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A Second Purine Nucleoside Phosphorylase in *Escherichia coli* K-12

I. Xanthosine Phosphorylase Regulatory Mutants Isolated as Secondary-Site Revertants of a *deoD* Mutant

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Summary. Enzyme purification studies have indicated the existence in *E. coli* of only a single species of purine nucleoside phosphorylase. Strains mutated in the gene (*deoD*) specifying this enzyme cannot grow on purine nucleosides such as inosine, adenosine and guanosine as carbon source. We have selected secondary-site revertants of a *deoD* strain by plating on adenosine or inosine as carbon source and we have shown that the site of the mutation enabling growth on adenosine or inosine in these revertants, termed *xapR*, lies between *nupC* and *ptsI* at 51 min, almost exactly 180° from the *deoD* gene on the *E. coli* chromosome. In some *xapR* mutants there is a constitutive synthesis of a second purine nucleoside phosphorylase; in other *xapR* mutants, this enzyme is induced by inosine. The properties of this enzyme in the *xapR* mutants are very similar to that of xanthosine phosphorylase found in wild-type cells induced with xanthosine, and we thus consider that *xapR* mutants are altered in the regulation of xanthosine phosphorylase.

From an *xapR* strain mutants were isolated which lacked this second purine nucleoside phosphorylase. The site of this mutation, *xap*, was 90% co-transducible with *xapR*. Such strains could not grow on xanthosine as sole carbon source. The rate of mutation to $xapR^-$ was very low (3×10^{-8}). Also studies with an F-prime covering the $xapR^+$ gene revealed that *xapR2* was partially dominant to $xapR^+$. We therefore suggest that the $xapR^+$ gene product is an inducer protein for the gene specifying xanthosine phosphorylase, which is inactive until converted by the inducer into a form able to switch on the operon, i.e. there is positive control of the *xap* gene.

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Introduction

The isolation of secondary-site revertants can be a powerful method for revealing the existence of previously undiscovered enzymes which in wild-type strains are inducible by unknown compounds, or whose induction requires special conditions. Thus such revertants should often occur in regulatory genes, resulting in a constitutive phenotype (for reviews see Clarke, 1974; Riley and Anilionis, 1978). In this paper we describe the isolation of secondary-site revertants of a *deoD* mutant deficient in purine nucleoside phosphorylase, which have revealed the existence of a second, quite distinct, enzyme with a different specificity. In the accompanying paper (Hammer-Jespersen, Buxton and Hansen, 1980) we describe the properties of this enzyme and its induction in the wild-type strain.

Purine nucleoside phosphorylase (purine nucleoside: orthophosphate ribosyl-transferase, EC 2.4.2.1) catalyses the reaction:

purine-(deoxy)nucleoside + phosphate $\rightleftharpoons$ purine + (deoxy)ribose-1-phosphate.

Mutants blocked in the *deoD* gene, mapping at 0 minutes, lack any detectable purine nucleoside phosphorylase activity (Ahmad, Barth and Pritchard, 1968; Karlström, 1968) and cannot grow on the nucleosides adenosine, inosine or guanosine, or their respective deoxyribonucleosides. In *Bacillus subtilis*, two such enzymes with differing specificities have been found (Senesi et al., 1976; Jensen, 1978). Enzyme purification experiments with *Escherichia coli* however, have revealed only the existence of one enzyme (Jensen and Nygaard, 1975). In an attempt to determine if there is a second purine nucleoside phosphorylase in *E. coli* we have selected for revertants of a *deoD* mutant and we show that these strains have mutations in a gene involved in the regulation of such an enzyme. The particular *deoD* mutation chosen

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was caused by the insertion of phage lambda (Buxton et al., 1978). The mutation is therefore non-leaky and does not revert easily, and moreover the presence of phage lambda is an independent test of *deoD* dysfunction. The strain was thus an ideal candidate in which to isolate the secondary-site revertants.

Materials and Methods

Bacterial Strains. The bacterial strains, all derivatives of *E. coli* K-12, are listed in Table 1.

Media. Complex media (L-broth) and minimal media (M9 salts) have been described previously (Buxton et al., 1978).

Genetic Crosses. Transduction was performed with phage *Plclm clr100* (Rosner, 1972) and bacterial matings were carried out as described by Miller (1972).

Introduction of the recA56 Marker. A Plclm clr lysate prepared on strain 6659 (*slr1300* (::Tn10) *recA56*) was used to transduce recipient strains to tetracycline-resistance (25 μg tetracycline hydrochloride; Sigma Chemical Co., Poole, Dorset) on LB plates (see Kleckner et al., 1977). These transductants were tested for co-inheritance of *recA56* by screening for those sensitive to ultraviolet light. Transductants were cured of *Plclm clr* by plating at 42° C.

