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Deoxythymidine Nucleotide Metabolism in *Bacillus subtilis* W23 Infected with Bacteriophage SP10c: Preliminary Evidence that dTMP in SP10c DNA Is Synthesized by a Novel, Bacteriophage-Specific Mechanism

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Despite the fact that mature SP10c DNA contains dTMP, the acid-soluble fraction of infected cells contained no dTTP during the interval of phage replication. However, infected cells contained normal cellular levels of dATP, dGTP, and dCTP. Upon infection of deoxythymidine-starved *Bacillus subtilis* M160 (a deoxythymidine-requiring mutant of *B. subtilis* W23), mature phage DNA with a normal dTMP content was made. SP10c codes for an enzyme that seems to catalyze the tetrahydrofolate-dependent transfer of 1-carbon fragments to the 5 position of dUMP. The transfer of 1-carbon fragments is not accompanied by oxidation of tetrahydrofolate to dihydrofolate, implying that the enzyme in question is not a dTMP synthetase. It is proposed that dTMP in mature SP10c DNA is derived by the postreplicational modification of some other nucleotide and not by the direct incorporation of dTTP into DNA.

SP10, a pseudolysogenic phage that productively infects the "Marburg" and W23 strains of *Bacillus subtilis* (3, 24, 29), contains an as yet unidentified pyrimidine in its DNA that replaces about 40% of the thymine (17; M. Mandel, personal communication). Interestingly, intracellular development of SP10 has several unusual features for a virus that contains thymine in its DNA (17). These unusual features include (i) an inability to incorporate significant amounts of exogenous deoxythymidine (dThd) into phage DNA, even under conditions that should maximize incorporation of this nucleoside; (ii) an inability to significantly inhibit phage DNA synthesis with 5-fluorodeoxyuridine (FdUrd), an inhibitor of the de novo synthesis of dTMP (13); and (iii) rapid appearance of a potent deoxynucleoside di- and triphosphatase that converts the di- and triphosphates of dThd and deoxyuridine to the corresponding monophosphates; typically, such enzymes occur in phage systems in which the conventional nucleotide is completely replaced by an unusual analog (11, 19).

This communication deals with dThd nucleotide metabolism in SP10c phage-infected cells. The results indicate that dTMP in SP10 DNA is derived by a novel phage-specific mechanism.

MATERIALS AND METHODS

Materials. Nonradioactively labeled materials were purchased from Sigma Chemical Co. Radioactively labeled materials were purchased from Schwarz/Mann, except $^{32}P_i$, which was obtained from New England Nuclear Corp.

Phage and bacteria. Phage SP10c, a clear-plaque variant of SP10 (17), was used in all experiments. *B. subtilis* W23 is ATCC 23059 (17). *B. subtilis* M160 is a mutant of W23 that requires low concentrations (2 μg/ml) of thymine or dThd for growth.

Media. Nutrient broth-salts (NBS) was described previously (3, 17). For labeling with $^{32}P_i$, NBSP was used. NBSP was prepared with nutrient broth (Difco) from which endogenous phosphate was first removed by precipitation as $MgNH_4PO_4$ (25); growth of bacteria became dependent upon readdition of phosphate (data not shown). In addition to the usual ingredients, NBSP contained 0.1 mM KH_2PO_4. NLM was a modified version of the defined medium used by Neubort and Marmur (20). It contained 100 mM Tris-hydrochloride (pH 7.5), 50 mM glucose, 35 mM NaCl, 7.5 mM $(NH_4)_2SO_4$, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM KH_2PO_4, 2 μM $FeCl_3$, and 10 μg each of the 20 common amino acids per ml. NLMT contained 2 μg of dThd per ml. When $^{32}P_i$ was used, the KH_2PO_4 concentration in NLM was reduced to 0.1 mM. When radioactively labeled dThd was employed, 125 μg of deoxyadenosine per ml was added 5 min before infection (5, 8, 35).

