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Altered Deoxyribonucleotide Pools in P2 Eductants of *Escherichia coli* K-12 due to Deletion of the *dcd* Gene

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Deletions of the *Escherichia coli* K-12 chromosome associated with P2-mediated eduction extend through the structural gene for uridine kinase, *udk*, and the *dcd* gene encoding 2'-deoxycytidine 5'-triphosphate deaminase. The lack of uridine kinase makes a positive selection possible for these strains. Due to the *dcd* mutation, P2 eductants show large alterations in their deoxyribonucleoside triphosphate pools.

Escherichia coli K-12 lysogenic for P2 in the H site segregates histidine-requiring eductants, which contain deletions extending from the $attP2_H$ gene through the *sbcB, his, gnd,* and *rfb* genes into the *mglP* gene (13).

Recently we isolated mutants of *Salmonella typhimurium* lacking 2'-deoxycytidine 5'-triphosphate (dCTP) deaminase (EC 3.5.4..) activity, i.e., *dcd* mutants. Transduction studies, using P22, showed that *dcd* cotransduces 95% with the structural gene *udk*, coding for uridine kinase (EC 2.7.1.48) (C.F. Beck, J. Neuhard, and E. Thomassen, unpublished data). On the *S. typhimurium* chromosome, *udk* is located between *metG* and *hisE*. With phage P1, *udk* cotranduces 23% with *metG* and about 12% with *hisE* (3). In *E. coli*, P1 transduction has indicated about 10% cotransduction between *udk* and *his* (7). These results suggest that the host deletions obtained by P2-mediated eduction in *E. coli* might extend through the *udk* and *dcd* genes. Mutants of *E. coli* defective in dCTP deaminase have been described; however, they were never characterized genetically (10).

Mutants defective in dCTP deaminase have no easily detectable phenotype (10). However, *udk* mutants are resistant to 5-fluorouridine, provided that the strain is already resistant to 5-fluorouracil, i.e., is defective in uracil phosphoribosyltransferase (EC 2.4.2.9) (*upp*) (4).

E. coli K-12 strain SØ864 (F^-, *trp, lacZ, strA, thi, upp*) was infected with P2 and plated in LB soft agar (0.7% agar) on LB agar plates (1.5% agar). Cells from the center of six plaques were combined and grown overnight in LB. Aliquots of this culture were spread on minimal agar plates containing basal salts (5), 0.2% glucose, tryptophan (40 μg/ml), histidine (40 μg/ml), thiamine (5 μg/ml), 5-fluorouridine (10 μg/ml) (F. Hoffman-La Roche & Co., Ltd., Basel) and 1.5% agar (Difco). The plates were incubated for 36 h at 37 C, and the resistant colonies appearing were tested for histidine requirement. Most of the resistant colonies had simultaneously acquired a histidine requirement. Five were kept for further studies (SØ874, SØ875, SØ876, SØ877, and SØ878). All five had become sensitive to P2 and did not produce P2 by spotting on susceptible indicator strains.

Table 1 shows the results of enzyme assays

TABLE 1. *Uridine kinase and dCTP deaminase activities in extracts[a] of different E. coli K-12 strains*

Strain	His phenotype	Sp act[b]	
		Uridine kinase[c]	dCTP deaminase[d]
SØ864	His^+	12.2	2.03
SØ874	His^-	<0.1	<0.05
SØ75	His^-	<0.1	<0.05
SØ876	His^-	<0.1	<0.05
SØ877	His^-	<0.1	<0.05
SØ878	His^-	<0.1	<0.05
HfrH *thi*	His^+	19.5	2.10
QE1	His^-	<0.1	<0.05

