JOURNAL OF BACTERIOLOGY, Sept. 1975, p. 851-854

Vol. 123, No. 3
Printed in U.S.A.

pyrR Identical to *pyrH* in *Salmonella typhimurium*: Control of Expression of the *pyr* Genes

JUST JUSTESEN AND JAN NEUHARD*

Enzyme Division, University Institute of Biological Chemistry B, Copenhagen, Denmark

Received for publication 2 June 1975

Mutants of *Salmonella typhimurium* showing constitutive synthesis of the pyrimidine biosynthetic enzymes coded for by the *pyrA-F* genes (G. A. O'Donavan and J. C. Gerhart, 1972) have been reinvestigated. The high rate of expression of the *pyrB-F* genes in these mutants as well as their pyrimidine excretion is shown to be due to mutations in the gene *pyrH* encoding uridine 5'-monophosphate kinase. Thus, the term *pyrR* used for these mutants should be replaced by the designation *pyrH*.

In *Salmonella typhimurium* and in *Escherichia coli* the expression of the nonlinked *pyr* genes, *pyrA-F*, is regulated by the endogenous pool of pyrimidine nucleotides (1, 4, 11, 12, 14). Recently an attempt was made by O'Donovan and Gerhart (10) to isolate regulatory mutants in *S. typhimurium* showing constitutive synthesis of the six pyrimidine biosynthetic enzymes coded for by *pyrA-F*. Two selections were used: (i) they isolated mutants that would feed the pyrimidine requirement of a pyrimidine requiring tester strain, and (ii) they selected mutants that were resistant to the two pyrimidine analogues 5-fluouracil (FU) and 5-flurouridine (FUR). Among the mutants they obtained were some that contained high levels of the enzymes responsible for the biosynthesis of uridine 5'monophosphate (UMP) even when the cells were grown in the presence of exogenous uracil. Since the mutation responsible for this phenotype was not co-transducible with any of the genes *pyrA-F*, it was tentatively classified as being in a regulatory gene, which they termed *pyrR*. Preliminary mapping of three *pyrR* mutants located *pyrR* around 10 min on the *Salmonella* chromosome (10).

S. typhimurium strains containing mutations in the structural gene, *pyrH*, for UMP kinase (EC 2.7.4.-) have been characterized (7). Since UMP kinase is essential for growth, only conditional *pyrH* mutants can be isolated. Owing to decreased UMP kinase activity *pyrH* mutants grow with greatly reduced endogenous levels of uridine-5'-triphosphate (UTP). This leads to derepression of the rate of synthesis of the pyrimidine biosynthetic enzymes (7, 12), which in turn results in overproduction of UMP and excretion of pyrimidines into the growth medium. As a result of these alterations in pyrimidine metabolism *pyrH* strains are resistant to FU and FUR. The gene *pyrH* is located at 12 min on the *Salmonella* chromosome (7).

The similarities in phenotypes between the *pyrR* and *pyrH* mutants as well as the chromosomal localization of the two genes led to the present investigation, which shows that the *pyrR* mutants contain partially defective UMP kinases. Thus the term *pyrR* for these mutations is misleading and should be replaced with the term *pyrH*.

MATERIALS AND METHODS

Chemicals. Nucleic acid bases, nucleosides, and nucleotides were the products of Sigma Chemical Co. (St. Louis, Mo.), [U-^{14}C]UMP was from The Radiochemical Center (Amersham, England), and carrier-free [^{32}P]orthophosphate in 0.1 N HCl was obtained from Atomenergikommissionens Forsøgsanlaeg (Risø, Denmark).

Bacterial strains and growth conditions. All strains used are derivatives of *S. typhimurium* LT-2. They are listed in Table 1. Cells were grown with vigorous aeration at 37 C in tris(hydroxymethyl)aminomethane (Tris) minimal medium (5) containing uracil (50 μg/ml), 0.2% Norite-treated vitamin-free casein hydrolysate (Difco), and 0.2% glucose. Cytidine was used in concentrations of 20 μg/ml. Growth was monitored at 436 nm in an Eppendorf photometer model 1101M.

Nucleoside triphosphate pools. Nucleoside triphosphate pools were determined in cultures grown for two generations in liquid medium in the presence of [^{32}P]orthophosphate (specific activity in the medium: 10 μCi per μmol). Extraction and chromatographic separation of the nucleoside triphosphates has been described by Neuhard and Thomassen (9).

