with Coomassie blue and by autoradiography.

Primer extension experiments. Total RNA was extracted from strains grown in AB glucose minimal medium (9) with or without xanthosine (1 mg/ml) as indicated in Results. The cultures were harvested in exponential growth at an OD_{436} of 0.5, and RNA was extracted as described by Sørensen and Neuhard (39).

The primers used for primer extension were 5′ end labelled with T4 polynucleotide kinase and [γ-^{32}P]ATP (New England Nuclear). All primer extension reactions were carried out as described by Sørensen and Neuhard (39). The XapA primer (5′-CAGCGGGTTATGAGAAAATTGAACC) was used to synthesize cDNA from RNA extracted from SØ6436(pGD111) and SØ6444 (pGD111). The XapA primer extensions were applied alongside a DNA se-

TABLE 2. Xanthosine phosphorylase activity expressed from plasmid pCC1 (*xapA*$^+$ *xapR*$^+$)[a]

Strain(plasmid)	With xanthosine		Without xanthosine	
	XapA (U/OD_{436} U)	t_D[b] (min)	XapA (U/OD_{436} U)	t_D (min)
RB400(pBR322)	117	70	<0.2	65
RB400(pCC1)	2,200	185	<0.2	120

[a] *E. coli* cells containing pBR322 or pCC1 were grown exponentially at 32°C in AB glucose minimal medium with or without xanthosine as indicated.
[b] t_D, doubling time.

quencing ladder of pCS59, using the XapA primer, on a 6% denaturing polyacrylamide gel. The pGD56 primer (5′-GCCAGCATTTCATAACCAACC, complementary to pCP10 downstream of the cloning sites starting 73 nucleotides downstream of the XapR ATG start codon) was used to synthesize cDNA from RNA extracted from SØ6444(pCP10) and SØ6444(pCP19). The pGD56 primer extensions were applied alongside a sequencing ladder of plasmid pCP10, using the pGD56 primer, on a 6% denaturing polyacrylamide gel.

Nucleotide sequence accession number. The DNA sequence has been deposited in the EMBL database with the accession number X73828.

RESULTS

Cloning of the *xap* genes. The proposal by Brun et al. (6) that ORF294 might encode the XapR protein led us to isolate an 8-kbp *Hin*dIII fragment carrying 4 kbp of DNA clockwise of ORF294 (Fig. 1). Since the DNA sequence counterclockwise of ORF294 did not suggest any reading frames that could be *xapA* and since *xapA* and *xapR* cotransduce with a frequency of 90%, we expected *xapA* to be located clockwise of ORF294. To ensure that the *Hin*dIII fragment carried *xapA*, we measured the XapA activity in strain RB400 containing pCC1 or pBR322. RB400 carries the wild-type *xap* genes on the chromosome (Table 2). RB400 harboring pCC1 or pBR322 was grown exponentially in minimal medium with or without xanthosine. From the results in Table 2 it can be seen that in the absence of xanthosine, there was no detectable XapA activity in the two strains used. In the presence of xanthosine, RB400 (pBR322) gave rise to 117 U per OD_{436} unit, while RB400 (pCC1) gave rise to a 19-fold-higher activity, thus indicating that the 8-kbp *Hin*dIII fragment carries a functional *xapA* gene.

DNA sequencing and analysis of the *xap* region. To identify the *xapA* gene in pCC1, we determined the DNA sequence clockwise of ORF294. The DNA sequence was determined on both strands in the whole 3.5-kbp fragment and with an overlap of 142 bp into the ORF294 sequence, which was identical to the sequence determined by Brun et al. (6). Analysis of the 3,462-bp *Hin*dIII-*Bgl*II nucleotide sequence clockwise of the proposed *xapR* gene revealed three ORFs with nucleotide sequences longer than 750 bases (Fig. 1 and 2). ORF290, which can encode a polypeptide of 290 amino acids, shows homology with human purine nucleoside phosphorylase gene (43). Of the 290 amino acids, 41% were identical to those of human purine nucleoside phosphorylase. On this basis, we believe that ORF290 is the *xapA* gene. ORF418, immediately downstream of ORF290 (*xapA*), can encode a polypeptide of 418 amino acids. The search for homology between the ORF418 product and other proteins in the EMBL database revealed a strong homology to the NupG protein (42), with 56% identity. ORF418 is called *xapB*. The last reading frame, ORF254, is transcribed divergently from ORF290, ORF418, and ORF294 (Fig. 1). The ORF254 product shows no homology with any other proteins in the EMBL database.

Construction and analysis of chromosomal *xap*::*kan* insertions and deletions. In order to be able to identify the functions of the *xap* genes, we made a series of mutants by insertion of the kanamycin gene into the *xap* region at different positions, as outlined in Fig. 3 (see Materials and Methods). The mutants were tested for growth on different nucleosides as the sole carbon source. As shown in Fig. 3, all of the mutants grew well on cytidine, but when either *xapA* or *xapR* was mutated, they could not grow on xanthosine. The *xapB* mutant behaved differently, as it grew very slowly on xanthosine and colonies were visible only after 4 to 5 days of incubation, indicating that *xapB* is involved in the metabolism of xanthosine.

To further characterize these mutants, we determined the activity of xanthosine phosphorylase in extracts of cells grown in the absence or presence of xanthosine (Table 3). In the wild type (SØ6436), XapA activity can be measured only if the cells are grown in the presence of xanthosine; under this condition, 214 U/mg of protein was found. If either the *xapA* or *xapR* gene was interrupted, no XapA activity could be detected. In SØ6686, in which *xapB* is deleted and which still carries the intact *xapA* and *xapR* genes, the XapA activity was 10-fold lower than that in the wild type, confirming the results for the growth phenotype of the *xapB* mutant.

