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RNA polymerase involvement in the regulation of expression of *Salmonella typhimurium pyr* genes. Isolation and characterization of a fluorouracil-resistant mutant with high, constitutive expression of the *pyrB* and *pyrE* genes due to a mutation in *rpoBC*

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A mutant of *Salmonella typhimurium* with a defect in the regulation of *pyr*-gene expression was obtained during a selection for mutants resistant to a combination of the two pyrimidine analogs, 5-fluorouracil and 5-fluorouridine. The mutant possesses 4-fold elevated pools of the pyrimidine nucleoside triphosphates, UTP and CTP. The specific activities of aspartate transcarbamylase and orotate phosphoribosyltransferase are 40-fold and 7-fold higher in the mutant than in the parent strain when grown in minimal media. Furthermore, the synthesis of the two enzymes in the mutant is not repressed following addition of exogenous pyrimidines. The levels of carbamoylphosphate synthase and orotidine 5'-monophosphate decarboxylase are ~3-fold enhanced, while the activities of dihydroorotase and dihydroorotate oxidase appear largely unaffected by the mutation. The mutation responsible for these effects was shown to map between two known point mutations in the *rpoBC* gene cluster encoding the β and β' subunits of RNA polymerase. These observations indicate a regulatory function of RNA polymerase in the control of *pyr*-gene expression in *S. typhimurium*.

Key words: pyr-genes/RNA polymerase/pyrimidine biosynthesis

Introduction

The *de novo* biosynthesis of UMP involves six consecutive enzymatic reactions catalyzed by carbamoylphosphate synthase *(pyrA)*, aspartate transcarbamylase *(pyrB)*, dihydroorotase *(pyrC)*, dihydroorotate oxidase *(pyrD)*, orotate phosphoribosyltransferase *(pyrE)*, and orotidine 5'-monophosphate decarboxylase *(pyrF)*. The first enzyme, carbamoylphosphate synthase, is also required for the synthesis of arginine (Figure 1). The genes coding for these enzymes are scattered on the chromosomes of both *Salmonella typhimurium* and *Escherichia coli* and thus do not constitute an operon (O'Donovan and Neuhard, 1970; Beck and Ingraham, 1971).

Little is known about the factors involved in the control of synthesis of the pyrimidine biosynthetic enzymes. The rate of synthesis of all six enzymes is reduced following addition of preformed pyrimidines to the growth medium, and it is enhanced during starvation of pyrimidine auxotrophic mutants for uracil (Beckwith *et al.*, 1962). Under both conditions, however, a noncoordinated response of the six enzymes was observed.

To identify the low molecular weight effectors participating in this control, attempts were made to correlate the rates of synthesis of the various enzymes with the pools of different pyrimidine nucleotides, in mutants where pyrimidine nucleotide interconversion was blocked by mutation at different positions. It was concluded that the expression of *pyrC* and *pyrD* is repressed primarily by a cytidine nucleotide other than CMP, whereas the expression of *pyrB*, *pyrE*, and *pyrF* is repressed by a uridine nucleotide other than UMP (Schwartz and Neuhard, 1975; Kelln *et al.*, 1975). The synthesis of carbamoylphosphate synthase is subject to cumulative repression by arginine and a pyrimidine compound (Abd-El-Al and Ingraham, 1969; Pierard *et al.*, 1976). In addition, *pyr*-gene expression in *S. typhimurium* appears to be under the control of a purine nucleotide, since manipulations of the guanine nucleotide pools create great variations in the rate of synthesis of the pyrimidine biosynthetic enzymes (Jensen, 1979).

A major difficulty in studying the regulation of *pyr*-gene expression is the lack of regulatory mutants. By selecting for resistance to pyrimidine analogs or for pyrimidine overproduction, mutants have been isolated which contained high, non-repressible levels of the pyrimidine biosynthetic enzymes. Initially these mutants were thought to lack a functional "pyrimidine repressor" (O'Donovan and Gerhart,

