Regulation of *Salmonella typhimurium* *pyr* Gene Expression: Effect of Changing Both Purine and Pyrimidine Nucleotide Pools

By KAJ FRANK JENSEN

Enzyme Division, Institute of Biological Chemistry, Sølvgade 83, DK-1307 Copenhagen K, Denmark

(Received 29 September 1988; revised 26 December 1988; accepted 4 January 1989)

The synthesis of the pyrimidine biosynthetic enzymes is repressed by the pyrimidine nucleotide end-products of the pathway. However, purine nucleotides also play a role. In this study, I have measured expression of the *pyr* genes (*pyrA–E*) in *Salmonella typhimurium* strains harbouring mutations that permit manipulation of the intracellular pools of both pyrimidine and purine nucleotides. The results identify the effectory purine compound as being a guanine nucleotide; it is probably GTP, but it may be GDP or GMP. The synthesis of carbamoylphosphate synthase, encoded by *pyrA*, and particularly dihydroorotase, encoded by *pyrC*, and dihydroorotate dehydrogenase, encoded by *pyrD*, is stimulated by the guanine nucleotide, while the synthesis of aspartate transcarbamoylase, encoded by *pyrBI*, and orotate phosphoribosyltransferase, encoded by *pyrE*, is inhibited by guanine nucleotides. The regulatory pattern of each *pyr* gene is discussed in relation to present knowledge on gene structure and regulatory mechanism.

INTRODUCTION

The biosynthesis of UMP is catalysed by six enzymes (Fig. 1) encoded by the genes *pyrA–F*, which are unlinked on the chromosome of *Salmonella typhimurium*. The synthesis of these enzymes is under repressive control by pyrimidine compounds, but the levels of the different enzymes do not change in parallel. Schwartz & Neuhard (1975) suggested that the cellular concentration of CTP regulated the levels of dihydroorotase (*pyrC*) and dihydroorotate dehydrogenase (*pyrD*) while the cellular concentration of UTP regulated the synthesis of aspartate transcarbamoylase (*pyrBI*), orotate phosphoribosyltransferase (*pyrE*) and OMP decarboxylase (*pyrF*). However, Kelln *et al.* (1975) were unable to find any clear correlations between the level of the individual enzyme and the absolute concentration of any individual pyrimidine nucleoside triphosphate. Thus, these authors suggested that pool ratios rather than the concentrations of individual compounds might regulate *pyr* gene expression.

In a selection for mutants resistant to the toxic pyrimidine analogues 5-fluorouracil and 5-fluorouridine, a mutant with a leaky requirement for guanine was obtained. The mutation responsible (*guaB235*) caused the formation of a defective GMP synthase (Jensen, 1979). In the absence of guanine supplement the mutant grew exponentially, but slowly. At the same time it contained a perturbed pattern of purine nucleotide pools relative to the parental strain: the pool of GTP was reduced fivefold, the ATP pool was twice normal, the CTP pool was twofold reduced, while the UTP pool was slightly above normal. Furthermore, the levels of pyrimidine biosynthetic enzymes in the *guaB235* mutant were abnormal relative to the results of previous studies (Schwartz & Neuhard, 1975), since *pyrC* and *pyrD* gene expression was tenfold reduced while the expression of *pyrBI*, *pyrE* and *pyrF* was considerably elevated (Jensen, 1979). These observations indicated that purine nucleotides play an important role in regulating *S. typhimurium pyr* gene expression. Here, I have characterized the effect of purine nucleotides on *pyr* gene expression in more detail.

