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Relationship of *Bacillus subtilis* DNA Polymerase III to Bacteriophage PBS2-Induced DNA Polymerase and to the Replication of Uracil-Containing DNA

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In vivo studies of PBS2 phage replication in a temperature-sensitive *Bacillus subtilis* DNA polymerase III (Pol III) mutant and a temperature-resistant revertant of this mutant have suggested the possible involvement of Pol III in PBS2 DNA synthesis. Previous results with 6-(*p*-hydroxyphenylazo)-uracil (HPUra), a specific inhibitor of Pol III and DNA replication in uninfected cells, suggest that Pol III is not involved in phage DNA replication, due to its resistance to this drug. Experiments were designed to examine possible explanations for this apparent contradiction. First, assays of the host Pol III and the phage-induced DNA polymerase activities in extracts indicated that a labile Pol III did not result in a labile phage-induced enzyme, suggesting that this new polymerase is not a modified HPUra-resistant form of Pol III. Indeed the purified phage-induced enzyme was resistant to the active, reduced form of HPUra under all assay conditions tested. Since in vitro Pol III was capable of replicating the uracil-containing DNA found in this phage, the sensitivity of the purified enzyme to reduced HPUra was examined using phage DNA as template-primer and dUTP as substrate; these new substrates did not affect the sensitivity of the host enzyme to the drug.

PBS2 (40) is a *Bacillus subtilis* bacteriophage which contains uracil as a complete substitute for thymine in its DNA (41). After infection, host thymine-DNA synthesis stops; however, host DNA is not degraded (44). Concomitant with PBS2 uracil-DNA synthesis, a large increase in DNA polymerase activity is induced (31). Since the induction of this polymerase activity is prevented by inhibitors of RNA and protein synthesis (31), probably at least a part of the polymerase or a protein modifying a host polymerase is made de novo after infection. Price and Fogt (33) have demonstrated that PBS2 phage production and DNA synthesis are resistant to 6-(*p*-hydroxyphenylazo)-uracil (HPUra), a specific inhibitor of *B. subtilis* DNA synthesis (3, 4). Gass et al. (14) and Mackenzie et al. (25) have determined in vitro that the reduced form of this drug, 6-(*p*-hydroxyphenylhydrazino)-uracil, specifically inhibits *B. subtilis* Pol III. Mutants of *B. subtilis* that are resistant to this drug also have a Pol III which is resistant to reduced HPUra, suggesting that Pol III is the "replicase" for *B. subtilis* DNA synthesis (5, 7, 20). The fact that PBS2 DNA synthesis is resistant to HPUra is strongly suggestive that host Pol III is not involved in phage-DNA synthesis. Thus, the phage-induced DNA polymerase activity might alone be responsible for the synthesis of uracil-containing PBS2 DNA.

Replication of *B. subtilis* phages SP8 (3), ϕe (26), and SPO1 (45), whose DNAs contain hydroxymethyluracil instead of thymine, as well as the thymine-containing DNA phages SP3 (3) and SPO2c$_1$ (3), is also unaffected by HPUra. SPO1 (45), SPO2c$_1$ (39), and ϕe (19) induce a large increase in DNA polymerase activity, as does PBS2 (31). The SPO1-induced DNA polymerase has been purified and is resistant to reduced HPUra in vitro (46). In contrast, the thymine-containing DNA phage ϕ105 is inhibited by HPUra and does not induce a new DNA polymerase activity (39). Furthermore, not only are ϕ105 and SPP-1 (another thymine DNA phage) sensitive to HPUra, but this sensitivity also results from their use of host Pol III (38). Rowley and Brown (38) have also demonstrated that the host Pol III does not appear to be modified by the phage. Thus, it appears that phages that are HPUra resistant must induce a new DNA polymerase activity (39), since these phages probably do not use host Pol III for their DNA replication.

There is, however, one report contradictory to

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the idea that host Pol III is not required by phages which have HPUra-resistant replication. Using a temperature-sensitive (*ts*) Pol III mutant of *B. subtilis*, strain 2355 *polA06 mut-1* (2), Lavi et al. (19) showed that phage ϕe DNA synthesis does not occur in this mutant at 48°C (the restrictive temperature for the host) but does occur at the nonrestrictive temperature of 37°C. Phage DNA synthesis does occur at both temperatures in the wild-type strain (2344 *polA06*) and in a spontaneous temperature-resistant (*tr*) revertant of 2355 (R2355 *polA06*). The burst size of ϕe is reduced at 48 versus 37°C by 75 and 71%, respectively, in both the wild type and revertant; however, the burst size is reduced by nearly 100% for phage grown at 48°C in the mutant strain. Temperature shift-down experiments with the ϕe-infected *mut-1* show that Pol III is irreversibly inactivated at 48°C and requires de novo synthesis for restoration of phage DNA synthesis after shift down to 37°C. Temperature shift-up experiments with ϕe-infected *mut-1* cells suggested that ϕe might require the host Pol III for DNA synthesis throughout the phage replication cycle. Lavi et al. (19) have suggested that host Pol III is modified by ϕe phage so that it replicates phage DNA instead of host DNA and becomes insensitive to reduced HPUra. They further suggested that the *bda*$^+$ (bacterial DNA arrest) gene product, coded for by the ϕe genome, may be involved in this modification (18).

However, it should be noted that Montenegro et al. (27) have demonstrated that the mutator phenotype of the *mut-1* host is not expressed during ϕe infection but is expressed during SPP-1 infection. This suggests the involvement of host Pol III in SPP-1 DNA synthesis, as has been discussed, but its noninvolvement in ϕe DNA synthesis. Nevertheless, it is likely that the modification of the *mut-1* Pol III may also result in the loss of its mutator character.

Therefore, we considered the possibility that PBS2-infected cells might also require the host Pol III as suggested above for ϕe (19). Our results indicated that the *ts mut-1* host does restrict PBS2 DNA synthesis. We also examined the relationship of host Pol III to the phage-induced DNA polymerase activity (31), to investigate whether this new activity is a modified form of host Pol III. We further determined the specificity of these two enzymes for uracil- versus thymine-containing DNAs and for dUTP versus dTTP. Finally, the effect of reduced HPUra on these two enzymes was investigated using various assay conditions. The data indicate that the PBS2-induced DNA polymerase and *B. subtilis* Pol III are distinct and unrelated enzymes.

This work comprises part of a thesis submitted by R. A. H. for the degree of Doctor of Philosophy in the Rackham School of Graduate Studies, University of Michigan.

MATERIALS AND METHODS

Materials. The following reagents were obtained from the indicated sources: lysozyme, deoxyribonuclease, and unlabeled deoxyribonucleoside triphosphates from Sigma; yeast extract, tryptone, and tryptose blood agar base from Difco; HAWP membrane filters from Millipore; Metricel GA-6 membranes from Gelman; N-ethylmaleimide (NEM) from Calbiochem; [8-^{3}H]dATP (11 Ci/mmol) and [6-^{3}H]uracil (23 Ci/mmol) from Amersham; and HPUra and fraction V-purified (6) *B. subtilis* DNA polymerase from Neal Brown.