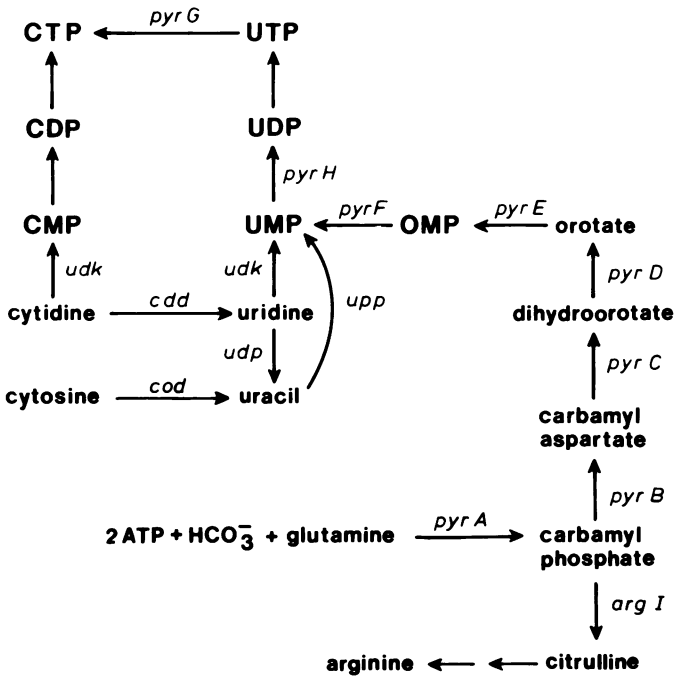

Fig. 1. Pathways for the biosynthesis of pyrimidine nucleoside triphosphates in *S. typhimurium*. The enzymes are identified by their corresponding gene designations as follows: *argI*, ornithine transcarbamylase (EC 2.1.3.3); *cdd*, cytidine deaminase (EC 3.5.4.5); *cod*, cytosine deaminase (EC 3.5.4.1); *pyrA*, carbamoylphosphate synthase (EC 2.7.2.5); *pyrB*, aspartate transcarbamylase (EC 2.1.3.2); *pyrC*, dihydroorotase (EC 3.5.2.3); *pyrD*, dihydroorotate oxidase (EC 1.3.3.1); *pyrE*, orotate phosphoribosyltransferase (EC 2.4.2.10); *pyrF*, OMP decarboxylase (EC 4.1.1.23); *pyrG*, CTP synthetase (EC 6.3.4.2); *pyrH*, UMP kinase (EC 2.7.4.14); *udk*, uridine kinase (EC 2.7.1.48); *udp*, uridine phosphorylase (EC 2.4.2.3); *upp*, uracil phosphoribosyltransferase (EC 2.4.2.9).

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Table I. Strains used.

Strain[a]	Genotype	Relevant phenotype[b]	Source or reference
KP-1469	*cdd-9 cod-8 deoD201 udp-11*	AR^-, UR^-	Jensen, 1979
KP-1475[c]	KP-1469 *rpo-FUR*	FU^R, Cs^-, high OPRT	This work
KP-1492[c]	KP-1469 *rpo-FUS*	High OPRT	This work
KP-1494[d]	KP-1469 *rpoB4 rpo-ts13*	Rif^R, Ts^-	This work
KP-1495[d]	KP-1469 *rpoB4*	Rif^R	This work
KP-1496[d]	KP-1469 *rpo-ts13 arg-1824::tet137*	Ts^-	This work
KP-1497[e]	KP-1469 *rpoB4 rpo-FUR*	Rif^R, FU^R, Cs^-, high OPRT	This work
TT-137[f]	*arg-1824::tet137*	Tet^R, Arg^-	J. Roth
KR-45[g]	*pyrC20 zcc::Tn10*	Pyr^-, Tet^R	R. Kelln
SDB-223[h]	*cysA his argE55 rpoB4 rpo-ts13*	Rif^R, Ts^-	D. Boyd
JL-84	HfrA,*metB406 hisD23 gal-50*	clockwise from 83 min	C. Beck
SA-828	HfrK25,*serA15 rfx*	clockwise from 34 min	J. Roth

[a]All strains having KP-numbers are derivatives of KP-1469, i.e., they contain *cdd-9,cod-8,deoD201,* and *udp-11* mutations.
[b]Phenotypes: AR^- and UR^- indicate that the strain is unable to use adenosine and uridine as carbon source, respectively. FU^R, 5-fluorouracil-resistant; Cs^-, unable to grow on L-broth at 20°C; Rif^R, rifampicin-resistant; Ts^-, unable to grow at 42°C; Tet^R, tetracycline-resistant; high OPRT, elevated orotate phosphoribosyltransferase level.
[c]See **Results.**
[d]The construction of these strains is described under **Materials and methods.**
[e]Progeny of cross 2 in Table VIII.
[f]*Tn10* element inserted in the *argEBCH* gene cluster at 89 min on the *Salmonella* map (Sanderson and Hartman, 1978).
[g]*Tn10* element cotransducible ~40% with the *pyrC20* mutation (R. Kelln, pers. comm.).
[h]P22 lysate of this strain was obtained from D. Boyd. The *rpo-ts13* mutation is most likely located in the *rpoC* gene, or alternatively (and less likely) in the promoter-distal part of the *rpoB* gene (Boyd *et al.*, 1979; D. Boyd, pers. comm.).