Growth of Phage Mucts62. The growth of this phage and its use in mutagenesis, have been described previously (Buxton, 1975).

Enzyme Assays. Cells were grown, extracts prepared and xanthosine phosphorylase was assayed as described in the accompanying paper (Hammer-Jespersen, Buxton and Hansen, 1980). Assay B was used when deoxynucleosides were used as substrates, assay A was used when inosine or xanthosine were substrates.

Results

Isolation and Characterisation of Purine Ribonucleoside Catabolising Revertants of a deoD Strain

Strain RB400 is a *deoD* mutant isolated after lysogenisation of a *Δattλ* strain with the heat-inducible, lysis defective lambda phage, *λcI857S7*. It lacks any detectable purine nucleoside phosphorylase activity (Buxton et al., 1978). It cannot grow on adenosine or inosine as sole carbon source, but can grow with thymidine as carbon source, indicating that the other *deo* genes are intact. The *deoD* mutation was caused by the insertion of the lambda phage since heat-pulse cured cells regained the ability to grow on adenosine, and there was only one site of lambda insertion on the chromosome, because phage P1 transductants, selected on inosine, became sensitive to *λb2c*. Experiments have shown that *deoD* is the last of the four *deo* genes to be transcribed (Baumanis et al., 1974; Buxton, 1975) and hence insertion of lambda into *deoD* would not be expected to block transcription of the other *deo* genes.

The secondary-site revertants, which had regained the ability to utilise purine nucleoside as the sole source of carbon, were selected by plating thick LB cultures of strain RB400, resuspended in phosphate buffer, onto minimal plates containing adenosine or inosine (1 mg/ml) as carbon source, at 32° C. The frequency of colonies on such plates was 3.3×10^{-8} (calculated from ten independent cultures, plated on adenosine). All the mutants isolated were still heat-sensitive and were therefore still lysogenised by phage *λcI857S7*. To test that lambda was still inserted in *deoD*, heat-resistant derivatives of two of the purine ribonucleoside catabolising revertants, RB450 and RB451, were isolated and tested for the loss of genetic markers adjacent to *deoD*. Some of these were Ser^- Thr^- and were thus probably deletions removing *serB* and *thr*, linked to *deoD*. These Ser^- Thr^- derivatives could still grow on inosine (IR^+) providing they could grow on thymidine (TdR^+). In contrast Ser^- TdR^- heat-resistant derivatives, in which *deoB* was deleted, were IR^-. This seemed to indicate that the purine ribonucleoside catabolising revertants were degrading these compounds via a phosphorolytic rather than hydrolytic reaction, since the *deoB* gene product, deoxyribomutase, was required for growth on inosine.

The purine ribonucleoside catabolising revertants could be divided into three classes based on their growth on nucleosides and deoxynucleosides (Table 2). Class I mutants grew well on all purine nucleosides tested viz., adenosine, deoxyadenosine, guanosine and deoxyguanosine (RB451, 452, 454, 457 and Sø1125). Class II mutants grew on adenosine, deoxyadenosine and inosine but failed to grow on guanosine or deoxyguanosine (RB450, 453 and 455). Class III mutants (RB456 and Sø1129) grew very poorly on adenosine, although they were actually selected on adenosine, but grew well on the other compounds, including deoxyadenosine. (The class III mutants probably grew initially on adenosine plates since enough adenosine deaminase would be present when large numbers of cells are plated, so that inosine would be formed). All the mutants grew reasonably well on uridine (RB453 and RB456 were somewhat slower), and all grew equally well on xanthosine.

Mapping of the xapR Mutation

The genetic site conferring ability of the *deoD* (::λ) mutant to grow on purine nucleosides is called *xapR* (for reasons which will be made clear later). Thus

