5-Methyl-dCTP Deaminase Induced by Bacteriophage XP-12

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Bacteriophage XP-12, whose DNA contains 34 mol% 5-methylcytosine, induces the synthesis of a unique enzyme, 5-methyl-dCTP deaminase. The substrate for this enzyme, 5-methyl-dCTP, is produced by reactions catalyzed in part by other phage-induced enzymes, and the product of the reaction is dTTP. The deaminase therefore provides a novel pathway for biosynthesis of thymine residues for phage XP-12 DNA. Evidence is presented that this pathway is used for dTTP synthesis in XP-12-infected *Xanthomonas oryzae*.

DNA from the *Xanthomonas* phage XP-12 (3, 11, 12) is the only known naturally occurring DNA in which the cytosine (Cyt) residues are virtually completely replaced by 5-methylcytosine (m^5 Cyt) residues (34 mol% m^5 Cyt, <1 mol% Cyt). The methylation takes place at the mononucleotide level (7) with serine as the methyl donor, probably via N^5,N^{10} -methylene-tetrahydrofolate (3). The m^5 Cyt residues of XP-12 can, therefore, be distinguished from those present in many other types of procaryotic DNA and from those in eucaryotic DNA (5, 8) by the following three properties: only XP-12 DNA has m^5 Cyt as a major base; only in the biosynthesis of XP-12 DNA does methylation take place at the mononucleotide level rather than after polymerization; only the m^5 Cyt residues of XP-12 DNA derive their methyl group from serine rather than from methionine.

m^5 Cyt and thymine (Thy) both have a methyl group at the 5-position. Although m^5 Cyt is a major base in XP-12 DNA, thymidine (dThd) is not a precursor for biosynthesis of its m^5 Cyt residues (3). Instead, dCMP is the substrate for a phage-induced methyltransferase that yields m^5 dCMP (7). The converse remained possible, that m^5 Cyt nucleotides could be converted to Thy nucleotides. Several lines of evidence suggested that there is a new pathway for Thy nucleotide biosynthesis in XP-12-infected *X. oryzae*. When a Thy auxotrophic host is transferred to a medium deficient in dThd and then infected with XP-12, a normal yield of phage is obtained. Nonetheless, in this medium the uninfected host is committed to thymineless death (4). When XP-12 phage is propagated on this Thy auxotroph in medium containing [2- 14 C]uracil and 50 μ g of dThd per ml, the resulting phage DNA contains m^5 Cyt and Thy residues labeled to equal specific activity. In contrast, the uninfected *thy*⁻ host is able to convert [2- 14 C]uracil

to Cyt residues but not to Thy residues in DNA (4).

Kuo and co-workers found evidence for a phage-induced pathway for synthesis of Thy nucleotides from m^5 Cyt nucleotides (T.-T. Kuo, T.-Y. Chow, and Y.-T. Lin, Abstr. Fourth Int. Congr. Virol., 1978). We show in this report that infection of *X. oryzae* with phage XP-12 induces the formation of a unique enzyme, m^5 dCTP deaminase. This enzyme may be responsible for the ability of phage XP-12 to synthesize normal amounts of phage DNA in the absence of host thymidylate synthetase activity and exogenous dThd (Fig. 1).

MATERIALS AND METHODS

Phage, bacteria, and DNA. Phage XP-12 was propagated on *X. oryzae* 507 as previously described (3). Phage-infected bacteria were harvested 45 min postinfection in XPS medium (3) according to previous methods (6). *X. oryzae* [14 C]cytosine DNA was prepared from the Thy auxotroph *X. oryzae thyH* (4) grown in medium containing 1 μ Ci of [2- 14 C]uracil per ml (30 mCi/mmol, ICN Pharmaceuticals, Irvine, Calif.) and 5 μ g of unlabeled dThd per ml. XP-12 [14 C]pyrimidine DNA, that is, DNA labeled to equal specific activity in both pyrimidine residues, was obtained from XP-12-infected *X. oryzae* propagated in the same medium containing [2- 14 C]uracil without dThd. The DNA was extracted by standard methods (15), except that after the cells were disrupted by lysozyme, sodium dodecyl sulfate, and heating, the sample was incubated at 37°C with 100 μ g of proteinase K per ml (EM Biochemicals, Darmstadt, Germany) for 16 h. Then the DNA was extracted with phenol and subsequently with chloroform/isoamyl alcohol (24:1, vol/vol).

