 α -Putrescinylothymine and the sensitivity of bacteriophage ϕ W-14 DNA to restriction endonucleases

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ABSTRACT

The modified base α -putrescinylothymine (putT) in ϕ W-14 DNA blocks cleavage of the DNA by 17 of 32 Type II restriction endonucleases. The enzymes cleaving the DNA do so to widely varying extents. The frequencies of cleavage of three altered forms of the DNA show that putT blocks recognition sites either when it occurs within the site or when it is in a sequence flanking the site. The blocking is dependent on both charge and steric factors. The charge effects can be greater than the steric effects for some of the enzymes tested. All the enzymes cleaving ϕ W-14 DNA release discrete fragments, showing that the distribution of putT is ordered. The cleavage frequencies for different enzymes suggest that the sequence CAPutTG occurs frequently in the DNA. Only TaqI of the enzymes tested appeared not to be blocked by putT, but it was slowed down. TaqI generated fragments are joinable by T4 DNA ligase.

INTRODUCTION

Many bacteriophage DNAs contain modified bases. Such DNAs can be divided into two types: those in which a given base is replaced completely and those in which a given base is replaced only partially by a modified base (1). As might be expected, since methylation of specific cytosines (C) or adenines (A) within the palindromic recognition sequences of Type II restriction endonucleases will prevent cleavage by certain of the enzymes, the modified phage DNAs of both types are either resistant to, or are cleaved only slowly by, many of these enzymes (2-6). Since sequences lying outside the recognition site influence the cleavage of DNA by EcoRI (7,8), it is not surprising that the modified bases can, in at least some instances, protect sites which do not contain the base in question (4-6).

The modifying groups in those DNAs examined vary considerably in size, and the majority of them are uncharged (1-6). The DNA of Bacillus subtilis phage SP15 is unusual in that 62% of its thymine (T) residues are replaced by phosphoglucuronated and glucosylated 5-(4',5'-dihydroxypentyl)uracil (hp⁵U; 9,10). SP15 DNA is resistant to

digestion by many Type II enzymes with 4, 5 or 6 base recognition sites, including some without T in those sites (4). The bulky, negatively charged substituents on the hp^5U residues obviously interfere strongly with cleavage at both modified and unmodified recognition sites.

This paper deals with the sensitivity of another unusual phage DNA to cleavage by Type II restriction endonucleases. The DNA of Pseudomonas acidovorans phage $\phi\text{W-14}$ contains 51 moles per cent guanine (G) + C, and has a molecular weight of 1.16×10^8 ; half its thymine residues are replaced by 5-(4-aminobutylaminomethyl)uracil, the trivial name for which is α -putrescinythymine (putT; 11, 12). Thus, $\phi\text{W-14}$ DNA is similar to SP15 DNA in having about half of its T residues replaced by a base carrying a bulky, charged substituent. The substituent on $\phi\text{W-14}$ DNA is smaller than that on SP15 DNA, however, and it is positively rather than negatively charged. The positive charges on putT can be removed by acetylation (13), and mutants of $\phi\text{W-14}$ have been isolated in which the putT content of the DNA is reduced relative to that of wild-type DNA (14,15). These different forms of $\phi\text{W-14}$ DNA can be used to examine both charge and steric effects of putT on the sensitivity of the DNA to Type II enzymes. Since putT accounts for some 12 percent of the total bases, the cleavage patterns should give information on the distribution of putT in $\phi\text{W-14}$ DNA.

MATERIALS AND METHODS

DNAs

am42 is a mutant of $\phi\text{W-14}$ that, in the non-permissive host, produces DNA containing 6 moles percent putT rather than the 12 moles percent found in the wild-type (15); this DNA is packaged into particles which are infectious but unable to produce progeny (15). am42rev4 is a revertant of am42 that produces progeny in the non-permissive host; its DNA contains 9 moles per cent putT (15). Phage preparations were purified by differential centrifugation. The final phage pellets were resuspended in 0.01M Tris.HCl-0.15M NaCl-0.01M EDTA, pH 7.4. Pronase was added to a final concentration of 2 mg ml^{-1} , the suspension made 0.5 per cent in SDS, and then incubated overnight at 37°C . The DNAs were purified by extraction once with phenol, once with phenol-chloroform (1:1) and once with chloroform. The final preparations were stored at 4°C over chloroform. DNAs were dialyzed against an appropriate buffer before use (16). Wild-type DNA was acetylated with acetic anhydride (13). Although

all of the putresciny groups react, the reaction does not go to completion under the conditions employed: about 60 per cent of the groups are diacetylated and 40 per cent monoacetylated (13).