Table 1. *E. coli* K-12 strains

Strain	Mating type	Relevant genotype	Origin/Reference
RB400	HfrH	*thi tyrT*$^+$ *Δ(gal-attλ-bio-uvrB-deoR) upp deoD* (::*λcI857S7*)	Buxton et al., 1978
NF709	F$^-$	*thi leu Δdeo-11 rpsL*	N. Fiil; Jørgensen et al., 1977
AB1157	F$^-$	*thi thr leu proA his argE rpsL*	B.M. Wilkins
Sø1161	F$^-$	*thi leu proA his argE deoD* (::*λS7*) *rpsL*	RB400 (*λcI*$^+$) × AB1157 → Thr$^+$Str^R
ctr-7(=CGSC 4801)	HfrKL16	*thi ptsI7*	B.J. Bachman; Morse et al., 1971
AT978	HfrKL16	*dapE9*	A.L. Taylor; Bukhari and Taylor, 1971
RB685	F$^-$	*thi leu proA argE deoD* (::*λS7*) *rpsL ptsI*	*ctr.7* × Sø1180 → His$^+$ Str^R
RB688	F$^-$	*thi leu proA argE deoD* (::*λS7*) *rpsL ptsIxapR2* (P1*clm clr*)	P1.Sø1180 × RB685 → IR$^+$
RB693	F$^-$	As RB688, P1$^-$	Heat^R derivative of RB688
RB689	F$^-$	*thi leu proA argE deoD* (::*λS7*) *rpsL dapE9*	*AT978* × Sø1180 → His$^+$Str^R
B189	HfrKL16	*thi nupC*	B. Mygind
RB701	F$^-$	*thi leu proA argE deoD* (::*λS7*) *nupC rpsL*	P1.B189 × RB693 → Mtl$^+$
F198/FF7040	F198	F198 *supN*$^+$ *nadB*$^+$ *ptsI*$^+$/*proC43 ptsI40 recA1 rpsL150*	W. Epstein (via B.J. Bachmann)
RB686	F$^-$	*thi leu proA argE deoD* (::*λ*) *xapR2 rpsL dapE9*	AT978 × Sø1180 → His$^+$Str^R
E11002	F′*lac*	F′*lacYA694*/*lacYA694 rpsE*	J. Scaife (via W.J. Brammar)
RB710	F$^-$	*thi leu proA argE deoD* (::*λ*) *xapR2 rpsE dapE9*	P1. E11002 × RB686 → Spc^R
6659	Hfr	*slr1300* (::Tn10) *recA56*	D. Botstein
RB717	F$^-$	As RB710, *slr1300* (::Tn10) *recA56*	P1. 6659 × RB710 → Tet^R
Sø1180	F$^-$	*thi leu proA argE his ara deoD* (::*λS7*) *upp xapR2 rpsL*	Sø1161 × RB451 → IR$^+$

Table 2. Growth patterns of purine ribonucleoside catabolising revertants of RB400 (*deoD*) on nucleosides and deoxynucleosides

Strain	Selected on	*xapR* allele	Growth on:							
			glucose	adenosine	deoxy-adenosine	inosine	guanosine	deoxy-guanosine	uridine	xanthosine
RB400		*xapR*$^+$	++++	−	−	−	−	−	++	++++
RB450	AR	*xapR1*	++++	+++	++++	++++	−	−	++	++++
RB451	AR	*xapR2*	++++	++++	++++	++++	++++	++++	++	++++
RB452	AR	*xapR3*	++++	++++	++++	++++	++++	++++	++	++++
RB453	AR	*xapR4*	++++	+++	++++	++++	−	−	+	++++
RB454	AR	*xapR5*	++++	++++	++++	++++	++++	++++	++	++++
RB455	AR	*xapR6*	++++	+++	++++	++++	−	−	++	++++
RB456	AR	*xapR7*	++++	(+)	++++	++	+++	+++	+	++++
RB457	AR	*xapR8*	++++	++++	++++	++++	++++	++++	++	++++
Sø1125	IR	*xapR9*	++++	++++	++++	++++	++++	++++	++	++++
Sø1129	AR	*xapR10*	++++	(+)	++++	+++	+++	+++	++	++++

The bacteria were suspended in buffer and streaked out on minimal agar plates containing the carbon sources indicated (glucose at 2 mg/ml; others at 1 mg/ml) and incubated at 32° C. − =no growth, ++++=good growth after two days

deoD xapR$^+$ strains cannot grow on inosine (IR$^-$) whereas *deoD xapR* mutants can do so.

The strain RB400 and its derivatives had apparently changed from an HfrH to become an F-prime. To expediate the mapping of *xapR* therefore, an F$^-$ strain carrying *deoD* (::*λ*) was constructed by transferring *deoD* (::*λcI*$^+$*S7*) (a heat-resistant revertant of *deoD* (::*λcI857S7*)) into strain AB1157 by conjugation (see Table 1). The resulting strain lost the F-prime, and was designated Sø1161. This was mated with strain RB451 and the *xapR2* allele transferred to give strain Sø1180. Using strain Sø1180 as recipient, it was shown that *xapR*$^+$ was transferred early by HfrKL16, the order being *upp-xapR* → KL16, and using strain *ctr-7* (Hfr KL16 *ptsI*), very close linkage of *xapR* to *ptsI* was indicated.

ptsI is closely linked to *dapE* (see Bachmann, Low and Taylor, 1976) and therefore transducing phage

Table 3. Three-point crosses to ascertain the position of *xapR* relative to *dapE*, *ptsI* and *nupC*[a]