Nucleotides. [14 C]dTMP and [14 C] m^5 dCMP were obtained from XP-12 [14 C]pyrimidine DNA digested with nuclease P1 (21). [14 C]dCMP was obtained from nuclease P1 hydrolysates of *X. oryzae* [14 C]cytosine DNA. Both [14 C] m^5 dCDP and [14 C] m^5 dCTP were prepared by incubation of [14 C] m^5 dCMP with an m^5 dCMP ki-

FIG. 1. Postulated scheme for biosynthesis of pyrimidine deoxymononucleotides in XP-12-infected *X. oryzae*. As discussed in the text, the pathway from m^5dCMP to dTTP might be the main one used by XP-12 for synthesis of dTTP. The dashed arrow represents the action of a hypothetical phage-induced dCTP nucleotidohydrolase. A dCTPase/dUTPase is encoded by *E. coli* phage T4 and provides dCMP for 5-hydroxymethylation catalyzed by another T4-encoded enzyme (16). The other pathways in this scheme are depicted as they are found in enteric bacteria in general and in *E. coli* in particular (1, 9, 16, 18). None of these enzymes from uninfected *X. oryzae* have been well characterized, although precursor incorporation studies are consistent with their occurrence as shown (4). The salvage pathways are indicated. The other reactions are part of the de novo synthetic pathways. The names of enzymes mentioned in the text are noted in the diagram. Enzymes which have been found only in XP-12-infected *X. oryzae* are underlined, as are nucleotides which are novel precursors for DNA synthesis in these cells.

nase fraction from XP-12-infected cells, which can convert m⁵dCMP to m⁵dCTP (R. Wang, L.-H. Huang, and M. Ehrlich, *Biochim. Biophys. Acta*, in press). After the enzyme reaction, m⁵dCTP was purified by DEAE-Sephadex column chromatography and then concentrated and desalted (Wang et al., in press).

Deaminase assay. The standard assay mixture consisted of 1 nmol of [^{14}C]m⁵dCTP (or [^{14}C]m⁵dCMP when assaying crude extracts), 33 nmol each of ATP and creatine phosphate, 1 U of creatine kinase (Sigma Chemical Co., St. Louis, Mo.), and 1 to 2 μl of enzyme (crude extract or fraction I, see below) in a total of 10 μl of 50 mM Tris-acetate (pH 7.6)–5 mM MgCl_2 –5 mM mercaptoethanol. After incubation for 1 h at 37°C, the mixture was chilled, EDTA was added to a final concentration of 10 mM, and dTTP, dTMP, m⁵dCMP, dThd, and 5-methyldeoxycytidine (m⁵dCyd) were added as markers. This mixture was applied to a cellulose

thin-layer chromatography (TLC) plate (Avicel, 250 μm ; Brinkman Instruments, Inc., Westbury, N.Y.). The plate was developed with isobutyric acid-ammonium hydroxide-0.5 M EDTA (pH 8.0)-H₂O (66:2.7:0.2:25, vol/vol), and the radioactivity in the scraped spots was determined. One unit of m⁵dCTP deaminase activity refers to the amount of enzyme which converts 1 μmol of m⁵dCTP to dTTP in 1 h under standard conditions.

Preparation of crude extract and fraction I. A 4-g amount of wet, packed XP-12-infected *X. oryzae* was sonicated in 25 ml of 50 mM Tris-hydrochloride (pH 7.6)–10 mM mercaptoethanol–0.1 mM EDTA and centrifuged at 27,000 $\times$ g for 30 min. The supernatant is referred to as the crude extract. Nucleic acids were removed from the crude extract by precipitation with streptomycin sulfate (0.5% [wt/vol], final concentration), and the supernatant was brought to a concentra-

tion of 10 mM Tris-hydrochloride (pH 7.6)–2 mM mercaptoethanol–0.1 mM EDTA–10% glycerol (buffer A) and applied to a DEAE-cellulose (DE-52, Whatman, Inc., Clifton, N.J.) column (1 by 25 cm) equilibrated with the above buffer. The flow-through fraction was subsequently applied to a carboxymethyl-cellulose (CM-52, Whatman) column equilibrated with buffer A. Again the m^5dCMP kinase activity eluted in the flow-through. The sample was then concentrated by ultrafiltration and dialyzed against 10 mM Tris-hydrochloride (pH 7.6)–2 mM mercaptoethanol–0.1 mM EDTA–10% glycerol. This fraction, which was stored at -40°C , is referred to as fraction I. It was stable under these conditions for at least 6 months.