Enzymes. *RsaI*, *XmaI*, *SspI*, *AhaIII* and T4 DNA ligase were from New England Biolabs. All other enzymes were from Bethesda Research Laboratories. The enzymes were used according to the manufacturers' directions. Reaction mixtures contained 5-10 units of enzyme μg^{-1} of DNA and were incubated overnight. Enzymes showing little or no activity on $\phi\text{W-14}$ DNAs were checked for activity on *Acinetobacter calcoaceticus* DNA.

Agarose gel electrophoresis

Digests were analyzed by electrophoresis through agarose gels. The conditions used varied according to the extent of digestion by a given enzyme. Tris-acetate buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) and 0.4 or 0.7% agarose were used for digests containing fragments largely > 10kb; Tris-phosphate buffer (40 mM Tris-phosphate, 1 mM EDTA, pH 8.0) and 1.0 or 1.4% agarose were used for digests containing predominantly smaller fragments.

Ligation of *TaqI* digested DNA

TaqI digests of wild-type DNA were ligated with T4 DNA ligase under standard conditions. Reaction mixtures contained 2 μg DNA fragments and 400 units of enzyme, and were incubated for 2 hr. The products were examined by electrophoresis through agarose gels.

RESULTS

Properties of $\phi\text{W-14}$ DNA

Theoretically, a DNA of 175 kbp and 51 moles percent G + C could be cleaved more than 600 times and about 40 times by restriction endonucleases with 4- and 6-base recognition sites, respectively. Most of the enzymes tested had 6-base recognition sites because it simplified analysis of the fragment patterns. Digests were analyzed after incubation overnight because it was the frequency and not the rate of cleavage which was of interest. Restriction fragments from $\phi\text{W-14}$ DNA will migrate slower than expected during electrophoresis because the putresciny groups reduce the net negative charge on the DNA by almost 25 percent, and increase the molecular weight by about 0.8 percent. Acetylation removes the positive charges on the putresciny groups but increases the molecular weight by about another 2.4 per cent. am42 and am42rev4 DNAs have fewer

putrescinyI groups and lower molecular weights than wild-type DNA. This means that the net negative charges on the same fragment from the four DNAs are acetylated > am42 > am42rev4 > wild-type, whereas the molecular weights of the fragment are acetylated > wild-type > am42 rev4 > am42. These differences make it difficult to identify with certainty common fragments released from the DNAs by a given enzyme, and to assign molecular weights to fragments. The numbers of fragments released by a given enzyme could not always be determined accurately; bands were counted without regard to relative fluorescent intensities; fragments of molecular weight greater than 20 kb were not clearly resolved. Nonetheless, the gels indicated clearly the relative sensitivities of the four DNAs to the enzymes tested. The results are summarized in Table 1.

Cleavage by enzymes with recognition sites of composition 2G:2C

Wild-type DNA was cleaved into more than 30 fragments by HhaI (GCG↓C) HpaII (C↓CGG), MspI (C↓CGG), FnuDI (GG↓CC), FnuDII (CG↓CG) and HaeIII (GG↓CC). Two of these enzymes, HpaII and MspI, were tested against the four DNAs. They appeared to cleave the altered DNAs more frequently than the wild-type DNA, since the former seemed to give smaller fragments. However, this may reflect the properties of the fragments rather than their sizes (see previous section).

Cleavage by enzymes with recognition sites of composition 1G:1C:1A:1T

Wild-type DNA was cleaved to slightly varying extents by the enzymes with recognition sites of composition 1G:1C:1A:1T. Three of the enzymes seemed to cleave the altered DNAs more frequently than wild-type DNA (MboI appeared to release smaller fragments from the modified DNAs than from wild-type DNA). The frequency of cleavage appeared to be am42 > am42rev4 > acetylated > wild-type. RsaI was different in that it appeared to cleave acetylated DNA more frequently than am42 rev4 DNA. TaqI cleaved all four DNAs with similar, apparently identical, high frequencies.

Cleavage by enzymes with recognition sites of composition 3G:3C

As expected, 3G:3C enzymes cleaved the DNAs less frequently than the 2G:2C enzymes. SmaI and XmaI cleaved all the DNAs much more frequently than SstII. However, the relative frequencies of cleavage for all three enzymes were am42 ≈ acetylated > am42rev4 ≈ wild-type.