Cross	Donor	Recipient	Selected marker	Unselected markers				Indicated order
1.	RB688 (*ptsI xapR2*)	RB689 (*dapE*)	$dapE^+$		*xapR2*	$xapR^+$		$xapR^-$ $ptsI^-$ $dapE^+$
				ptsI	4	5	D	+ + −
				$ptsI^+$	0	124	R	
2.	B189 (*nupC*)	RB693 (*ptsI xapR2*)	$ptsI^+$		*xapR2*	$xapR^+$		$nupC^-$ $xapR^+$ $ptsI^+$
				nupC	1	71	D	+ − −
				$nupC^+$	25	18	R	
3.	RB688 (*ptsI xapR2*)	RB701 (*nupC*)	*xapR2*		*ptsI*	$ptsI^+$		$nupC^+$ $xapR^-$ $ptsI^-$
				nupC	21	11	D	− + +
				$nupC^+$	80	13	R	
4.	Sø1180 (*xapR2*)	RB685 (*ptsI*)	*xapR2*		*ptsI*	$ptsI^+$		
					25	62		
5.	B189 (*nupC*)	RB685 (*ptsI*)	$ptsI^+$		*nupC*	$nupC^+$		
					69	31		

[a] P1*clm clr* was grown on the donor strains indicated and the resulting lysates were used to transduce the recipient strains. Cross 1 was performed on minimal medium containing glucose-6-phosphate as carbon source (sodium salt, 1.5 g/litre), and lacking diaminopimelic acid; $ptsI^+$ and *ptsI* strains grow equally well on this medium (Epstein, Jewett and Fox, 1970). Selection for $ptsI^+$ (cross 2) was performed with mannitol as carbon source (Epstein et al., 1970), and selection for *xapR* (cross 3) was with inosine as carbon source. The unselected markers were tested as follows: *xapR2*, growth on inosine; *nupC*, growth on 0.16 mM showdomycin (Komatsu and Tanaka, 1972; B. Mygind, personal communication); *ptsI*, no growth with mannitol as carbon source. All the recipients are *deoD* (::λ*S7*)

P1*clm clr* grown on Sø1180 was used to transduce RB689 (*dapE deoD* (::λ)) to Dap^+ (Table 3). From the least frequent recombinant class of this cross, the order was indicated as *xapR-ptsI-dapE*, and similar three-point crosses involving *ptsI* and *nupC* (Table 3) indicated the order to be *nupC-xapR-ptsI*. The genetic map obtained, together with linkage values, is given in Fig. 1.

To test whether the *xapR* mutation in the other mutants was located in a similar place to *xapR2*, phage P1 lysates were prepared on them and used to transduce RB685 (*deoD* (::λ*S7*) *ptsI*) to $PtsI^+$, and these were tested for co-inheritance of the IR^+ property. Twelve such recombinants from each cross were tested, and co-inheritance of IR^+ ranged from 7/12 to 11/12. Thus all the other *xapR* mutations were linked to *ptsI*.

As a control, it was thought desirable to eliminate the possibility that the particular *deoD* (::λ) mutation was in some way affecting the expression of the *xapR* mutations. Sø1180 (F^- *deoD* (::λ) *ara leu rpsL xapR2*) was therefore mated with RB391 (HfrH *deoD391*), selection being made for Ara^+ Leu^+ Str^R recombinants, which were tested for inheritance of *deoD391* (sensitivity to λ*b2c*) and also their growth pattern on inosine and adenosine was determined. No difference could be seen between *deoD* (::λ*S7*) and *deoD391* recombinants, thus confirming that the particular *deoD* allele did not affect the *xapR* phenotype.

Enzyme Levels in xapR Mutants

A representative of each of the three classes of *xapR* mutants was grown on different carbon sources and the deoxyguanosine splitting activity was measured (Table 5). A class I mutant, RB451 showed high levels on all carbon sources, with a variation from 500 to 2,400 units/mg. Another class I mutant Sø1125 gave the same result (data not shown). RB453, a class II mutant, had low levels on all carbon sources except on most purine nucleosides. It seemed to contain a purine nucleoside phosphorylase inducible by all purine nucleosides except guanosine and deoxyguanosine. RB455, also a class II mutant, gave the same result (data not shown). Sø1129, a class III mutant, had high levels on acetate and on most purine nucleosides as carbon source, and showed intermediate enzyme activity on ribose and glycerol; the enzyme levels seemed however, to be very sensitive to the addition of glycerol (*cf.* guanosine with glycerol+guanosine). In the glycerol-overnight cultures we have found levels ranging from 30 to 500 units/mg; therefore the results of two independent experiments a and b are

