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Mutants Constitutive for Nucleoside-Catabolizing Enzymes in *Escherichia coli* K12

Isolation, Characterization and Mapping

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Regulatory mutants of *Escherichia coli*, which synthesize cytidine deaminase and uridine phosphorylase constitutively have been isolated by different selection procedures and have been characterized. These mutants were also found to synthesize four other nucleoside-catabolizing enzymes, thymidine phosphorylase, deoxyriboaldolase, phosphodeoxyribomutase and purine nucleoside phosphorylase in amounts 3- to 10-fold in excess of wild-type levels.

This phenotype was found to be caused by mutations in one regulatory gene designated *cytR*.

Another class of regulatory mutants containing levels of deoxyriboaldolase, thymidine phosphorylase, phosphodeoxyribomutase and purine nucleoside phosphorylase 10- to 100-fold above wild-type levels have been isolated by similar selection procedures. These mutants are a result of a mutation in another regulatory gene, designated *deoR*, and were shown to contain low, inducible levels of cytidine deaminase and uridine phosphorylase.

The *cytR* and *deoR* genes have been found by P1-mediated transductions to be located at 76.5 min and 18.5 min, respectively, on the *E. coli* chromosome.

In *Escherichia coli*, catabolism of cytidine proceeds through the reactions 1–3 in Fig. 1 [1,2]. The two initial reactions are catalyzed by the enzymes cytidine deaminase and uridine phosphorylase, in this paper designated the cyt-enzymes, which are present in low amounts in wild-type cells, but induced to high levels if cytidine is added to the medium [3]. Uridine does not act as inducer in *E. coli* [4,5].

Further catabolism of cytidine, and of deoxycytidine as well, requires the action of another group of nucleoside-catabolizing enzymes, thymidine phosphorylase, deoxyriboaldolase, phosphodeoxyribomutase (for further references see [6]). These enzymes, together with purine nucleoside phosphorylase, in the following are designated the deo-enzymes.

Although the genes specifying the deo-enzymes have been shown to be closely linked and regulated by a common regulatory factor [7,8], evidence has been presented which indicates that they belong to at least two different regulatory units. Thus, addition of deoxyribonucleosides causes induction of all four enzymes while inosine or guanosine induce purine nucleoside phosphorylase and phosphodeoxyribomutase only [6,7]. Furthermore, cytidine and under certain conditions also adenosine, cause increased levels of the four enzymes as well as of cytidine deaminase and uridine phosphorylase [6].

This paper describes the isolation and characterization of regulatory mutants which synthesize either the cyt-enzymes or the deo-enzymes in constitutive amounts. These results suggest a relationship between the regulation of the genes which code for the deo-enzymes and for the cyt-enzymes. This relationship is also indicated by the complex pattern of induction by the various nucleosides and will be further discussed.

Abbreviations and Symbols. Genes coding for: purine nucleoside phosphorylase, *pup*; thymidine phosphorylase, *tpp*; gene coding for phosphodeoxyribomutase, *drm*; gene coding for uridine phosphorylase, *udp*; gene coding for cytidine deaminase, *cdd*; gene coding for deoxyriboaldolase, *dra*. Regulatory gene for: cytidine deaminase and uridine phosphorylase, *cytR*, for thymidine phosphorylase, deoxyriboaldolase, phosphodeoxyribomutase and purine nucleoside phosphorylase, *deoR*. Enzymes coded for by *tpp*, *dra*, *drm* and *pup*, deo-enzymes; enzymes coded for by *cdd* and *udp*, cyt-enzymes.

Enzymes. Purine nucleoside phosphorylase or purine-nucleoside: orthophosphate (deoxy)ribosyltransferase (EC 2.4.2.1); thymidine phosphorylase or thymidine: orthophosphate deoxyribosyltransferase (EC 2.4.2.4); uridine phosphorylase or uridine: orthophosphate ribosyltransferase (EC 2.4.2.3); cytidine deaminase or (deoxy)cytidine aminohydrolase (EC 3.5.4.5); deoxyriboaldolase or 2-deoxy-D-ribose-5-phosphate: acetaldehyde-lyase (EC 4.1.2.4).

MATERIALS AND METHODS

Chemicals

Ribo- and deoxyribonucleosides and other fine chemicals were purchased from Sigma Chemical Company (St. Louis, Missouri, U.S.A.). 2′-Deoxycytidine was obtained from Calbiochem (Luzern, Schweiz). 5-Fluoro-2′-deoxycytidine was a generous gift from F. Hoffmann La Roche & Co. Ltd, (Basle, Schweiz) through the courtesy of Dr A. V. Sprecher (Roche A/S, Copenhagen). Rifampicin (sodium-salt) was a gift from Ciba (Basle, Schweiz).