Cleavage by enzymes with recognition sites of composition 2G:2C:1A:1T

Only KpnI of the seven enzymes tested cleaved wild-type DNA, but infrequently; the frequency of cleavage was increased slightly by acetylation and to about the same extent in am42 and am42rev DNAs. Of the

Table 1. Cleavage of ϕ W-14 DNA by restriction endonucleases

General Recognition Site	Enzyme	Specific Recognition Site	Number* of fragments released from DNA			
			Wild-type	Acetylated	<u>am42</u>	<u>am42rev4</u>
2G:2C	HpaII	C↓CGG	>40	>40	>40	>40
	HspI	C↓CGG	>40	>40	>40	>40
1G:1C:1A:1T	TaqI	T↓CGA	>>40	>>40	>>40	>>40
	Sau3a	↓GATC	≈30	>40	>40	>40
	MboI	↓GATC	>40	>40	>40	>40
	RsaI	GT↓AC	≈15	≈30	>40	≈20
	AluI	AG↓CT	≈30	>40	>40	>40
3G:3C	XmaI	C↓CCGGG	20	≈25	≈20	≈20
	SmaI	CCC↓GGG	14	20	20	15
	SstII	CCGC↓GG	0	4	3	2
2G:2C:1A:1T	XhoI	C↓TCGAG	0	0	0	0
	SstI	GAGCT↓C	0	≈5	≈20	≈5
	SalI	G↓TCGAC	0	0	≈13	≈8
	NcoI	C↓CATGG	0	0	≈30	0
	BamHI	G↓GATCC	0	0	4	2
	KpnI	GGTAC↓C	5	9	8	8
	SphI	GCATG↓C	0	0	≈30	0
1G:1C:2A:2T	HindIII	A↓AGCTT	0	0	2	0
	ClaI	AT↓CGAT	0	0	12	6
	XbaI	T↓CTAGA	0	0	1	0
	BclI	T↓GATCA	0	0	≈30	1
	BglII	A↓GATCT	0	2	1	1
	HpaI	GTT↓AAC	2	2	6	3
	EcoRI	G↓AATTC	7	15	≈30	≈15
	NdeI	CA↓TATG	0	0	10	0
	EcoRV	GAT↓ATC	2	3	14	8
3A:3T	SspI	AAT↓ATT	5	6	≈20	6
	AhaIII	TTT↓AAA	0	0	0	0
Miscellaneous	HinfI	G↓AATC	>40	>40	>40	>40
	AccI	GT↓(A)(T)AC	1	≈25	≈30	≈18
	BglI	GCCN ₄ ↓NGGC	0	0	0	0
	MboII	GAAGAN ₈ ↓	≈15	≈20	>40	>40

*Only clearly discernible bands below the very high molecular weight material at the top of the gel were scored.

other enzymes, only SstI cleaved acetylated DNA, but infrequently; three of them, including SstI, cleaved am42 and am42rev4 DNAs to varying extents, with am42 DNA being cleaved more frequently than am42rev4 DNA. NcoI and SphI cleaved only am42 DNA, but quite frequently. XhoI did not cleave any of the DNAs.

Cleavage by enzymes with recognition sites of composition 1G:1C:2A:2T

Three of the nine enzymes tested, HpaI, EcoRI and EcoRV, cleaved wild-type DNA, but at low frequency. Acetylated DNA was cleaved more frequently than wild-type by EcoRI, but this DNA appeared to be cleaved at

about the same frequency as wild-type DNA by HpaI and EcoRV. BglII may have cleaved acetylated DNA to give a single fragment, but the remaining enzymes did not cleave it. All of the enzymes cleaved am42 DNA to varying extents, and am42rev DNA to a lesser extent.

Cleavage by enzymes with recognition sites of composition 3A:3T

SspI cleaved all the DNAs at a low frequency; the relative frequencies were am42 > wild-type $\approx$ acetylated $\approx$ am42rev4. AhaIII cleaved none of the DNAs.

Cleavage by other enzymes

All four DNAs were cleaved extensively by HinfI. AccI cleaved wild-type DNA very infrequently, but was much more active, to about an equal extent, with the three altered DNAs. MboII cleaved all four DNAs, the frequency being am42 > am42rev4 > wild-type. BglI cleaved none of the DNAs.