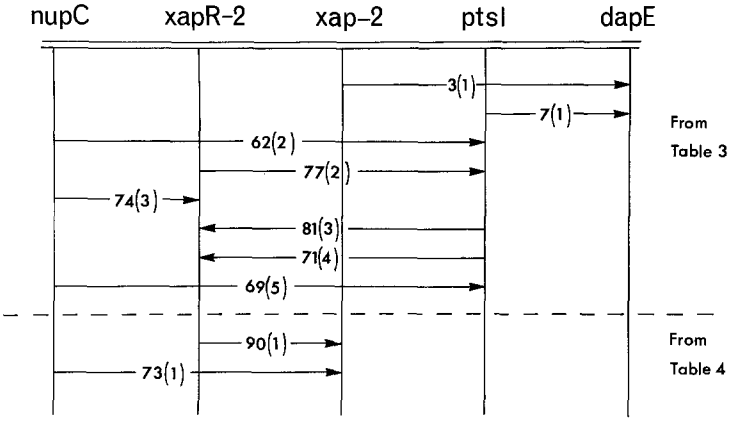

Fig. 1. Linkage map obtained from experimental results. Arrowheads point in the direction of selected markers. The numbers in brackets after each of the co-transduction percentages refer to the cross number in the appropriate Table from which the percentages were calculated

Table 4. Three-point crosses to determine the position of *xap* relative to *xapR* and *nupC*[a]

Cross	Donor	Recipient	Selected marker	Unselected markers				Indicated order
1.	B189 (*nupC*)	RB719 (*xap-2 xapR2*)	*xap*$^+$		*xapR*	*xapR*$^+$		*nupC*$^-$ *xapR*$^+$ *xap*$^+$
				nupC	1	122	D	+ + +
				nupC$^+$	15	30	R	+ − −
2.	B189	RB718 (*xap-1 xapR2*)	*xap*$^+$		*xapR*	*xapR*$^+$		
				nupC	0	21		
				nupC$^+$	2	7		
3.	B189	RB720 (*xap-3 xapR2*)	*xap*$^+$		*xapR*	*xapR*$^+$		
				nupC	0	20		
				nupC$^+$	4	6		

[a] P1*clm clr* grown on the donor strain B189 was used to transduce the XR$^-$ mutants to XR$^+$ on minimal plates with xanthosine as sole carbon source. Inheritance of the unselected *xapR* marker was tested with inosine as carbon source, *xapR* being IR$^+$, xapR$^+$ being IR$^-$. Inheritance of *nupC* was tested by resistance to showdomycin (Table 3)

From cross 1, 36 recombinants were tested for *deoD* (::λ*S7*) by checking for resistance to λ*b2*c. All were λ*b2c*^{R}. Similarly all the recombinants from cross 2 were λ*b2*c resistant

Table 5. Induction of xanthosine phosphorylase in *xapR* mutants

Carbon source	Xanthosine phosphorylase activity (deoxyguanosine units/mg protein)			
	Strain:	RB451	RB453	Sø1129
	Genotype:	*deoD xapR2*	*deoD xapR4*	*deoD xapR10*
	Phenotpye:	class I	class II	class III
				a	*b*
Glycerol		1240	4	470	37
Glycerol + Adenosine		1004	1080	310	
Glycerol + Guanosine		1147	10	155	228
Glycerol + Inosine		1128	1030	34	
Glycerol + Xanthosine		2043	3080	3280	4460
Glycerol + Uridine		549	6	46	
Deoxyadenosine		723	991		2130
Guanosine		1431			1239
Inosine		1130	1570		800
Xanthosine		1796	4271		5811
Ribose		1744	5	280	
Acetate		2463	11	2848	807

Table 6. Substrate specificity of xanthosine phosphorylase in *xapR* mutants compared with *xap* and *deoD* activities

Strain and relevant genotype	Carbon source in growth media	Activity relative to deoxyinosine (%)				
		Deoxyinosine[a, c]	Inosine[a]	Xanthosine[a]	Deoxyguanosine[b]	Deoxyadenosine[b]
RB451 (*deoD xapR2*) (class I)	acetate	100 (4960)	79	31	72	1
RB453 (*deoD xapR4*) (class II)	inosine	100 (2020)	81	29	77	1
Sø1129 (*deoD xapR10*) (class III)	acetate	100 (4000)	80	29	78	1
RB400 (*deoD xapR*$^+$)	xanthosine	100 (3450)	77	30	80	1
RB595 (*deoD*$^+$ *deoR xapR*$^+$)	glycerol	100 (4000)	45	<0.04	57	30

[a] Assayed using assay A (xanthine oxidase). 2 mM inosine was used, the other nucleosides were 1 mM

[b] Assayed using the thiobarbituric acid colour reaction for deoxyribose (assay B). Substrate concentrations of 1 mM were used; activity was expressed relative to deoxyinosine activity

[c] Numbers in parenthesis give units/mg protein

given in Table 5. Such a variation in enzyme level might indicate that the level of the new purine nucleoside phosphorylase in this strain is dependent upon the metabolic state of the cell. RB456, the other class III mutant behaved similarly to Sø1129 (data not shown).