Assay of nucleoside and nucleotide kinase activity. Crude extracts of uninfected and XP-12-infected *X. oryzae* harvested 45 min after infection were assayed for various kinase activities by incubating 1 μl of extract with 33 nmol of ATP, 33 nmol of creatine phosphate, 1 U of creatine kinase, and 1 nmol of the indicated nucleoside or mononucleotide in a total of 10 μl of 50 mM Tris-acetate (pH 7.6)–5 mM MgCl_2 –5 mM mercaptoethanol. The reaction was conducted at 37°C for 1 h and terminated by cooling and adding EDTA to 10 mM. The reaction mixtures were analyzed by TLC as described for the deaminase assay.

Analysis of incorporation of uracil into host DNA. *X. oryzae* was grown on 100 ml of XMS medium as described previously (3) with the substitution of potato dextrose broth (Difco Laboratories, Detroit, Mich.) for potato extract. When the cells reached late logarithmic phase, the culture was diluted 15-fold with the above medium, and dThd was added to half to give a final concentration of 50 $\mu\text{g/ml}$. Three hours later, 10 μCi of $[2\text{-}^{14}\text{C}]\text{uracil}$ was added to each 50 ml of culture. After 16 h of incubation, the cells were harvested and DNA was isolated. The purified DNA was hydrolyzed with nuclease P1, and the radioactivity in the resulting mononucleotides was determined after TLC as previously described (21).

RESULTS

m^5dCTP deaminase activity in crude extracts. Crude extracts of XP-12-infected cells catalyzed the conversion of m^5dCMP to dTTP as well as to m^5dCTP (Table 1). These activities were not detectable in analogous extracts of uninfected cells. To detect the deaminase activity in infected cell extracts, ATP, creatine phosphate, and creatine kinase had to be included in the reaction mixture. In the absence of this ATP regenerating system, almost all of the $[^{14}\text{C}]m^5dCMP$ was converted to $[^{14}\text{C}]m^5dCyd$; whereas, in its presence, approximately half of the $[^{14}\text{C}]m^5dCMP$ was converted to a mixture of $[^{14}\text{C}]m^5dCTP$ plus $[^{14}\text{C}]dTTP$ (Table 1).

Since this crude extract from XP-12-infected cells phosphorylated m^5dCMP , deamination might have occurred after phosphorylation. When $[^{14}\text{C}]m^5dCTP$ was used as the substrate, the yield of $[^{14}\text{C}]dTTP$ increased twofold, but small amounts of $[^{14}\text{C}]m^5dCMP$ and $[^{14}\text{C}]dTMP$ were also formed by phosphatase activity in the crude extract, even in the presence of the ATP

regenerating system. When $[^{14}\text{C}]m^5dCTP$ was incubated with crude extracts of uninfected cells under the same conditions, <1% of the radioactivity was converted to $[^{14}\text{C}]dTTP$ even though 61% of the radioactivity was still present as $[^{14}\text{C}]m^5dCTP$.

In an attempt to determine whether $[^{14}\text{C}]m^5dCMP$ could be directly converted to $[^{14}\text{C}]dTMP$, the former was used as a substrate for the infected cell extract under conditions which prohibit the phosphorylation but minimize conversion to the nucleoside. A 70-fold excess of dCMP was added to try to saturate the phosphatase activity. The crude extract was incubated in the absence of ATP, creatine phosphate, and creatine kinase, but in the presence of a 70-fold excess of dCMP to overwhelm the phosphatase activity. Under these conditions, 42% of the $[^{14}\text{C}]m^5dCMP$ remained monophosphorylated but still there was no detectable formation of dTMP (Table 1). The addition of excess dGMP, dTMP, or dUMP instead of dCMP gave similar results.

m^5dCTP deaminase activity in fractionated extracts from XP-12-infected cells. By chromatographing extracts from infected cells on DEAE-cellulose column, we were able to separate the m^5dCMP kinase activity, which in the presence of creatine phosphate and creatine kinase converted m^5dCMP to m^5dCTP , from the deaminase activity. The latter eluted in the pass-through fraction of a column rinsed with buffer A, whereas the former was retained under these conditions. However, this chromatography did not separate most of the nucleotide phosphatase activity from the deaminase activity. Furthermore, both the deaminase and phosphatase activity coeluted in the pass-through fraction of a carboxymethyl-cellulose column, fraction I. We obtained approximately 9 U of deaminase activity in fraction I from 4 g of wet packed cells.