Influence of putT content on the rate of cleavage

TaqI was chosen to examine the effect of putT content on the rate of cleavage of DNA. This enzyme seems to be impervious to a wide variety of base modifications (14,17; and Discussion); it gave very similar, if not identical, cleavage patterns with all four DNAs (Table 1). However, am42rev4 DNA was cleaved much faster than wild-type DNA by this enzyme. After 2 hr at 37°C, all the am42rev4 DNA was cleaved to fragments of less than 23 kbp, whereas wild-type DNA was mostly fragments larger than this (data not shown).

Ligation of ϕ W-14 DNA fragments

Fragments released from wild-type DNA by TaqI could be joined with T4 DNA ligase (data not shown).

DISCUSSION

The C-5 positions of the pyrimidines lie to one side of the major groove in B-form DNA. The effects of modifications at these positions depend on the sizes, the charges and the frequencies of occurrence of the modifying groups. In T4 DNA, every C is modified, so that a large, uncharged substituent occurs on average once in every six bases. T4 DNA is resistant to all but one of the type II enzymes tested against it (2,4,29); the exception is TaqI, which cleaves it slowly (4). In SP15 DNA, in which 62 percent of the Ts are modified, a very large, negatively charged substituent occurs once in every 5 bases. SP15 DNA is resistant to most of the enzymes tested against it, but it is cleaved very slowly

and incompletely by three enzymes, and slowly but completely by *TaqI* (4). The enzymes tested against T4 and SP15 DNAs include ten with 4-base and sixteen with 6-base recognition sites.

In wild-type ϕ W-14 DNA, compared to T4 and SP15 DNAs, a smaller, positively charged substituent occurs once in every eight bases. ϕ W-14 DNA was cleaved extensively by all seven enzymes with 4-base recognition sites which were tested against it; it was cleaved extensively by two of the three enzymes with 6-base sites of composition 3G:3C; and it was cleaved, but infrequently, by five of the eighteen enzymes with 6-base sites containing 1, 2 or 3Ts. This greater susceptibility of ϕ W-14 DNA to Type II enzymes must reflect the less frequent occurrence of its smaller modified base, as well as the distribution of the modification. This conclusion is supported by the effects of 5-(allylamino)uracil (aaU) on the susceptibility of DNA to Type II enzymes; aaU is structurally similar to putI, but carries only a single positive charge. The complete substitution of aaU for T in one strand of *fd* double-stranded DNA blocks cleavage by *AluI*, *DdeI* and *RsaI*, but not by *HinfI* or *TaqI*; it also markedly slows the rate of cleavage by *HaeIII* (17). ϕ W-14 DNA was cleaved extensively by *AluI* and *RsaI*, as well as by *HinfI* and *TaqI*.

Type II restriction endonucleases are affected by the sequences flanking their recognition sites (4-8, 17-24), by DNA conformation (25-27), and by base modification (2-6,17,20,24,28-31). *EcoRI* is, perhaps, the best understood of these enzymes. The major determinant of site recognition by *EcoRI* is the formation of a tight, complementary interface between the enzyme and the major groove of DNA (32), an interaction which distorts the helix within the recognition site (32,33). The interaction of the enzyme with DNA is dependent on lysine residues (7), an indication that charge density on the DNA could affect the interaction. Although there appear to be no contacts between the enzyme and bases outside the recognition site (32), the flanking sequences do influence the enzyme (7,8,18). Methylation protection and phosphate ethylation of DNA suggest that *EcoRI* interacts with a minimum of 10 nucleotide pairs (22), in spite of the lack of close contacts outside the recognition site (32). Modifications of the T methyl group at C-5 either block cleavage (2,4,28,29) or reduce its rate (28). Even though their polypeptide structures are varied, some, for example, being monomeric and others oligomeric (34), it is probable that the interactions of other Type II enzymes with DNA will be somewhat similar to that of *EcoRI*. The

susceptibilities of the four ϕ W-14 DNA to the enzymes tested here confirm and extend these conclusions.