Representatives of each of the three classes of *xapR* mutants were grown on a carbon source giving high levels of xanthosine phosphorylase. Xanthosine was avoided because it is inducer of xanthosine phosphorylase in *xapR*$^+$ strains. Such a strain served as control and also a *deoR deoD*$^+$ strain having high levels of the normal purine nucleoside phosphorylase was included. Extracts were prepared and the substrate specificity of the different purine nucleoside phosphorylase activities was measured in the extracts (Table 6). The substrate specificity was found to be the same for all the *xapR* mutants and the xanthosine induced *xapR*$^+$ (RB400), but clearly different from the *deoD*$^+$ encoded enzyme.

The difference in nucleoside growth pattern exhibited by the different *xapR* mutants thus does not seem to reflect the presence of different purine nucleoside phosphorylases in these strains but rather different mutations involved in the regulation of xanthosine phosphorylase expression, hence the name *xapR*. Class I mutants express xanthosine phosphorylase constitutively on all carbon sources and they are therefore able to grow on all purine nucleosides as carbon source, including adenosine and deoxyadenosine because adenosine deaminase will provide inosine and deoxyinosine which are substrates for the xanthosine phosphorylase. Class II mutants presumably contain a changed *xapR* protein, so in these strains the xanthosine phosphorylase is not only inducible by xanthosine but also by inosine, deoxyinosine, adenosine and deoxyadenosine (probably only after deamination to inosine and deoxyinosine) but not by guanosine or deoxyguanosine. Group II mutants thus cannot grow on guanine nucleosides as carbon source because the xanthosine phosphorylase is not induced. Group III mutants can grow on all nucleosides except adenosine; the xanthosine phosphorylase level is mainly constitutive in these strains but may be very sensitive to catabolite repression.

Gel Electrophoretic Analysis of xapR Mutants

The crude extracts from the *xapR* mutants, strain RB400 grown with xanthosine and the *deoD*$^+$ strains which were used for the substrate specificity studies (Table 6) were also subjected to polyacrylamide gel electrophoresis. Immediately after the electrophoresis the gel was washed and incubated with arsenate and deoxyguanosine for 30 min at 37° C. Any purine nucleoside phosphorylase that degraded deoxyguanosine would then form free deoxyribose under these conditions. The deoxyribose was visualised by the colourimetric method described by Gabriel and Wang (1969). Each extract gave rise to one red band after staining. The mobility of the deoxyguanosine activity in the

xapR mutants coincided with that in the xanthosine grown RB400, but these bands clearly had a lower mobility than the *deoD*$^+$ encoded purine nucleoside phosphorylase. These results demonstrate that the xanthosine phosphorylase activity resides in a protein different from the normal purine nucleoside phosphorylase. Furthermore the results strongly support the idea that the *xapR* mutants contain the same xanthosine phosphorylase protein as the *xapR*$^+$ strains i.e. no change in the structural gene (*xap*) has occurred.

Dominance Studies

If the *xapR* gene product were negatively controlling the synthesis of xanthosine phosphorylase, it would be expected that the *xapR* alleles, at least from the constitutive mutants (class I), would be recessive to the wild-type *xapR*$^+$. In contrast, positive control by the *xapR* gene product would be expected to result in a dominance of *xapR* over *xapR*$^+$.

To try to answer this question, preliminary dominance tests have been performed with an F-prime, F198, covering the *xapR* region of the chromosome. It should be noted however, that it is an assumption that F198 carries this region, since faster growing derivates of strains carrying this F-prime often have deletions of part of the episome (B. Bachmann, personal communication). The F$^-$ strain RB686 (*dapE xapR2*), carrying an *xapR* allele typical of class I mutants, was converted to Spc^R by P1 grown on strain E11002 (*rpsE*) and then the *recA56* marker was transduced in as described in Methods. The resulting strain RB717 was mated with an F-prime strain carrying F198 (see Table 1) and selection for inheritance of the F-prime was made by isolating Dap$^+$ Spc^R recombinants. After purification by single colony isolation, these were tested for their growth on xanthosine and on inosine, in the absence of diaminopimelic acid. All the recombinants grew slowly on xanthosine, but grew as well on inosine; this suggests that *xapR2* is dominant to *xapR*$^+$, consistent with the idea that *xapR* specifies an inducer protein for the *xap* gene specifying xanthosine phosphorylase, which is inactive until converted by the inducer into a form able to switch on the operon. Acridine orange curing was performed on two such recombinants, resulting in the isolation of DAP-requiring clones; it thus seemed certain that the recombinants were genuine F-prime carrying strains.

Assays of xanthosine phosphorylase activity gave a more quantitative picture (Table 7). Whilst the F-prime *xapR*$^+$/*xapR2* heterozygotes had reasonably high constitutive levels of xanthosine phosphorylase (77 and 49 units/mg protein), these values were nevertheless considerably lower than the 607 units/mg protein of the F$^-$ *xapR2* strain. Putative F$^-$ segregants largely regained the high constitutive levels of xanthosine phosphorylase typical of *xapR2* containing strains (324 and 273 units/mg protein). These results seem to indicate a partial dominance of *xapR2* over *xapR*$^+$, but further analysis, including placing the *xapR2* allele on the episome, is required before a positive control system is established.