Table 1. *S. typhimurium* strains used

Strain	Genotype	Source or reference
KP1001	<i>Salmonella typhimurium</i> LT2	J. L. Ingraham
KP1294	<i>guaC234</i>	Hoffmeyer & Neuhard (1971)
KP1469	<i>cdd-9 cod-8 deoD201 udp-11</i>	Jensen (1979)
KP1470	<i>cdd-9 cod-8 deoD201 udp-11 guaB235(leaky)</i>	Jensen (1979)
KP1474	<i>metB406 guaB235</i>	Own collection
KP1476	<i>cdd-9 cod-8 deoD201 udp-11 guaC236(ts)</i>	Jensen (1979)
KP1478	<i>zag-207::Tn10</i>	Own collection
KP1479	<i>purE1522 pyrH1631</i>	
KP1482	<i>purE1522 pyrH1631 zag-207::Tn10</i>	Neuhard <i>et al.</i> (1982)
KP1489	<i>cdd-9 cod-8 deoD201 udp-11 guaA</i>	
KP1535	<i>purE1522</i>	Hoffmeyer & Neuhard (1971)
KP1548	<i>purE1522 deoD203</i>	Hoffmeyer & Neuhard (1971)
KP1722	<i>cdd-9 cod-8 deoD201 udp-11 guaB235 zag-207::Tn10</i>	
KP1723	<i>cdd-9 cod-8 deoD201 udp-11 guaB235 pyrH1631 zag-207::Tn10</i>	
KP1724	<i>cdd-9 cod-8 deoD201 udp-11 zag-207::Tn10</i>	
KP1725	<i>cdd-9 cod-8 deoD201 udp-11 pyrH1631 zag-207::Tn10</i>	

METHODS

Strains used. The strains used are all derivatives of *Salmonella typhimurium* LT2 and are given in Table 1. Strain KP1479 was selected in strain KP1535 by selecting for the ability to use 2,6-diaminopurine (200 µg ml⁻¹) as purine source on minimal agar plates. For unknown reasons approximately one-third of all clones in this type of selection are altered in UMP kinase (K. F. Jensen & P. Nygaard, unpublished results). The *pyrH* mutation of KP1479 was transduced into KP1470 (via KP1482) by using phage P22 and selecting for resistance to tetracycline (10 µg ml⁻¹) on agar plates containing glucose (0.2%), Casamino acids (0.2%), guanine (30 µg ml⁻¹) and thiamin (1 µg ml⁻¹). This resulted in formation of KP1722 and KP1723. KP1724 and KP1725 were made by a similar transduction using KP1469 as recipient.

Cell growth and media. Liquid cultures were grown at 37 °C in the Tris-buffered medium of Edlin & Maaløe (1966) with the phosphate concentration reduced to 0.3 mM. The medium was supplemented with glucose (0.2%) and thiamin (1 µg ml⁻¹) as well as amino acids, purines and pyrimidines as indicated. Solid media contained 1.5% (w/v) Difco agar and were based on the phosphate-buffered medium of Clark & Maaløe (1967) or L-broth (Miller, 1972). Transductions using phage P22HT/*int-201* were done as described previously (Neuhard *et al.*, 1982).

Determination of nucleoside triphosphate pools. [³²P]Orthophosphate (25 µCi) was added to 2 ml exponentially growing culture at OD₄₃₆ = 0.2. Growth was continued to OD₄₃₆ = 0.8. Then the cells were extracted by pipetting 0.5 ml culture into 0.1 ml 2 M-formic acid. Cell debris was removed by centrifugation after 30 min incubation in a melting icebath. The content of nucleoside triphosphates was determined by two-dimensional chromatography on polyethyleneimine-impregnated cellulose thin-layer plates on plastic sheets as described by Jensen *et al.* (1979).

Determination of pyrimidine biosynthetic enzymes. The cells were harvested in the exponential phase at OD₄₃₆ = 0.8. For determination of carbamoylphosphate synthase activity by the procedure of Abdelal & Ingraham (1975) the cells were resuspended in 0.1 M-potassium phosphate pH 7.1 and disrupted by ultrasonic treatment. For determination of the other enzymes the cells were suspended and disrupted in 0.1 M-Tris/HCl pH 7.5, 2 mM-EDTA. Aspartate transcarbamoylase activities were determined by the colorimetric procedure of Gerhart & Pardee (1962). Dihydroorotase and dihydroorotate dehydrogenase activities were measured as described by Schwartz & Neuhard (1975), while the activity of orotate phosphoribosyltransferase was measured according to Poulsen *et al.* (1983). As a control the activity of glucose-6-phosphate dehydrogenase was assayed according to Kornberg & Horecker (1955).