In order to verify that both *xapA* and *xapR* are required for XapA expression and that they function in *trans*, we transformed SØ6447 with pCS63 (which is a pACYC plasmid that carries the *xapR* gene), with pGD225 (which is a pBR322 derivative that carries the *xapA* gene), with pCS14 (which carries both *xapA* and *xapB*) or with combinations of two of these plasmids. As shown in Table 3, the *xapA* gene was expressed in only two situations, namely, when both the *xapR* (pCS63) and *xapA* (pGD225 or pCS14) genes were present (there was a large variation in the XapA activity in these two strains from one experiment to another, probably because of copy number variations). When SØ6447(pCS14, pCS63) was grown in the presence of xanthosine, the growth was strongly inhibited and the copy number of pCS14 was reduced 5- to 10-fold (data not shown). This might be the reason for the relatively low XapA expression compared with that in SØ6447(pGD225, pCS63).

We also measured the XapA activity in SØ6436 transformed with pCS63 and pGD225 to see the effect of *xapA* and *xapR* when they are overexpressed independently. When *xapA* was overexpressed, the XapA activity decreased fourfold compared with that in the wild type, while the activity increased eightfold when *xapR* was overexpressed. When both *xapA* and *xapR* were overexpressed, the XapA activity increased 75-fold. These results indicate that the XapR concentration in wild-type cells is limiting for *xapA* expression.

Expression and cellular location of the *xapA*, *xapB*, and *xapR* gene products. To show that the proposed *xapA*, *xapB*, and *xapR* genes in fact encode proteins, we carried out a minicell experiment. The minicell-forming strain BD1854 was transformed with derivatives of pGD216 containing different combinations of the *xap* genes. From cultures of these transformants, minicells were isolated and allowed to synthesize proteins in the presence of [^{35}S]methionine. The labelled proteins were analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 4A). On this gel one protein band with a molecular mass of approximately 30.1 kDa was expressed from the *xapA*$^+$ plasmids (lanes 2 and 4) but not from the *xapA*$^-$ plasmid (lane 3). This molecular mass of XapA (30.1 kDa) is about the value predicted by the nucleotide sequence (31,451 Da). A band corresponding to a molecular mass of 31.8 kDa was seen only in extracts from cells expressing the *xapR* gene (Fig. 4A, lanes 3 and 4). When the *xapR* gene was mutated, this band disappeared (lane 2). The different XapA levels in lanes 2 and 4 of Fig. 4A could be a reflection of the different XapR levels. In lane 4, a very weak band at 36.6 kDa which was not visible in

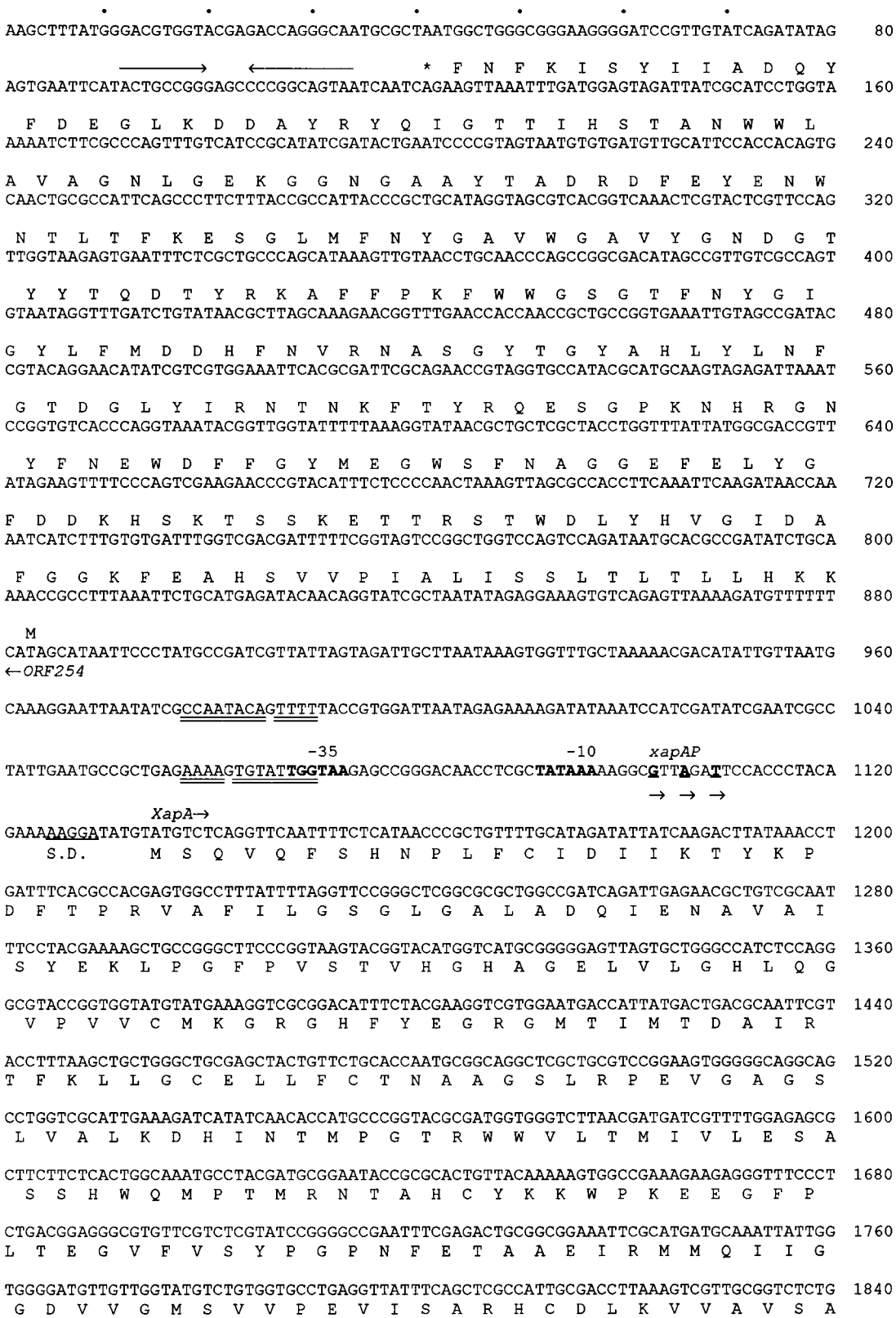

FIG. 2. Nucleotide and predicted amino acid sequences of ORF254 and the *xapA* and *xapB* genes. To read the *xapABR* genes from left to right, the orientation of the DNA sequence is inverted relative to that deposited in the EMBL database and to the 52-min region shown in Fig. 1. The −10 and −35 regions of the *xapA* and *xapR* promoters are shown in boldface, and the mapped transcription start sites are shown underlined and in boldface with an arrow below the sequence. S.D., Shine-Dalgarno sequence. The two double-underlined sequences indicate two inverted complementary sequences. Potential transcriptional terminators after ORF254 and *xapB* are indicated by arrows.