Bacterial strains. All strains used are listed in Table 1.

Extracts. Gentle-lysis extracts (essentially free of endogenous DNA) were prepared as described previously (36). Extracts of sonically disrupted cells were prepared as described by Gass and Cozzarelli (12).

***B. subtilis* and PBS2 DNA preparations.** These DNAs were purified as described elsewhere (manuscript in preparation).

Reduction of HPUra. HPUra was reduced by using dithiothreitol according to the method of Gass et al. (14). This reduction was necessary for in vitro inhibition of *B. subtilis* Pol III (14, 25).

DNA activation. Native DNAs were activated according to the method of Gass and Cozzarelli (12) by treatment with deoxyribonuclease I to 6 to 12 % acid solubility. DNA treated to this extent contains many single-stranded, gapped, and nicked regions with 3′-hydroxyl primers available for the initiation of DNA synthesis.

Determination of acid-insoluble, alkali-stable radioactivity. Samples (50-μl) of cultures labeled with [^{3}H]uracil were added to 1 ml of 10% trichloroacetic acid containing 100 μg of salmon sperm DNA as carrier. After 30 min on ice, the tubes were centrifuged at 10,000 × *g* for 10 min, and the pellets were dissolved in 0.3 ml of 0.3 N NaOH. The tubes were incubated for 20 h at 37°C to hydrolyze RNA; then 1 ml of 10% trichloroacetic acid was added. After 30 min at 0°C, the contents of the tubes were filtered on 4.5-μm HA or GA-6 membrane filters. After the tubes were rinsed with ice-cold 5% trichloroacetic acid, the filters were washed six times with 5 ml of 5% trichloroacetic acid, followed by two times with ice-cold 95% ethanol. The dried disks were counted as previously described (31).

PBS2 titering. Infected cells were shaken vigorously for 2 h at the indicated temperatures, and the lysates were then incubated at 37°C for 15 min in the presence of lysozyme at 100 μg/ml to complete the lysis. Titering was done as previously described (31).

PBS2 phage-induced DNA polymerase assay. Assay mixtures contained 73 mM Tris-hydrochloride buffer (pH 8.5); 70 mM $MgCl_2$; 60 mM NaCl; 1 mM 2-mercaptoethanol (MSH); 0.1 mg of bovine serum albumin per ml; 2.3% glycerol; heat-denatured (10 min at 100°C followed by quick cooling in ice) salmon sperm DNA at 318 μg/ml (0.98 mM in nucleotide

TABLE 1. *Strains of B. subtilis used*

Strain	Genotype	DNA polymerase phenotype[a]	Isolated by	Obtained from
SB19E	*str*^{r} *ery*^{r}	Wild type	I. Takahashi (43)	I. Takahashi
1306	*polA06 leu met*	Pol I$^-$ Pol III$^+$	P. Hempstead[b]	J. Gross
F2	*polA59 met his leu*	Pol I$^-$ Pol III$^+$	N. Cozzarelli (7)	N. Cozzarelli
F25	*polA59 polC25 met his leu*	Pol I$^-$ Pol III^{ts} (from F2 but has HPUra-resistant DNA polymerase III that is also temperature sensitive [7, 8, 13])	N. Cozzarelli (11)	N. Cozzarelli
2344	*polA06 thy*	Pol I$^-$ Pol III$^+$	J. Gross (2)	M. Marcus
2355	*polA06 mut-1 met ileu*	Pol I$^-$ Pol III^{ts} (mutagenic and temperature-sensitive DNA polymerase III [2, 18, 20])[c]	J. Gross (2)	M. Marcus
R2355	*polA06 met ileu*	Pol I$^-$ Pol III^{R+} (revertant of 2355 to temperature resistance)	M. Marcus (19)	M. Marcus
BD318	*polA59 arg*	Pol I$^-$ Pol III$^+$	N. Cozzarelli	N. Cozzarelli
BD317	*polA59 mut-1 arg*	Pol I$^-$ Pol III^{ts} (from BD318, mutagenic and temperature-sensitive DNA polymerase III)	N. Cozzarelli	N. Cozzarelli
BD313	*polA59 dnaF arg*	Pol I$^-$ Pol III^{ts} (from BD318, temperature-sensitive DNA polymerase III)	N. Cozzarelli, *dnaF* by Gross et al. (15)	N. Cozzarelli
JH158	*mut-1*	Pol I$^+$ Pol III^{ts}	N. Cozzarelli (21)	N. Cozzarelli

[a] Mutations *polC25, mut-1,* and *dnaF* all map very close together and near other mutations known to affect DNA polymerase III activity (20). It is likely that they all map in the structural gene for this enzyme.

[b] Ph.D. dissertation, University of London, 1968.

[c] Strain 2355 was derived from strain 1443 (168 *polA06 leu met ileu*) by transformation of *mut-1* (2) using the donor strain 2175 (168 *mut-1 met ileu*). Strain 2344 was derived by transformation from 1443 (1). Thus 2344 and 2355 have somewhat different genetic backgrounds.

equivalents); 37 μM each dGTP, dCTP, dTTP, and [8-^{3}H]dATP (88,000 cpm); plus enzyme in a total volume of 220 μl. The enzyme was added, followed by incubation at 37°C. At 5, 10, and 15 min, a 50-μl sample was processed for acid-insoluble counts as previously described (31). One unit for this assay was 1 nmol of [^{3}H]dAMP incorporated into acid-insoluble material (DNA) per min at 37°C.

Host Pol III assay. These assays were done by a method similar to that of Gass and Cozzarelli (12), except a 220-μl assay contained final concentrations of: 65 mM Tris-hydrochloride (pH 7.5); 6.5 mM $MgCl_2$; 3 mM MSH; 0.1 mg of bovine serum albumin per ml; 2.3% glycerol; activated salmon sperm DNA (treated with pancreatic deoxyribonuclease to 5.7% acid solubility) at 225 μg/ml (0.70 mM in nucleotide equivalents); and 37 μM each dGTP, dCTP, dTTP, and [^{3}H]dATP (506,000 cpm). Samples of 100 μl were removed at 4 and 9 min, and acid-insoluble counts were obtained on membrane filters.

Other assays. The PBS2-induced dTMP phosphohydrolase converting dTMP to thymidine and P_i, was assayed colorimetrically as previously described (32). The *B. subtilis* deoxyuridine triphosphatase activity converting [^{3}H]dUTP to [^{3}H]dUMP and PP_i was assayed at pH 8.5 as previously described (35). Protein concentrations were determined by the method of Lowry et al. (24) using trypsin as a standard.