One enzyme unit is defined as the amount of enzyme that converts 1 nmol substrate to product min⁻¹.

Protein concentrations were determined by the Lowry method.

RESULTS AND DISCUSSION

Effects of purine nucleotides on pyr gene expression

I wanted to test if the levels of pyrimidine nucleotide biosynthetic enzymes characteristic for the *guaB235* mutant could be reproduced by other means of manipulating the purine nucleotide pools. Thus, a sample of mutants with other defects in purine nucleotide biosynthesis or interconversion (Table 1) were analysed for content of nucleoside triphosphates and pyrimidine biosynthetic enzymes (see Table 2). The two strains KP1535 and KP1548 require purines due to

Fig. 1. Pathways for the biosynthesis and interconversion of pyrimidine nucleotides in *S. typhimurium*. The enzymes are identified by their corresponding gene designations as follows: *argI*, ornithine transcarbamoylase (EC 2.1.3.3); *cdd*, cytidine deaminase (EC 3.5.4.5); *cod*, cytosine deaminase (EC 3.5.4.1); *pyrA*, carbamoyl-phosphate synthase (EC 6.3.5.5); *pyrB*, aspartate transcarbamoylase (EC 2.1.3.2); *pyrC*, dihydroorotase (EC 3.5.2.3); *pyrD*, dihydroorotate dehydrogenase (EC 1.3.3.1); *pyrE*, orotate phosphoribosyltransferase (EC 2.4.2.10); *pyrF*, orotidine 5'-phosphate (OMP) decarboxylase (EC 4.1.1.23); *pyrG*, CTP synthase (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).

mutations in *purE* (see Fig. 2). This requirement can be satisfied by adenine, hypoxanthine or guanine. However, the strains contain widely different pools of purine nucleotides depending on the added purine. Following growth on adenine the cells contained high pools of adenine nucleotides (exemplified by ATP) and low pools of guanine nucleotides (exemplified by GTP). The nucleotide pools were more perturbed from the wild-type (KP1001) pattern with KP1548 than with KP1535. This is because KP1548 carries a *deoD* mutation that destroys one of two pathways for the conversion of adenine nucleotides to guanine nucleotides (see Fig. 2, and Hoffmeyer & Neuhaard, 1971). Furthermore, I used a strain (KP1294) where the conversion of guanine nucleotides to IMP (and hence to adenine nucleotides) is prevented due to a *guaC* mutation, as well as strain KP1474, which carries the leaky *guaB235* mutation. The results are collected in Table 2.

The data clearly demonstrate that purine nucleotides play an important role in the regulation of the synthesis of pyrimidine biosynthetic enzymes in *S. typhimurium*. As exemplified for dihydroorotase in Fig. 3, the specific activity of this enzyme is linearly proportional to the intracellular concentration of GTP and inversely correlated with the ATP pool under these conditions, where the pyrimidine nucleotide pools remained relatively constant. Very similar curves are obtained when the specific activity of dihydroorotate dehydrogenase (of Table 2) is plotted against the pools of the individual nucleoside triphosphates (not shown). In contrast, the levels of aspartate transcarbamoylase or orotate phosphoribosyltransferase are high when the GTP pool is low and vice versa.

Identification of the purine nucleotide effector as a guanine nucleotide

In the experiments described above an inverse correlation between the ATP and GTP pools was always seen. To break this correlation, cultures of a *guaA* and a *guaB* mutant were allowed to exhaust a limiting supply of guanine (Fig. 4). The GTP pool fell abruptly following the break in

Fig. 2. Pathways for the biosynthesis and interconversion of purine nucleotides in *S. typhimurium*. The enzymes are identified by the corresponding gene symbols as follows: *apt*, adenine phosphoribosyltransferase (EC 2.4.2.7); *deoD*, purine-nucleoside phosphorylase (EC 2.4.2.1); *gpt*, guanine phosphoribosyltransferase (EC 2.4.2.-); *gsk*, guanosine kinase (2.7.1.-); *guaA*, GMP synthase (EC 6.3.4.1); *guaB*, IMP dehydrogenase (EC 1.1.1.205); *guaC*, GMP reductase (EC 1.6.6.8); *purE*, phosphoribosylaminoimidazole carboxylase (EC 4.1.1.21); *purF*, amidophosphoribosyltransferase (EC 2.4.2.14); *hpt*, hypoxanthine phosphoribosyltransferase (EC 2.4.2.8). Other abbreviations: AIR, phosphoribosyl-5-aminoimidazole; AICAR, phosphoribosyl-5-amino-4-imidazolecarboxamide.