Bacterial Strains and Growth Media

The strains employed are presented in Table 1. In transduction experiments bacteriophage P1v, a virulent derivative of P1kc, selected as able to grow on *recA* strains, was used throughout. In experiments where enzyme levels were determined, bacteria were grown in phosphate-buffered minimal medium supplemented with the necessary nutritional requirements and with glycerol or glucose as carbon source. When inosine was added as a carbon source, thiamine (1 μg/ml) was added to the growth media.

For mating or transduction experiments the strains were grown in L-broth [12].

Genetic Methods

Matings were performed with cultures growing exponentially in L-broth. At densities of approximately 2×10^8 cells per ml, 0.5 ml donor culture was added to 2 ml recipient culture in a 500 ml erlenmeyer flask. After gentle mixing, the flask was left without shaking

Table 1. *Bacterial strains used and their origin*

Strain	Sex	Genotype	Preparation and/or source
SØ003	F⁻	*metB*	K12 58—161 from Institute of Microbiology, Copenhagen
SØ102	F⁻	*metB thy drm*	isolated from SØ003 in the same way as SØ103
SØ103	F⁻	*metB thy dra*	isolated from SØ003 [9].
SØ205	F⁻	*metB thy*	from SØ003; trimethoprim selection [10].
SØ270	F⁻	*metB cytR1*	from SØ003; rapid growth on uridine
SØ274	F⁻	*metB deoR1 thy drm*	from SØ205; very low thymine selection
SØ275	F⁻	*metB cytR2 thy drm*	from SØ205; very low thymine selection
SØ283	F⁻	*metB thy pup*	isolated from SØ103 as able to grow on dAdo + Glc. After this selection *dra* was removed by transduction with P1v (SØ003)
SØ303	F⁻	*metB deoR1*	from SØ274; two transductions with P1v (SØ003), selected first for drm^+ and then for thy^+
SØ330	F⁻	*metB cdd*	from SØ003 by mutagenization and selection for resistance to 5-fluoro-2′-deoxycytidine [11]
SØ331	F⁻	*metB deoR2*	from SØ003; rapid growth on inosine
SØ335	F⁻	*metB cytR3*	from SØ003; rapid growth on inosine
SØ337	F⁻	*metB cdd cytR7*	from SØ330; rapid growth on uridine
SØ343	F⁻	*metB cytR2*	from SØ275; two transductions with P1v (SØ003); selected first for drm^+ then for thy^+
SØ365	F⁻	*cytR2 thy drm*	from SØ275 by transduction with P1v (NF400) selected for met B^+
SØ384	F⁻	*cytR2*	from SØ003 by transduction with P1v (SØ365)
SØ385	F⁻	*cytR2 thy drm*	from SØ102 by transduction with P1v (SØ365)
SØ389	F⁻	*metB deoR1 thy pup*	from SØ274 by transduction with P1v (SØ283)
SØ392	F⁻	*metB cytR2 thy pup*	from SØ275 by transduction with P1v (SØ283)
KL16—99	Hfr	*recA1 thi*	from Brooks Low through Frode Engbæk
RE103	F⁻	*cmlA1 proB trp his* str^r	from E.C.R. Reeve
NF400	F⁻	*argH* rif^r *metA malB1* str^r	rif^r-derivative of PA505 from F. Jacob
NF413	F⁻	*metB cytR2 recA1 drm*	from SØ275 by mating with KL16—99
NF431	F′14/	*metB cytR2 recA1 drm*	from NF413 by sexduction with AB1206 F′14
AB1206	F′14/Δ	*ilvnarg thi his pro* str^r	from Brooks Low

for 1 h; appropriate dilutions were then plated on selective media.

Transductions

The standard procedures for P1-transduction [12] were followed. To avoid getting lysogenic transductants, a low multiplicity of phage was used, and sodium citrate (0.02 M) was included in the selective plates to prevent re-infections of the transduced cells. Recombinants were purified by restreaking once on selective plates, before being tested for unselected markers.

Patches of the recombinants were replicated onto plates containing 40 μg/ml rifampicin to test for rifampicin-resistance. To identify the *recA* mutation, patches of the recombinants were replicated on minimal plates and irradiated with ultraviolet light for 10 sec at a distance of 50 cm from a 15-watt germicidal ultraviolet lamp. This procedure killed all *recA* cells, but allowed growth of *recA*$^+$ cells.

Acridine-orange curing of episomes was carried out at acridine concentrations of 20 μg/ml as described by Hirota [13]. Donor characteristics were checked by replication onto a lawn of F$^-$ cells on selective plates, and by cross-streaking with the male-specific phage R17.

Enzyme Assays

Preparation of dialyzed cell extracts for enzyme assay was performed as previously described [6].