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CGATTACCAATATGGCGGAAGGTTTAAGCGATGTGAAATTGTCACATGCGCAAACGCTGGCGGCTGCGAGCTCACAAGCA 1920
  I  T  N  M  A  E  G  L  S  D  V  K  L  S  H  A  Q  T  L  A  A  A  S  S  Q  A

GAACCTCATCAACCTTATTTGTGGCTTTCTGCGCAAAATTGCCTGAGAAAAACAATAAGAAACGGCCTCGCGGGTGAGGC 2000
E  P  H  Q  P  Y  L  W  L  S  A  Q  N  C  L  R  K  T  I  R  N  G  L  A  G  E  A
                    XapB→
CAAATGATGCGACAAGGAAACGATTATGAGCATCGCGATGCGCTTAAAGGTAATGTCCTTTTTGCAATATTTTATCTGGG 2080
 K  *        S.D.       M  S  I  A  M  R  L  K  V  M  S  F  L  Q  Y  F  I  W  G

GGAGCTGGCTGGTTACCCTCGGCTCTTACATGATTAATACTCTTCATTTCACCGGCGCTAATGTTGGCATGGTTTACAGT 2160
  S  W  L  V  T  L  G  S  Y  M  I  N  T  L  H  F  T  G  A  N  V  G  M  V  Y  S

TCCAAAGGGATCGCCGCGATTATTATGCCTGGTATAATGGGGATCATCGCAGTACAAATGCGTGCGCGCAGAACGTGCAT 2240
S  K  G  I  A  A  I  I  M  P  G  I  M  G  I  I  A  V  Q  M  R  A  R  R  T  C  I

ACATGCTGTGTCACCTGGTGTGTGCGGCGTACTTTTTTATGCGGCATCCGTAACTGATCCGGATATGATGTTTTGGGTGA 2320
 H  A  V  S  P  G  V  C  G  V  L  F  Y  A  A  S  V  T  D  P  D  M  M  F  W  V  M

TGTTAGTCAATGCGATGGCGTTTATGCCGACTATTGCGTTATCGAACAGCGTCTCTTATTCCTGTCTTGCCCAGGCAGGG 2400
  L  V  N  A  M  A  F  M  P  T  I  A  L  S  N  S  V  S  Y  S  C  L  A  Q  A  G

CTTGACCCGGTGACCGCTTTCCCGCCCATTCGCGTTTTTGGTACGGTGGGGTTCATTGTCGCGATGTGGGCAGTAAGCCT 2480
L  D  P  V  T  A  F  P  P  I  R  V  F  G  T  V  G  F  I  V  A  M  W  A  V  S  L

GCTGCATCTGGAATTGAGTAGTCTGCAGCTGTATATCGCGTCCGGTGCGTCATTGCTGCTGTCGGCTTATGCGCTGACTT 2560
 L  H  L  E  L  S  S  L  Q  L  Y  I  A  S  G  A  S  L  L  L  S  A  Y  A  L  T  L

TGCCGAAGATTCCGGTTGCGGAGAAAAAAGCGACCACATCGCTTGCCAGCAAGCTGGGTCTGGATGCCTTCGTGCTGTTT 2640
  P  K  I  P  V  A  E  K  K  A  T  T  S  L  A  S  K  L  G  L  D  A  F  V  L  F

AAAAATCCACGCATGGCCATCTTTTTCCTCTTTGCCATGATGCTGGGTGCGGTACTGCAAATTACCAACGTTTTTGGTAA 2720
K  N  P  R  M  A  I  F  F  L  F  A  M  M  L  G  A  V  L  Q  I  T  N  V  F  G  N

TCCGTTCCTACATGATTTCGCCCGTAACCCGGAGTTTGCTGACAGTTTTGTGGTGAAATATCCCTCCATTTTACTGTCAG 2800
 P  F  L  H  D  F  A  R  N  P  E  F  A  D  S  F  V  V  K  Y  P  S  I  L  L  S  V

TTTCACAGATGGCAGAAGTGGGCTTTATACTGACTATCCCATTCTTTTTAAAGCGATTTGGCATTAAAACCGTCATGCTG 2880
  S  Q  M  A  E  V  G  F  I  L  T  I  P  F  F  L  K  R  F  G  I  K  T  V  M  L

ATGAGTATGGTGGCCTGGACGTGCGCTTTGGCTTCTTCGCCCTATGGCGATCCGTCAACAACCGGATTTATTTTGCTGCT 2960
M  S  M  V  A  W  T  C  A  L  A  S  S  P  Y  G  D  P  S  T  T  G  F  I  L  L  L

GCTGTCGATGATTGTTTATGGCTGTGCATTCGATTTCTTCAATATTTCTGGTTCGGTATTTGTCGAACAGGAAGTTGATT 3040
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CCAGCATTCGTGCCAGCGCGCAGGGGCTCTTTATGACCATGGTAAATGGTGTCGGCGCATGGGTTGGCTCGATTCTGAGT 3120
  S  I  R  A  S  A  Q  G  L  F  M  T  M  V  N  G  V  G  A  W  V  G  S  I  L  S

GGCATGGCAGTAGATTACTTTTCGGTGGATGGCGTAAAAGACTGGCAAACTATCTGGCTGGTGTTTGCAGGATATGCTCT 3200
G  M  A  V  D  Y  F  S  V  D  G  V  K  D  W  Q  T  I  W  L  V  F  A  G  Y  A  L