Table 2. Pools of nucleoside triphosphates and levels of pyrimidine biosynthetic enzymes in strains of *S. typhimurium* with defects in purine biosynthesis or interconversion

Strain (and relevant mutations)	Purine* (0.1 mM)	Pool of: [$\mu\text{mol (g dry weight)}^{-1}$]				Specific activity of†: [units (mg protein) $^{-1}$]				
		GTP	ATP	CTP	UTP	ATCase (<i>pyrBI</i>)	DHOase (<i>pyrC</i>)	DHOox (<i>pyrD</i>)	OPRTase (<i>pyrE</i>)	G-6-P dehyd
KP1001 (wt)	—	2.6	5.5	1.1	1.4	37	88	29	50	ND
KP1535 (<i>purE</i>)	Ad	1.1	12.7	0.8	1.6	87	35	13	160	233
	Hx	3.6	7.5	1.4	1.7	55	90	40	60	292
	Gu	4.3	4.3	1.4	1.8	20	127	44	33	301
KP1548 (<i>purE deoD</i>)	Ad	0.3	14.1	0.8	1.1	157	12	10	246	ND
	Hx	2.7	5.4	1.1	1.1	45	93	35	52	333
	Gu	3.1	2.6	1.2	1.4	16	93	43	58	292
KP1294 (<i>guaC</i>)	—	3.3	6.5	1.4	2.1	24	122	54	43	239
	Gu + GR	5.0	2.9	1.5	1.8	20	163	67	36	328
KP1474 (<i>guaB</i>)	—	0.6	14.1	1.0	1.8	807	21	15	648	204
	Gu	4.8	5.9	1.9	2.1	70	141	58	80	372

ND, Not determined.

* Purines: Ad, adenine; Hx, hypoxanthine; Gu, guanine; GR, guanosine.

† Enzymes: ATCase, aspartate transcarbamoylase; DHOase, dihydroorotase; DHOox, dihydroorotate dehydrogenase; OPRTase, orotate phosphoribosyltransferase; G-6-P dehyd, glucose-6-phosphate dehydrogenase.

Fig. 3. Specific activity of dihydroorotase as a function of individual nucleoside triphosphate pools. Points obtained with different strains are shown with different symbols: KP1001 (●), KP1535 (×), KP1548 (○), KP1294 (□), and KP1474 (△). The data are from Table 2.

the growth curves for both strains, whereas the CTP and the UTP pools remained unchanged or increased slightly (Fig. 4). However, the two strains behaved differently with respect to ATP. The ATP pool of KP1476 (*guaB*) began to accumulate immediately after the exhaustion of guanine (Fig. 4c) while the ATP pool of strain KP1489 (*guaA*) decreased initially and only later began to increase (Fig. 4d). In both strains the levels of aspartate transcarbamoylase and orotate phosphoribosyltransferase increased considerably, while the levels of dihydroorotase and dihydroorotate dehydrogenase remained constant or decreased slightly (Fig. 4a, b). Thus, these data show that a guanine nucleotide (GTP, GDP or GMP) rather than an adenine nucleotide plays a role as effector of *pyrBI* and *pyrE* expression. IMP and XMP have no effect since a very high pool of IMP accumulated in KP1476 (*guaB*) and a very high XMP pool accumulated in KP1489 (*guaA*) during guanine starvation (not shown). The data are not as conclusive for *pyrC* and *pyrD* expression as they are for *pyrBI* and *pyrE*, since repression of the synthesis of dihydroorotase and dihydroorotate dehydrogenase requires bacterial growth in order to be reflected in the enzyme levels.