RESULTS

A *B. subtilis ts* mutant defective in host Pol III restricts PBS2 DNA replication. Since wild-type PBS2 did not produce phage in any strains tested at 48.5°C (the restrictive temperature for *mut-1*), it was necessary to isolate a temperature-resistant mutant of PBS2 for this higher-temperature work. PBS2 phage were plated on tryptose blood-agar base plates on a lawn of *B. subtilis* 1306 at 50°C. Spontaneous mutant plaques resistant to this high temperature were observed at a frequency of 2×10^{-8}. Phage isolated from one plaque (*tr*-1) were used to make more phage in medium at 49°C for use in the following in vivo experiments. The nature of the *tr*-1 mutation(s) is not known. However, this mutant phage retained its resistance to rifampin (added 5 min before infection at 100 μg/ml), a characteristic unique to PBS2 (34) among all other known *B. subtilis* phages. PBS2 is known to induce its own rifampin-resistant RNA polymerase (5).

The *B. subtilis* strains used by Lavi et al. (19) were obtained (see Table 1) and studied. Note that all three are *polA* (lacking DNA polymerase

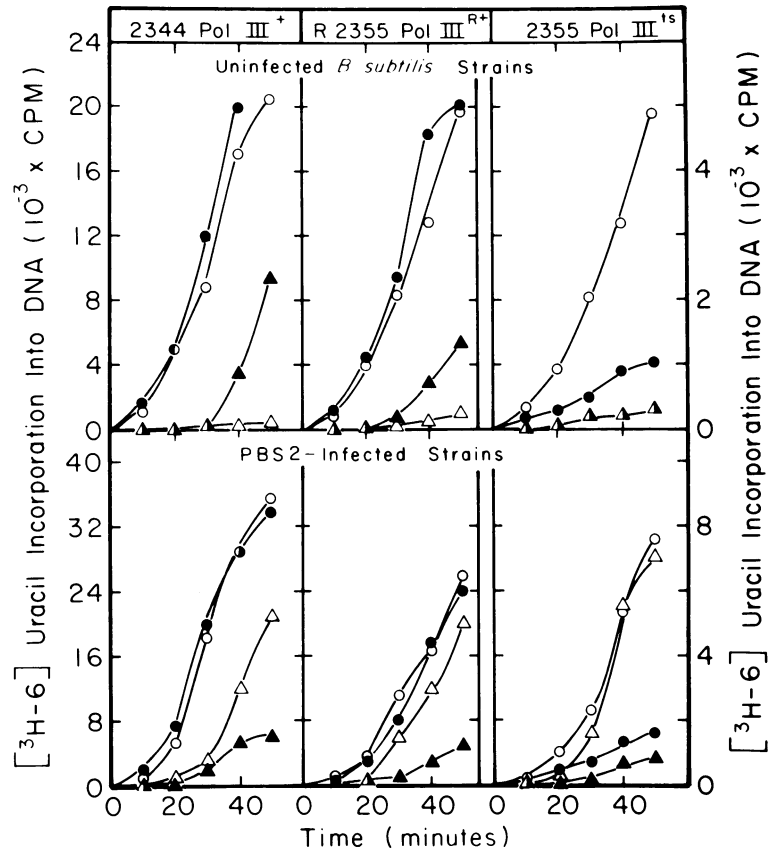

FIG. 1. *DNA synthesis in uninfected and phage-infected B. subtilis mutants at 37 and 48.5°C. B. subtilis strains 2355 mut-1 (Pol III^{ts}), 2344 Pol III^{+}, and R2355 Pol III^{R+} cells were grown to an absorbance at 660 nm (1-cm path) of 1 at 37°C in TY broth (17), and each culture was divided into two portions. One portion remained uninfected, and one portion was infected with PBS2 tr-1 phage at an MOI of 10 (infection occurred at zero time). Two minutes after infection, the two portions were each divided into four tubes containing [6-^{3}H]uracil at a final concentration of 48 μM and 12 μCi/ml. In two of the tubes HPUra was also present at a final concentration of 400 μM. The tubes were immediately blended in a Vortex mixer, and two (one with and one without HPUra) tubes were put in a 37°C shaking water bath; the other two tubes went into a 48.5°C bath. At 10-min intervals, 50-μl samples were removed to determine the alkali-stable, acid-insoluble counts per minute in DNA as described in the text. DNA synthesis (○, △) at 37°C; (●, ▲) at 48.5°C; (○, ●) in the absence of HPUra; and (△, ▲) in the presence of HPUra. Note that the values for the ts strain are on a different scale.*

I activity). Using 2344 Pol III^{+}, 2355 Pol III^{ts} (*mut-1*), and R2355 Pol III^{R+} (*mut-1* revertant) strains, both uninfected and PBS2-infected cells were tested in vivo for DNA synthesis by [6-^{3}H]uracil incorporation into alkali-stable, acid-insoluble material. Uninfected strains 2344 and R2355 both demonstrated similar rates of DNA synthesis at 37 and 48.5°C (see Fig. 1); however, strain 2355 Pol III^{ts} (*mut-1*) showed a nearly complete turnoff of DNA synthesis at 48.5°C. HPUra completely inhibited host DNA synthesis at 37°C for all three strains, but HPUra gave less than complete inhibition at 48.5°C. This HPUra resistance at 48.5°C may be due to the rich TY medium (17) used in these experiments; Lavi et al. (19) did not see such an effect using minimal medium. Nevertheless, at 37°C, HPUra inhibition can be used as an indication of the amount of host DNA synthesis in uninfected and infected cells. Analyses by CsCl equilibrium centrifugation have demonstrated that only phage DNA (density of 1.722 g/cm^{2} and 28% guanine and cytosine content [41]) synthesis occurred in the presence of this concentration of HPUra at 37°C (data not shown).

All of the DNA synthesis at 37°C in PBS2 *tr*-1-infected strain 2355 (*mut-1*, Pol III^{ts}) appeared to be phage DNA synthesis, since little inhibi-

tion by HPUra was seen (Fig. 1). Furthermore, nearly all this phage DNA synthesis was inhibited at 48.5°C in strain 2355 Pol III^{ts}. On the other hand, DNA synthesis in infected strains 2344 Pol III^{+} and revertant R2355 Pol III^{R+} were the same at 37 and 48.5°C, demonstrating that phage-DNA synthesis was not temperature sensitive in these two strains. The partial HPUra resistance of DNA synthesis of these two infected strains indicated that most of the DNA synthesis being observed was of phage origin. Thus, PBS2 *tr*-1 phage DNA synthesis is temperature sensitive in strain 2355, apparently due to the *mut-1* mutation, which produces a *ts* mutant Pol III (2, 19, 20). The revertant of *mut-1* (strain R2355 Pol III^{R+}), however, did not show temperature sensitivity in PBS2 DNA synthesis, suggesting that the *mut-1* mutation in Pol III is causing this effect.

At 48.5°C a nonspecific effect of HPUra on phage DNA synthesis was observed for all three PBS2-infected strains. HPUra also reduced phage titers in the 2 h lysates by 80% for all three. These same effects were also observed for PBS2-infected strains BD313, BD317, and BD318 (see Table 1) during growth at 49.5°C. Thus, these effects appear to be unrelated to the *mut-1* mutation in strain 2355.