TTTTCTCGCAGTGATATTTTTCTTTGGGTTTAAATATAATCATGACCCTGAAAAGATAAAGCATCGAGCGGTGACTCATT 3280
 F  L  A  V  I  F  F  F  G  F  K  Y  N  H  D  P  E  K  I  K  H  R  A  V  T  H  *

AAAAGGATTCGCGGCTCTGCTCTTCAGAGCTGCTTTTATGATAAAGGTTAATTAGTGAAAGATATTTTATTTTCTTCCGG 3360
          ————————→     ←————————
                                                    -35                    -10
CGTTGGTTTTGGTATTGGTGCTTTGTTTACAATCGTGCGCTTGCCTATTCCTGTACCAAATGTATTACCGGGTATATTAT 3440

   xapRP2                          -35                -10       xapRP1
CCATTGTGTTTATGTATGTCGGATATCTGGTGGTGAAATACTTTATGCCATGATAATTTAATACGATGTATTTATTATAT 3520
       →                                                          →
              XapR→
GGAGCACTTAATTATGGAACGCGTATACAGAACAGATCTTAAGTTGCTCCGTTATTTTCTTGCCGTAGCGGAAGAGTTGC 3600
S.D.        M  E  R  V  Y  R  T  D  L  K  L  L  R  Y  F  L  A  V  A  E  E  L  H