Purine nucleoside phosphorylase was assayed spectrophotometrically with xanthine oxidase. Thymidine phosphorylase was assayed by colorimetric determination of the deoxyribose phosphate formed. Deoxyribomutase activity was determined as the disappearance of thiobarbituric-acid-reacting deoxyribose. Uridine phosphorylase and cytidine deaminase were determined by measuring changes in absorbance at 290 nm. These assays have been described in detail elsewhere (for further references see [6]).

For determination of deoxyriboaldolase a slightly modified procedure was followed. Assay mixtures contained in a volume of 0.25 ml: 10 mM Tris-Cl, pH 7.3, 2 mM EDTA, 1.2 mM deoxyribose-5-phosphate and 20—100 units per ml of enzyme. At 0, 2, 5 and 10 min, 0.05 ml samples were pipetted into 0.4 ml 7% (w/v) perchloric acid. Deoxyribose-5-phosphate was determined with diphenylamine [14]. Inhibition of enzyme activity by one of the reaction products, acetaldehyde, was avoided by addition of alcohol dehydrogenase (3000 units per ml) and NADH (1.5 mM).

RESULTS

ISOLATION OF *cytR* MUTANTS

Isolation on Uridine as Carbon Source

The two cytidine-catabolizing enzymes, cytidine deaminase and uridine phosphorylase, are induced, when cytidine is added to the growth medium of wild-type cells (Table 2). In cells lacking cytidine deaminase, uridine phosphorylase is induced when cytidine is added. Neither uridine nor deoxycytidine gives rise to induction of these enzymes; deoxycytidine will, however, like other deoxyribonucleo-

Table 2. *Induced and constitutive levels of nucleoside-catabolizing—enzymes at various growth conditions*
Cultures were grown in minimal medium with carbon source and additional compounds as indicated. Cells were harvested by filtration, usually at a density of approximately 3×10^8 cells/ml. Added inducers were allowed to act for 2 h before harvesting. Cell extracts were prepared, dialyzed and assayed for enzymes as described [6]. n.d. = not determined. The enzymes are represented by the three letter code ordinarily used to designate the corresponding genes

Strain	Relevant genotype	Carbon source	Addition (2 mM)	Enzyme levels					
				cdd	udp	dra	tpp	drm	pup
				U/mg					
SØ003	wild	glycerol		45	90	29	46	87	180
SØ003	wild	glycerol	Cyd	1390	1030	286	500	200	280
SØ003	wild	glycerol	dCyd	66	128	470	1146	n.d.	340
SØ003	wild	glycerol	Urd	43	113	35	58	65	81
SØ330	*cdd*	glycerol		<5	125	39	68	49	170
SØ330	*cdd*	glycerol	Cyd	<5	1840	351	762	n.d.	372
SØ330	*cdd*	glycerol	Urd	<5	100	46	63	n.d.	200
SØ270	*cytR1*	glycerol		4350	1910	205	460	215	415
SØ275	*cytR2,drm,thy*	glycerol		2630	1790	330	650	16[a]	346
SØ331	*deoR2*	glycerol		38	98	1432	1823	548	907
SØ335	*cytR3*	glycerol		3515	2130	372	875	211	380
SØ274	*deoR1,drm,thy*	glycerol		65	107	1900	4140	7[b]	1145
SØ331	*deoR2*	glucose		29	83	n.d.	1828	n.d.	736
SØ270	*cytR1*	glucose		700	348	96	121	n.d.	147

[a] The corresponding *drm*$^+$ strain SØ343 had phosphodeoxyribomutase levels of 150 units/mg protein.
[b] The corresponding *drm*$^+$ strain SØ303 had phosphodeoxyribomutase levels of 900 units/mg protein.

sides, induce the deo-enzymes. The rationale for the isolation of mutants, constitutive for uridine phosphorylase was, therefore, that since uridine is the only nucleoside which does not cause induction of any of the enzymes which catabolize its degradation, then mutants constitutive in one or more of these enzymes would grow with increased rate on uridine as sole carbon source.

The slow growth of wild-type cells on uridine as sole carbon source is in accordance with this (Table 3). On plates, faster growing colonies appear, the majority of which are constitutive for uridine phosphorylase. These mutants are represented by strain SØ270. The finding that they are also constitutive for cytidine deaminase indicates that these two enzymes are regulated together as was suggested by the induction of both enzymes by cytidine.