Toluene treatment and extraction of cells. Toluene treatment. Glucose-limited overnight cultures (0.05% glucose) were harvested by centrifugation, washed once with 0.9% NaCl, and resuspended in one-twentieth the volume of 0.1 M Tris-hydrochlo-

TABLE 1. *Bacterial strains*

Strains[a]	Genotype[b]	Selection method	Reference
KP1001	Wild type		
JL1018	*pyrF146*		Beck et al. (2)
JL1269	*cdd-7 pyrH1609*		Ingraham and Neuhard (7)
HD-11 (KP1378)	*pyrR* (*pyrH1614*)	FU^r, FUR^r [c]	O'Donovan and Gerhart (10)
HD-12 (KP1380)	*pyrR* (*pyrH1616*)	FU^r, FUR^r	O'Donovan and Gerhart (10)
HD-46 (KP1381)	*pyrR* (*pyrH1617*)		O'Donovan and Gerhart (10)
HD-47 (KP1379)	*pyrR* (*pyrH1615*)	Excretion[d]	O'Donovan and Gerhart (10)
HD-58 (KP1263)	*pyrR* (*pyrH1613*)	Excretion	O'Donovan and Gerhart (10)
HD-87 (KP1382)	*pyrR* (*pyrH1618*)	FU^r, FUR^r	O'Donovan and Gerhart (10)
HD-11 *cdd* (KP1407)			R. Kelln[e]

[a] Strain numbers in parenthesis are the numbers in the collection of J. Neuhard.

[b] Genotypes in parenthesis are assigned by the present authors.

[c] Selected as mutants resistant to 1 μg/ml of each of the two pyrimidine analogues FU and FUR (10).

[d] Selected as mutants capable of forming a halo of secondary growth surrounding the colony, when plated on minimal plates seeded with 10^8 cells of a pyrimidine-requiring indicator strain (10).

[e] This mutant was isolated by R. Kelln, Texas A & M University, Tex., as a 5-fluorodeoxycytidine-resistant derivative of KP1378.

ride, pH 7, 8, 10 mM $MgCl_2$ (about 6×10^8 to 8×10^8 cells per ml). Toluene was added to the suspensions (10 μl/ml suspension) and they were incubated at 37 C for 15 min in stoppered tubes followed by 15 min of incubation at 0 C with shaking. The toluene-treated suspensions were used for assays within a few hours.

Sonic disruption. Two hundred milliliters of stationary night cultures were harvested by centrifugations, washed once with 0.9% NaCl, and resuspended in 4 ml 0.1 M Tris hydrochloride, pH 7.8, 10 mM $MgCl_2$, and 2 mM mercaptoethanol. The suspensions were sonically oscillated three times for 30 s (Branson Sonifier) and centrifuged for 30 min at $20,000 \times g$ in a Sorvall refrigerated centrifuge at 0 C. The supernatant fluids were treated with one-fifth volume of 10% streptomycin sulfate. After 30 min at 0 C the suspensions were centrifuged and the supernatant fluids were dialyzed for 2 h against 200 volumes of 0.1 M Tris-hydrochloride, pH 7.8, 10 mM $MgCl_2$, and 2 mM mercaptoethanol. The dialyzed extracts were used directly for enzyme assays.

Enzyme assays. For assays either toluene-treated cells or streptomycin-treated sonic extracts were used. They gave similar results.

ATCase. Aspartate transcarbamylase (ATCase) was assayed according to Gerhart and Pardee (6).

UMP kinase. UMP kinase activity was determined as described by Ingraham and Neuhard (7). One unit of enzyme is the amount that catalyzes the conversion of 1 nmol of substrate per min at 37 C. Specific activities are expressed as units per milligram of protein.

Protein. Proteins were determined by the method of Lowry et al. (8).

Heat treatment. One milliliter of streptomycin-treated sonic extract (3 to 6 mg of protein/ml) was placed in a glass tube in a waterbath at 62 C. At times, 0, 2, 5, 10, and 20 min, 100-μl samples were transferred to centrifuge tubes kept in an ice bath. After centrifugation the supernatant fluids were assayed for UMP kinase activity.