Uptake and phosphorylation of exogenous dThd. Cultures (10 ml) were incubated with [*methyl*-^{3}H]dThd (50 μCi/ml) for 6 min. The cells were harvested by centrifugation at 9,000 × *g* for 1 min, washed twice with 20 ml of ice-cold 100 mM phosphate buffer (pH 6.8)-100 mM NaCl, and resuspended into 2 ml of ice-cold 1 M formic acid. After 15 min on ice, the cells were removed by centrifugation, and the supernatant was applied to a 0.5-cm column of Norit A in a Pasteur pipette plugged at the narrow end with glass wool.

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The column was washed with 2 ml of water and eluted with 2 ml of 60% ethanol-0.3% NH_4OH. The eluate was lyophilized, and the residue was dissolved in about 50 μl of 60% ethanol-0.3% NH_4OH. Duplicate 20-μl samples were applied to a glass-backed cellulose thin-layer plate (20 by 20 cm) that was developed in the ascending mode at room temperature with 200 mM ammonium acetate (pH 5.0)-67% ethanol (26). Optical markers (dTTP, dTDP, dTMP, and dThd) were run in parallel. Panels on the plate that received radioactively labeled material were divided into fractions (1 by 1 cm). The cellulose from each fraction was scraped free of the plate and drawn by suction into a Pasteur pipette loosely plugged at the narrow end with glass wool (2). The cellulose was eluted with 1 ml of 60% ethanol-0.3% NH_4OH, and the amount of label in each fraction was determined.

Deoxynucleoside triphosphate pools. Cultures (10 ml) were labeled with $^{32}P_i$ (10 μCi/ml) for 6 min and then extracted as described above except that unlabeled carrier dTTP, dUTP, dCTP, dATP, and dGTP (10 μg each) were added to the Norit A column eluate. Deoxynucleoside triphosphates were resolved by two-dimensional chromatography on polyethyleneimine-cellulose thin layers (Fig. 5b of reference 9). Spots were identified, and the amount of label in each spot was determined.

Other methods. Conditions of infection are described elsewhere (17). Unless stated otherwise, the multiplicity of infection was 10 PFU/cell. Protein determinations were made by the method of Lowry et al. (16).

RESULTS

Uptake and phosphorylation of exogenous dThd. Exogenous dThd and FdUrd are taken up and phosphorylated by the same enzyme system (13, 35). Consequently, inability to effectively label SP10*c* DNA with dThd or to significantly inhibit phage replication with FdUrd (17) could simply mean that this virus somehow obviates uptake and phosphorylation of these deoxynucleosides. To test this hypothesis directly, uninfected and infected cultures were pulse-labeled with high-specific-activity [*methyl*-^{3}H]dThd, and the amount of radioactivity recoverable from cells as dTMP, dTDP, and dTTP was determined.

When uninfected cells were pulse-labeled for 6 min, virtually all the intracellular, acid-soluble label was recovered as dTDP and, especially, dTTP (Fig. 1A). Basically the same pattern was observed when cells were labeled for the first 6 min postinfection by SP10*c* (Fig. 1B). At later times, however, acid-soluble label was recovered almost exclusively as dTMP (Fig. 1C and D); presumably, induction of the phage-coded deoxynucleoside di- and triphosphatase (17) is responsible for the reduced levels of dTDP and dTTP. Because label administered as dThd was recovered as dTMP, it is concluded that salvage of this nucleoside (and, probably, of FdUrd as well) proceeded throughout infection.