Isolation as Minimal Thymine Requirers

thy,drm mutants will form colonies when supplemented with 2 μg/ml thymine, while *thy,drm* strains, which in addition are constitutive for the deo-enzymes, will grow on 0.5 μg/ml thymine. This has been used by Ahmad and Pritchard [15] to select for such constitutive mutants. The same selection procedure was used for the isolation of mutants constitutive for cytidine deaminase and uridine phosphorylase in the following way. Thymine auxotrophs were spread on glucose-minimal plates containing 1 μg/ml thymine and the colonies appearing were further tested for their ability to form single colonies on 0.5 μg/ml thymine. 13 mutants thus obtained were analyzed and all were found to lack phosphodeoxyribomutase. 9 of these had raised levels of thymidine phosphorylase. Of these 9 strains, 4 showed highly increased levels of thymidine phosphorylase, deoxyriboaldolase and purine nucleoside phosphorylase and were clearly of the *deoR* type described by Ahmad and Pritchard [15]. These strains are in the following represented by strain SØ274. These mutants have wild-type levels of cytidine deaminase and uridine phosphorylase. Three other mutants, however, were found to contain constitutive levels of the cyt-enzymes and thus were of the *cytR* type. The high growth rates of these strains (represented by strain SØ275) on inosine or uridine as carbon sources were characteristic for *cytR* strains, while *deoR* strains showed increased growth rate on inosine only (Table 3). The two remaining mutants were not further characterized.

Isolation on Inosine as Carbon Source

The finding that *cytR* mutants readily arise if wild-type cells are spread on uridine as a carbon source suggested that a similar procedure could be used for the isolation of mutants constitutive for the deo-enzymes. Growth rates of such mutants (*e.g.* strain SØ303) as compared to wild-type cells on inoside as carbon source supported this assumption (Table 3). In control experiments where mixtures of wild-type cells and a known *deoR* strain were spread on plates with inosine as carbon source it proved difficult to distinguish fast growing colonies, if the total cell number spread on the plates exceeded 10^4. Before spreading on the selective plates the cultures were therefore enriched for constitutive mutants by growth for 10—12 generations in liquid cultures with inosine as sole carbon source. 10^3—10^4 cells were then spread on the selective plates. Approximately 0.3% of the colonies appearing were fast growing. When analyzed the majority of these proved to be *cytR* mutants (represented by strain SØ335 in Table 2) while a few were characterized as *deoR* strains (strain SØ331).

Table 3. *Growth rate on different carbon sources*
Growth on glucose and glycerol was determined by diluting overnight cultures in fresh medium containing the same carbon source. In the experiments with inosine and uridine, exponentially growing cultures on glycerol were filtered and resuspended in media containing inosine or uridine as carbon source. Growth was followed over a 10-fold increase in mass

Strain	Selected by growth on	Relevant genotype	Generation time on			
			Glycerol	Glucose	Inosine	Uridine
			min	min	min	min
SØ003	—	wild	66	51	204	188
SØ270	uridine	*cytR1*	76	58	82	62
SØ343	low thymine	*cytR2*	90	60	74	65
SØ303	low thymine	*deoR1*	82	60	78	140
SØ331	inosine	*deoR2*	76	53	86	152
SØ335	inosine	*cytR3*	86	51	76	66

Properties of cytR *and* deoR *Mutants*

cytR *Mutants.* It was reported previously [6] that addition of cytidine to the growth medium of wild-type cells induces the synthesis of cytidine deaminase and uridine phosphorylase to high levels and at the same time causes elevated levels of the four deo-enzymes, deoxyriboaldolase, thymidine phosphorylase, phosphodeoxyribomutase and purine nucleoside phosphorylase (Table 2). When assayed for these four enzymes, the mutants constitutive for cytidine deaminase and uridine phosphorylase, were found to contain raised levels of the four deo-enzymes, levels which corresponded to the induction level caused by cytidine in wild-type strains (Table 2).

These raised levels of the deo-enzymes may explain why selection procedures originally designed for isolation of *deoR* mutants render *cytR* mutants.

Table 4. *Induction of nucleoside-catabolizing enzymes in different constitutive strains of* E. coli *K12*
The cells were grown on glycerol as a carbon source. After addition of the various inducers, growth was continued for 120 min. The cells were then harvested and enzymes were extracted and assayed as described in Materials and Methods. The enzymes are represented by the three letter code ordinarily used to designate the corresponding genes

E. coli K12 strain	Genotype	Present during incubation	Enzyme levels			
			cdd	udp	tpp	pup
			U/mg			
SØ343	*cytR2*	none	2630	1570	567	335
		Cyd	2170	1460	575	324
		dThd	2880	1663	4600	1480
		Ino	1786	946	466	564
SØ331	*deoR2*	none	38	98	1823	907
		Cyd	610	869	2295	1100
		dThd	30	87	1779	830
		Ino	30	74	1813	864
SØ392	*cytR2, pup thy*	none	2280	1390	786	<5
		Ado	1690	1026	540	<5
SØ389	*deoR1, pup, thy*	none	53	61	4240	<5
		Ado	470	392	5040	<5

In the case of a thymine-requiring strain, raised levels of thymidine phosphorylase probably offer selective advantages in the utilization of exogenous thymine, when the concentration of this compound is limited; therefore, both *deoR* and *cytR* mutants are obtained in this selection procedure. Likewise, the raised levels of purine nucleoside phosphorylase and phosphodeoxyribomutase in *cytR* strains permit these strains to grow faster on inosine as carbon source, although it is not clear why the majority of mutants obtained by this isolation procedure were of the *cytR* type and not the *deoR* type with considerably higher enzyme levels.