1972), but they turned out to contain leaky mutations in the *pyrH* gene encoding UMP kinase (Justesen and Neuhard, 1975). Such mutants contain high levels of the enzymes as a consequence of their reduced capacity to convert UMP to UDP and UTP, the presumed corepressors (Ingraham and Neuhard, 1972; Schwartz and Neuhard, 1975).

We describe the selection and characterization of a different type of mutant, KP-1475, isolated as resistant to a combination of the two pyrimidine analogs, 5-fluorouracil and 5-fluorouridine. This mutant contains high, irrepressible levels of aspartate transcarbamylase and orotate phosphoribosyltransferase. In contrast to what is found for the *pyrH* mutants, the UTP and CTP pools of KP-1475 are 4-fold higher than in the parent strain. Thus, KP-1475 shows a phenotype expected for a true regulatory mutant: elevated expression of the *pyr* genes in the presence of high intracellular concentrations of the presumed corepressors. Genetic mapping of the mutation responsible for this phenotype shows that it is located in the *rpoBC* gene cluster, encoding the β, β'-subunits of RNA polymerase, either in the *rpoC* gene or in the promoter distal part of the *rpoB* gene. This indicates a regulatory function for RNA polymerase in the control of expression of the *pyr* genes of *S. typhimurium.*

Results

Isolation of a fluorouracil-resistant mutant with defective regulation of pyr-gene expression

During a search for pyrimidine regulatory mutants, samples (10^8 cells) of KP-1469 were plated on glucose media containing 0.2% casamino acids, 5-fluorouracil (5 μg/ml), and 5-fluorouridine (5 μg/ml). After 3 days at 37°C, resistant colonies were picked and analyzed for their content (i) of ribonucleoside triphosphates using the one-dimensional chromatographic system of Cashel *et al.*, 1969, and (ii) of aspartate transcarbamylase. One mutant, KP-1475, had very high pools of UTP and CTP and substantially elevated levels of aspartate transcarbamylase. The mutant grows considerably slower than the parent strain regardless of nutritional supplement (Table II), indicating that it harbors a mutation in an essential gene. At 20°C the growth of KP-1475 is extremely poor, particularly on L-broth, where visible colonies are formed only after 6–7 days.

A pseudorevertant of KP-1475, i.e., KP-1492, is also used in this study. KP-1492 was isolated on L-broth at 20°C. It grows with near wild-type rates (Table II), but contains somewhat elevated levels of aspartate transcarbamylase. It has, however, lost the resistance to pyrimidine analogs.

Table II. Growth characteristics of KP-1469 and the two regulatory mutants, KP-1475 and KP-1492.

Strain	Generation time at 37°C (min)		
	L-broth[a]	Glucose + casamino acids[b]	Glucose minimal[c]
KP-1469	26	34	58
KP-1475	66	79	100
KP-1492	32	39	61

[a]Miller, 1972.
[b]AB medium (Clark and Maaløe, 1967) containing 0.2% casamino acids.
[c]Tris-minimal medium.

Table III. Specific activities of six UMP biosynthetic enzymes in KP-1469 and the two regulatory mutants KP-1475 and KP-1492.

Strain	Addition to growth medium	Specific activity (units/mg)[a]					
		CPS (*pyrA*)	ATC (*pyrB*)	DHO (*pyrC*)	DHOox (*pyrD*)	OPRT (*pyrE*)	OMPdec (*pyrF*)
KP-1469	None	18	24	64	48	31	7
	Uracil[b]	5	10	40	32	8	ND
	Cytidine[c]	15	21	30	23	30	ND
KP-1475	None	48	915	44	68	229	24
	Uracil	20	892	46	54	220	ND
	Cytidine	50	834	26	50	231	ND
KP-1492	None	24	180	49	56	218	7
	Uracil	8	111	37	42	199	ND
	Cytidine	19	162	20	31	215	ND

[a]CPS, carbamoylphosphate synthetase; ATC, aspartate transcarbamylase; DHO, dihydroorotase; DHOox, dihydroorotate oxidase; OPRT, orotate phosphoribosyltransferase; OMPdec, orotidine 5′-monophosphate decarboxylase; ND, not determined.
[b]Uracil is present at a concentration of 10 μg/ml.
[c]Cytidine concentration is 20 μg/ml.