ATTTTGGCCGCGCAGCAGCGCGTTTAAA 3628
  F  G  R  A  A  A  R  L  N
```

FIG. 2—*Continued.*

any other lanes was seen. This is likely to be the *xapB* gene product, although the M_r deviates significantly from the value derived from the amino acid sequence (see below).

The predicted amino acid sequence of XapB indicates that it is very hydrophobic. Moreover, it shows a high degree of similarity to the NupG protein, which is a membrane-associated protein. Since XapB was hardly visible in total protein fractions in Fig. 4A (lane 4), another set of minicell experiments, in

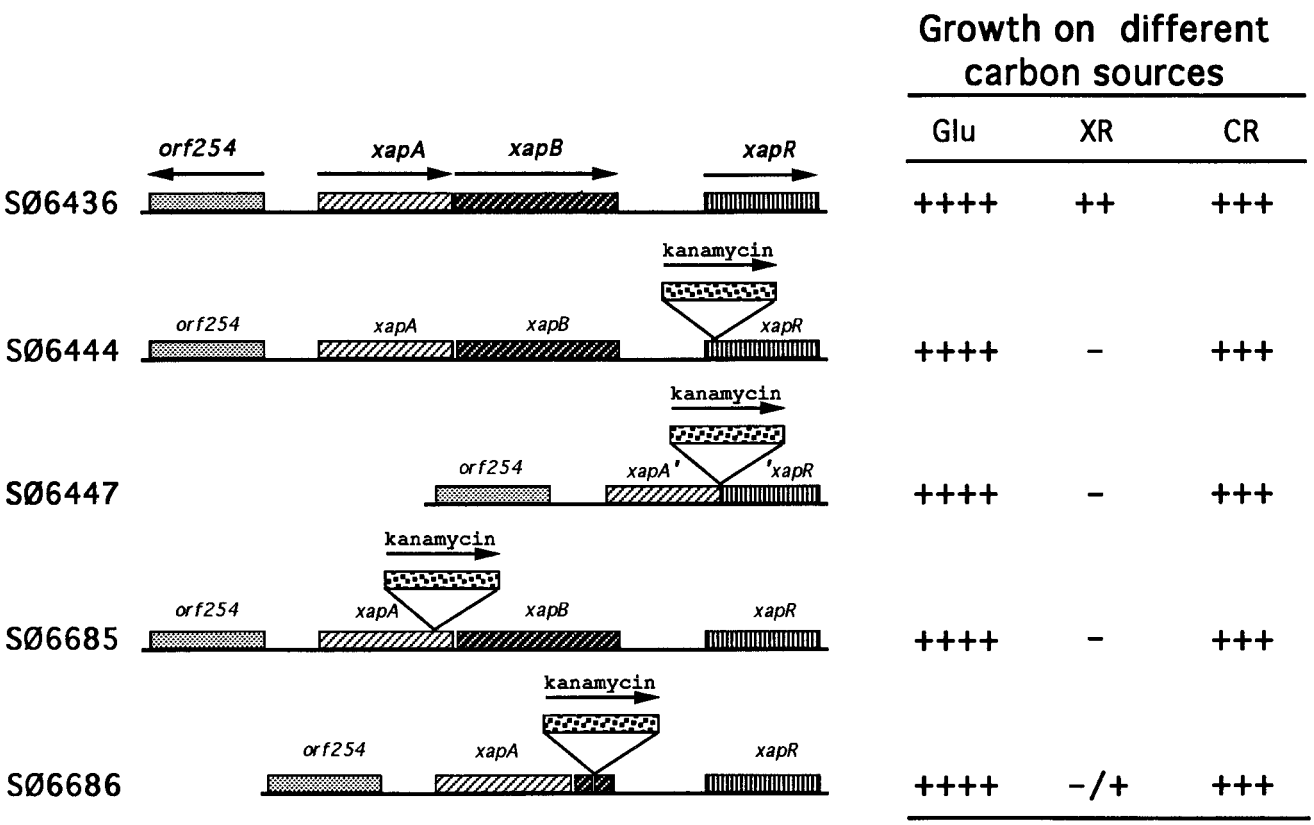


FIG. 3. Structures and growth of the mutants containing the chromosomal kanamycin insertions. Growth was monitored on AB minimal medium plates supplemented with 0.2% glucose (Glu), 1 mg of xanthosine (XR) per ml, or 1 mg of cytidine (CR) per ml as the sole carbon source. −, no growth after 3 days; + to ++++, weak to very good growth; −/+, small colonies were visible after 5 days of incubation at 37°C.

which the cell extract was enriched in the membrane fraction, was done (Fig. 4B). A significant enrichment of the 36.6-kDa protein band (Fig. 4B, lane 6) compared with those of the other plasmid-encoded proteins was seen. The product of the *tet* gene (37 kDa), which is known to reside in the cell membrane, was also enriched in the membrane fraction (compare lanes 1 and 5).

Transcription start sites for *xapA* and *xapR*. The 5′ ends of the *xapA* and *xapR* mRNAs were identified by primer extension mapping. A 25-nucleotide synthetic primer that hybridized to the DNA 9 nucleotides downstream of the ATG codon of *xapA* was used to direct synthesis of DNA complementary to the *xapA* mRNA. Total RNA was prepared from SØ6436 (pGD111) [*xapR*$^+$/Φ(*xapA-lacZ*)] and SØ6444(pGD111) [*xapR*/Φ(*xapA-lacZ*)] grown in the absence or presence of xanthosine. As shown in Fig. 5A, three major transcriptional start sites are present. By comparing the lengths of the products with the DNA sequence constructed by the same primer, the start sites are mapped to 26, 29, and 32 nucleotides upstream of the ATG codon of *xapA*. All three start sites were present in SØ6436, and only when the cells are grown in the presence of xanthosine (Fig. 5A, lane 2), and were not present in SØ6444, in which *xapR* was destroyed (lanes 3 and 4). Upstream of the three start sites is a very AT-rich region in which the sequence TATAAAAA is positioned favorably relative to the two strongest transcripts (32 and 29 nucleotides upstream of *xapA*). The third start site is probably too far away from the −10 region (TATAAA); however, it is possible that the sequence TAAA AA (2 bp closer) functions as a −10 region. At 18 bp upstream of the −10 region, there is a potential −35 region (TGGTAA) that shows some homology to the consensus sequence.

TABLE 3. Xanthosine phosphorylase activity in wild-type and *xap* mutant strains

Strain	Genotype	XapA (U/mg of protein)[a]	
		With xanthosine	Without xanthosine
SØ6436	Wild type	214	<1
SØ6444	*xapR*::*kan*	<1	<1
SØ6685	*xapA*::*kan*	<1	<1
SØ6686	*xapB*::*kan*	20	<1
SØ6447	Δ(*xapABR*)::*kan*	<1	<1
SØ6436(pGD225)	Wild type/*xapA*$^+$	57	2
SØ6436(pCS63)	Wild type/*xapR*$^+$	1,760[b]	1
SØ6436(pCS63, pGD225)	Wild type/*xapA*$^+$ *xapR*$^+$	15,980[b]	71[b]
SØ6447(pGD225)	Δ(*xapABR*)::*kan*/*xapA*$^+$	<1	<1
SØ6447(pCS63)	Δ(*xapABR*)::*kan*/*xapR*$^+$	<1	<1
SØ6447(pCS63, pGD225)	Δ(*xapABR*)::*kan*/*xapA*$^+$ *xapR*$^+$	3,430[b]	18
SØ6447(pCS14)	Δ(*xapABR*)::*kan*/*xapA*$^+$*B*$^+$	<1	<1
SØ6447(pCS14, pCS63)	Δ(*xapABR*)::*kan*/*xapA*$^+$*B*$^+$*xapR*$^+$	1,696[b,c]	6

[a] *E. coli* cells were grown exponentially at 37°C in AB glucose minimal medium with or without xanthosine as indicated. The values are the averages from two to four independent determinations.

[b] The activity varied from experiment to experiment, probably because of copy number variations.

[c] Very strong growth inhibition.

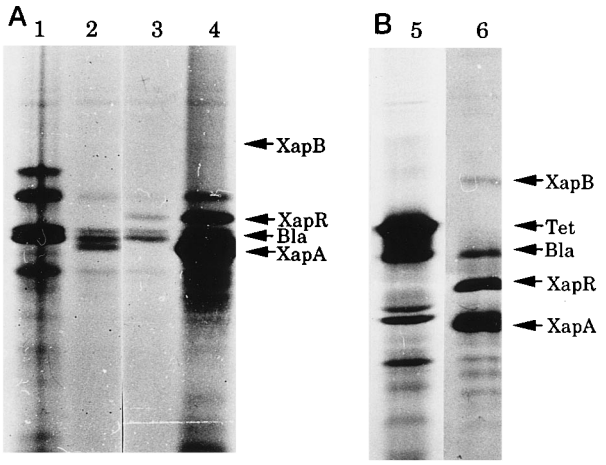


FIG. 4. Identification and cellular locations of the *xapA*, *-B*, and *-R* gene products. (A) Total protein; (B) membrane fraction. Autoradiograms of SDS–12.5% polyacrylamide gels with [^{35}S]methionine-labelled extracts of minicells containing plasmids pBR322 (lanes 1 and 5), pCS59 ($xapA^+B^+R^+$) (lanes 4 and 6), pCS60 ($xapA^-B^-R^+$) (lane 3), and pCS64 ($xapA^+B^+R^-$ (lane 2) are shown.