Table 2 demonstrates that a great reduction (94%) occurred in phage production for PBS2-infected strain 2355 (*mut-1*, Pol III^{ts}) at 48.5°C as compared to 37°C. A much less severe reduction in lysate titer occurred in PBS2-infected 2344 Pol III^{+} or R2355 Pol III^{R+}. Thus both phage DNA synthesis and phage production are made temperature sensitive in *mut-1* due to a *ts* mutation in the host Pol III. This is evidence that Pol III may be required for PBS2 DNA synthesis. However, it should be noted that *mut-1* grows slowly and replicates DNA slowly even at 37°C, and may thus contain other phenotype defects besides the *mut-1* lesion. The R2355 reversion may therefore involve more than a reversion of Pol III^{ts} to Pol III^{R+}.

Attempts to show this effect with other temperature-sensitive *B. subtilis* Pol III mutants have not been very successful, because DNA synthesis in the available F25 *polC25* (Pol III^{ts}) and BD313 *dnaF* (Pol III^{ts}) strains was not completely turned off by temperature shift up to 50°C. Above 50°C in liquid culture, wild-type *B. subtilis* strains grow poorly. Cozzarelli et al. (8) showed that DNA synthesis in F25 was more temperature sensitive than wild type at 51°C, but this demonstration required very special growth conditions (N. R. Cozzarelli, personal communication). Nevertheless, using growth at 37 versus 49.5°C, strains BD318, BD317, and BD313 were examined for PBS2 *tr*-1 phage production. Lysate titers for 49.5°C infections were reduced 55% for strain BD318 Pol III^{+}, 84% for BD317 Pol III^{ts}, and 73% for BD313 Pol III^{ts}, as compared to infections at 37°C. PBS2 DNA synthesis was not significantly reduced for BD313 *dnaF* (Pol III^{ts}) at high temperature, but was reduced about 40% for BD317 *mut-1* (Pol III^{ts}) (data not shown). These results are consistent with those obtained with strain 2355 *mut-1*, again suggesting a role for host Pol III in PBS2 replication.

Stability of the PBS2-induced DNA polymerase activity is not affected by a mutation making the host Pol III unstable. Considering the previous discussion and having demonstrated that PBS2 behaves similar to ϕe in *mut-1 ts* cells, it is possible that the apparent induction by PBS2 of a new DNA polymerase activity might result from the modification of host Pol III. Since this hypothetical modified form of Pol III retains its *ts* character in PBS2-infected *mut-1* cells, it is possible that this *ts* character might also be observed for the phage-induced DNA polymerase in these cells.

The following experiments were done to test the above model. A certain *B. subtilis* mutant (F25 *polA59 polC25*), selected for its resistance to HPUra, has a defective Pol III which was reported to be very labile in extracts of cells (21). If the phage-induced DNA polymerase were also more labile in F25 *polC25*-infected extracts, it would provide good evidence that Pol III is being modified by the phage. But if the phage DNA polymerase activity were normal in these mutant cells, it would suggest that the host Pol III is not part of the phage-induced DNA polymerase activity.

TABLE 2. *PBS2 phage production in related B. subtilis mutants at 37 and 48.5°C*[a]

Host strain	Phenotype	PBS2 titers (PFU/ml) after lysis at:		% Reduction of titer at high temp
		37°C	48.5°C	
2344	Pol I^{-} Pol III^{+}	4.5×10^{10}	2.5×10^{10}	44
2355	Pol I^{-} Pol III^{ts}	1.0×10^{10}	0.06×10^{10}	94
R2355	Pol I^{-} Pol III^{R+}	4.3×10^{10}	2.4×10^{10}	44

[a] Host cells were grown at 37°C and infected with PBS2 *tr*-1 phage as indicated in Fig. 1, shaken vigorously at 37 or 48.5°C for 2 h, lysed, and titered at 30°C on a lawn of SB19E as described in the text.

To test the heat stability of DNA-free preparations of PBS2 phage-induced DNA polymerase in wild-type and mutant cells, gentle-lysis extracts were prepared from PBS2-infected and uninfected strains (F2 *polA* and F25 *polA polC25*). Part of these 37°C-infected cultures were allowed to go to lysis; both F2 and F25 were lysed normally by PBS2, giving titers of 1 $\times 10^{10}$ to 2 $\times 10^{10}$ phage per ml, corresponding to a burst of 50 to 100 phage per cell. Then host Pol III assays were done on the uninfected extracts to determine whether Pol III had been lost in the strain F25 (*polA polC25*) gentle-lysis extracts (as it had in the sonic extracts made by Low et al. [21]). Since both Pol II and Pol III are present in these extracts and since Pol II also has activity in the Pol III assay, 4.5 mM NEM was used to differentiate between the two polymerases (MSH was omitted in these assays since it reversed the NEM effect). Gass and Cozzarelli (11) have already established that, at this NEM concentration in identical assays, purified *B. subtilis* Pol II is only inhibited 5% whereas Pol III is inhibited 95%. Thus, the amount of activity inhibited by NEM should represent the amount of Pol III present in the extract.

Table 3 shows the results of such assays on gentle-lysis extracts. The polymerase activities of uninfected F2 and F25 *polC25* were essentially the same, suggesting no gross deficiency in the level of Pol III. Another *B. subtilis* enzyme, deoxyuridine triphosphatase, was assayed as a control for host enzyme levels; this enzyme was also present at the same level in the two uninfected extracts. The NEM inhibition of DNA polymerase activity was also essentially the same for both extracts, suggesting that about three-fourths of the activity in each extract was due to Pol III. Thus, in contrast to the sonic extracts made by Low et al. (21), our gentle-lysis extracts of F25 *polC* did not seem deficient in Pol III.

Further evidence that the NEM inhibition seen in the above gentle-lysis extracts represents the Pol III contribution may be seen from inhibition by two concentrations of reduced HPUra (Table 3). At 190 μM reduced HPUra, the extract from F2 *polC*$^+$ was inhibited 41%, in contrast to F25 *polC25*, which was inhibited by only 3%. This is consistent with the fact that F25 *polC25* contains a reduced HPUra-resistant Pol III (7, 8), whereas F2 contains a Pol III which is sensitive to this compound. Our finding that the Pol III activity of F25 *polC25* is not lost during extract preparation by gentle lysis may be valuable to those interested in purifying a host Pol III mutated in an essential region of the chain.

Table 3 also shows that there was normal induction of the PBS2 DNA polymerase activity in F25 *polC25* and F2 *polC*$^+$. Another phage-induced enzyme, deoxythymidylate phosphohydrolase, was assayed as a control and was found at the same level in gentle-lysis extracts of both strains.