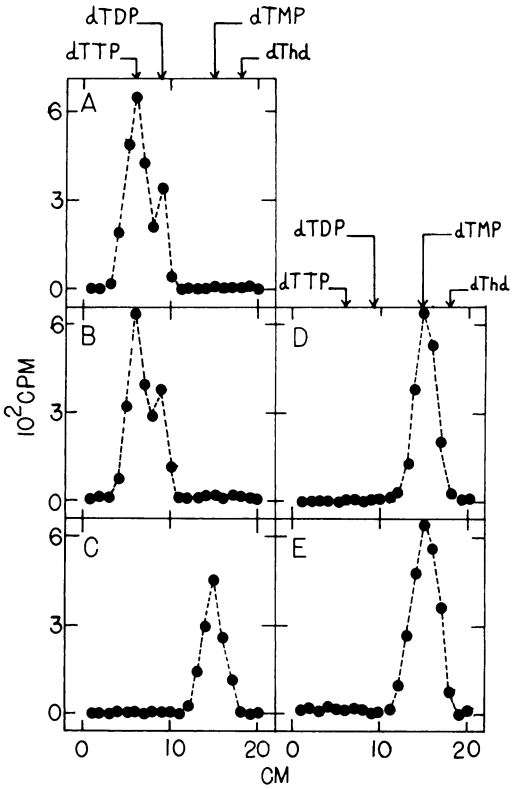

FIG. 1. *Uptake and phosphorylation of [methyl-^{3}H]dThd by uninfected and SP10c phage-infected B. subtilis W23. Cells were grown and infected in NBS as described in the text. At various times postinfection, 10-ml aliquots were transferred to fresh tubes containing 0.5 mCi of [methyl-^{3}H]dThd. The cells were labeled for 6 min, and the amount of label recovered from cells as dThd, dTMP, dTDP, and dTTP was determined as described in the text. (A) Uninfected cells. (B) through (E), SP10c phage-infected cells labeled from 0 to 6, 7 to 13, 21 to 27, and 39 to 45 min postinfection, respectively.*

Deoxynucleoside triphosphate pools. Little, if any, dTTP could be generated via the salvage pathway in SP10*c* phage-infected cells (Fig. 1), and this virus codes for a deoxynucleoside di- and triphosphatase (17). Therefore, some information about the effect of infection on deoxynucleoside triphosphate levels, in general, would be beneficial. In these experiments, cells were labeled with $^{32}P_i$, and the amount of radioactivity recovered as various deoxynucleoside triphosphates was measured.

Relative to uninfected cells, there was no marked change in the amount of radioactivity recovered as dATP, dGTP, and dCTP during an SP10*c* infection (Fig. 2). Intracellular levels of dTTP remained at about the preinfection value for the first 6 min of the SP10*c* program; there-

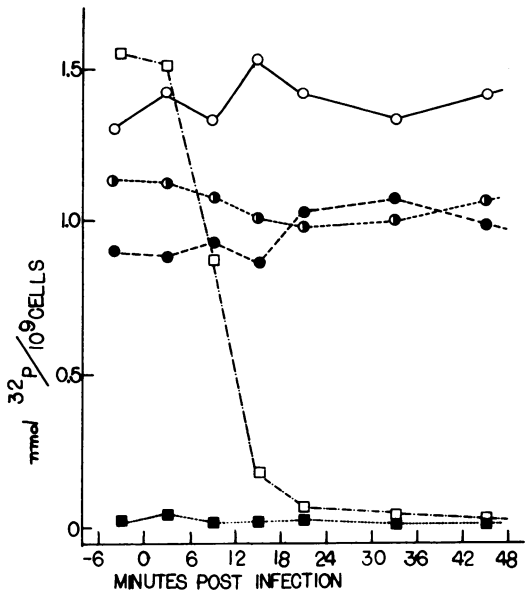

FIG. 2. *Distribution of $^{32}P_i$ among deoxynucleoside triphosphates in uninfected and infected B. subtilis W23. All protocols are given in the text. Cells were pulse-labeled with $^{32}P_i$ (10 μCi/ml) for 6 min. Symbols are placed at the midpoint of the pulse. Symbols: ○, dATP; ●, dGTP; ◑, dCTP; □, dTTP; ■, dUTP.*

after, the amount of label recovered from infected cells as dTTP decreased to less than 5% of the preinfection level. In both uninfected and infected cells, only background levels of radioactivity were recovered as dUTP (Fig. 2), implying that this nucleotide is not a substrate for either bacterial or SP10c DNA synthesis; the absence of dUTP in uninfected *B. subtilis* has been noted before (21).