RESULTS AND DISCUSSION

FUR is toxic to *S. typhimurium* because it is converted by the cells to 5-fluorouridine-5'-triphosphate which may be incorporated into ribonucleic acid rendering it nonfunctional. Thus, mutants unable to convert FUR to 5-fluorouridine-5'-monophosphate are resistant to this analogue. It was previously shown (3) that FUR may be converted to F-UMP by two pathways in *S. typhimurium*: (i) by direct phosphorylation catalyzed by uridine kinase (EC 2.7.1.48), and (ii) by phosphorolysis to FU followed by conversion of FU to 5-fluorouridine-5'-monophosphate by the action of uracil phosphoribosyltransferase (EC 2.4.2.9). Thus, two mutations, *udk* and *upp*, are required to make the cells resistant to FUR. Recently, however, it was observed, independently by O'Donovan and Gerhart (10) and by Ingraham and Neuhard (7) that single mutations, resulting in overproduction of pyrimidines may also cause resistance to FUR. In one class (7) the mutation causing pyrimidine overproduction was shown to be in the structural gene, *pyrH*, coding for the essential enzyme UMP kinase (EC 2.7.4.-). Cells containing a partially defective UMP kinase contain a low pool of UTP and, as a result, they contain high levels of the pyrimidine biosynthetic enzymes (7, 12). The derepressed synthesis of the biosynthetic enzymes explains the excess production and excretion of pyrimidines by these mutants. Co-transduction studies, using the general transducing phage P1, showed 30% linkage between the *pyrH* and the *pan* loci on the *Salmonella* chromosome (7).

The second class of mutants resistant to FU and FUR due to overproduction of pyrimidines has also high endogenous levels of the pyrimidine biosynthetic enzymes, even when grown in the presence of uracil in the growth medium. The affected gene was shown by P22 transduction to be unlinked to any of the structural genes, *pyrA-F*. Preliminary mapping studies by conjugation placed the mutations between *leu* and *pro* on the *Salmonella* chromosome ([10]; J. Roth, personal communication). It was suggested that this class of mutants were mutated in a gene, *pyrR*, coding for a protein involved in the control of expression of the *pyr* genes (10). Since recent evidence indicate that the six *pyr* genes do not belong to one single regulon (12, 13) this possibility seems at present unlikely. An alternative explanation is that they all, like the first class of mutants, are mutated in the structural gene for UMP kinase, and thus are *pyrH* mutants.

Table 2 (columns 3 and 4) gives the levels of ATCase and UMP kinase in the six HD strains investigated. For comparison we have included the data obtained with a known *pyrH* mutant JL1269. In all the *pyrR* strains the specific activities of UMP kinase are less than 15% of that in the wild type. Three alternative explanations should be considered to explain the simultaneous appearance of high levels of ATCase (as well as the other pyrimidine biosynthetic enzymes [12]) and the low levels of UMP kinase: (i) the mutation affects a gene (*pyrR*) involved in the regulation of expression of one or more of the *pyrA-F* genes, and that the gene product(s) of one or more of these genes inhibits the expression of the *pyrH* gene; (ii) the high levels of the pyrimidine biosynthetic enzymes interfere with the in vitro assay of UMP kinase; (iii) the *pyrR* gene is identical to *pyrH*, which means that the HD strains, like JL1269, are mutated in the structural gene for UMP kinase.

The first alternative cannot, at present, be discarded. However, the physiological significance of such a regulatory mechanism is not apparent. The second alternative mentioned above is ruled out by an experiment in which the specific activities of ATCase and UMP kinase were determined in a pyrimidine auxotrophic strain, JL1018, after 2 h of pyrimidine starvation. The results obtained (Table 3) show that derepression of the synthesis of the pyrimidine biosynthetic enzymes (represented by the ATCase values in Table 3) does not affect the specific activity of UMP kinase.

According to the third explanation, the low UMP kinase activities of the HD strains are the result of mutations in *pyrH* rendering the UMP kinases of the strains partially defective. If so, it would be highly probable that the structurally altered enzymes show increased temperature sensitivity in vitro, as was found for the UMP kinase of JL1269 (7). The stability of UMP kinase activity to heating at 62 C was tested using crude extracts from HD-11, HD-12, HD-47, HD-87, and the wild-type KP-1001. The UMP kinases of the mutants are significantly more labile to heating than is the wild-type enzyme, indicating that the HD strains contain structurally altered UMP kinases (Fig. 1).