In order to map the 5′ ends of the *xapR* transcripts, we used pCP10, which contains the *xapR* promoter region inserted in the *galK* vector pGD56 upstream of the promoterless *galK* gene. In pCP10, the pGD56 primer maps 73 nucleotides downstream of the *xapR* ATG codon. From the autoradiogram shown in Fig. 5B, two transcripts were seen (lanes 1 and 2). A strong start site maps 28 nucleotides upstream of the ATG codon ($xapRp_1$), while a much weaker start site maps 86 nucleotides upstream of ATG ($xapRp_2$). The two transcripts had similar relative intensities when SØ6444(pCP10) was grown in the absence (Fig. 5B, lane 1) and in the presence (lane 2) of xanthosine. In order to verify that both transcripts originate from the plasmid-encoded *xapR* gene, we made a 4-bp insertion in the beginning of the *xapR* gene by filling in the *Bgl*II site (pCP19). When RNA was isolated from SØ6444(pCP19) grown with or without xanthosine, both transcripts were shifted by 4 bp (Fig. 5B, lanes 3 and 4) as expected. The −10 region of $xapRp_1$ (TGATAATTT) shows good homology to the extended consensus promoter, where the TGn motif is expected to make the promoter less dependent on the presence of a −35 region (18, 33), while the −35 region (TGGTGA) is identical to the consensus sequence in 3 of 6 nucleotides. The weak promoter ($xapRp_2$) (−35, TGTACC; −10, TATTAT) also shows reasonable homology to the consensus sequence.

Construction of translational fusions to *lacZ*. To study the regulation of the *xapABR* genes and to show that *xapA* and *xapB* form an operon, we constructed translational fusions between the *xap* genes and *lacZ* as outlined in Fig. 6. The fusions were constructed in pRAK plasmids (19) and were consequently transferred to the single-copy plasmid pOU250. The different fusions cannot be compared with respect to promoter strength because they have different translational start sites. Expression of the *xapA-lacZ* fusion in SØ6436(pGD111) strictly depended on the presence of xanthosine in the growth

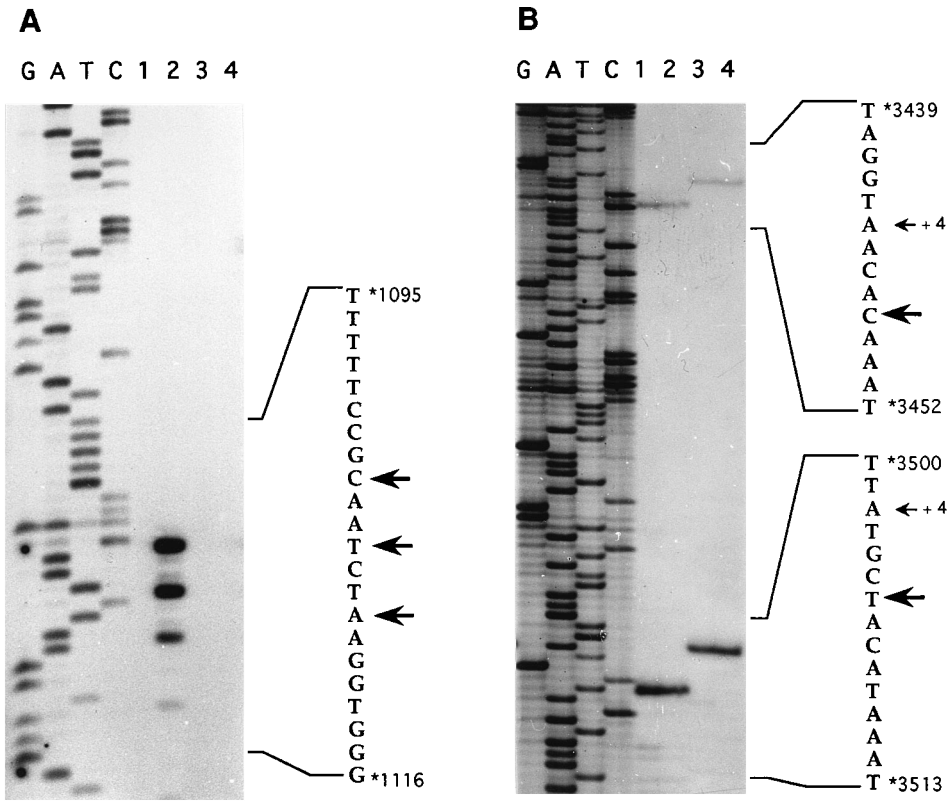
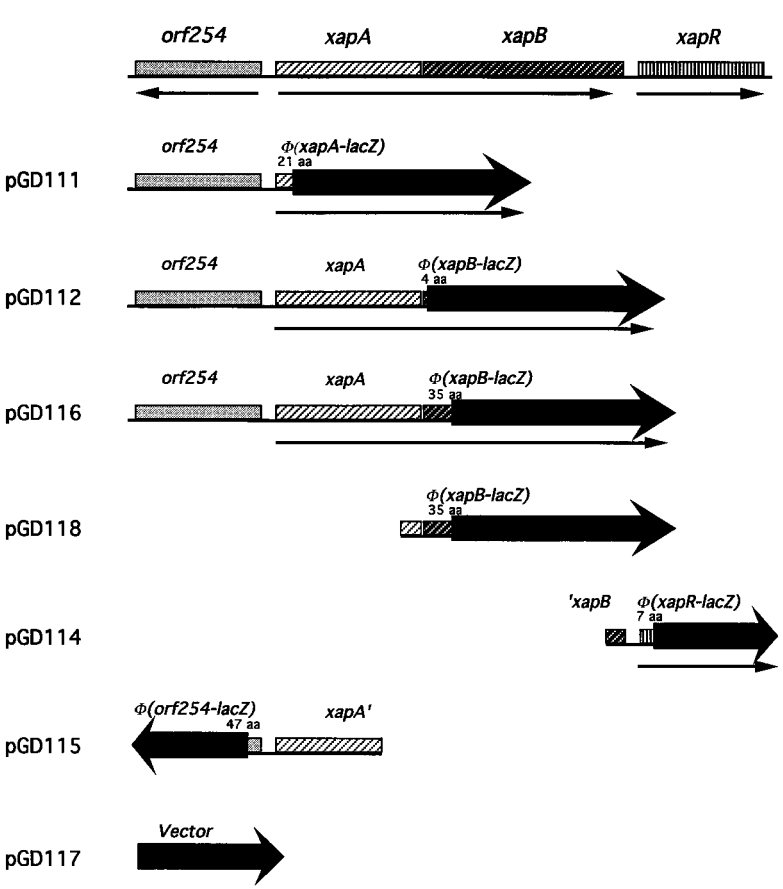


FIG. 5. Primer extension mapping of the *xapAp* (A) and $xapRp_1$ and $xapRp_2$ (B) promoters. (A) Lane 1, SØ6436(pGD111) without xanthosine (XR); lane 2, SØ6436(pGD111) with XR; lane 3, SØ6444(pGD111) without XR; lane 4, SØ6444(pGD111) with XR. The DNA sequence was produced with the same primer on pCS59 DNA (the noncoding strand is shown). (B) Lane 1, SØ6444(pCP10) without XR; lane 2, SØ6444(pCP10) with XR; lane 3, SØ6444(pCP19) without XR; lane 4, SØ6444(pCP19) with XR. The DNA sequence was produced with the pGD56 primer on pCP10 DNA. The arrows indicate the transcription start sites. Numbers indicate nucleotide positions.



ß-galactosidase(units/mg protein)

	SØ6436 wt		SØ6444 xapR::kan		SØ6447 Δ(xapABR)::kan	
	+ XR	-	+ XR	-	+ XR	-
pGD111	11890	2.5	12	4.2	2.4	2.0
pGD112	30	0.8	0.4	0.5	0.7	0.5
pGD116	124	0.6	0.4	0.4	0.8	0.6
pGD118	0.6	1.3	0.5	0.4	0.6	0.6
pGD114	1680	1496	1680	1504	1751	1323
pGD115	3.5	1.0	1.4	1.6	1.6	1.2
pGD117	0.5	0.7	0.5	0.6	0.4	0.4

FIG. 6. Structures and specific activities of various translational *lacZ* fusions. The number of amino acids (aa) in the N-terminal portion is indicated over the fusion point. SØ6436, SØ6444, and SØ6447 containing the different *lacZ* fusions on low-copy-number plasmids were grown exponentially in AB glucose minimal medium at 32°C in the absence (−XR) or in the presence (+XR) of 1 mg of xanthosine per ml. The values are averages from two to four independent experiments.

medium, with a fold induction of almost 5,000 (11,890 versus 2.5 U/mg). This expression also depended on the presence of the XapR protein, since only a two- to threefold induction took place in SØ6444(pGD111) and no induction was found in SØ6447(pGD111). Expression of the *xapB-lacZ* fusion also strictly depended on the presence of both xanthosine and XapR (pGD112 and pGD116). In SØ6436(pGD112), the β-galactosidase activity was induced from 0.8 to 30 U/mg of protein, while that in SØ6436(pGD116) was induced from 0.6 to 124 U/mg of protein. With pGD118, in which the *xapA* promoter was deleted (from pGD116), expression of the *xapB-lacZ* fusion no longer took place, indicating that *xapA* and *xapB* were both transcribed from the *xapA* promoter and form an operon. In contrast to the case for *xapA* and *xapB*, the expression of the *xapR-lacZ* fusion (pGD114) was not dependent on either xanthosine or XapR protein, since the same level of β-galactosidase activity (ranging from 1,323 to 1,751 U/mg of protein) was found in all three strains. These results agree well with those of the primer extension experiments, in which *xapA* promoter transcripts were seen only in the presence of both XapR protein and xanthosine while *xapR* promoter transcripts were seen in the absence of XapR protein and xanthosine. We have also constructed a fusion between ORF254 and *lacZ* to see if expression of ORF254 was somehow associated with the expression of the *xap* genes. The β-galactosidase activity in SØ6436 (pGD115) was induced three- to fourfold by xanthosine, but the level was only slightly above the basal level of the vector construction (pGD117) and probably was not important for the expression of the *xap* genes.

DISCUSSION