Since the Pol III was not labile in a F25 *polC25* gentle-lysis extract, extracts of these same strains were prepared by sonic disruption as described by Gass and Cozzarelli (12). The DNA polymerase activities of uninfected and infected extracts were measured using phage-induced DNA polymerase assay conditions. Uninfected extracts of F2 *polA* and F25 *polA polC25* gave 0.13 and 0.15 nmol/min per mg, respectively, whereas PBS2-infected extracts of F2 and F25 gave 3.5 and 3.4 nmol/min per mg, respectively. The PBS2 DNA polymerase activity was thus induced to an equal extent in both strains, to a level of 23 to 27 times the level of DNA polymerase activity in uninfected extracts assayed under the same conditions. Thus, the level of phage-induced DNA polymerase activity was not reduced in an F25-infected sonic extract versus an F2-infected extract.

To determine whether the host Pol III activity was indeed reduced in F25 versus F2 sonic ex-

TABLE 3. *Enzyme levels in gentle-lysis extracts of PBS2-infected and uninfected B. subtilis strains*[a]

Strain	Uninfected cells					PBS2-infected cells	
	Host DNA polymerase (nmol/min/mg)	% Inhibition by reduced HPUra at (concn):		% Inhibition by NEM at 4.5 mM	dUTPase (nmol/min/mg)	Phage DNA polymerase (nmol/min/mg)	dTMPase (nmol/min/mg)
		19 μM	190 μM				
F2 *polA59*	0.090	11	41	77	25	2.0	217
F25 *polA59 polC25*	0.092	2	3	72	28	1.8	217

[a] Gentle-lysis extracts (free of endogenous DNA) were made, and enzyme assays were performed as described in the text. Uninfected and PBS2 phage-infected (for 45 min at 37°C) cells of strains F2 and F25 were employed. Strain F25 *polC25* had been described by Low et al. (21) to contain undetectable levels of DNA polymerase III in sonic extracts; however, we found normal activity in these gentle-lysis extracts (see text for discussion). dUTPase, Deoxyuridine triphosphatase: dTMPase, deoxythymidine monophosphatase.

tracts, as indicated by Low et al. (21), the uninfected extracts were also tested under the host Pol III assay conditions (Table 4). Assuming that 4.5 mM NEM inhibits all of the Pol III activity (11), the F25 *polA polC25* extract had only 10% NEM-inhibitable activity, in contrast to F2 *polA*, which had 50% of its activity NEM inhibitable. Thus, F25 *polC25* had a greatly reduced Pol III level as compared to F2 *polC*$^+$, demonstrating that the F25 *polC25* Pol III activity was lost during sonic extract preparation. This difference was substantiated in that both extracts had the same *B. subtilis* deoxyuridine triphosphatase activity (23 nmol/min per mg).

These PBS2-infected sonic extracts were also assayed using the host Pol III conditions (Table 4). For infected extracts, it is still possible to examine the host Pol II and III levels since the phage-induced DNA polymerase activity is not detectable under these assay conditions (see below). The data show that almost half of the activity in an extract of PBS2-infected F2 *polC*$^+$ was Pol III (NEM and reduced-HPUra inhibitable), but an infected F25 *polC* extract contained only 16% of its activity as Pol III (Table 4). Thus, the F25 *polC25* Pol III appeared to be unstable in an infected as well as an uninfected extract prepared by sonic disruption. Evidence that this activity really represents Pol III and not interfering phage-induced activity is indicated by the effect of reduced HPUra. Both uninfected F2 *polC*$^+$ and PBS2-infected F2 extracts show that 74% of the amount of NEM-inhibitable activity is also inhibitable by reduced HPUra. As will be discussed, reduced HPUra does not inhibit the phage-induced DNA polymerase, using either host Pol III or phage DNA polymerase assay conditions. Therefore, we conclude that Pol III activity is nearly absent in both PBS2-infected and uninfected sonic extracts of F25 *polA polC*, without reducing specific activity of the phage-induced DNA polymerase in infected F25 extracts. These results indicate that the phage-induced DNA polymerase is not a modified form of host Pol III.

An alternative possiblity is that PBS2 phage induces a modification of the host Pol III to make it more stable. We found that the phage-induced DNA polymerase activity in extracts from infection of either F2 *polC*$^+$ or F25 *polC25* was much more stable than the host DNA polymerase activities (II and III) seen in uninfected

TABLE 4. *Host DNA polymerase activities in extracts of sonically treated cells of uninfected and PBS2 phage-infected strains F2 polA59 and F25 polA59 polC25*

Extract[a]	Inhibitor added[b]	Host DNA polymerase activity[c] (nmol/min/mg)	% Inhibition	
			+MSH	−MSH
Uninfected F2 *polA59*	None	0.062	0	
	HPUra	0.038	39	
	None	0.036		0
	NEM	0.017		53
Uninfected F2 *polA59 polC25*	None	0.049	0	
	HPUra	0.046	6	
	None	0.031		0
	NEM	0.028		10
PBS2-infected F2 *polA59*	None	0.096	0	
	HPUra	0.062	35	
	None	0.092		0
	NEM	0.049		47
PBS2-infected F2 *polA59 polC25*	None	0.058	0	
	HPUra	0.054	7	
	None	0.054		0
	NEM	0.045		16

[a] Sonic extracts were prepared of uninfected and PBS2 phage-infected (45 min at 37°C) cells of strains F2 and F25. Low et al. (21) had indicated that strain F25 *polC25* contained undetectable levels of DNA polymerase III in sonic extracts; our data here agree with this conclusion.

[b] Inhibitor concentrations were 190 μM for reduced HPUra (see the text for reduction procedure) and 4.5 mM for NEM. Incubations using NEM had to be done with no added MSH; thus a separate "no inhibition" control was needed.

[c] All assays were done using host DNA polymerase III assay conditions (see the text) where the PBS2-induced DNA polymerase activity is essentially undetectable (less than 1% of the activity seen under the phage DNA polymerase assay conditions) (see the text and Table 7).

extracts of either cell type. When DNA-free gentle-lysis extracts were incubated at 55°C for 5 min and then assayed at 37°C, both F2- and F25-infected extracts lost only about 10% of their phage-induced DNA polymerase activity, in contrast to the uninfected extracts of both cell types, which lost over 90% of their activities. Thus, the phage-induced activity is indeed much more heat stable than both host DNA polymerases II and III. However, F2 *polA* cells seem to retain after phage infection the normal level (Tables 3 and 4) of host Pol II and III (with normal drug sensitivities). We previously showed that DNA polymerase I levels also remain high 15 min after PBS2 infection of wild-type cells. Thus, it does not appear that appreciable amounts of host Pol III (and II or I) could be normally modified by PBS2 infection.