Turnbough (1983) observed that the accumulation of ppGpp, induced by partial amino acid starvation, prevented derepression of aspartate transcarbamoylase synthesis in *Escherichia coli*,

Fig. 4. Pyrimidine nucleotide biosynthetic enzymes and nucleoside triphosphate pools as a function of time after onset of guanine starvation of a *guaB* strain (KP1476; a and c) and a *guaA* strain (KP1489; b and d). The cells were grown in a minimal medium supplemented with adenine ($15 \mu\text{g ml}^{-1}$) and guanine ($6 \mu\text{g ml}^{-1}$) and allowed to exhaust the guanine supply (time equals zero). At various times, samples were withdrawn for determination of nucleotide pools and pyrimidine nucleotide biosynthetic enzymes. In (c) the ATP pool is depicted as one-quarter of its actual size.

and similarly, a repressive effect of ppGpp on *carAB* expression was noticed by Bouvier *et al.* (1984). However, the concentration of ppGpp was considered unlikely as the primary cause of the phenomena observed here, because it appeared low [$\leq 0.1 \mu\text{mol (g dry weight)}^{-1}$] and constant in all experiments. This was judged by inspection of the autoradiograms. The magic spot (ppGpp) could easily be seen in these pictures. However, as it migrated at a position close to the origin where background is high and variable it was unreliable to determine the content of

Table 3. Pools of nucleoside triphosphates and levels of pyrimidine biosynthetic enzymes in strains of *S. typhimurium* with defects in either purine or pyrimidine interconversion

The background for all experiments was that of KP1469 (*cdd-9 cod-8 deoD201 udp-11*).

Strain (and relevant mutations)	Addn* (0.1 mM)	Pool of: [$\mu\text{mol (g dry weight)}^{-1}$]				Specific activity of†: [units (mg protein) $^{-1}$]				
		GTP	ATP	CTP	UTP	CPSase (<i>pyrA</i>)	ATCase (<i>pyrBI</i>)	DHOase (<i>pyrC</i>)	DHOox (<i>pyrD</i>)	OPRTase (<i>pyrE</i>)
KP1723	–	0.59	8.45	0.48	0.55	44	1860	45	50	826
(<i>pyrH1631</i>	CR	0.57	7.89	1.92	0.52	27	1955	14	25	702
<i>guaB235</i>)	Ura	0.68	8.39	0.48	0.54	42	2411	46	55	879
	Gu	3.53	3.45	0.95	0.60	48	826	74	126	420
	Gu, CR	3.60	3.42	7.0	0.28	61	2702	28	74	518
KP1722	–	0.36	9.78	0.63	1.24	9	134	21	13	413
(<i>guaB235</i>)	CR	0.30	9.80	0.78	1.20	7	184	17	9	454
	Ura	0.27	10.27	0.61	1.48	5	104	15	16	327
	Gu	2.67	4.17	1.12	1.24	18	33	65	78	38
	Gu, CR	2.25	3.37	2.10	1.16	15	28	32	34	34
	Gu, Ura	2.26	4.27	1.56	1.67	5	17	43	33	11
KP1725	–	1.62	3.12	0.45	0.43	40	992	73	133	401
(<i>pyrH1631</i>)	CR	2.78	4.67	4.32	0.23	40	2662	29	69	439
KP1724	–	1.67	4.08	0.87	1.49	15	34	58	39	37
	CR	1.37	3.57	1.40	1.35	14	52	37	23	26
	Ura	1.61	4.51	1.16	1.63	5	11	46	25	13

* Additions: –, none; CR, cytidine; Ura, uracil; Gu, guanine.

† Enzymes: CPSase, carbamoylphosphate synthase; ATCase, aspartate transcarbamoylase; DHOase, dihydroorotase; DHOox, dihydroorotate dehydrogenase; OPRTase, orotate phosphoribosyltransferase. The level of glucose-6-phosphate dehydrogenase was measured in all experiments; the lowest value was 398 units mg^{-1} and the highest value was 550 units mg^{-1} .

radioactivity in the spot. Further evidence that ppGpp plays at most a minor role under these conditions stems from the fact that *guaB* mutants of *E. coli* which are relaxed (*relA*) behave like the *guaB* mutants in the stringent (*relA*⁺) *Salmonella* strain used herein with regard to *pyrE* expression (Poulsen & Jensen, 1987).