In spite of the presence of phosphatase activity in fraction I, deaminase activity was clearly detectable if an ATP regenerating system was included in the reaction mixture. Table 2 shows that in this case the phosphatase activity was counteracted so that <3% of the m^5dCTP was dephosphorylated. In the absence of creatine kinase, creatine phosphate, and ATP, m^5dCTP could still be converted to dTTP but with a yield of only ~6% (Table 2). When only creatine kinase and creatine phosphate were omitted from the standard reaction mixture, ~27% of the m^5dCTP was deaminated to dTTP and 7% was converted to dTDP. These data were derived from TLC analysis of the reaction mixture before and after treatment with alkaline phosphatase.

Since the m^5dCMP kinase activity is missing from fraction I of the carboxymethyl-cellulose

TABLE 1. Phosphorylation, deamination, and dephosphorylation of [¹⁴C]m⁵dCMP by crude extracts of XP-12-infected and of uninfected *X. oryzae*

Substrate ^a	Temp (°C)	ATP rege- nerating system	% of total recovered radioactivity in the region of: ^b						
			m ⁵ dCyd	dThd	m ⁵ dCMP	dTMP	m ⁵ dCDP	m ⁵ dCTP	dTTP
Infected cell extracts									
[¹⁴ C]m ⁵ dCMP	37	+	8.1	9.9	7.4	8.6	13	26	27
[¹⁴ C]m ⁵ dCMP	0	+	3.0	<1	95	1.4	<1	<1	<1
[¹⁴ C]m ⁵ dCMP + 7 mM dCMP	37	—	53	1.4	42	<1	<1	<1	<1
[¹⁴ C]m ⁵ dCMP	37	—	95	2.4	2.3	<1	<1	<1	<1
[¹⁴ C]m ⁵ dCMP	0	—	5.3	<1	93	1.4	<1	<1	<1
Uninfected cell extracts									
[¹⁴ C]m ⁵ dCMP	37	+	71	<1	27	<1	<1	<1	<1

^a Reactions were conducted under standard conditions as described in the text, except where indicated the ATP regenerating system (ATP, creatine phosphate, and creatine kinase) was omitted. The 0°C incubation mixtures were controls in which [¹⁴C]m⁵dCMP was added to enzyme reaction mixtures at 0°C instead of at 37°C.

^b Separation and quantitation of nucleosides and nucleotides was described in the text. The *R_f* values were as follows: m⁵dCyd, 0.72; dThd, 0.63; m⁵dCMP, 0.51; dTMP, 0.39; m⁵dCDP, 0.35; dTDP, 0.22; m⁵dCTP, 0.22; dTTP, 0.13.

chromatogram, the m⁵dCMP could not be converted to m⁵dCDP or m⁵dCTP by this fraction. Therefore, we could test directly whether m⁵dCMP itself is a substrate for the deaminase using fraction I in the presence of a 30-fold excess of unlabeled dCMP, dTMP, or dUMP to limit breakdown of the [¹⁴C]m⁵dCMP. Less than 2% of the [¹⁴C]m⁵dCMP was converted to [¹⁴C]dTMP (Table 2). In contrast, under the same conditions but in the presence of an ATP regenerating system, 51% of the [¹⁴C]m⁵dCTP

was converted to [¹⁴C]dTTP; addition of a 30-fold excess of dCMP, dTMP, or dUMP had no effect upon this reaction.

Since we were not able to remove most of the phosphatase activity from the deaminase preparation, the possibility remained that m⁵dCDP which formed transiently from m⁵dCTP was the substrate for the deaminase. Subsequent rephosphorylation of the resulting dTDP by creatine kinase and creatine phosphate in the reaction mixture could have yielded the final dTTP prod-

TABLE 2. Relative deamination of [¹⁴C]m⁵dCMP and [¹⁴C]m⁵dCTP by the deaminase activity in fraction I.