In the *cytR* mutants the levels of the deo-enzymes may be raised further by addition of appropriate inducers (Table 4). Thus, addition of thymidine induces the four deo-enzymes while inosine causes an increase in purine nucleoside phosphorylase and phosphodeoxyribomutase alone. When cytidine is added, no significant changes are seen in the level of any of the enzymes (Table 4).

Adenosine was previously reported to cause an "unspecific" induction of the cyt-enzymes as well as of the deo-enzymes, when added to the growth medium of a strain lacking purine nucleoside phosphorylase [6]. The rise in enzyme levels corresponds to the rise of enzyme levels in wild-type strains after addition of cytidine and to the enzyme levels in a *cytR* strain (*e.g.* strain SØ270 in Table 2).

If adenosine is added to a *cytR* strain lacking purine nucleoside phosphorylase (strain SØ392 in Table 4), the already raised enzyme levels do not change significantly. It seems likely, therefore, that the inducing effect of both cytidine and adenosine depends on the *cytR* gene product.

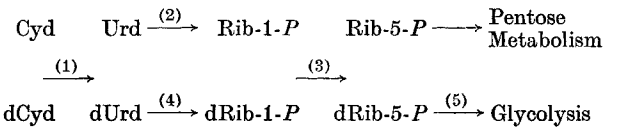

Fig. 1. *Schematic representation of the catabolism of cytidine and deoxycytidine.* (1) (Deoxy) cytidine deaminase; (2) uridine phosphorylase; (3) phosphodeoxyribomutase; (4) thymidine phosphorylase; (5) deoxyriboaldolase

Two other enzymes, adenosine deaminase and cytosine deaminase, which are involved in nucleotide metabolism in the cells, have been determined before and after addition of cytidine to a wild-type strain or of adenosine to a strain which lacks purine nucleoside phosphorylase. No significant changes were seen (data not shown). This indicates that the inducing effect of cytidine and adenosine may be quite specific for the deo-enzymes and the cyt-enzymes in the cell.

cytR MUTANTS WITH A BLOCK IN DEOXYRIBONUCLEOSIDE CATABOLISM

Wild-type cells as well as mutants lacking deoxyriboaldolase have low levels of thymidine phosphorylase suggesting that such strains only to a small extent degrade deoxyribonucleotides to deoxyribose-5-phosphate [9]. This latter compound is reported to be the inducer of the deo-enzymes [16].

Deoxycytidine might give rise to the formation of deoxyribose-5-phosphate through the reactions 1, 4, 3 in Fig. 1 and is in fact an even better substrate for cytidine deaminase than is cytidine. In the cell deoxycytidine might be formed as a breakdown product of deoxycytidine nucleotides. The finding that cells deficient in cytidine deaminase excrete substantial amounts of deoxycytidine monophosphate in the medium (Munch-Petersen, unpublished observation) supports this contention. It seemed possible, therefore, that the high levels of cytidine deaminase in the *cytR* strains might increase the flow of deoxyribosyl groups from deoxycytidine nucleotides through deoxycytidine to deoxyribose-5-phosphate, thus causing increased levels of the deo-enzymes.

If this was the case, *cytR* mutants with an additional mutation in deoxyribonucleoside catabolism, rendering them unable to form deoxyribose-5-phosphate, should not contain increased levels of deo-enzymes. Comparison of strain SØ275, *cytR2, thy,drm* (Table 2) and strain SØ343, *cytR2* (Table 4) indicated no difference in enzyme levels. Furthermore, two different *cytR* mutants were constructed by transducing the *cytR* gene into a wild-type strain and into a *drm,thy* strain. Comparison of enzyme

Table 5. *Effect of a block in deoxyribonucleoside catabolism on the levels of nucleoside-catabolizing enzymes*
The cells were grown on glycerol as a carbon source. After several generations of exponential growth the cells were harvested and the enzymes were extracted and assayed as described in Materials and Methods. n.d. = not determined. The enzymes are represented by the three letter codes ordinarily used to designate the corresponding genes

Strain	Relevant genotype	Enzyme levels				
		cdd	dra	tpp	drm	pup
		U/mg				
SØ384	*cytR2*	3100	260	820	290	300
SØ385	*cytR2, thy, drm*	3100	350	730	7	340
SØ337	*cytR7, cdd*		280	604	n.d.	236

levels in these two strains showed no difference in the levels of deo-enzymes (strains SØ383 and SØ384 in Table 5). Likewise, when a *cytR* mutation was introduced into a cytidine-deaminase-negative strain the levels of the deo-enzymes did increase (Table 5). Thus, there is no indication that the raised levels of the deo-enzymes in *cytR* strains are due to internal induction by deoxyribose-5-phosphate.