Table 7. Xanthosine phosphorylase activity in partial diploid strains

Strain and relevant genotype	Carbon source	Xanthosine phosphorylase activity (deoxyguanosine units/mg protein)
RB689 (F$^-$ *deoD xapR*$^+$ *recA*$^+$)	acetate	<1
RB717 (F$^-$ *deoD xapR2 recA*)	acetate	607
RB732 (F198/*deoD xapR2 recA*)	acetate	77
RB733 (F198/*deoD xapR2 recA*)	acetate	49
RB748 (F$^-$ *deoD xapR2 recA*)	acetate	324
RB749 (F$^-$ *deoD xapR2 recA*)	acetate	273

RB732 and RB733 were derived from RB717 after introducing F198 selecting for Dap$^+$ Spc^R recombinants. RB748 and RB749 are Dap$^-$ segregants (presumably F$^-$) obtained from RB732 and RB733 respectively after acridine orange curing

In the accompanying paper evidence is presented which suggests that xanthosine itself is the inducer of xanthosine phosphorylase.

Isolation, Characterisation and Mapping of xap Mutants

The *deoD* mutant RB400 was isolated (Buxton et al., 1978) as a colony of a *upp* strain resistant to a mixture of 5-fluorouracil (FU) and deoxyadenosine (AdR) (see Buxton et al., 1977 for an explanation of this selection). The *xapR* mutants were sensitive to FU + AdR; this suggested therefore that *xap* mutants, defective in the structural gene for xanthosine phosphorylase, could be selected as FU + AdR resistant colonies of a *upp xapR* strain. In fact selection was made for colonies resistant to FU (2 μg/ml) + deoxyguanosine (GdR) (150 μg/ml) since deoxyguanosine is a better substrate for xanthosine phosphorylase than deoxyadenosine (see accompanying paper). Strain Søl180 was mutagenised with Mu*cts62* and selection made for FU + GdR resistance on agar

plates with glycerol (0.2% w/v) as carbon source, at 32° C. The resulting colonies were tested for their growth on xanthosine, inosine, uridine and thymidine. Of 50 mutants (10 from each of 5 independent cultures) 3 grew very poorly on xanthosine and inosine, but as well as their parent on uridine and thymidine. The other mutants grew equally well on all four carbon sources.

Assays for xanthosine phosphorylase activity, with deoxyguanosine as substrate, were performed on crude extracts of the XR^- mutants and their parent. When grown with acetate as carbon source, Søl180 had a specific activity of 688 units/mg protein, whereas no activity was detectable in the two XR^- mutants tested, RB718 and 719.

Time of entry experiments indicated that the *xap*$^+$ gene, specifying growth on xanthosine, was transferred early by Hfr KL16, and using P1 transduction it was ascertained that the *xap-2* mutation, resulting in the failure to grow on xanthosine (XR^-), was closely linked to *nupC* (Table 4), and from the least frequent recombinant class, the order was deduced to be *nupC-xapR-xap*.

We cannot be certain that the *xap-2* mutation actually lies within the structural gene for xanthosine phosphorylase. Thus if, for example, the *xapR* gene product is actually required for *xap* gene expression, then some XR^- mutants should have lesions in *xapR*, and this could be the case for *xap-2*. If this were so, such that *xapR2* and *xapR2* were very closely linked, i.e. lying within the same gene, then nearly all recombinants inheriting *xap*$^+$ (i.e. XR^+) would also inherit *xapR*$^+$ (i.e. IR^-). In fact only 90% were IR^-. This level of co-transduction suggests, but does not prove that recombination is occurring between genes rather than by intragenic recombination, thus placing *xap-2* in a separate gene to *xapR2*.

Discussion

The secondary-site revertants of a *deoD* strain described in this paper have served to identify a gene, *xapR*, which in wild-type *xapR*$^+$ strains controls the expression of a second purine nucleoside phosphorylase, termed xanthosine phosphorylase. All the *xapR* mutations were found to be linked to *ptsI*.

In the accompanying paper the properties of this enzyme and its induction by xanthosine in wild-type strains are described. In the present paper, enzyme assays on extracts from the different *xapR* mutants show that they contain a purine nucleoside phosphorylase with the same substrate specificity as the xanthosine phosphorylase found in wild-type cells grown in the presence of xanthosine. This idea was further supported by the finding that the purine nucleoside phosphorylase present in the *xapR* mutants had the same mobility on polyacrylamide gels as the xanthosine phophorylase from wild-type cells. Thus we conclude that the *xapR* mutants are altered in the regulation of the xanthosine phophorylase.