To obtain further evidence that the phage-induced enzyme is not a modified form of the host Pol III, the following in vitro experiment was performed. Sonically disrupted cell extracts of PBS2-infected strains F2 *polA* and F25 *polA polC25* (Pol III deficient, see above) were incubated at 55°C; at various times, samples were removed for assay at 37°C under the phage-induced DNA polymerase assay conditions, to determine whether there was any difference in the heat-inactivation curves of the phage-induced DNA polymerase activity present in F2 versus F25 due to the *polC25* mutation (Fig. 2). Both activities displayed parallel heat inactivation, suggesting that the phage-induced DNA polymerase of infected F25 *polC25* does not have increased lability due to the host Pol III *polC25* mutation. Furthermore, heat-inactivation curves for extracts of PBS2-infected strain F2 *polA59* versus strain BD317 *polA59 mut-1* also displayed parallel heat inactivation, similar to Fig. 2. Thus, neither of the Pol III *ts* mutations in F25 *polC25* nor BD317 *mut-1* resulted in increased lability of the phage-induced DNA polymerase. We conclude that the phage-induced DNA polymerase is probably not a modified form of host Pol III.

Purified host Pol III can use dUTP as a substrate and uracil-containing DNA as a template-primer. If host Pol III were involved in PBS2 uracil-DNA synthesis, then this enzyme must be capable of using dUTP as a substrate. We have demonstrated that the dTTP pool disappears and dUTP accumulates after PBS2 infection, thus changing the substrate available for phage DNA synthesis (16). Table 5 demonstrates that purified *B. subtilis* Pol III uses either dUTP or dTTP, with either uracil-containing PBS2 DNA or thymine-containing *B. subtilis* or salmon sperm DNA as template-primer. Note that the purified PBS2-induced DNA polymerase can also use both dUTP and dTTP, as well as both uracil- and thymine-containing DNAs. (A more detailed study of the specificity of purified phage-induced DNA polymerase is in preparation.) For Table 5 it was necessary to use deoxyribonuclease-activated DNAs for comparison, since purified host Pol III will not use denatured DNA as a template, unlike the purified phage-induced enzyme, which uses denatured DNA as well as activated DNA.

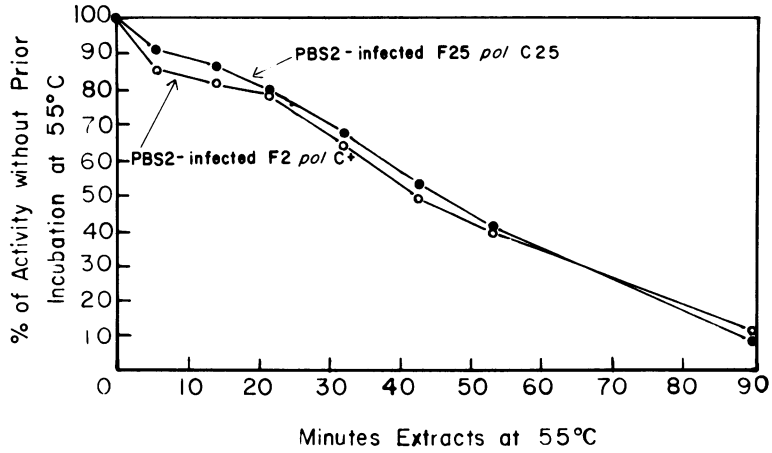

FIG. 2. *Heat-inactivation curves of the PBS2-induced DNA polymerase activities present in extracts of sonically disrupted cells of PBS2-infected F2 polA59 and PBS2-infected F25 polA59 polC25. Both sonic extracts (see Table 4) were incubated in parallel at 55°C, and at the indicated times samples were withdrawn and assayed immediately at 37°C for 10 min using the standard phage-induced DNA polymerase assay procedure (see the text).*

TABLE 5. *Comparison of purified B. subtilis DNA polymerase III and PBS2 DNA polymerase for use of uracil- versus thymine-containing DNAs and deoxyribonucleoside triphosphates*

DNA template-primer	dNTP[a]	Relative DNA polymerase activity (%)	
		B. subtilis DNA polymerase III[b]	PBS2 DNA polymerase[c]
Activated salmon sperm[d]	dUTP	81	
Activated salmon sperm	dTTP	100	
Denatured PBS2 DNA	dUTP	1	
Denatured PBS2 DNA	dTTP	3	
Native PBS2 DNA	dUTP	1	
Native PBS2 DNA	dTTP	3	
Activated PBS2 DNA[d]	dUTP	70	100
Activated PBS2 DNA	dTTP	134	101
Activated *B. subtilis* DNA[d]	dUTP	56	48
Activated *B. subtilis* DNA	dTTP	69	45
None	dUTP	0	0
None	dTTP	0	0

[a] In either the phage-induced DNA polymerase assay or the host DNA polymerase III assay (see the text), either dUTP or dTTP was present with [^{3}H]-dATP, dGTP, and dCTP. dNTP, Deoxyribonucleoside triphosphate.

[b] The host DNA polymerase III assay was used with 60 mM NaCl and 10 μl of a 10-fold dilution of purified *B. subtilis* DNA polymerase III (fraction V activity [6] was obtained from Neal Brown) per 110-μl assay. One hundred percent activity for this assay was the conversion of 84,700 cpm of [^{3}H]dATP to acid insolubility in 12 min out of a possible 600,000 total cpm.

[c] Alterations of the standard phage-induced DNA polymerase assay were 7 mM $MgCl_2$, 360 mM NaCl, each DNA at 200 μg/ml, and 2 μl of DNA-cellulose-purified PBS2 DNA polymerase (27 nmol/min per ml) per 110-μl assay. One hundred percent activity for this assay was the conversion of 80,000 cpm of [^{3}H]dATP to acid insolubility in 6 min out of a possible 144,000 cpm.

[d] Native salmon sperm, phage PBS2, and *B. subtilis* DNAs were activated by deoxyribonuclease treatment to 6, 12, and 10% acid solubility, respectively.

Thus it appears that neither *B. subtilis* Pol III nor the PBS2-induced enzyme has much specificity for dUTP versus dTTP or for uracil- versus thymine-containing DNAs. Thus, both enzymes are capable of carrying our PBS2 DNA synthesis, so there is no apparent necessity for the induction of a new DNA polymerase uniquely capable of replicating uracil-containing DNA. Of course, phage DNA replication in vivo may well take place in a "complex" where only phage-induced proteins might be functional, as indicated for *Escherichia coli* phage T4 (9, 28, 37; G. R. Greenberg and C. Chiu, Fed. Proc. **37**:768, 1978). This hypothetical complex for PBS2 DNA replication might have the selectivity necessary for the switch from thymine-DNA synthesis to uracil-DNA synthesis.

Effects of reduced HPUra on purified *B. subtilis* Pol III using uracil- and thymine-containing substrates. The observed resistance of PBS2 infection to HPUra (33) might result from the induction (31) of a new phage-coded DNA polymerase, by the modification of host Pol III, or by a combination of both. However, an alternative model would be that the presence of dUTP instead of dTTP and the use of a uracil-containing PBS2 DNA instead of thymine-containing *B. subtilis* DNA could prevent reduced HPUra from inhibiting Pol III. Although this seemed unlikely in view of the known interaction of reduced HPUra in base pairing with DNA-cytosine (14, 25) to form a long-lived ternary complex with the enzyme (6, 8, 22), this model was nonetheless tested in vitro. The data in Table 6 show that purified *B. tilis* Pol III was inhibited to about the same extent by reduced HPUra with all possible combinations of uracil- versus thymine-containing DNAs and with dUTP versus dTTP. Thus, the above alternative model may be eliminated.