Development of SP10c in dThd-starved *B. subtilis* M160. The foregoing results indicated that most dTMP in SP10c DNA was probably derived by some virus-specific pathway. To confirm this conclusion, experiments were done with M160 as host. In principle, these experiments should consist simply of infecting dThd-starved and dThd-supplemented cells with SP10c and showing that viral development is normal under both conditions. Unfortunately, SP10c cannot, for some reason, infect cells grown in defined media, and NBS medium contains sufficient endogenous thymine or dThd to support the growth of M160 (data not shown). Consequently, the experiments described below were performed according to the following protocol. Cells were grown and infected in NBS. At 12 min postinfection (i.e., just before the onset of viral DNA synthesis), cells were rapidly harvested by centrifugation, washed once with NLM, and resuspended to their original concentration in either NLM or NLMT. As a control, parallel cultures were resuspended into NBS. It should be noted that all dTTP in uninfected *B. subtilis* M160 is lost within 2 min of the transfer to NLM (data not shown).

A variety of experiments were performed to study SP10c phage development upon shifting to NLM, NLMT, and NBS. The results presented in Fig. 3 show that the eclipse and latent periods of SP10c were somewhat longer after shifting to the chemically defined media but that essentially normal phage yields were obtained. In separate experiments (data not shown), it was shown that normal amounts of SP10c phage-coded enzymes were made in both defined media tested. Consequently, starvation for dThd did not affect SP10c phage development in any major way. In both minimal media employed, there was no detectable dTTP in phage-infected cells (data not shown).

In all three media tested, SP10c DNA was readily labeled with [5-^{3}H]uridine ([5-^{3}H]Urd), [6-^{3}H]Urd, and [*G*-^{3}H]cytidine ([*G*-^{3}H]Cyd); moreover, similar amounts of each precursor were incorporated in the case of all three media, indicating that the level of phage DNA synthesis was not affected by the absence of exogenous dThd (Table 1). In either NLMT or NBS, there was no measureable incorporation of [*methyl*-^{3}H]dThd or [6-^{3}H]dThd into SP10c DNA, whereas *B. subtilis* M160 DNA was effectively labeled by both precursors (Table 1). As expected, there was no detectable incorporation of [^{3}H]Urd or [^{3}H]Cyd into M160 DNA in NLM (Table 1). In independent experiments, M. Mandel (personal communication) has also found that [*methyl*-^{3}H]thymine or [*methyl*-^{3}H]dThd, in the presence of excess deoxyadenosine, are not incorporated into SP10c DNA, whereas label administered as [6-^{3}H]uracil or [6-^{3}H]Urd was incorporated and appeared in all three pyrimidine nucleotides in SP10c DNA.

Label administered as [5-^{3}H]Urd and [*G*-^{3}H]-Cyd was recovered from SP10c DNA as dCMP, whereas all pyrimidine nucleotides in SP10c DNA were labeled in the presence of [6-^{3}H]Urd (Table 2). In NLMT medium, dTMP in M160 DNA was not labeled with [6-^{3}H]Urd, whereas the same precursor labeled dTMP in W23 DNA (Table 2), thereby supporting the conclusion that de novo synthesis of dTMP did not occur in M160.

Finally, Table 3 shows the composition of phage DNA isolated from SP10c propagated in various media. The results clearly indicate that the nucleotide composition of SP10c DNA was unaffected by the medium employed.

Tetrahydrofolate-dependent transfer of 1-carbon fragments to the 5 position of

dUMP. Extracts of *B. subtilis* M160 and W23 cells were tested for dTMP synthetase activity by the tetrahydrofolate-dependent displacement of tritium on [5-^{3}H]dUMP (15, 33) and a standard spectrophotometric procedure (30) which monitors the oxidation of tetrahydrofolate to dihydrofolate. Extracts of M160 showed no activity with either assay employed.

With respect to tritium release from [5-^{3}H]-dUMP, a severalfold increase in activity was evident upon infection of either *B. subtilis* W23 or M160, but there was no concomitant increase in activity as judged by the spectrophotometric assay (Table 4). Coliphage T4 codes for its own dTMP synthetase (19), and extracts of T4-infected *Escherichia coli* B3 (a dThd-requiring