The more frequent cleavage of am42 and am42rev4 DNAs relative to wild-type DNA shows that putT blocks cleavage at many potentially susceptible sites. The blocking depends on both positive charge and steric hindrance, and the increased frequency of cleavage of acetylated relative to wild-type DNA by many of the enzymes indicates that for putT charge can be more significant than size. This is to be expected if interaction of the enzymes with DNA depends on positively charged groups on the enzyme surfaces. These effects will be greatest when putT occurs in a recognition site. However, it is clear from the results with XmaI, SmaI and SstII that putT also affects site sensitivity when it is in the flanking sequences, and again the effect of acetylation indicates that charge can be more significant than size. The inability of a given enzyme to cleave wild-type DNA could result from putT occurring in all potential recognition sites, or from putT blocking some sites internally but others from flanking sequences, or from an absence of potential sites. In the first instance, am42 DNA might be, but acetylated DNA should not be, cleaved more frequently than wild-type DNA. In the second instance, acetylated and am42 DNAs might be cleaved more frequently than wild-type DNA. In the third instance, none of the DNAs should be cleaved. Note that analysis of am42 can show the presence of sites but not their total absence because it still contains some putT. It is unlikely that the conformation of ϕ W-14 DNA affects its susceptibility to the enzymes because wild-type DNA in solution appears to be B-form (35).

The distribution of putT in ϕ W-14 DNA appears to be ordered because it occurs more than twice as frequently as T in the sequence purine-pyrimidine-purine (36). Because putT in the recognition site of an enzyme blocks cleavage at that site, it is improbable that discrete fragments would be released from ϕ W-14 DNA if the putT is distributed randomly. The release of discrete fragments by all of the enzymes cleaving it shows that the distribution must be ordered. The release of discrete fragments from am42rev4 and am42 DNAs, in which the putT contents are 6 and 9 moles percent, respectively, shows that the distribution is ordered in these DNAs, too. Thus, the product of the gene defined by am42 determines, or is intimately involved in, the sequence-specific conversion of hmU to putT at the polynucleotide level (15,37). The mutation in am42 obviously reduces, but does not altogether eliminate, the sequence

specificity of the modification reactions. The differences in frequencies of cleavage between different enzymes and between the different DNAs do not absolutely define any sequence distribution of putT. However, the cleavage of only am42 DNA by NcoI (C↓CATGG) and SphI (GCATG↓C) suggests that the sequence CAPutTG may be frequent in wild-type DNA; the lack of cleavage am42rev4 DNA by these enzymes suggests that it may be a major site of modification. This is significant because of the distribution of T and putT in the sequence purine-pyrimidine-purine: the ratio putT/T in this sequence is 2.4 in wild-type DNA but only 0.4 in am42 DNA (18,37). Furthermore, NdeI (CA↓TATG) also cleaves only am42 DNA, but at a lower frequency than NcoI and SphI. The NdeI recognition site contains CATA and TATG. BclI (T↓GATCA) cleaves am42 DNA frequently, am42rev4 DNA only once, wild-type and acetylated DNAs not at all. Here the recognition site contains NNTG.

T4 (4), SP15 (4), aaU-containing fd (17) and ϕ W-14 DNA are all cleaved extensively by TaqI, albeit slowly. This means that the enzyme binds to recognition sites containing substituents varying widely in size and charge. The interaction of this DNA with its recognition site must present some novel features. The modifications on T4, Sp15 and ϕ W-14 DNAs do slow the enzyme down (4, this paper).

Modifications of T residues do interfere with the joining of TaqI generated fragments by T4 DNA ligase (38). Nonetheless, TaqI fragments of ϕ W-14 wild-type DNA can be joined with T4 ligase. The susceptibility of the DNA to certain Type II restriction endonucleases and the ligation of restriction fragments with T4 ligase show that it will not be necessary to use putT-free ϕ W-14 DNA in cloning experiments.

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REFERENCES

1. Warren, R.A.J. (1980). *Ann. Rev. Microbiol.* 34, 137-158.
2. Kaplan, D.A. and Nierlich, D.P. (1975). *J. Biol. Chem.* 250, 2395-2397.
3. Ito, J., Kawamura, F. and Duffy, J.J. (1975). *FEBS Letters* 55, 278-281.
4. Huang, L.-H., Farnet, C.M., Ehrlich, K.C. and Ehrlich, M. (1982). *Nucleic Acids Res.* 10, 1579-1591.
5. Bron, S., Luxen, E. and Venema, G. (1983). *J. Virol.* 46, 703-708.
6. Wiatr, C.L. and Witmer, H. (1984). *J. Virol.* 52, 47-54.