If the increased rate of synthesis of the pyrimidine biosynthetic enzymes of the HD strains is a result of their low UMP kinase

TABLE 2. *ATCase, UMP kinase, and pyrimidine nucleoside triphosphate contents of the pyrR mutants*

Strain	Genotype	ATCase activity[a]	UMP kinase activity[b]	Pools (μmol/g [dry wt])	
				UTP	Cytidine 5'-triphosphate
KP1001	Wild type	1	26	1.24	1.09
HD-11	*pyrR*	19	3.5	0.41	1.31
HD-12	*pyrR*	30	5.2	0.70	0.89
HD-46	*pyrR*	21	2.3	0.46	1.18
HD-47	*pyrR*	32	6.6	0.63	1.37
HD-58	*pyrR*	35	ND[c]	0.52	0.67
HD-87	*pyrR*	22	4.7	0.66	0.95
JL1269	*pyrH*	175	3.0	0.20	0.75

[a] ATCase values are given relative to the fully repressed value observed in KP1001 grown in the presence of uracil in the growth medium.

[b] Nanomoles of UMP phosphorylated per minute per milligram of protein at 37 C, as measured in sonicated, streptomycin-treated extracts (see Materials and Methods).

[c] ND, Not determined.

TABLE 3. *Effect of pyrimidine starvation on ATCase and UMP kinase activities in the pyrimidine requiring strain JL1018*[a]

Exp no.	Growth conditions[b]	Relative ATCase activity	UMP kinase activity[c]
1	Glucose limitation	1	1780
2	Uracil limitation	11	1950

[a] Enzyme activities were determined in toluenized cell suspensions containing in both experiments 6×10^9 cells per ml.

[b] Cells were grown overnight in Tris minimal medium containing in experiment 1: 0.05% glucose and 50 μg of uracil per ml. and in experiment 2: 0.2% glucose and 4 μg of uracil per ml.

[c] UMP kinase activities are expressed as counts per minute of [^{14}C]UDP + [^{14}C]UTP formed per minute from [^{14}C]UMP in a 10-μl aliquot of a standard assay.

activity, we would expect these strains to contain subnormal levels of UTP even when grown with uracil in the medium. Table 2 column 5 shows that this expectation is fulfilled; the UTP pools of the mutants are between 25 and 50% of that found in the wild type. Such a decrease is sufficient to cause the observed derepression of the biosynthetic enzymes (12).

The rate of synthesis of two of the enzymes of the de novo pathway, i.e., dihydroorotase and dihydroorotate dehydrogenase are specifically repressed when cells are grown with high endogenous cytidine-5'-triphosphate pools, independently of the size of the UTP pool (12). Such a condition may be created by growing mutants lacking cytidine deaminase (EC 3.5.4.5) (*cdd*) in a cytidine-containing medium. A *cdd* derivative of HD-11 has been constructed (KP1407) and assayed for dihydroorotase and dehydroorotate dehydrogenase after growth in the presence or in the absence of cytidine. Cytidine is fully capable of repressing the rate of synthesis of these two enzymes in KP-1407 (R. Kelln, personal communication).

From the results presented we conclude that pyrimidine excretion, resistance to FU and FUR, and "constitutivity" of the pyrimidine biosynthetic enzymes (10) of the HD strains investigated in this study, are the results of mutations in *pyrH*, the structural gene coding for UMP kinase. We suggest that the gene designation *pyrR* should be reserved for a gene coding for a regulatory protein directly involved in the control of expression of one or more of the genes *pyrA*, *B*, *C*, *D*, *E*, and *F*.