[a] Cells were grown overnight at 37 C with aeration in AB medium (5) containing 0.2% glucose, 0.2% Casamino Acids (Difco), thiamine (5 μg/ml), and tryptophane (40 μg/ml). They were harvested by centrifugation, washed once with 0.9% NaCl, and kept frozen until assayed. For uridine kinase assays, cells were suspended in a solution of 0.1 M tris(hydroxymethyl)aminomethane (Tris)-chloride (pH 7.8) and 1 mM ethylenediaminetetraacetate (EDTA), sonically disrupted, and centrifuged. The supernatants were dialyzed for 2 h against 100 volumes of the sonication buffer and assayed for uridine kinase activity. For dCTP deaminase assays, cells were suspended in a solution of 0.05 M potassium phosphate buffer (pH 6.8) and 2 mM EDTA, sonically disrupted, and centrifuged. The supernatants were treated with streptomycin sulfate at 4 C (final concentration, 3%), left standing for 30 min,

TABLE 1—*Continued*

and centrifuged. The supernatants were made 55% saturated with solid ammonium sulfate, left standing for 30 min at 4 C, and centrifuged again. The precipitates were dissolved in a solution of 0.05 M potassium phosphate buffer (pH 6.8), 2 mM EDTA, and 2 mM mercaptoethanol and dialyzed for 2 h against 100 volumes of the same buffer.

[b] Nanomoles of substrate converted per minute per milligram of protein. Proteins were determined by the method of Lowry (8).

[c] Uridine kinase assay. Reaction mixtures of 40 μl contained 0.05 M Tris-chloride (pH 7.8), 2.5 mM $MgCl_2$, 3 mM guanosine 5'-triphosphate, 1 mM [2-^{14}C]uridine (specific activity, 0.5 μCi/μmol), and extract. Reactions were started by the addition of extracts and were performed at 37 C. At 5, 10, and 15 min, 10-μl samples were applied to the "starting" spots of poly(ethyleneimine)-impregnated cellulose thin-layer plates (12) and dried with hot air. The chromatograms were developed for 12 cm with water. After the chromatograms were dry, the starting spots containing phosphorylated uridine compounds were cut out and counted in a Packard Tri-Carb liquid scintillation spectrometer.

[d] dCTP deaminase activity was determined by the spectrophotometric assay described by Beck et al. (2).

performed on SØ864 and the five eductants SØ874 through 878. All five mutants are deficient in both uridine kinase and dCTP deaminase. Similarly, QE1, which is a P2 eductant of *E. coli* HfrH *thi*, isolated by Sunshine and Kelly (13), is devoid of both activities (Table 1). Since QE1 was isolated as a *his*$^-$ eductant without employing 5-fluorouridine, we conclude that the *udk* and *dcd* genes are located on that piece of deoxyribonucleic acid which is lost concomitant to the exit of the P2 phage from its H site.

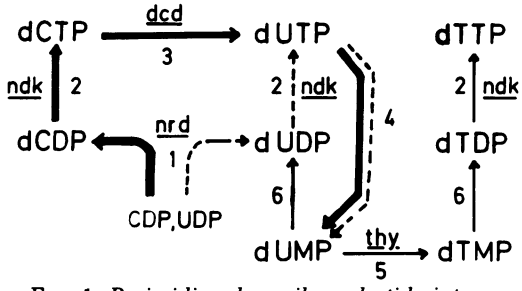

FIG. 1. *Pyrimidine deoxyribonucleotide interconversion in E. coli. Symbols:* →, *main pathway for dUMP synthesis;* ⇢, *secondary pathway for dUMP synthesis. The enzymes are identified by numbers as follows: 1, ribonucleoside diphosphate reductase (EC 1.8..); 2, nucleoside diphosphate kinase (EC 2.7.4.6); 3, dCTP deaminase (EC 3.5.4. .); 4, deoxyuridine 5'-triphosphatase (EC 3.1. . .); 5, thymidylate synthetase (EC 2.1.1.b); 6, thymidylate kinase (EC 2.7.4.9). The gene designations for the corresponding structural genes, where known, are underlined.*

dCTP deaminase is the key enzyme of the main pathway for the synthesis of 2'-deoxyuridine 5'-monophosphate (dUMP), the ultimate precursor for thymidine nucleotide synthesis (2) (Fig. 1). Thus, *dcd* mutants can only synthesize dUMP via the second pathway, which in-