Substrate ^a	Temp (°C)	ATP regenerating system	<i>E. coli</i> phosphatase ^b	% of total recovered radioactivity in the region of:						
				m ⁵ dCyd	dThd	m ⁵ dCMP	dTMP	m ⁵ dCDP	m ⁵ dCTP	dTTP
[¹⁴ C]m ⁵ dCTP	37	+	—	1.8	<1	<1	<1	2.8	44	51
[¹⁴ C]m ⁵ dCTP	0	+	—	<1	<1	<1	<1	2.6	92	3.3 ^d
[¹⁴ C]m ⁵ dCTP	37	—	—	30	10	24	1.8	<1	27	5.9
[¹⁴ C]m ⁵ dCTP	0	—	—	<1	<1	<1	1.6	17	77	3.5 ^d
[¹⁴ C]m ⁵ dCTP ^c	37	+	+	42	57					
[¹⁴ C]m ⁵ dCTP ^c	0	+	+	99	<1					
[¹⁴ C]m ⁵ dCMP	37	—	—	96	2.7 ^e	<1	<1			
[¹⁴ C]m ⁵ dCMP + 3 mM dUMP	37	—	—	62	2.1 ^e	34	1.0 ^e			
[¹⁴ C]m ⁵ dCMP	0	—	—	2.0	<1	96	1.5 ^e			

^a Enzyme assays were carried out under standard conditions, except where indicated the ATP regenerating system was omitted or the temperature was lowered to 0°C during the deaminase reaction.

^b Alkaline phosphatase from *E. coli* was added to the reaction mixture after incubation with fraction I.

^c Chromatography was also performed on cellulose TLC plates in *n*-butanol-acetic acid-H₂O (80:12:30) as well as in the standard assay system. The *R_f* values in this solvent system were 0.46 for m⁵dCyd and 0.60 for dThd. The results obtained from both chromatographs were essentially the same.

^d This radioactivity probably represents trailing from [¹⁴C]m⁵dCTP.

^e This radioactivity also may be due to trailing from [¹⁴C]m⁵dCMP or [¹⁴C]m⁵dCyd.

uct. That this was not the case was shown by the finding that <2% of [^{14}C]m 5 dCDP was deaminated in the standard reaction mixture lacking ATP, creatine kinase, and creatine phosphate but containing an additional 9 mM dUMP. During this incubation, the presence of excess dUMP prevented 60% of the [^{14}C]m 5 dCDP from being dephosphorylated. Incubation of [^{14}C]m 5 dCTP under the same conditions resulted in dephosphorylation of most of the nucleoside triphosphate to the diphosphate, but when the nucleotides were subsequently hydrolyzed to deoxynucleosides with *Escherichia coli* alkaline phosphatase, 11% of the radiolabel cochromatographed with dThd. Therefore, there was some deamination of [^{14}C]m 5 dCTP before dephosphorylation. In the complete reaction mixture, deamination of [^{14}C]m 5 dCTP was predominant and dephosphorylation was negligible in the presence or absence of 9 mM dUMP.

That [^{14}C]dTTP is the product of the deaminase reaction was confirmed by hydrolysis of the standard reaction mixture with alkaline phosphatase and subsequent TLC in two different solvent systems (Table 2). Approximately the same percentage of radioactivity that cochromatographed with dTTP before alkaline phosphatase treatment also cochromatographed with dThd after incubation with alkaline phosphatase. The possibility that the deaminase is subject to end product inhibition by dTTP was tested. At concentrations of dTTP less than or equal to that of m 5 dCTP, little or no inhibition of the deaminase reaction was observed. When a 20-fold excess of dTTP was added, the deaminase activity decreased twofold. Similar concentrations of dATP, dGTP, or dCTP had no effect on the reaction.

We tested whether the deaminase could deam-

inate m 5 Cyt residues in XP-12 DNA. Less than 0.2% of m 5 Cyt residues in the DNA were converted to Thy residues under conditions in which 46% of m 5 dCTP was converted to dTTP. Also, under these conditions, less than 0.3% of [^{14}C]dCTP was converted to [^{14}C]dUTP.