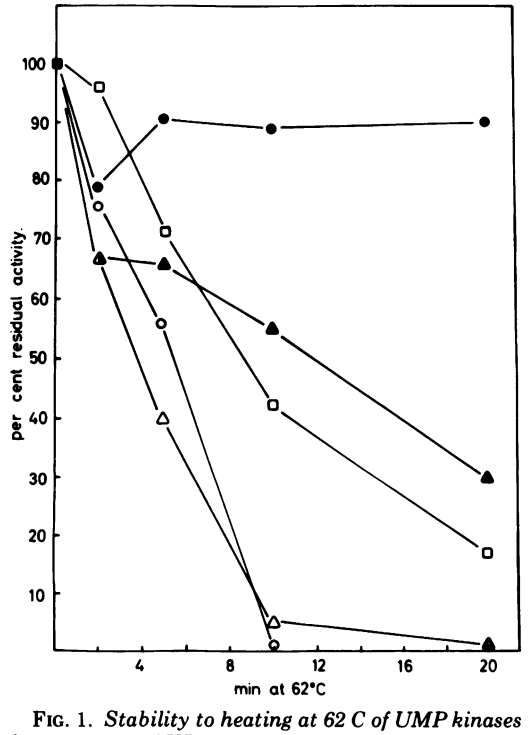

FIG. 1. *Stability to heating at 62 C of UMP kinases from extracts of KP1001 (●), and the "pyrR" mutants HD-11 (○), HD-12 (△), HD-47 (▲), and HD-87 (□). The activity of UMP kinase in each extract before heat treatment is arbitraraly set at 100%. The specific activities are listed in Table 2.*

ACKNOWLEDGMENTS

Part of this work was done in the laboratory of G. A. O'Donovan, Texas A & M University, College Station, Tex. We would like to express our gratitude to Dr. O'Donovan for his hospitality during the stay, for making the HD strains available to us, and for numerous stimulating discussions.

This work was supported by a NATO grant (no. 629) to J. Neuhard and G. A. O'Donovan.

LITERATURE CITED

1. Abd-El-Al, A., and J. L. Ingraham. 1969. Control of carbamylphosphate synthesis in *Salmonella typhimurium*. J. Biol. Chem. **244**:4033-4038.
2. Beck, C. F., J. L. Ingraham, and J. Neuhard. 1972. Location on the chromosome of *Salmonella typhimurium* of genes governing pyrimidine metabolism. II. Uridine kinase, cytosine deaminase and thymidine kinase. Mol. Gen. Genet. **115**:208-215.
3. Beck, C. F., J. L. Ingraham, J. Neuhard, and E. Thomassen. 1972. Metabolism of pyrimidines and pyrimidine nucleosides by *Salmonella typhimurium*. J. Bacteriol. **110**:219-228.
4. Beckwith, J. R., A. B. Pardee, R. Austrian, and F. Jacob. 1962. Coordination of the synthesis of the enzymes in the pyrimidine pathway of *E. coli*. J. Mol. Biol. **5**: 618-634.
5. Edlin, G., and O. Maaloe. 1966. Synthesis and breakdown of messenger RNA without protein synthesis. J. Mol. Biol. **15**:428-434.
6. Gerhart, J. C., and A. B. Pardee. 1962. The enzymology of control by feedback inhibition. J. Biol. Chem. **237**:891-896.
7. Ingraham, J. L., and J. Neuhard. 1972. Cold-sensitive mutants of *Salmonella typhimurium* defective in uridine monophosphate kinase (*pyrH*). J. Biol. Chem. **247**:6259-6265.
8. Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
9. Neuhard, J., and E. Thomassen. 1971. Turnover of the deoxyribonucleoside triphosphates in *Escherichia coli* 15T⁻ during thymine starvation. Eur. J. Biochem. **20**:36-43.
10. O'Donovan, G. A., and J. C. Gerhart. 1972. Isolation and partial characterization of regulatory mutants of the pyrimidine pathway in *Salmonella typhimurium*. J. Bacteriol. **109**:1085-1096.
11. O'Donovan, G. A., and J. Neuhard. 1970. Pyrimidine metabolism in microorganisms. Bacteriol. Rev. **34**:278-343.
12. Schwartz, M., and J. Neuhard. 1975. Control of expression of the *pyr*-genes in *Salmonella typhimurium*: effects of variations in uridine and cytidine nucleotide pools. J. Bacteriol. **121**:814-822.
13. Williams, J. C., and G. A. O'Donovan. 1973. Repression of enzyme synthesis of the pyrimidine pathway in *Salmonella typhimurium*. J. Bacteriol. **115**:1071-1076.
14. Yates, R. A., and A. B. Pardee. 1957. Control by uracil of formation of enzymes required for orotate synthesis. J. Biol. Chem. **227**:667-692.