MAPPING OF THE *cytR* GENE

Preliminary mapping by uninterrupted mating indicated that the *cytR* gene was linked to *metB*. This was confirmed by P1-transduction which showed approximately 80% cotransduction between *metB* and *cytR*. In order to determine the exact map position of *cytR* relative to other markers in the region around 77 min on the Taylor-map [17] the following four-factor cross was performed: P1v was grown on NF400 (*cytR*$^+$,*metB*$^+$,*rif*^{r},*argH*) and as recipient was used: *cytR*,*metB*,*rif*^{s},*argH*$^+$. *met*$^+$ recombinants were isolated and analyzed for the other three markers; the results are shown in Table 6 which demonstrates that the order is *cytR-metB-argH-rif* for both *cytR2* and *cytR3*. The cotransduction frequencies can be calculated to be 0.27 between *metB* and *argH*; 0.18 between *metB* and *rif*; 0.92 between *metB* and *cytR2* and 0.76 between *metB* and *cytR3*.

The level of enzymes specified by the *cdd* and the *tpp* genes were measured in all the transductants shown in Table 6 and in 70 recombinants from similar transductions involving independently isolated constitutives (117 transductants in total); no separation of control of the two genes was found. In 35 cases also purine nucleoside phosphorylase was measured and found to vary together with the thymidine phosphorylase and the cytidine deaminase.

The *cytR2* allele has been transferred by P1v transduction from SØ365 into strain SØ003 by selection for *met*$^+$. Among 20 transductants analyzed,

Table 6. *Linkage between* metB, argH, rif *and* cytR
Transductions were carried out as described in Materials and Methods. The donor strain used was NF400, recipients were SØ275 and SØ335; *met*$^+$ recombinants were selected. The *cytR* genotype of the transductants were deduced from the enzyme levels of cytidine deaminase and thymidine phosphorylase

Genotype of *met*$^+$ transductants			Number of recombinants	
cytR	*argH*	*rif*	SØ275 *cytR2*	SØ335 *cytR3*
+	+	r	2	2
+	+	s	36	29
+	−	r	7	5
+	−	s	10	7
−	+	r	0	1
−	+	s	3	10
−	−	r	2	3
−	−	s	0	0
			60	57

Table 7. *Dominance relationship between the* cytR$^+$ *and the* cytR *alleles*
The cells were grown on glycerol as a carbon source. After several generations of exponential growth, the cells were harvested and the enzymes extracted and assayed as described in Materials and Methods. Enzymes are represented by symbols ordinarily used to designate the corresponding genes

E. coli K12 strain		Enzyme levels			
		cdd	udp	tpp	pup
		U/mg			
NF413	(*recA1, cytR2, metB*)	3218	2530	875	475
NF431	(*recA1, cytR2, metB*/ F'14*cytR*$^+$, *metB*$^+$)	165	270	74	135

16 showed constitutive phenotype, demonstrating that no genetic change outside this region is needed for the expression of constitutivity. Our inability to separate genetically the control of the three different regulatory units makes it extremely likely that the constitutive phenotype results from mutation in a single gene, the *cytR* gene.

Enzyme levels have been measured in a strain heterozygotic for the *cytR* gene. The strain was constructed by transfer of F'14 carrying *cytR*$^+$ from AB1206 into NF413, a *recA1* derivative of SØ275 *cytR2,metB*. As shown in Table 7 the wild-type allele is dominant over the constitutive one. The presence of the recessive *cytR* allele in NF431 was demonstrated in acridine-orange-cured derivatives.

Pritchard and Ahmad [18] mapped *udp*, uridine phosphorylase, at approximately 75 min. From their cotransduction studies we concluded that this gene is separated from the *cytR* gene by at least 1 min. Furthermore, *cdd*, one of the other genes under *cytR* control, maps between 40 and 45 min (Fuchs and

Karlström, personal communication). Thus, the *cytR* gene product controls genes located in these widely separated regions of the *E. coli* chromosome.

deoR MUTANTS

Two such strains have been investigated in detail. One (SØ274) was isolated as a minimal thymine requirer and one (SØ331) as a fast growing colony on inosine as carbon source.