Relative phosphorylation of various pyrimidine mononucleotides and nucleosides by crude extracts. To better understand pyrimidine nucleotide metabolism in XP-12-infected cells, we tested the ability of crude extracts to phosphorylate m 5 dCMP, dCMP, dTMP, and dThd. Table 3 shows that crude extracts of both uninfected and infected cells converted dThd and dTMP to dTTP to similar extents. Only extracts from infected cells produced m 5 dCTP from m 5 dCMP. On the other hand, only extracts from uninfected cells can convert significant amounts of dCMP to dCTP. This lack of detectable formation of dCTP in infected cells might be due to a phage-induced dCTP nucleotidohydrolase. Such an enzyme has not yet been looked for in XP-12-infected cells but is postulated to exist in analogy to a T4-encoded enzyme (16).

Effect of exogenous thymidine on incorporation of uracil into phage and host DNA. It was shown previously that [^{14}C]uracil is converted with equal specific activity to Thy and m 5 Cyt residues in XP-12 DNA when a Thy auxotroph is used as the host for phage XP-12 in medium supplemented with dThd (4). In contrast, radiolabel from [^{14}C]uracil is incorporated into DNA only as Cyt residues in this uninfected host grown on the same medium (4). We have confirmed the former observation on a phage-infected prototrophic host. Also, we have shown that, as expected, addition of dThd to the medium depressed utilization of [^{14}C]uracil for synthesis of Thy residues in the DNA of the uninfected

TABLE 3. Relative phosphorylation of pyrimidine derivatives by extracts of uninfected and XP-12-infected *X. oryzae*

Source of crude enzyme ^a	Substrate	% of total recovered radioactivity			
		Deoxynucleoside	dNMP	dNDP	dNTP
Uninfected cells	[^{14}C]m 5 dCMP	69	29	<1	<1
	[^{14}C]dCMP	39	10	16	35
	[^{14}C]dTMP	53	17	10	20
	[^{14}C]dThd	58	24	6.0	12
XP-12-infected cells	[^{14}C]m 5 dCMP	28 ^b	13	8.7	51
	[^{14}C]dCMP	66	28	5.0	<1
	[^{14}C]dTMP	55	17	8.3	20
	[^{14}C]dThd	59	21	6.7	13

^a The crude enzyme was prepared from sonicated, uninfected or XP-12-infected cells 45 min after infection as described in the text. The protein concentration in these extracts was 11 mg/ml.

^b Because of the presence of m 5 dCTP deaminase as well as nucleotide kinase activities in crude extracts of XP-12-infected cells, a mixture of m 5 dCyd and dThd derivatives is present in this sample.

prototroph. The ratio of radioactivity in Thy residues in *X. oryzae* DNA compared to that in Cyt residues was ~4 times greater in the absence of exogenous dThd than in its presence.

DISCUSSION

A new pathway for dTTP synthesis has been revealed in phage XP-12-infected *X. oryzae*, namely, the enzymatically catalyzed deamination of m⁵dCTP (Fig. 1). Since m⁵dCTP but neither m⁵dCDP nor m⁵dCMP served as a substrate for the activity in fraction I from XP-12-infected cells, deamination took place at the triphosphate level (Table 2 and Results). In crude extracts from infected cells, [¹⁴C]m⁵dCTP was generated from [¹⁴C]m⁵dCMP by a phage-induced m⁵dCMP kinase, ATP, creatine phosphate, and host diphosphokinase (Wang et al., in press). This [¹⁴C]m⁵dCTP, in turn, served as the substrate for the deaminase. Under conditions in which m⁵dCTP did not form, deamination did not take place (Table 1).

The DNA from XP-12 is novel in having m⁵Cyt as a major base which is incorporated into the DNA as m⁵dCTP (7 and this paper). Since m⁵dCTP is not expected to be found in other studied organisms, the m⁵dCTP deaminase activity may be peculiar to XP-12-infected *X. oryzae*. Indeed, uninfected *X. oryzae* cells did not contain an activity capable of deaminating m⁵dCTP.

Although the occurrence of m⁵dCTP deaminase may be limited to XP-12-infected *X. oryzae*, dCTP deaminase is found in *Salmonella typhimurium*, *E. coli* (1), and phage PBS1-infected *Bacillus subtilis* (10, 20). Furthermore, dCMP deaminase is found in a wide range of eucaryotic (14) and in some procaryotic cells including T-even phage-infected *E. coli* (13) and phage SP8-infected *B. subtilis* (17). dCyd deaminase activity is also widely distributed among different kinds of organisms (9, 18).