Changing the pools of both purine and pyrimidine nucleotides

For the purpose of creating changes in both the pyrimidine and purine nucleotide pools a set of strains was constructed by introducing *guaB* and *pyrH* alleles in the background of KP1469 (Table 1). One strain (KP1724) contained none of the mutations. Another strain (KP1722) carried the leaky mutation *guaB235*. The third strain (KP1725) harboured a leaky mutation, *pyrH1631*, and grew exponentially with low pools of UTP. In the fourth strain (KP1723) both these mutations, *guaB235* and *pyrH1631*, were present.

This set of bacteria was cultivated either in unsupplemented minimal medium or in a medium containing guanine, cytidine or uracil. The cells were harvested and the content of nucleoside triphosphates and pyrimidine biosynthetic enzymes was determined. In these experiments the cultures were grown in the same batch of medium to minimize experimental uncertainty. The results are collected in Table 3.

Since each *pyr* gene shows its own characteristic regulatory pattern it seems most convenient to discuss the genes individually.

Regulation of *pyrC* expression

According to Schwartz & Neuhaud (1975) the level of dihydroorotase, encoded by *pyrC*, is negatively correlated with the intracellular concentration of CTP. The data in Fig. 3 and Table 2 indicated that the level of this enzyme is close to being proportional to the GTP pool. Thus it seemed reasonable to plot the levels of dihydroorotase, taken from Table 3, as a function of either the GTP pool or the CTP pool (Fig. 5*a, b*). It appears that both of these plots are quite

Fig. 5. Specific activity of dihydroorotase as a function of the GTP pool (a), the CTP pool (b), and the [GTP]/[CTP] pools ratio (c). Data from Table 3.

scattered. However, when the enzyme levels are depicted versus the pools ratio ([GTP]/[CTP]) the data points meet nicely on a curve with very little scatter (Fig. 5c). This indicates that the pools of GTP and CTP account for the majority of the variation in *pyrC* gene expression observed under these conditions. Accordingly, GTP stimulates *pyrC* expression, while CTP inhibits it.

Neuhard *et al.* (1986) and Kelln & Neuhard (1988) have found that *pyrC* gene expression in *S. typhimurium* is regulated primarily at the translation level. They found that the *pyrC* mRNA chain can form a secondary structure around the ribosome-binding site. Accordingly, the authors anticipated the existence of a regulatory protein which may bind to the mRNA chain and stabilize the conformation which inhibits protein synthesis (Kelln & Neuhard, 1988). If this explanation is valid the data in this paper suggest that GTP competes with CTP and makes the 'open' *pyrC* mRNA conformation favourable. Thereby a high intracellular GTP concentration promotes translation of the *pyrC* cistron even at high CTP pools.

Regulation of *pyrD* gene expression

The level of dihydroorotase dehydrogenase was previously found to be negatively correlated with the intracellular concentration of CTP, and to change in parallel with *pyrC* gene expression (Schwartz & Neuhard, 1975). According to the data in Table 2, dihydroorotase dehydrogenase, like dihydroorotase, is synthesized in proportion to the GTP pool when no deliberate changes are made in the pools of pyrimidine nucleotides. Thus it seemed reasonable to assume that the level of this enzyme would be explained by the [GTP]/[CTP] pools ratio as seen for dihydroorotase (Fig. 5). However, this turned out not to be the case: when the enzyme levels were depicted as a function of the [GTP]/[CTP] ratio all points obtained by analysis of cultures grown with low UTP pools fell above the curve defined by the other points (not shown). Instead I have plotted the levels of dihydroorotase dehydrogenase in Table 3 against the GTP pool, the UTP pool, or the [GTP]/[UTP] ratio (Fig. 6). The data points in the first two figures (6a and b) are scattered. However, in the last figure (6c) the data points meet on two curves: the upper curve is seen for the points obtained from cells grown with low or normal CTP pools, while the lower curve is seen for the points obtained from cultures grown in the presence of cytidine, which causes a very high concentration of CTP to accumulate in the cells. Thus, it appears that the synthesis of dihydroorotase dehydrogenase is influenced by three nucleotides: CTP and UTP inhibit *pyrD* expression, while GTP stimulates it.