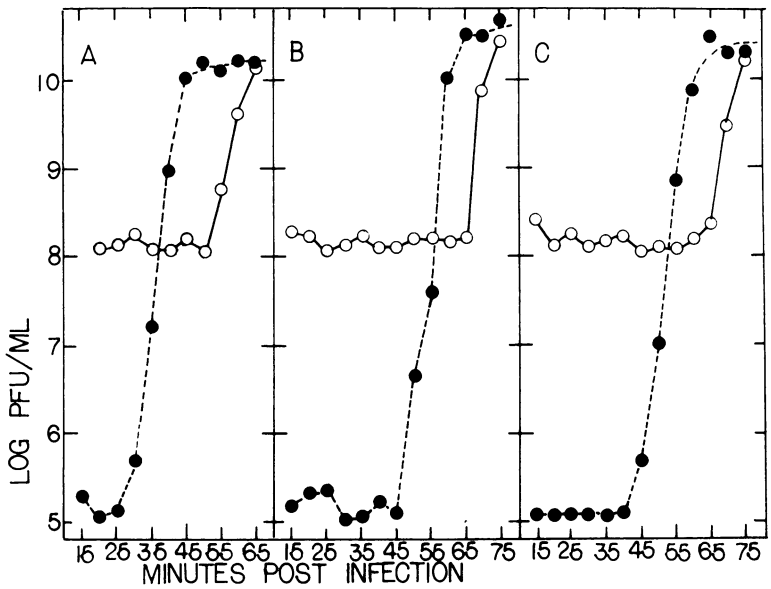

FIG. 3. *Eclipse and latent periods of SP10c upon transfer of infected cells to minimal medium. A culture of B. subtilis M160 was grown and infected in NBS. At 10 min postinfection, three 10-ml aliquots were harvested by centrifugation at 9,000 × g for 1 min, washed once with 25 ml of ice-cold NLM, and resuspended into 10 ml of warm (37°C) NBS (A), NLM (B), or NLMT (C). At the indicated times, duplicate 0.1-ml aliquots were removed. One aliquot was diluted and plated immediately. The other aliquot was diluted into cold medium containing $CHCl_3$ and stored on ice until the end of the experiment, whereupon the samples were diluted and plated. Symbols:* ○, *samples diluted and plated immediately;* ●, *$CHCl_3$-treated samples.*

TABLE 1. *Specific activities of SP10c and B. subtilis DNAs made in a variety of media*[a]

Precursor	Sp act (cpm/μg)					
	SP10c-infected			Uninfected M160		
	NLM	NLMT	NBS	NLM	NLMT	NBS
[5-^{3}H]Urd	12,010	10,090	14,162	5	13,150	17,021
[6-^{3}H]Urd	35,123	37,886	40,142	26	27,480	27,513
[*G*-^{3}H]Cyd	10,831	12,206	20,433	17	10,073	9,569
[*methyl*-^{3}H]dThd	—[b]	11	7	—	148,735	162,521
[6-^{3}H]dThd	—	5	2	—	126,612	178,173

[a] Uninfected or SP10c phage-infected cultures (25 ml) in NBS were transferred to NLM, NLMT, or NBS medium as described in the legend to Fig. 3. Phage-infected cultures received 200 μM 6-(*p*-hydroxyphenylazo)uracil (7, 22) 5 min preinfection to selectively inhibit bacterial DNA synthesis (17). Labeled precursors were present at 2 μCi/ml and minimal media (NLM and NLMT) also received 2 μg of the relevant unlabeled nucleoside per ml. Cultures were labeled from 12 min postinfection until 5 min before the onset of lysis (see Fig. 3). The cells were harvested by centrifugation, and total cellular DNA was extracted (18). In the case of infected cultures, DNA yields were corrected for the fact that about 10% of total cellular DNA present 5 min before lysis is bacterial (our unpublished data).

[b] —, Cultures were not tested for incorporation of labeled dThd.

TABLE 2. *Distribution of radioactivity in pyrimidine deoxynucleotides isolated from SP10c and bacterial DNA*[a]