The existence of an XP-12-induced m⁵dCTP deaminase implies that Thy residues in XP-12 DNA can arise by a de novo pathway independent of dTMP synthetase. Consistent with this prediction, DNA synthesis in XP-12-infected cells requires neither the host's thymidylate synthetase nor the salvage pathway for dTMP synthesis (4). Furthermore, the above described experiments on the effect of exogenous dThd on the incorporation of Ura into DNA are evidence for the existence of a new phage-induced pathway for the synthesis of Thy residues. In all studied eucaryotes and bacteria, dTMP synthetase (de novo pathways) or thymidine kinase (salvage pathway) is required for dTTP biosynthesis (9, 18; Fig. 1). Two cases of Thy nucleotide biosynthesis independent of thymidylate synthetase or of the salvage pathway have been

reported, namely, the synthesis of Thy residues for the DNA of *Pseudomonas* phage ϕ W14 (22) and for that of *Bacillus* phage SP10 (2). The biosynthesis of both of these DNAs seems to involve conversion of 5-hydroxymethyluracil residues in phage DNA to Thy residues, that is, the formation of Thy at the polynucleotide level. Furthermore, this unusual means of biosynthesis of Thy residues apparently completely replaces the normal pathway for dTTP production in these phage-infected cells and is also used for synthesis of the highly modified Thy residues found in the mature phage DNAs (2, 22).

Phage XP-12's novel pathway for production of Thy nucleotides might similarly be the major pathway for dTTP synthesis (Fig. 1) in infected prototrophic and Thy auxotrophic (4) host cells. Although the possibility has not been disproven, it is unlikely that XP-12 also encodes a thymidylate synthetase. We have been unable to test this directly because of unexplained difficulties in quantitating thymidylate synthetase activity in phage-infected or uninfected cells (J. Mayo, R. Wang, M. Ehrlich, unpublished data). Since XP-12 induces the synthesis of easily detectable amounts of m⁵dCTP deaminase, which catalyzes the formation of dTTP, and of enzymes necessary to produce the substrate, m⁵dCTP, it may have no additional need for a phage-induced thymidylate synthetase. Extensive attempts to isolate an XP-12 mutant which cannot grow on a Thy auxotrophic host (4) in the absence of exogenous dThd were unsuccessful. The same methods were used which had yielded T4 phage mutants deficient in a phage-induced thymidylate synthetase (19, 23).

It remains to be determined how the activity of the m⁵dCTP deaminase is regulated so that a majority of the m⁵dCTP is not deaminated in vivo. Because there are less than half as many Thy residues as m⁵Cyt residues in XP-12 DNA, most of the m⁵dCTP should escape the action of m⁵dCTP deaminase so that it can be incorporated into the DNA without alteration. The dCTP deaminase of *S. typhimurium* is specifically inhibited by dUTP and dTTP (1), which can explain the regulation of this enzyme in vivo. We could not push the m⁵dCTP deaminase reaction in vitro to more than 50% completion either by raising the enzyme concentration, decreasing the substrate concentration, or prolonging the reaction time (data not shown). These results cannot be explained simply by feedback inhibition by dTTP because significant inhibition of the deaminase activity was seen only at very high concentrations of dTTP, namely 1.7 mM. Differences between the in vivo structure or environment of m⁵dCTP deaminase and its in vitro counterpart might make dTTP a more effective inhibitor in vivo.

Incorporation of dThd by the salvage pathway (Fig. 1) into XP-12 DNA is only barely detectable despite the presence of kinase activity in XP-12-infected cells, which can convert dThd to dTTP (Table 3). In analogy to the model for phage T4 DNA synthesis (16), it is proposed that some of the enzymes involved in the synthesis of precursors for XP-12 DNA form a multienzyme complex at the replication fork that channels m⁵dCTP and dTTP (which is synthesized by deamination of part of the m⁵dCTP pool) into phage DNA synthesis. This complex might include the phage-induced m⁵dCTP deaminase, dCMP 5-methyltransferase (7), m⁵dCMP kinase, and the host's nucleoside diphosphokinase (Wang et al., in press).

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