Levels of pyrimidine nucleotide biosynthetic enzymes in wild-type and mutants

Table III shows the specific activities of the six UMP biosynthetic enzymes in KP-1469 and in the two regulatory mutants, KP-1475 and KP-1492, following growth in the absence or presence of pyrimidine supplements. The presence of pyrimidines does not alter the growth rate of the strains. In unsupplemented minimal medium the specific activities of aspartate transcarbamylase and orotate phosphoribosyltransferase are elevated 35- and 7-fold in KP-1475 relative to KP-1469. Furthermore, the synthesis of these two enzymes is not repressed in KP-1475 by addition of exogenous pyrimidines, while uracil addition causes 3 – 5-fold repression of their synthesis in KP-1469. Thus, in the presence of uracil the specific activity of aspartate transcarbamylase is elevated 80 – 100 times and the specific activity of orotate phosphoribosyltransferase is raised 25 – 30-fold in KP-1475 relative to KP-1469 (Table III). The carbamoylphosphate synthase and OMP decarboxylase activities are increased ~3-fold in KP-1475, while the activities of dihydroorotase and dihydroorotate oxidase seem only marginally affected by the defect in the strain.

In the pseudorevertant, KP-1492, the specific activities of the pyrimidine biosynthetic enzymes are partially normalized (Table III). The level of aspartate transcarbamylase has decreased ~5-fold relative to KP-1475 and it has partially regained the repressibility by uracil. In contrast, the level of orotate phosphoribosyltransferase is still as high in KP-1492 as in KP-1475, and it is still irrepressible by uracil. The other four pyrimidine biosynthetic enzymes seem to be present in near normal quantities in KP-1492 (Table III).

Although the repressibility of aspartate transcarbamylase and orotate phosphoribosyltransferase is lost in KP-1475, it was still possible that their levels might increase further by uracil starvation. To test this, pyrimidine-requiring derivatives (*pyrC20 zcc::Tn10*) of KP-1469 and KP-1475 were constructed by transduction, and the levels of the two enzymes were determined in uracil-grown and uracil-starved cells. As shown in Table IV pyrimidine starvation does not cause a further increase in the levels of the two enzymes in KP-1475 *pyrC20 zcc::Tn10*. Thus, they appear to be synthetized constitutively.

Table IV. Effect of uracil starvation on the levels of two pyrimidines biosynthetic in KP-1469 and KP-1475[a].

Strain[a]	Growth condition	Specific activity (units/mg)	
		ATC (*pyrB*)	OPRT (*pyrE*)
KP-1469, *pyrC20 zcc::Tn10*	exponential (+ uracil)	10	10
	starved (– uracil)	449	58
KP-1475, *pyrC20 zcc::Tn10*	exponential (+ uracil)	958	240
	starved (– uracil)	1227	223

[a]Pyrimidine-requiring derivatives of KP-1469 and KP-1475 were constructed by transduction with a P22-lysate grown on KR-45 (*pyrC20 zcc::Tn10*). Tetracycline resistance was selected and uracil auxotrophs were picked.

Table V. Enzyme levels in KP-1475 and KP-1469.

Enzyme	Specific activity (units/mg)	
	KP-1469	KP-1475
Ornithine transcarbamylase (EC 2.1.3.3)	481	551
Histidinol dehydrogenase (EC 1.1.1.23)	10	11
Glucose-6-phosphate dehydrogenase (EC 1.1.1.49)	328	431
5-Phosphoribosylpyrophosphate synthetase (EC 2.7.6.1)	65	149
Thymidine phosphorylase (EC 2.4.2.4)	28	46
Thymidine phosphorylase, induced[a]	487	465

[a]Cells were grown in the presence of thymidine (1 mg/ml) for 90 min before harvest (Hoffee and Robertson, 1969).

To test the specificity of the regulatory mutation we assayed five enzymes that are not involved in the UMP biosynthetic pathway. The results are shown in Table V. Only minor differences in the specific activities of these enzymes are seen between KP-1469 and KP-1475; also the induction of thymidine phosphorylase by thymidine occurs normally in KP-1475.

Nucleotide pools in KP-1469, KP-1475, and KP-1492

The pools of the eight nucleoside triphosphates and of UMP, UDP-glucose, ppGpp, and 5-phosphoribosyl-l-pyrophosphate in KP-1469, KP-1475, and KP-1492 grown in unsupplemented minimal medium are shown in Table VI. The regulatory mutation in KP-1475 results in a 4-fold increase in the UTP and the CTP pools. The ATP pool is doubled while the GTP pool is normal. The pools of ppGpp and 5-phosphoribosyl-l-pyrophosphate in KP-1475 are reduced to about one third of normal. The UMP pool is somewhat elevated in KP-1475, but it never reaches the extremely high levels found in *pyrH* mutants (Ingraham and Neuhard, 1972).

Table VI. Pools[a] of various nucleotides in KP-1469 and the two regulatory mutants grown in glucose minimal medium.