Medium	Precursor	DNA source	% of recovered label		
			dCMP	dTMP	dYMP[b]
NLM	[5-^{3}H]Urd	SP10c-in-	99.2	0.6	0.2
	[6-^{3}H]Urd	fected	38.3	33.7	28.0
	[*G*-^{3}H]Cyd	M160	98.3	0.9	0.8
NLMT	[5-^{3}H]Urd	SP10c-in-	99.0	0.4	0.6
	[6-^{3}H]Urd	fected	39.4	35.2	25.4
	[*G*-^{3}H]Cyd	M160	99.4	0.6	0
NLMT	[5-^{3}H]Urd	Uninfected	99.8	0.2	—[c]
	[6-^{3}H]Urd	M160	99.5	0.5	—
	[*G*-^{3}H]Cyd		99.6	0.4	—
	[6-^{3}H]dThd		0.7	99.3	—
NLM[d]	[5-^{3}H]Urd	Uninfected	99.5	0.5	—
	[6-^{3}H]Urd	W23	42.6	57.4	—
	[*G*-^{3}H]Cyd		99.1	0.9	—
	[6-^{3}H]dThd		0.3	99.7	—
NLMT	[5-^{3}H]Urd	Uninfected	99.4	0.6	—
	[6-^{3}H]Urd	W23	40.7	59.3	—
	[*G*-^{3}H]Cyd		99.9	0.1	—
	[6-^{3}H]dThd		0.2	99.9	—

[a] SP10c and *B. subtilis* M160 DNAs were the same as those described in Table 1. DNA of uninfected *B. subtilis* W23 was prepared in a separate run. The DNAs were denatured by dialysis against distilled water followed by heating at 60°C for 5 min. The denatured DNAs were hydrolyzed to mononucleotides by treatment with S1 nuclease of *Aspergillus oryzae* (1, 27, 34) and snake venom phosphodiesterase (31). Commercially available snake venom phosphodiesterase was purified further to remove residual phosphatase and 5'-nucleotidase activity (23). The deoxyribonucleoside monophosphates were resolved by two-dimensional chromatography on cellulose thin layers (10). The solvent for the first direction was isobutyric acid–water–NH_4OH (66:20:1); the solvent for the second direction was saturated $(NH_4)_2SO_4$–1 M sodium acetate–isopropanol (80:18:2). Unlabeled dAMP, dGMP, dTMP, dCMP, and dYMP (ca. 10 μg each) were run as optical markers. Spots containing the nucleotides were identified, and the amount of radioactivity in each spot was determined (2).

[b] dYMP is the novel pyrimidine nucleotide that partially replaces dTMP in SP10c DNA (M. Mandel, personal communication).

[c] —, dYMP does not occur in bacterial DNA.

[d] *B. subtilis* M160 does not replicate in NLM (Table 1).

TABLE 3. *Relative abundance of nucleotides in SP10c phage DNA*[a]

Medium	% of label in DNA				
	dAMP	dGMP	dCMP	dTMP	dYMP[b]
NLM	31	19	20	19	11
NLMT	30	20	20	18	12

[a] *B. subtilis* M160 were infected in NBSP and transferred to minimal medium as described in the footnotes to Table 1 and in the text. The minimal medium contained $^{32}P_i$ (10 μCi/ml), and labeling was continued until the time of cell lysis. To insure uniformity of labeling, the phage were passed three consecutive times through bacteria in labeled medium. The phage were purified by the salt–polyethylene glycol method (36) followed by banding in CsCl (4). DNA was isolated (28), and the amount of radioactivity in each of the five nucleotides was determined (see Table 2).

[b] dYMP is the hypermodified nucleotide that partially replaces dTMP in mature SP10c DNA (M. Mandel, personal communication).

mutant) showed about the same increase in activity when assayed by either tritium release or spectrophotometric procedures (Table 4). Table 5 shows the requirements for tritium release from [5-^{3}H]dUMP as catalyzed by extracts prepared 40 min after infection of *B. subtilis* M160 by SP10c. Maximum activity required tetrahydrofolate and formaldehyde. In other experiments (data not shown), we have shown that 40-min extracts of SP10c-infected *B. subtilis* M160 will catalyze the tetrahydrofolate-dependent transfer of [^{14}C]formaldehyde to dUMP. The nucleotide product of these latter reaction mixtures has not been identified unambiguously, but it is neither dTMP nor dYMP. Collectively, these results indicate that SP10c codes for an enzyme that catalyzes the tetrahydrofolate-dependent transfer of 1-carbon fragments to dUMP and that the enzyme in question is probably not a dTMP synthetase.