These strains contain high levels of the deo-enzymes but wild-type levels of the cyt-enzymes (Table 2). The deo-enzymes could not be induced further by addition of thymidine or inosine. Addition of cytidine induces cytidine deaminase and uridine phosphorylase as in the wild-type strain, but has little or no effect on the level of the deo-enzymes (Table 4). Likewise, addition of adenosine has only a slight effect on the levels of the deo-enzymes in a *deoR,pup* strain (SØ389) but causes a significant increase in cytidine deaminase and uridine phosphorylase (Table 4).

The fact that the mutase-negative strain (SØ274), which cannot produce deoxyribose-5-phosphate, has high levels of thymidine phosphorylase, deoxyriboaldolase and purine nucleoside phosphorylase indicates that the high enzyme levels are not caused by internal accumulation of deoxyribose-5-phosphate, as was also concluded by Ahmad and Pritchard [15].

Mapping of the deoR *Gene*

Preliminary mapping by uninterupted conjugation indicated that *deoR* mapped in the *gal,trp* region. Transduction with various markers in this region showed that *deoR* was very closely linked to *cmlA*. P1v phage grown on RE103, *cmlA1*, was used to infect SØ331 *deoR2* and SØ308, a derivative of strain SØ274 *deoR1*, selecting for resistance to 10 μg/ml of chloramphenicol, as described by Reeve and Doherty [19]. In the case of SØ331, 11 chloramphenicol resistant colonies were picked up and checked for thymidine phosphorylase and purine nucleoside phosphorylase levels. In all, 11 recombinants both enzyme levels were reduced to normal wild-type levels, indicating 100% cotransfer of $deoR^+$ allele with *cmlA1*. In the case of SØ308, 20 recombinants were analyzed for thymidine phosphorylase level. Again, all twenty recombinants had regained wild-type enzyme levels (four of the 20 recombinants were, in addition, analyzed for purine nucleoside phosphorylase, which also showed low, inducible levels).

Effect of Glucose

The *deoR* and the *cytR* strains differ in their response to glucose in the growth medium. When a *deoR* mutant is grown with glucose as a carbon source there is only a minor decrease in the deo-enzyme levels as compared to the levels on glycerol (Table 2). In contrast a 3- to 5-fold repression of both deo-enzyme and cyt-enzyme levels occurs when a *cytR* strain is grown in glucose instead of in glycerol.

DISCUSSION

Information concerning the control of cytidine catabolism in enteric bacteria has until recently been scarce and somewhat controversial [3]. The present results state more concisely that cytidine, but not uridine or deoxycytidine, in *Escherichia coli* will act as inducer of cytidine deaminase and uridine phosphorylase, and that uridine phosphorylase is induced by cytidine in strains lacking cytidine deaminase. Thus, either cytidine or possibly a phosphorylated derivative of cytidine seems to be the inducing compound. Inducing conditions in *Salmonella* are slightly different and will be dealt with in a separate report (Nygaard, unpublished observation).

Peterson *et al.* have reported [20] that strains of *E. coli* which were "adapted" to uridine by growth for several generations on this nucleoside as sole carbon source contained 10-fold raised levels of cytidine deaminase and uridine phosphorylase. The strains were not further characterized; it appears likely, though, that the high enzyme levels found in the strains were due to constitutivity rather than induction, since, as shown above, growth on uridine may be used to select for mutants, which synthesize these two enzymes in constitutive amounts.

The elevated levels of the deo-enzymes found in all *cytR* strains permit selection procedures for *cytR* strains that are based on these high levels, *i.e.* screening for rapid growth on inosine as a carbon source or for growth of thymine-requiring mutants on exceptionally low concentrations of thymine. The high levels of the deo-enzymes correspond approximately to those found in wild-type strains after addition of cytidine or in purine nucleoside phosphorylase negative strains after addition of adenosine. It is conceivable, therefore, that adenosine and cytidine, when added to wild-type cells, trigger the same induction mechanism, which, mediated by the *cytR* gene product, results in increased levels of all nucleoside catabolizing enzymes.

cytR mutations from strains, obtained by three different selection procedures, have all been shown to be cotransducible with *metB* (Table 6); this indicates that they represent the same mutation. On the basis of results from 187 transductants, it was concluded, that the constitutive phenotype is the result of one mutation.