Strain	GTP	ATP	CTP	UTP	dGTP	dATP	dCTP	dTTP	PRPP	ppGpp	UMP	UDPG
KP-1469	2.48	5.37	1.04	1.51	0.20	0.38	0.19	0.33	0.97	0.08	1.12	2.49
KP-1475	2.62	10.53	4.53	6.69	0.23	0.56	0.39	0.87	0.35	0.03	2.18	7.37
KP-1492	2.70	6.65	2.09	2.81	0.29	0.38	0.25	0.63	0.56	0.04	1.83	4.23

[a]Pools are given as micromoles per gram (dry weight) of bacteria grown in glucose minimal at 37°C. PRPP, 5-phosphoribosyl-l-pyrophosphate; ppGpp, guanosine-3′,5′-bis pyrophosphate; UDPG, uridine diphosphate glucose.

Table VII. Mapping of the regulatory mutation in KP-1492 by Hfr mating[a].

Donor	Recipient	Selected marker	No. of recombinants		Number
Hfr K25	KP-1492,*arg-1824* *udp arg deoD,FUS*⁻	*udp*⁺	124	*Arg*⁺ *FUS*⁺ *DeoD*⁺	20
				Arg⁺ *FUS*⁺ *DeoD*⁻	52
				Arg⁺ *FUS*⁻ *DeoD*⁺	1
				Arg⁺ *FUS*⁻ *DeoD*⁻	5
				Arg⁻ *FUS*⁺ *DeoD*⁺	0
				Arg⁻ *FUS*⁺ *DeoD*⁻	1
				Arg⁻ *FUS*⁻ *DeoD*⁺	6
				Arg⁻ *FUS*⁻ *DeoD*⁻	39

[a]Mating was performed as described in **Materials and methods.** The *udp*⁺ recombinants were selected on minimal agar containing uridine (1 mg/ml) as carbon source and arginine (50 μg/ml), and they were tested for arginine prototrophy (Arg⁺), for their ability to use adenosine as carbon source (DeoD⁺), and for orotate phosphoribosyltransferase activity.
FUS⁻ indicates high levels of orotate phosphoribosyltransferase.
FUS⁺ indicates low (wild-type) levels of this enzyme.

Table VIII. Mapping of the regulatory mutation(s) by P22-transductions[a].

Cross No.	Donor	Recipient	Selected phenotype[a]	No. of transductants	Unselected phenotypes	Number
1[b]	KP-1492 FUS⁻	KP-1494 (*rpoB4,rpo-ts13*)	Ts⁺	58	FUS⁺ Rif^S	0
					FUS⁺ Rif^R	2
					FUS⁻ Rif^S	38
					FUS⁻ Rif^R	18
2[c]	KP-1475 Cs⁻	KP-1494 (*rpoB4,rpo-ts13*)	Ts⁺	143	Cs⁺ Rif^S	1
					Cs⁺ Rif^R	7
					Cs⁻ Rif^S	68
					Cs⁻ Rif^R	67

[a]Temperature insensitivity (Ts⁺) was selected at 42°C on agar plates supplemented with glucose and casamino acids. The plates were incubated for 2 days.
[b]The FUS phenotype was scored by assaying orotate phosphoribosyltransferase activity.
[c]The Cs⁻ character was scored as inability to grow on L-broth agar plates at 20°C.

The UTP and CTP pools are also increased in KP-1492, but are not as high as in KP-1475 (Table VI).

Genetic mapping of the mutation affecting pyr-gene regulation

Preliminary matings using HfrA and HfrK25 strains revealed that the regulatory defect in KP-1492 is located in the region between *udp* (84 min) and *deoD* (99 min). Hence an arginine-requiring derivative (*arg-1824::tet137*) of KP1492 was constructed by transduction. The *Tn10* element of this strain is inserted in the *argECBH* gene cluster at 89 min. In a gradient of clockwise transmission done by mating Hfr K25 with this arginine-requiring derivative, *udp*⁺ recombinants were selected. The recombinants were then tested for arginine prototrophy, for recombination of the *deoD* marker, and for content of orotate phosphoribosyltransferase. By assuming that distal marker inheritance decreases exponentially with map distance, the results of this experiment (Table VII) indicate that the regulatory mutation is located within a minute clockwise to the *argECBH* genes, i.e., close to the *rpoBC* gene cluster. Thus, KP-1494 (*rpoB4 rpo-ts13*) was used for a more precise mapping of the regulatory mutation. This strain contains two independent mutations in the *rpoBC* gene cluster. One of the mutations, *rpoB4,* confers rifampicin resistance to strains, whereas the other mutation, *rpo-ts13,* results in the synthesis of a thermolabile RNA polymerase. This latter mutation is either located in *rpoC* or, less likely, in the promoter distal part of *rpoB* (Boyd *et al.,* 1979; D. Boyd, pers. comm.).