TABLE 4. *Tritium displacement from [5-^{3}H]dUMP and oxidation of tetrahydrofolic acid by extracts of phage-infected cells*[a]

Time after infection (min)	SP10c				T4[b]	
	B. subtilis W23		*B. subtilis* M160		*E. coli* B3	
	Assay A	Assay B	Assay A	Assay B	Assay A	Assay B
0	0.83	0.99	0	0	0	0
6	0.75	0.96	0	0	0.92	1.12
12	1.02	0.77	1.00	0	4.83	5.63
18	2.62	0.52	2.46	0	8.14	9.03
24	3.81	0.32	3.82	0	8.52	9.03
30	3.95	0.32	3.70	0	8.43	9.21
40	3.93	0.31	3.85	0	8.52	9.16

[a] A 1-liter culture of *B. subtilis* W23 or M160 was grown and infected with SP10c in NBS at 37°C (17). At the designated times, 100-ml samples were removed, and extracts were prepared (17). Assay A measured the displacement of tritium from [5-^{3}H]dUMP that was dependent upon the presence of tetrahydrofolate and formaldehyde (see Table 5). This displacement was measured by the method of Lomax and Greenberg (15) as modified by Wiberg et al. (33). The reaction mixture contained (in 0.5 ml) 50 μmol of Tris-acetate (pH 8), 40 nmol of [5-^{3}H]dUMP (ca. 250,000 cpm), 50 μmol of β-mercaptoethanol, 1 μmol of Na_2-EDTA (pH 8), 0.5 μmol of *d*,L-tetrahydrofolate, 0.5 μmol of formaldehyde, and 100 μg of extract proteins. Reaction mixtures were incubated at 37°C for 5 min (32) before addition of extract, after which addition the reaction mixtures were incubated at 37°C for 30 min. The product ($^3H_3O^+$) was separated from [5-^{3}H]dUMP by a combination of charcoal and anion-exchange chromatography (33). Assay B is the spectrophotometric assay of Wahba and Friedkin (30) that measures the oxidation of tetrahydrofolate to dihydrofolate in the presence of dUMP and formaldehyde; reaction mixtures that contained no dUMP or formaldehyde showed only 2 to 5% of maximum activity. In the case of both assays, we report nanomoles of product formed in 30 min at 37°C per milligram of extract proteins.

[b] *E. coli* B3 was infected with T4 in NBS at 30°C as described elsewhere (2).

TABLE 5. *Dependence of tritium displacement from [5-^{3}H]dUMP on the presence of tetrahydrofolate and formaldehyde*[a]

Reaction	Activity[b]
Complete	4.01[d]
Minus tetrahydrofolate[c]	0.08
Minus formaldehyde[c]	0.13

[a] Data are for extracts prepared 40 min postinfection of *B. subtilis* M160 by SP10c (Table 4).

[b] Activity is nanomoles of tritium displaced in 30 min at 37°C per milligram of protein.

[c] Missing ingredients were replaced with an equivalent volume of sterile, distilled water.

[d] In the case of enzymeless reaction mixtures, only 0.03 to 0.05 nmol of tritium was "displaced," and all activities are corrected for this value.

DISCUSSION

The results presented in this paper indicate that dTMP in SP10c DNA is synthesized by a virus-specific pathway because normal phage DNA was made in dThd-starved *B. subtilis* M160 (Table 3). Throughout phage DNA synthesis, infected cells contained no detectable dTTP (Fig. 1 and 2), implying that dThd nucleotides are not incorporated directly into phage DNA. Thus, dTMP present in mature SP10c DNA may be derived by post-replicational modification of some other nucleotide.

Extracts of SP10c phage-infected cells contain an enzyme that apparently catalyzes the tetrahydrofolate-dependent transfer of 1-carbon fragments to the 5 position of dUMP (Tables 4 and 5). This enzyme is probably not a dTMP synthetase, because there was no apparent oxidation of tetrahydrofolate to dihydrofolate (Table 4). Kelln and Warren (12) reported that *Pseudomonas acidovorans* phage φW-14, in which half the thymine in DNA was replaced by α-putrescinylthymine (14), directs the synthesis of an enzyme with some properties comparable to those of the SP10c enzyme described here. Kelln and Warren (12) proposed that the φW-14 phage-coded enzyme was a dUMP hydroxymethyltransferase. However, it seems premature to offer any strong conclusions about the actual nature of the SP10c enzyme in question until more definitive studies, currently in progress, have been completed.