TABLE 2. *Nucleoside triphosphate pools[a] in different E. coli K-12 strains*

Strain	Addition to the medium	Pool sizes[b]							
		ATP	GTP	CTP	UTP	dATP	dGTP	dCTP	dTTP
SØ864	None	4.84	2.59	1.07	1.24	0.23	0.08	0.17	0.20
	UdR[b] (100 μg/ml)	6.93	3.51	1.81	1.72	0.30	0.20	0.25	0.54
	Thymidine (100 μg/ml)	5.87	2.97	1.47	1.74	0.24	0.08	0.22	0.44
	Thymine (50 μg/ml)	7.34	3.43	1.87	2.14	0.30	0.12	0.30	0.30
SØ874	None	4.97	2.25	1.00	1.56	0.22	0.07	7.43	0.07
	UdR[b] (100 μg/ml)	3.87	2.06	1.27	1.13	0.17	0.11	0.79	0.72
	Thymidine (100 μg/ml)	4.28	2.10	1.23	1.35	0.11	0.05	1.07	0.89
	Thymine (50 μg/ml)	2.90	1.23	0.69	1.03	0.12	0.06	4.39	0.08

[a] SØ864 and SØ874 were grown at 37 C in low-phosphate medium (6) containing 0.2% glucose, tryptophan (40 μg/ml), histidine (40 μg/ml), thiamine (5 μg/ml), [^{32}P]orthophosphate (specific activity, 10 μCi/μmol), and the additions mentioned in the table. After two generations of exponential growth, 5 ml of each culture was filtered on membrane filters (Millipore Corp.), and the filters were extracted with ice-cold 0.3 N perchloric acid. The quantitative thin-layer chromatographic determination of individual nucleoside triphosphates in the extracts was described previously (9).

[b] Pools are given in micromoles per gram of bacterial dry weight. ATP, Adenosine 5'-triphosphate; UTP, uridine 5'-triphosphate; dATP, 2'-deoxyadenosine 5'-triphosphate; dGTP, 2'-deoxyguanosine 5'-triphosphate; UdR, 2'-deoxyuridine.

volves the reduction of uridine 5'-diphosphate (UDP) to dUDP (Fig. 1), and they are therefore stressed in their ability to synthesize thymidine nucleotides (10). The P2 eductant SØ874 is indeed limited in its 2'-deoxythymidine 5'-triphosphate (dTTP) pool (Table 2). Concomitantly with the low-dTTP pool the dCTP pool is increased about 20-fold. This agrees with the previous studies on the effect of thymine starvation on the deoxyribonucleoside triphosphate pools of *thy* mutants of *E. coli* (9).

Since the low-dTTP pool of the eductants is due to a decrease in the availability of the precursor dUMP, one would expect that the addition of either thymidine or deoxyuridine to the growth medium of the mutants would increase the dTTP pool and, as a result, restore the dCTP pool to normal values. This is, indeed, what is observed (Table 2). However, the addition of even high concentrations of thymine is not capable of normalizing the deoxyribonucleotide pools of SØ874 (Table 2). This latter result is to be expected, since the deoxyribose-1-phosphate required for the utilization of exogenous thymine by dcd^+ strains is derived from dUMP (1) which, in the eductants, is the limiting compound for thymidine nucleotide synthesis.

The somewhat lower growth rate observed with P2 eductants as compared with the parental strains is not normalized by the addition of either thymidine or deoxyuridine to the growth medium.

The present work has extended the number of genes known to be deleted from the *E. coli* K-12 chromosome after eduction of P2. Of particular interest is the finding that the deletion covers the *dcd* gene, resulting in partial thymidine nucleotide starvation of the eductants. This, in turn, causes alterations in the deoxyribonucleotide pools (Table 2), elongations of the cells (unpublished data), and, eventually, a decrease in the replication time of the chromosome (11).

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