Phage P22 grown on KP-1492 was used to transduce KP-1494 (*rpoB4 rpo-ts13*) to grow at 42°C. Fifty-eight Ts⁺ transductants were purified and analyzed for resistance to rifampicin and for their content of orotate phosphoribosyltransferase. From the results, shown in Table VIII, cross 1, it appears that the regulatory mutation of KP-1492 maps very closely to the *rpo-ts13* mutation. Most likely it is located between this mutation and *rpoB4,* since the two Ts⁺ transductants that had not obtained the regulatory mutation were resistant towards rifampicin.

Table VIII, cross 2, shows the results of a similar transduction where phage P22 grown on KP-1475 was used to transduce KP-1494 to grow at 42°C. Ts⁺ transductants were analyzed for resistance to rifampicin and for ability to grow on L-broth at 20°C, i.e., the Cs phenotype. A very high degree of linkage is evident between the mutation responsible for the cold sensitivity and the *rpo-ts13* mutation. Only eight of the 143 Ts⁺ transductants were Cs⁺. Furthermore, the results suggest that the mutation which causes the cold-sensitivity is located between *rpo-ts13* and *rpoB4* since only one of the eight Cs⁺ transductants was rifampicin-sensitive (Table VIII, cross 2). To assure that this low frequency of rifampicin-sensitive transductants in the Cs⁺ class is real, 23 additional Cs⁺ transductants were analyzed; only two of these were found to be sensitive to rifampicin.

To confirm that the Cs⁻ phenotype is caused by the same mutation as the one which is responsible for the altered *pyr*-gene expression in KP-1475, 32 of the Cs⁻ and 8 of the Cs⁺ transductants of cross 2 in Table VIII were assayed for aspartate transcarbamylase and orotate phosphoribosyltransferase and for resistance towards 5-fluorouracil. No segregation was observed between these phenotypic characters of KP-1475

(not shown). Hence, they seem to be caused by a single mutation, *rpo-FUR*. We did, however, notice that the *rpoB4* allele had a slightly beneficial effect on the growth rate of the recombinants containing the *rpo-FUR* mutation. Thus, while the *rpo-FUR, rpoB4*$^+$ transductants have a generation time in minimal medium of 100 min the *rpo-FUR, rpoB4* transductants have a generation time of 90 min. This may perhaps explain the observed difference in cotransduction frequencies between *rpoB4* and *rpo-ts13* in cross 1 and cross 2 of Table VIII.

In the transductions shown in Table VIII it was noticed that when the *rpoBC* region of KP-1492 was transduced into KP-1494 the recombinants obtained the phenotype of KP-1492 and not the phenotype of KP-1475. This means that the pseudoreversion of KP-1475 (*rpo-FUR*) which has generated KP-1492 (*rpo-FUS*) must have occurred in the *rpoBC* region very close to the site of the original mutation.

Discussion

In the present report we have described the isolation and characterization of a mutant, KP-1475, with a defect in the regulation of *pyr*-gene expression. The mutant was obtained by selecting for resistance to a combination of the two pyrimidine analogs, 5-fluorouracil and 5-fluorouridine. It possesses highly elevated, constitutive levels of aspartate transcarbamylase and orotate phosphoribosyltransferase, and contains very high pools of UTP and CTP. When grown in the presence of uracil the levels of aspartate transcarbamylase and orotate phosphoribosyltransferase are ~90-fold and 25–30-fold higher in KP-1475 than in the parent strain KP-1469.

KP-1475 grows considerably more slowly than the parent strain in all media (Table II). In L-broth at 20°C the mutant hardly shows any growth at all. A fast-growing pseudorevertant, KP-1492, was isolated from KP-1475. Phenotypically, KP-1492 is sensitive to 5-fluorouracil and 5-fluorouridine and its growth properties are nearly like wild-type *S. typhimurium*. However, the level of orotate phosphoribosyltransferase is still as high as in KP-1475, and it is still irrepressible by uracil. The level of aspartate transcarbamylase is 5-fold lower in KP-1492 than in KP-1475, but still considerably higher than in wild-type. The nucleotide pools of KP-1492 are between those found in wild-type and those of KP-1475.

By Hfr-matings and three-factor transductional crosses it was established that the mutation(s) responsible for the defective regulation of *pyr*-gene expression both in KP-1475 and in KP-1492 is located in the *rpoB4* and *rpo-ts13,* very close to the latter. The *rpo-ts13* mutation is most likely in the *rpoC* gene (Boyd *et al.,* 1979; D. Boyd, pers. comm.). Hence the regulatory *rpo* mutation may either be located in the *rpoC* gene, which encodes the β'-subunit of RNA polymerase, or in the promoter-distal part of the *rpoB* gene, encoding the β-subunit of RNA polymerase.