Reduced HPUra effects on the purified

TABLE 6. *Effects of reduced HPUra on purified B. subtilis DNA polymerase III activity using uracil- versus thymine-containing DNAs and deoxyribonucleoside triphosphates*

DNA[a]	dNTP[b]	Reduced HPUra added[c] (μM)	DNA polymerase activity[d] (%)	Percent inhibition by HPUra
Activated PBS2	dUTP	0	46	
Activated PBS2	dUTP	182	15	67
Activated PBS2	dTTP	0	100	
Activated PBS2	dTTP	182	19	81
Activated *B. subtilis*	dUTP	0	36	
Activated *B. subtilis*	dUTP	182	10	72
Activated *B. subtilis*	dTTP	0	51	
Activated *B. subtilis*	dTTP	182	10	80

[a] DNAs were activated as described in Table 5 and were used in the assays at 220 μg/ml.

[b] The host (*B. subtilis*) DNA polymerase III assay was done as described in the text but with 60 mM NaCl also present. The assays were done with 37 μM dUTP or dTTP, plus [^{3}H]dATP, dGTP, and dCTP present as described in the standard assay. dNTP, Deoxyribonucleoside triphosphate.

[c] Reduced HPUra (see the text) was added as indicated.

[d] Ten microliters of a 10-fold dilution of *B. subtilis* DNA polymerase III (obtained from Neal Brown as fraction V activity) was used per 110-μl assay. One hundred per cent activity was 135;000 cpm of [^{3}H]dATP becoming acid insoluble in 12 min out of 600,000 total cpm.

PBS2 phage-induced DNA polymerase activity. The purified PBS2-induced DNA polymerase was also examined for its sensitivity to reduced HPUra. Using dUTP versus dTTP and uracil- versus thymine-containing DNAs, the data in Table 7 were obtained. Lines 1 to 8 were obtained using the phage-induced DNA polymerase assay. The same reduced HPUra preparation as employed for Table 6 (experiments were done at the same time) was used here to insure the potency of this drug. In all cases the PBS2 DNA polymerase demonstrated no significant inhibition by the drug under these conditions.

Finally, the possibility existed that the lack of sensitivity to reduced HPUra by the PBS2 DNA polymerase was due to the differences in the standard assay conditions used for the phage enzyme as opposed to the host enzyme (compare these two assay procedures in Materials and Methods). To eliminate this possibility, the purified PBS2 phage-induced enzyme was assayed (Table 7, lines 12 and 13) using host Pol III assay conditions as employed in Table 6. About 20 times more phage DNA polymerase had to be used in the assays, since the phage enzyme gives very little activity under the host enzyme assay conditions (about 5% of the activity, comparing lines 10 and 12 and also lines 11 and 13, or about 1% of the activity, comparing lines 3 or 7 with 12). Again, the phage enzyme demonstrated negligible inhibition by reduced HPUra. Thus, under all the conditions tested, reduced HPUra had essentially no effect on the PBS2 DNA polymerase activity, in line with the in vivo results.

DISCUSSION

The results of Lavi et al. (19) concerning the *ts* character of phage ϕe DNA synthesis in *mut-1* (Pol III^{ts}) cells have been controversial in light of the finding that ϕe DNA synthesis is resistant to HPUra, a specific inhibitor of Pol III (26). The first results suggest that the host Pol III is involved in ϕe phage DNA synthesis, while the other results indicate that it is not. In our studies with PBS2, the same contradiction was observed and further investigated.

Although we have demonstrated that PBS2 DNA synthesis (Fig. 1) and burst size (Table 2) are reduced at the restrictive temperature for strain 2355 *mut-1* (Pol I$^-$ Pol III^{ts}), there still remains some doubt as to the validity of the conclusion that host Pol III is involved in PBS2 replication. First, the *mut-1* mutation in the Pol III gene is the only available *ts* mutation which displays a reasonable restrictive temperature for DNA synthesis (48.5°C) in liquid broth (19, 20).

TABLE 7. *Lack of effect of reduced HPUra on the purified PBS2 phage-induced DNA polymerase activity using uracil- versus thymine-containing DNAs and deoxyribonucleoside triphosphates*

DNA polymerase assay conditions[a]	DNA[b]	dNTP[c]	Reduced HPUra[d] (μM)	Relative activity[e] (%)	Percent inhibition by HPUra
1. Phage	Activated PBS2	dUTP	0	100	
2. Phage	Activated PBS2	dUTP	182	103	0
3. Phage	Activated PBS2	dTTP	0	101	
4. Phage	Activated PBS2	dTTP	182	97	4
5. Phage	Activated *B. subtilis*	dUTP	0	48	
6. Phage	Activated *B. subtilis*	dUTP	182	47	2
7. Phage	Activated *B. subtilis*	dTTP	0	45	
8. Phage	Activated *B. subtilis*	dTTP	182	44	2
9. Phage	Denatured salmon sperm	dTTP	0	14	
10. Phage	Denatured salmon sperm	dTTP	182	14	0
11. Phage	Denatured salmon sperm	dTTP	364	13	7
12. Host	Activated salmon sperm	dTTP	0	0.67	
13. Host	Activated salmon sperm	dTTP	182	0.64	4

[a] DNA-cellulose-purified PBS2 DNA polymerase (manuscript in preparation) was used at 2 μl (27 nmol/min per ml by standard phage-induced DNA polymerase assay) for lines 1 through 8, at 2.5 μl (27 units per min per ml) for lines 9 through 11, and at 15 μl (104 units per min per ml) for lines 12 and 13 per 100-μl assay. Either host DNA polymerase III or phage DNA polymerase assay conditions were used as described in the text; however, lines 1 through 8 were obtained using 7 mM $MgCl_2$ with 360 mM NaCl and lines 9 through 13 were obtained using 70 mM $MgCl_2$ with 60 mM NaCl.

[b] DNAs were activated as in Table 5 and used in each assay at a DNA concentration of 220 μg/ml. However, denatured DNA was present at 320 μg/ml.

[c] Assays contained either dUTP or dTTP plus [^{3}H]dATP, dGTP, and dCTP. dNTP, Deoxyribonucleoside triphosphate.

[d] HPUra was reduced as described in the text and used at the indicated concentrations.

[e] All lines are normalized to line 1; 100% activity was 80,000 cpm of [^{3}H]dATP becoming acid insoluble in 6 min out of 144,000 total cpm.