On the basis of these findings the growth pattern of the different *xapR* mutants on nucleosides as carbon source may be explained, when combined with the measured enzyme levels (Table 5). Class I mutants seem to synthesise xanthosine phosphorylase constitutively, and are therefore able to grow on all the common purine nucleosides. In class II mutants inosine and deoxyinosine induce xanthosine phosphorylase, while guanosine and deoxyguanosine cannot do so. These mutants are therefore able to grow on all the common purine nucleosides as carbon source except the guanine nucleosides. We thus postulate that *xapR* mutants belonging to class II contain an *xapR* protein with altered effector binding site(s). Whether adenine nucleosides will also serve as effectors for these mutants cannot be determined in these strains because of the presence of adenosine deaminase.

Similar mutants to our class II mutants, in which a mutation in a regulator gene has changed the inducer specificity so that a compound for which an enzyme has some affinity is now able to induce enzyme synthesis have been reported in the *ara* operon of *E. coli* (Le Blanc and Mortlake, 1971) and also in *Pseudomonas aeruginosa* (Brammar et al., 1967; for review see Clarke, 1974).

Class III mutants also contain constitutive levels of xanthosine phosphorylase, but the enzyme levels seemed to be very sensitive to glycerol and generally to the metabolic state of the cell. Class III mutants could grow on all purine nucleosides except adenosine. As adenosine is a metabolic precursor closely related to both the adenylate charge and the cAMP system of the cell, we suggest that the *xapR* gene in these mutants might have mutated so as to now recognise a normal cell metabolite as inducer (Greenblatt and Schlief, 1971) and thus become more sensitive to the metabolic state of the cell.

One particularly striking piece of evidence suggests that a purely negative control is not a sufficient explanation of the control of expression of the *xap* gene. This is the fact that the rate of mutation to *xapR*$^-$ was very low, approximately 3×10^{-8}. If a negative control system were operating, then any deleterious mutation in the *xapR* gene should result in a constitutive phenotype, and a mutation frequency of around 10^{-5} to 10^{-6} would be expected. (We cannot, however, rule out the possibility that complete loss of *xapR* is for some reason, lethal to the bacterium).

In contrast, if a system similar to the positive control system of the *ara* operon (Greenblatt and Schleif, 1971) were present, so that the *xapR* gene product was required as an inducer protein for the expression of the operon, then deleterious mutations in *xapR* would eliminate its inducer capacity, resulting in complete loss of xanthosine phosphorylase activity. Only rare constitutive mutations in *xapR* would retain this function.

The partial dominance of *xapR2* over $xapR^+$ is consistent with the notion that the $xapR^+$ gene product positively controls the xap^+ gene; in $xapR^+$/*xapR2* partial diploids, the *xapR2* protein induces the synthesis of the *xap* genes in the absence of xanthosine. Subunit interaction or a repressive activity of the wild-type activator protein could account for the partial nature of this dominance.

If such a positive control system were present, then selection for xanthosine non-utilising mutants should result in some mapping in the *xapR* gene rather than in the structural gene. As yet only 3 such mutants have been isolated and we cannot be certain whether they map in *xapR* or in the structural gene.

In connection with this it is interesting to note that Kocharian and Smirnov (1977) have independently isolated secondary-site revertants of a *deoD* strain which seem almost certainly to be *xapR* mutants although no detailed mapping was reported. In contrast to our results however, their parental strain could not utilise xanthosine, whereas their, presumed, *xapR* mutants could do so. We have tested many different 'wild-type' strains (see accompanying paper) and all can utilise xanthosine. It may be, therefore, that Kocharian and Smirnov's 'wild-type' strain contains an $xapR^-$ mutation, resulting in non-expression of xap^+, and that this is mutated in their secondary-site revertants so that *xap* is expressed, but expressed constitutively.

The map positions of *xap* and *xapR*, at approximately 51 min, 180° from the *deoD* gene, raises the intriguing possibility that *xap* and *deoD* have evolved from a gene duplication along the lines suggested by Zipkas and Riley (1975), i.e. that the *E. coli* genome might have undergone two genome doublings followed by evolutionary divergence of the different gene copies. A number of workers believe that such evolutionary divergence of duplicated pairs of genes could have been a major way in which new functions were acquired (Ohta and Kimura, 1971; Koch, 1972). A comparison of the *deoD* and *xap* DNA sequences may reveal some evidence for such a gene duplication.

The region of chromosome encoding *xap* and *xapR* appears to be one of those regions of the chromosome which is missing in *Salmonella typhimurium* but present in *E. coli* (Riley and Anilionis, 1978). This may account for the inability of *S. typhimurium* LT2 to grow on xanthosine and the absence of xanthosine phosphorylase activity when grown in glycerol plus xanthosine (Hammer-Jespersen, Buxton and Hansen, 1980).

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