Concomitantly to the characterization of KP-1475, we have isolated rifampicin-resistant mutants of *S. typhimurium* with pleiotropic defects in the regulation of *pyr*-gene expression (Neuhard *et al.,* 1982). About 7% of all rifampicin-resistant mutants exhibited an arginine requirement when grown in the presence of uracil, i.e., they are uracil-sensitive. This phenotype was shown to be due to hyperrepression of the synthesis of carbamoylphosphate synthase caused by uracil addition. In the same selection we also found one mutant that showed 6-fold elevated synthesis of orotate phosphoribosyltransferase when grown in the presence of uracil. This mutant was isolated following nitrosoguanidine mutagenesis, and it was shown by transduction that its phenotypes were caused by two closely linked mutations: *rpoB*110, which is responsible for the Rif^R phenotype; and *rpo*-1100, which is located between *rpoB*110 and *rpo*-ts13 and causes high levels of orotate phosphoribosyltransferase. Thus the *rpo*-1100 mutation causes alterations in *pyr*-gene expression similar to those seen in KP-1475.

From the data of Tables III and V it appears that the *rpo-FUR* mutation in KP-1475 preferentially affects the rate of expression of the uridine repressible *pyr* genes, particularly *pyrB* and *pyrE*. The levels of the two cytidine repressible enzymes, dihydroorotase (*pyrC*) and dihydroorotate oxidase (*pyrD*), as well as the levels of five control enzymes appear to be largely identical in KP-1475 and KP-1469. The low molecular weight effector involved in uridine repression of *pyr*-gene expression has been shown to be a uridine nucleotide, most likely UDP or UTP (Schwartz and Neuhard, 1975). Therefore, the three following hypotheses can be offered to explain the phenotype of KP-1475: (i) The *rpo-FUR* mutation results in the synthesis of an RNA polymerase with decreased affinity (K_S) for UTP. Such an enzyme would lack UTP for RNA polymerization despite the high pools of UTP (and CTP). The derepressed synthesis of the pyrimidine biosynthetic enzymes in KP-1475 would then be the result of such a permanent "pyrimidine starvation" of RNA polymerization. If this explanation is correct, it implies that RNA polymerase itself, and not a hypothetical repressor, is the receptor which senses the cellular supply of pyrimidine nucleotides, and that the *pyr* genes have evolved in such a way that the frequency of their transcription is modulated by changes in RNA polymerase induced by variation in the pyrimidine nucleotide pools. Variations in the nucleoside triphosphate concentrations have been shown to alter the degree of pausing made by the transcribing RNA polymerase *in vitro* (Kassavetis and Chamberlin, 1981). (ii) The altered RNA polymerase of KP-1475 is defective in a hypothetical regulatory (allosteric) binding site for UTP, and the degree of saturation of this site determines the frequency of *pyr* gene transcription. (iii) RNA polymerase of KP-1475 is unable to perform a specific regulatory control step, like attenuation (Yanofsky, 1981) or interaction with an accessory protein required for proper modulation of *pyr*-gene expression. Studies of the *in vitro* transcription kinetics of the mutated RNA polymerase may help to decide between these three alternatives.

We have previously shown that leaky *guaB* mutants may be obtained by selecting for resistance to the pyrimidine analogs used in this work (Jensen, 1979). When grown in the absence of exogenous guanine, the GTP pool of such a mutant is reduced to ~20% of normal. At the same time the rate of *pyr*-gene expression is highly abnormal: *pyrB, pyrE,* and *pyrF* show enhanced expression while *pyrA, pyrC,* and *pyrD* show decreased rates of expression (Jensen, 1979, and unpubl. obs.). This may reflect a regulatory mechanism exploited by the cells to balance the rate of pyrimidine nucleotide biosynthesis to the supply of purine nucleotides, and may indicate that the regulatory protein, which monitors the pyrimidine nucleotide concentration in the cell, is also able to recognize purine nucleotides. Thus, the behaviour of the *guaB* mutant agrees well with the notion that RNA polymerase is involved in the regulation of *pyr*-gene expres-

sion as indicated by the data presented in this communication.

Materials and methods

Bacterial strains

The strains used are all derivatives of *S. typhimurium* LT-2 and are listed in Table I.

Growth conditions

Unless otherwise specified the cells were grown in the Tris-buffered minimal medium of Edlin and Maaløe, 1966, with the phosphate content reduced to 0.3 mM. Growth was measured turbidometrically at 436 nm (Jensen, 1979).

Pyrimidine starvation

Cells were grown in glucose minimal medium supplemented with uracil (20 μg/ml). At OD_{436} 0.5 aliquots (30 ml) were harvested by filtration, washed and resuspended either in 60 ml glucose medium containing 20 μg/ml uracil (for exponential growth) or in 30 ml glucose minimal medium (for uracil starvation). After 2h incubation cells were harvested for enzyme assays.