Although it is not possible, at present, to talk in specific terms about the origin of dYMP and dTMP in SP10c mature DNA, two testable models are shown in Fig. 4. According to both schemes, dYMP and dTMP are ultimately derived from dUMP (Tables 1 and 4). In scheme I, dYMP (the hypermodified nucleotide in mature SP10c DNA) is synthesized de novo, phosphorylated to dYTP, and incorporated into DNA; subsequently, a portion of the dYMP in SP10c DNA is modified to dTMP. In scheme II,

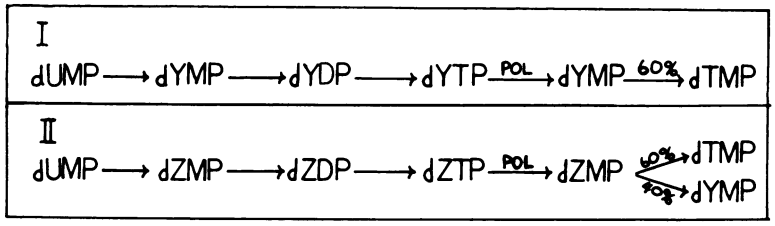

FIG. 4. *Hypothetical schemes for production of dYMP and dTMP in SP10c mature DNA. See text for explanation. All events to the right of the arrow marked pol (polymerization) are viewed as post-replicational modifications of DNA; dYMP designates the unidentified nucleotide that partially replaces dTMP in mature SP10c DNA.*

dUMP is converted to some nucleotide, dZMP, that is phosphorylated and incorporated into DNA; thereafter, dZMP residues in DNA are modified to either dYMP or dTMP. Both hypothetical mechanisms envision dTMP in SP10*c* mature DNA as being a post-replicational modification of another nucleotide.

Both the hypothetical schemes predict that the base composition of newly replicated SP10*c* DNA will differ from that of mature DNA. At 37°C, newly replicated (pulse-labeled) SP10*c* DNA has a base composition similar, if not identical, to that of mature SP10*c* DNA (unpublished data). However, this could simply mean that, at 37°C, the interval between polymerization and modification is shorter than the pulse time used.

As mentioned above, dUMP is viewed as the ultimate source of both dYMP and dTMP in SP10*c* DNA. If so, phage infection should generate conditions that augment the amount of dUMP available for the synthesis of SP10*c*-specific nucleotides. The combined activities of the SP10*c* deoxynucleoside di- and triphosphatase could, in principle, help generate such conditions. The phage deoxyuridine diphosphatase (17) would dephosphorylate dUDP, produced by cellular ribonucleoside diphosphate reductase (13), to dUMP. The deoxythymidine diphosphatase-deoxythymidine triphosphatase activities would generate dTMP (17), and the latter is an inhibitor of dTMP synthetase (21). Considering the amount of dTTP present in cells at the time of infection (Fig. 2, reference 21), the amount of dTMP generated by deoxythymidine triphosphatase could inhibit both cellular dTMP synthetases (21); therefore, the amount of dUMP "wasted" on the de novo synthesis of dTMP in phage-infected cells would be minimal.

SP10 apparently belongs to a small group of viruses in which a hypermodified pyrimidine replaces approximately half the thymine in DNA. Other members of this group are *B. subtilis* phage SP15 and *P. acidovorans* phage φW-14 in which half the thymine in DNA is replaced by 5-(4′,5′-dihydroxypentyl)uracil (6) and α-putrescinylthymine (14), respectively. In the case of SP15, thymine and the hypermodified base are synthesized de novo; i.e., they are assembled as the free mononucleotide and subsequently inserted into DNA (31). On the other hand, cells infected with φW-14 contain no dTTP (R. A. J. Warren, personal communication). Thus, φW-14 and SP10*c* may be special cases in the sense that dTMP in mature phage DNA may be derived by post-replicational modification of another nucleotide.

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