As for the *pyrC* gene, the ribosome-binding sites of the *pyrD* gene of *E. coli* (Larsen & Jensen, 1985) and *S. typhimurium* (R. Kelln, personal communication) are preceded by secondary structures in the mRNA chain. Therefore it is possible that the CTP and GTP effects on the

Fig. 6. Specific activity of dihydroorotate dehydrogenase as a function of the GTP pool (a), the UTP pool (b), and the [GTP]/[UTP] pools ratio (c). Data from Table 3.

pyrD gene are exerted by a mechanism involving the mRNA translation efficiency, as for *pyrC*. However, in addition *pyrD* gene expression (in *E. coli*) is regulated at the level of transcription and sensitive to changes in the UTP pool (Jensen *et al.*, 1984).

Regulation of *pyrA* gene expression

The synthesis of carbamoylphosphate synthase, which participates in the synthesis both of pyrimidine nucleotides and of arginine (Fig. 1), appears to be stimulated by a guanine nucleotide. The UTP pool as well as the CTP pool seems to repress the synthesis of this enzyme (Table 3). These pyrimidine effects are in agreement with recent results of Kilstrup *et al.* (1988).

Control of *pyrBI* and *pyrE* gene expression

Expression of *pyrBI* and *pyrE* is influenced by the pools of UTP and GTP, both causing repression (Tables 2 and 3). However, neither the individual pools nor any ratio between these satisfactorily correlate with the enzyme levels (Table 3).

The *pyrBI* and *pyrE* genes encode the two subunits of aspartate transcarbamoylase and the subunit of orotate phosphoribosyltransferase, respectively. The expression level of both genes is regulated by transcription attenuation and is inversely correlated with the pool of UTP (Turnbough *et al.*, 1983; Roland *et al.*, 1985; Clemmesen *et al.*, 1985; Michaels *et al.*, 1987; Poulsen *et al.*, 1983, 1984; Bonekamp *et al.*, 1984; Neuhard *et al.*, 1985; Jensen *et al.*, 1986a; Poulsen & Jensen, 1987). Since attenuation is regulated by the mRNA chain elongation rate, rather than by the free UTP pool (Jensen *et al.*, 1982, 1986b), it may not be surprising that *pyrBI* and *pyrE* expression also responds to changes in the intracellular concentration of GTP. The K_m values for the interaction between the elongating RNA polymerase and both UTP and GTP are high relative to the intracellular concentrations of these compounds, while the intracellular concentrations of ATP and CTP are substantially above the concentrations needed for half-maximal saturation of RNA polymerase (Kingston *et al.*, 1981; Jensen *et al.*, 1986b). Therefore it is reasonable that the UTP and GTP pools, rather than the ATP and CTP pools, are of importance for *pyrBI* and *pyrE* expression.

Expression of the *pyrF* gene

The levels of OMP decarboxylase were not measured in these experiments. However, from previous studies (Jensen, 1979) it is known that the activity of this enzyme is high in a leaky *guaB* mutant grown with a low pool of GTP. Therefore, a guanine nucleotide appears to inhibit *pyrF* gene expression.

Conclusion

The present paper is rather descriptive and does not provide any clues to the understanding of *pyr* gene control at the molecular and mechanistic level. However, the data show that the *pyr* genes are controlled in a highly complex manner by the nucleotide pools in *S. typhimurium* and that each gene shows its own characteristic regulatory pattern. Hopefully, the data may be useful in the design of future experiments aimed at gaining a complete understanding of the control of pyrimidine nucleotide biosynthesis at the molecular level.

I appreciate the technical help of Lise Schack and thank André Piérard and Jan Neuhard for suggestions to improve the manuscript. The work received financial support from the Danish Natural Science Foundation.

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