Nucleotide pool determinations

For nucleotide pool determinations the minimal medium was supplemented with [^{32}P]orthophosphate (25 μCi/ml). Nucleoside triphosphates and 5-phosphoribosyl-l-pyrophosphate were separated by t.l.c. as described by Jensen *et al.*, 1979. For the isolation of ppGpp the nucleoside triphosphate system of Neuhard *et al.*, 1965, was employed. UMP and UDP-glucose were isolated using the chromatographic system of Randerath and Randerath, 1965.

Preparation of extracts for enzyme assays

Exponentially growing cultures at a density of 3 x 10^8 cells/ml were harvested by centrifugation, washed with 0.9% chloride, and stored frozen at −20°C. For carbamoylphosphate synthase assays the cells were resuspended in 0.1 M potassium phosphate pH 7.5 containing 0.5 mM EDTA (10^{10} cells/ml), and disrupted by sonication for 1 min. After centrifugation 0.55 ml of the supernatant was desalted by passage through a 1.5 cm^3 column of Sephadex G-25 (Jensen *et al.*, 1979). For other assays the extracts were prepared in 0.10 M Tris-HCl, 2 mM EDTA as described (Jensen, 1979). Extracts were always assayed on the day of their preparation.

When a large number of mutants were screened for content of aspartate transcarbamylase and orotate phosphoribosyltransferase activities, 5-ml cultures of each were grown overnight with 0.04% glucose. In the morning the cells were harvested by centrifugation and disrupted by sonication in 0.5 ml, 0.10 M Tris-HCl, pH 7.6, 2 mM EDTA.

Enzyme assays

Unless otherwise stated, all assays were carried out at 37°C. One unit of activity is defined as the amount of enzyme that utilizes 1 nmol of substrate or produces 1 nmol of product per min under standard assay conditions. Specific activities are given as units per mg protein (Lowry *et al.*, 1951).

Aspartate transcarbamylase activity was determined by the method of Gerhard and Pardee, 1962. Orotate phosphoribosyltransferase, dihydroorotase, and dihydroorotate oxidase assays were performed as described by Schwartz and Neuhard, 1975. Carbamoylphosphate synthase activity was determined as described by Abdelal and Ingraham, 1975.

OMP decarboxylase activity was determined as follows: 25 μl of a mixture containing 0.20 M Tris-HCl pH 8.8, 12 mM $MgCl_2$, and 1 mM [6-^{14}C]OMP (0.5 Ci/mol) was prewarmed at 30°C. The reaction was initiated by adding 25 μl of bacterial extract containing ~2 mg protein/ml. At 10 s, 3.5 min, and 7 min 10 μl samples were withdrawn, mixed with 5 μl formic acid (1 M), and applied to polyethyleneimine impregnated cellulose thin-layer plates, containing 10 nmol UMP and 50 nmol EDTA in each start spot. The chromatograms were developed 10 cm in 0.9 M HCOOH - 0.3 M LiCl. The positions of UMP and OMP on the chromatograms were located in u.v. light, the areas were cut out and radioactivity was determined by liquid scintillation counting.

The following enzyme activities were determined by published procedures: 5-phosphoriboyl-l-pyrophosphate synthetase (Jensen *et al.*, 1979), ornithine transcarbamylase (Abdelal *et al.*, 1977), glucose 6-phosphate dehydrogenase (Kornberg and Horecker, 1955), thymidine phosphorylase by the periodate-thiobarbituric acid procedure (Schwartz, 1971), and histidinol dehydrogenase by the spectrophotometric assay of Martin *et al.*, 1971.

Transductions

In all transductions the integration deficient *Salmonella* phage p22 HT 105/*int201* was used. All transductants were purified to single colonies before analysis.

Hfr-matings

Equal volumes of a fullgrown L-broth culture of the recipient (~10^9 cells/ml) and of an exponential L-broth culture of the Hfr-strain (~10^8 cells/ml) were mixed and incubated at 37°C for 100 min without shaking. After this time the cells were pelleted by centrifugation, resuspended by vigorous shaking in the same volume of minimal medium and spread on selective plates.

Construction of strains containing the rpoB4, rpo-ts13 mutations

KP-1469 was transduced to rifampicin resistance with a P22 lysate prepared on SDB-223 (*rpoB4, rpo-ts13*) obtained from D. Boyd. A temperature-sensitive transductant, KP-1494 (*rpoB4, rpo-ts13*) and a temperature-insensitive transductant, KP-1495 (*rpoB4*) were left. Subsequently, KP-1494 was transduced to tetracycline resistance with a P22 lysate prepared on TT-137 (*arg-1824::tet137*). One transductant that had lost the rifampicin resistance but retained temperature sensitivity KP-1496 (*arg-1824::tet137, rpo-ts13*) was kept.

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