We have identified three genes involved in the metabolism of xanthosine and have visualized their gene products in minicells. Two of these genes, *xapA* and *xapR*, have previously been characterized genetically (8), and xanthosine phosphorylase (XapA) has been purified and characterized (12, 24). The finding of the *xapB* gene, however, was quite unexpected. When the amino acid sequences of the four reading frames in pGD216 were searched for in the EMBL database, all except that of ORF254 showed sequence homology to known proteins. XapA shows strong similarity to the human purine nucleoside phosphorylase, while there is very little similarity to the *E. coli* purine nucleoside phosphorylase encoded by *deoD*. This is in agreement with earlier results showing that the substrate specificity of XapA resembles that of the mammalian enzyme rather than that of the *E. coli* purine nucleoside phosphorylase (12). XapB shows strong similarity to the nucleoside transport protein NupG (see below), and XapR shows homology to the other members of the LysR family of transcriptional activators, as already described by Brun et al. (6).

Several *xap* mutants have previously been characterized, but these were mainly regulatory *xapR* mutants that were affected in inducer binding so that other nucleosides could substitute

for xanthosine (7, 20, 21). We therefore mutated each of the *xap* genes (Table 3). Both *xapA* and *xapR* are required for expression of the *xapA* gene, and expression takes place only when the cells are grown in the presence of xanthosine. When *xapB* is mutated, *xapA* is only partially expressed, suggesting that XapB is involved in the expression of *xapA* (see below). In SØ6447, in which all three genes are mutated, xanthosine phosphorylase activity can be restored only if both *xapA* and *xapR* are supplied in *trans*. It is interesting that a high level of expression can be achieved in SØ6447(pCS63, pGD225) [Δ(*xapABR*) *xapA*$^+$ *xapR*$^+$]. In this strain there is no XapB protein, and one would expect XapB to be a limiting factor for XapA expression when the levels of expression in SØ6436 and SØ6686 are compared. In SØ6436(pCS63, pGD225) (wild type; *xapA*$^+$ *xapR*$^+$), in which the wild-type *xapB* gene is present on the chromosome, a level of expression fivefold higher than that in SØ6447(pCS63, pGD225) is found, indicating that XapB is important. In SØ6447(pCS14, pCS63), in which XapB is expressed on pCS14, one would expect a higher level of expression than in SØ6447(pCS63, pGD225) or SØ6436(pCS63, pGD225). The relatively low level of XapA expression in SØ6447 (pCS14, pCS63) is probably a consequence of the growth inhibition and the very low plasmid copy number of pCS14. A likely explanation could be that overexpression of XapB protein becomes toxic to the cell. Such an observation is often made when membrane proteins are overexpressed, and a similar toxicity was found with pCC1 and pGD216 and with other multicopy plasmids that express *xapB* (Table 2). When *xapR* is overexpressed in wild-type cells, the XapA activity increases eightfold, indicating that the concentration of XapR is not sufficient for full induction of *xapA*. A fourfold decrease in the XapA activity caused by the presence of *xapA* in multicopy is more difficult to explain. However, if full induction of *xapA* requires the binding of two or more XapR molecules, then a high concentration of *xapA* genes could titrate XapR.

In the absence of xanthosine, transcription of the *xapA* promoter is shut off, as no transcription was detectable. Transcription was observed only in the presence of both xanthosine and XapR. This is evident from the hardly detectable xanthosine phosphorylase activity in wild-type cells (SØ6436) grown in the absence of xanthosine (Table 3), from the β-galactosidase activity of the *xapA*-lacZ fusion (which is induced almost 5,000-fold by xanthosine [from 2.5 to 11,890 U/mg of protein] [Fig. 6]), and from the primer extension mapping (Fig. 5A). This tightness of the *xapAB* operon compared with other systems involved in nucleoside catabolism is unusual. Often, a low basal level of enzyme activity is maintained to ensure that nucleosides can be taken up and metabolized. This utilization is important for the formation or accumulation of the inducers which then turn on the transcription apparatus. The *xapA-lacZ* fusion and primer extension data indicate that the *xapA* promoter is a strong promoter. The −10 region (TATAAA) is close to the consensus sequence, but there is no good −35 region. The most obvious −35 region (TGGTAA) is 18 bp upstream of the −10 region. The absence of a good −35 region is often seen for promoters that require an activator protein. The DNA upstream of XapA is very AT rich, and a Shine-Dalgarno sequence (AAGGA) with homology to the 16S RNA is upstream of the ATG codon of XapA.

Only 18 bp separates *xapA* and *xapB*; this 18 bp includes a Shine-Dalgarno sequence (AAGGA) upstream of the ATG codon of *xapB*, while no obvious promoter sequences are found (Fig. 2). The *xapB* gene seems to be transcribed only from the *xapA* promoter and thus follows the same tight induction pattern as *xapA* (Fig. 6). It is not possible to directly compare different protein fusions because of differences in both mRNA translatability and the stabilities of mRNAs and fusion proteins. It is remarkable, however, that the induction ratios for cotranscribed genes are so different.