The *mut-1* mutation, isolated by Hempstead (Ph.D. dissertation, University of London, 1968), is highly mutagenic, and care must be taken that other deleterious mutations do not occur during its handling (2). It is possible that this strain may have another mutation in it that is causing the observed phenotype during PBS2 phage infection. However, the behavior (Fig. 1 and Table 2) of strain R2355, a spontaneous revertant of 2355 to temperature resistance (19), gave results similar to that with 2344 *polA polC*$^+$, strongly suggesting that *mut-1* is the effectual mutation. Furthermore, revertant R2355 no longer has the *ts* and mutagenic Pol III phenotypes (19). Unfortunately, Lavi et al. (19) did not indicate the frequency of this spontaneous reversion.

Since the above in vivo experiments suggested that the *mut-1* strain maintained its *ts* character during a possible involvement with PBS2 DNA replication, a proposed phage-induced modification of the host Pol III (to make it HPUra resistant for phage DNA synthesis in vivo) would not eliminate the *ts* character of the modified form. Using another strain with a *ts* Pol III mutation (*polC25*), we have demonstrated (Table 4) that Pol III activity, but not the phage-induced DNA polymerase activity, is diminished in sonic extracts of uninfected and phage-infected cells. When heat stress was applied to phage-induced DNA polymerase activities from the above strain and from the wild-type strain, both showed parallel heat inactivation (Fig. 2). Similar results were also observed during comparison of extracts from a *mut-1* strain and its Pol III$^+$ phage-infected parental strain. Thus, it appears that the *ts* character of mutant host Pol III is not reflected in the PBS2 DNA polymerase activity induced in these mutant strains. Therefore, this PBS2 activity is probably not a converted form of host Pol III.

Another important observation made in comparing F2 *polC*$^+$ and F25 *polC25* extracts was that the host Pol III could be assayed in the presence of the phage-induced DNA polymerase (Tables 4 and 7). The latter activity is essentially inactive under host Pol III conditions, apparently due to an aggregation phenomenon with PBS2 DNA polymerase accompanied by an associated loss of activity that occurs at low ionic strength (manuscript in preparation). This allowed us to determine that host DNA polymerases II and III were present 30 min after PBS2 infection, at about the same levels and in about the same ratio as found in uninfected extracts. In extracts of uninfected and phage-infected F2 *polA59* cells, host Pol III represents about 50% of the activity as determined by NEM inhibition. Furthermore, the Pol III present in both uninfected and PBS2-infected F2 cell extracts demonstrated identical sensitivity to reduced HPUra. This is interesting since Pol III must be modified in some way so as to lose its sensitivity to this drug if it is to be directly involved in phage DNA replication in vivo. However, a change in a hypothetical replication complex which might result in reduced HPUra resistance by a "loose" association, might be very labile and easily disrupted in vitro (37).

Using our purified PBS2-induced DNA polymerase, N. C. Brown and M. Barnes (personal communication) demonstrated that antibody made against purified *B. subtilis* Pol III did not affect the activity of the PBS2-induced DNA polymerase, under conditions in which Pol III was inhibited over 85%. Nor was the phage-induced enzyme retained on a Sepharose column to which this antibody was covalently attached, under conditions in which Pol III was retained.

Since it appeared that the phage-induced DNA polymerase and the host Pol III were two distinct enzymes, their abilities to synthesize uracil-containing DNA using PBS2 DNA as template and dUTP instead of dTTP as substrate were compared. Results show that either enzyme appeared to be quite capable of uracil-DNA synthesis in vitro (Tables 5 to 7). These results are consistent with the recent in vivo results of Tamanoi and Okazaki (42) demonstrating uracil incorporation during DNA replication in a *B. subtilis* mutant.

As expected, the purified PBS2 phage-induced DNA polymerase was shown to be insensitive to reduced HPUra (Table 7), even when assayed using host DNA polymerase conditions, where *B. subtilis* Pol III is inhibited over 80% by this drug. Finally, the sensitivity of purified *B. subtilis* Pol III to reduced HPUra was examined in vitro, to determine whether the new substrates, uracil-DNA and dUTP, present in phage-infected cells could make Pol III resistant to reduced HPUra. This was not the case (Table 6), since HPUra had the same effect with uracil or thymine-containing substrates.

Further evidence that the purified PBS2 phage-induced enzyme is different from *B. subtilis* DNA polymerases I, II, and III is summarized in Table 8. The PBS2 DNA polymerase's molecular weight and subunit sizes are completely different from those of the host enzymes. Besides the high ionic strength required for the PBS2 enzyme's activity, it differs from the other enzymes in its extensive aggregation at low salt. The phage enzyme's template-primer preference is for both denatured DNA and activated DNA; however, activated DNA is used only in the presence of high ionic strength. Only host Pol I shares this property of using denatured DNA, since the other two host enzymes will not use

TABLE 8. *PBS2 phage DNA polymerase compared with B. subtilis DNA polymerases I, II, and III*

DNA polymerase[a]	Mol wt	Subunit size	Optimal NaCl concn (mM)[b]	DNA template preference	Associated exonuclease activity	Percent inhibition by			State in *polA polC*^{ts} extracts
						ara-CTP[c]	NEM[d]	HPUra[e]	
PBS2	155,000	69,000 +78,000 +27,000(?)	350	Denatured or activated	3′ → 5′	73	87	0	Stable
Host I	115,000	One chain	250	Denatured or activated	None known	0	0	0	Absent
Host II	170,000	Unknown	100	Activated	None known	52	0	0	Present
Host III	166,000	One chain	15	Activated	3′ → 5′	15	90	99	Unstable

[a] *B. subtilis* DNA polymerase data are from Gass and Gozzarelli (11, 23) and others (6, 10). Some of PBS2 DNA polymerase data are to be presented elsewhere (in preparation).

[b] With $MgCl_2$ at 7 mM in the assay.

[c] With dCTP at 40 μM, ara-CTP was used as an inhibitor at 50 μM.

[d] Without added 2-mercaptoethanol, NEM was added as an inhibitor at 2 mM.

[e] With dGTP at 40 μM, HPUra was tested as an inhibitor at 100 μM.

and are even inhibited by this template-primer (11). An associated 3′ → 5′-exonuclease has only been demonstrated for the PBS2 enzyme (manuscript in preparation) and host Pol III (23); however, host Pol I has not been thoroughly investigated as to a possible exonuclease (29, 30). The differences between ara-CTP, NEM, and reduced HPUra sensitivities also are good indications for different properties of the phage versus the host enzymes (11). The PBS2-induced DNA polymerase was purified from strain 1306 (see Table 1), which is deficient in host Pol I activity. Furthermore, host Pol II and III activities were separated from the phage-induced DNA polymerase on phosphocellulose chromatography (R. A. Hitzeman, Ph.D. dissertation, University of Michigan, Ann Arbor, 1978). Therefore, the PBS2-induced DNA polymerase appears to be distinct and unrelated to the *B. subtilis* Pol III or to the other two host DNA polymerases.

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