Similar experiments were carried out with strains constitutive for the deo-enzymes. Such strains were

Table 8. *Induction pattern of ribo- and deoxyribonucleoside catabolizing enzymes*

The table contains a compilation of results from a number of induction experiments. In all cases the cells were grown on glycerol as a carbon source and the various (deoxy)-nucleosides (2 mM) were added 120 min before harvesting. Cell extraction and enzyme assays were carried out as described [6]. Induced enzyme levels are given as a multiple of basal enzyme levels as determined in uninduced cells. Enzymes are represented by symbols ordinarily used to designate the corresponding genes

Enzymes	Inducing compounds			
	dRib-5-*P*	Ino or Guo [a]	Ado added to *pup*-deficient strain	Cyd
dra tpp	15—20	[b]	5—7	5—7
drm pup	3—6	2—4	2—4	2—3
cdd udp	[b]	[b]	7—20	7—20

[a] Ado has the same effect as Ino because it is deaminated to this compound.
[b] No induction.

isolated in two different ways (*i.e.* by faster growth on inosine as a carbon source or by selection as minimal thymine requirers); both mutations were shown to be more than 96% transducible with *clmA*, which is located at 18.5 min (Table 7).

This cotransduction is consistent with the findings of Ahmad and Pritchard [15], that the regulatory gene for the deo-enzymes is located 1—2 min after *galE*, and that this gene is responsible for the control of all four enzymes.

The raised levels of the deo-enzymes which were found in all *cytR* strains are most likely not due to internal induction by raised intracellular pools of deoxyribose-5-phosphate. They rather seem to indicate the existence of a mutual regulatory mechanism for the cyt-enzymes and the deo-enzymes.

Mutual as well as independent control mechanisms are probably also indicated by the very complex induction pattern found for these nucleoside catabolizing enzymes. Table 8 summarizes the induction results from this and previous papers, concerning the induction of cyt- and deo-enzymes. The six enzymes seem to be controlled through three different regulatory units in the folloing way.

In the first unit, cytidine deaminase and uridine phosphorylase are regulated by at least one gene product, the *cytR* product, with cytidine (and possibly adenosine) as effector. This induction is strongly repressed by glucose.

In the second unit, thymidine phosphorylase and deoxyriboaldolase are regulated by at least two genes. One is the *deoR* gene product, which in this case has deoxyribose-5-phosphate as effector. An additional control is exerted by the *cytR* gene product, again with cytidine (adenosine) as effector. Also, here the *cytR*-gene-mediated regulation is strongly influenced by glucose; in contrast glucose has little or no effect on the control exerted by the *deoR* gene.

In the third unit, phosphodeoxyribomutase and purine nucleoside phosphorylase are regulated by the *cytR* and *deoR* genes, but since the degree of induction found for these enzymes usually is rather low the results are less clearcut. In this case, the effector for the *deoR* product may be either deoxyribose-5-phosphate or inosine or guanosine; the effector for the *cytR* product again is cytidine or adenosine. The effect of glucose appears similar to that which was found for the two other deo-enzymes.

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REFERENCES

1. Wang, T. P., Sable, H. Z. & Lampen, J. O. (1950) *J. Biol. Chem. 184*, 17.
2. Paege, L. M. & Schlenk, F. (1952) *Arch. Biochem. Biophys. 40*, 42.
3. O'Donovan, G. A. & Neuhard, J. (1970) *Bacteriol. Rev. 34*, 278.
4. Razzel, W. E. & Casshyap, P. (1964) *J. Biol. Chem. 239*, 1789.
5. Saunders, P. P., Wilson, B. A. & Saunders, G. F. (1969) *J. Biol. Chem. 244*, 3691.
6. Hammer-Jespersen, K., Munch-Petersen, A., Nygaard, P. & Schwartz, M. (1971) *Eur. J. Biochem. 19*, 533.
7. Robertson, B. S., Jargiello, P., Blank, J. & Hoffee, P. A. (1970) *J. Bacteriol. 102*, 628.
8. Ahmad, S. J. & Pritchard, R. H. (1969) *Mol. Gen. Genet. 104*, 351.
9. Munch-Petersen, A. (1970) *Eur. J. Biochem. 15*, 191.
10. Stacey, K. A. & Simson, E. (1965) *J. Bacteriol. 90*, 554.
11. Karlström, O. (1968) *J. Bacteriol. 95*, 1069.
12. Lennox, E. J. (1955) *Virology, 1*, 190.
13. Hirota, Y. (1960) *Proc. Nat. Acad. Sci. U. S. A. 46*, 57.
14. Burton, K. (1956) *Biochem. J. 62*, 315.
15. Ahmad, S. J. & Pritchard, R. H. (1971) *Mol. Gen. Genet. 111*, 77.
16. Breitman, T. R. & Bradford, R. M. (1967) *Biochim. Biophys. Acta, 138*, 217.
17. Taylor, A. L. (1970) *Bacteriol. Rev. 34*, 155.
18. Pritchard, R. H. & Ahmad, S. J. (1971) *Mol. Gen. Genet. 111*, 84.
19. Reeve, E. C. R. & Doherty, P. (1968) *J. Bacteriol. 96*, 1450.
20. Peterson, R. N., Boniface, J. & Koch, A. L. (1967) *Biochim. Biophys. Acta, 135*, 771.

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