The *xapR* gene was expressed constitutively from two promoters. In the primer extension experiment, both promoters were active in the absence of XapR and xanthosine (Fig. 5B), and the *xapR-lacZ* fusion gave the same level of β-galactosidase activity in the presence or absence of xanthosine in the wild type and in SØ6444 or SØ6447, in which no XapR protein is synthesized. One of the common features in the LysR family is that the regulatory protein activates the transcription of a neighboring divergently oriented gene, while it represses the transcription of its own gene. In the *xap* region, *xapA* and *xapR* are transcribed in the same orientation, and there is no indication of any regulation of *xapR* transcription. We cannot offer any explanation for why *xapR* is transcribed from two promoters. We cannot exclude the possibility that *xapR* is also transcribed from the *xapA* promoter. However, a stem-loop structure followed by T residues is found immediately after the stop codon of the *xapB* gene, which is likely to encode a rho-independent transcription terminator.

The region upstream of the ATG codon of the *xapA* gene contains several sequences resembling the consensus sequence (ATATTGTTT) proposed by Bohannon and Sonenshein (4) to be involved in the binding of LysR family proteins. At 120 bp upstream of the transcription start, the sequence CCAAT ACAGTTTT is found (double underlined in Fig. 2). At 80 bp downstream of this sequence and overlapping the −35 region, the inverted complementary sequence (AAAACTGTATTGG) is found. These sites are centered at positions −40 and −120 relative to the transcription start site of *xapAp* and are good candidates for the target of the XapR protein. The identification of the XapR binding sites is currently under investigation, but this has proved to be difficult because the XapR protein aggregates when it is overproduced. It has not yet been possible to purify enough soluble XapR protein for in vitro studies.

Is XapB a transport protein? *xapB* encodes a polypeptide that has a high degree of hydrophobicity (58% hydrophobic amino acids) and is homologous to the *E. coli* nucleoside transport protein NupG. The hydropathy profile of the XapB amino acid sequence reveals that the hydrophobicity is located in 12 hydrophobic segments, presumptive α-helices each of which is able to traverse the cytoplasmic membrane once (data not shown). The finding of 12 putative membrane-spanning segments in XapB is in accordance with a model for many membrane proteins (29). In minicells, XapB is enriched in the membrane fraction, although the molecular mass is significantly lower than that calculated from the amino acid sequence of the polypeptide (45,705 Da) (Fig. 4B). However, integral membrane proteins tend to migrate faster in SDS-polyacrylamide gel electrophoresis than expected from their molecular mass (15). The use of a more concentrated SDS-polyacrylamide gel (20%) changed the migration rate of the *xapB* band considerably, to an apparent molecular mass of 41 kDa (data not shown). A similar variation in the molecular mass was found with the NupG protein (42).

Earlier studies have shown that induction of xanthosine phosphorylase requires the presence of one of the nucleoside transport proteins NupC and NupG (12). Mutants defective in either *nupC* or *nupG* grow normally on xanthosine as the sole carbon source, but a *nupC nupG* double mutant cannot grow. When Hammer-Jespersen et al. (12) studied the kinetics of xanthosine induction, they found that induction of xanthosine phosphorylase increased with time and full induction occurred after three generations. They suggested that another transport system had to be expressed and incorporated into the cell

membrane before xanthosine could fully induce *xapA* expression. This gene could be *xapB*. In SØ6686, in which the *xapB* gene is deleted, the induction of *xapA* expression is only 10% of the induction in the wild type (SØ6436) (Table 3), and the *xapB* mutant hardly grows on xanthosine as a carbon source (Table 2). The requirement for either NupC or NupG indicates that the basal level of XapB in the absence of xanthosine is too low for xanthosine to be taken up when added to the growth medium. On the other hand, the requirement for XapB for full induction indicates that NupC and NupG cannot transport xanthosine very efficiently but can do so just enough to weakly induce the *xapA* promoter, resulting in low levels of XapA and XapB. This low level of XapB could be sufficient to transport xanthosine and cause a full induction of *xapA* and *xapB*. On the basis of these results, we propose that XapB is a xanthosine transport protein.

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