7. Woodhead, J.L. and Malcolm, A.D.B. (1980). *Nucleic Acids Res.* 8, 389-402.
8. Jack, W.E., Terry, B.J. and Modrich, P. (1982). *Proc. Natl. Acad. Sci. U.S.A.* 79, 4010-4014.
9. Hayashi, H., Nakanishi, K., Brandon, C. and Marmur, J. (1973). *J. Amer. Chem. Soc.* 95, 8749-8757.
10. Ehrlich, M. and Ehrlich, K. (1981). *J. Biol. Chem.* 256, 9966-9972.
11. Kropinski, A.M.B., Bose, R.J. and Warren, R.A.J. (1973). *Biochemistry* 12, 151-157.
12. Scraba, D.G., Bradley, R.D., Leyritz-Wills, M. and Warren, R.A.J. (1983). *Virology* 124, 152-160.
13. Gerhard, B. and Warren, R.A.J. (1982). *Biochemistry* 21, 5458-5462.
14. Miller, P.B., Maltman, K.L. and Warren, R.A.J. (1982). *J. Virol.* 43, 67-72.
15. Miller, P.B., Scraba, D.G., Leyritz-Wills, M., Maltman, K.L. and Warren, R.A.J. (1983). *J. Virol.* 47, 399-405.
16. Marusyk, R. and Sergeant, A. (1980). *Anal. Biochem.* 105, 403-404.
17. Bodmar, J.W., Zempsky, W., Warder, D., Bergson, C. and Ward, D.C. (1983). *J. Biol. Chem.* 258, 15206-15213.
18. Thomas, M. and Davis, R.W. (1975). *J. Molec. Biol.* 91, 315-328.
19. Forsblum, S., Rigler, R., Ehrenberg, M., Pettersson, U. and Philipson, L. (1976). *Nucleic Acids Res.* 3, 3255-3269.
20. Marchionni, M.A. and Roufa, D.J. (1978). *J. Biol. Chem.* 253, 9075-9081.
21. Halford, S.E., Johnson, N.P. and Grinstead, J. (1980). *Biochem. J.* 191, 581-592.
22. Lu, A.-L., Jack, W.E. and Modrich, P. (1981). *J. Biol. Chem.* 256, 13200-13206.
23. Armstrong, K. and Bauer, W.R. (1982). *Nucleic Acids Res.* 10, 993-1007.
24. Berkner, K.L. and Folk, W.R. (1983). *Anal. Biochem.* 129, 446-456.
25. Vardimon, L. and Rich, A. (1984). *Proc. Natl. Acad. Sci. U.S.A.* 81, 3268-3272.
26. Zacharias, W., Larson, J.E., Kilpatrick, M.W. and Wells, R.D. (1984). *Nucleic Acids Res.* 12, 7677-7692.
27. Azorin, F., Hahn, R. and Rich, A. (1984). *Proc. Natl. Acad. Sci. U.S.A.* 81, 5714-5718.
28. Berkner, K.L. and Folk, W.R. (1977). *J. Biol. Chem.* 252, 3185-3193.
29. Berkner, K.L. and Folk, W.R. (1979). *J. Biol. Chem.* 254, 2551-2560.
30. Petruska, J. and Horne, D. (1983). *Nucleic Acids Res.* 11, 2495-2510.
31. Hofer, B. and Köster, H. (1980). *Nucleic Acids Res.* 8, 6143-6162.
32. Frederick, C.A., Grable, J., Melia, M., Samudzi, C., Jen-Jacobson, L., Wang, B.-C., Greene, P., Boyer, H.W. and Rosenberg, J.M. (1984). *Nature* 309, 327-331.
33. Kim, R., Modrich, P. and Kim, S.-H. (1984). *Nucleic Acids Res.* 12, 7285-7292.
34. Modrich, P. and Roberts, R.J. (1982). In "Nucleases", Linn, S.M. and Roberts, R.J., Eds. (Cold Spring Harbor Laboratory), 109-154.
35. Spetter, S., Chen, C., Warren, R.A.J. and Hanlon, S. Accepted for publication in *Biochim. Biophys. Acta*.
36. Warren, R.A.J. (1981). *Curr. Microbiol.* 6, 185-188.
37. Maltman, J.L., Neuhaard, J., Lewis, H.A. and Warren, R.A.J. (1980). *J. Virol.* 34, 354-359.
38. Cao, X.-M., Huang, L.-H., Farnet, C.M. and Ehrlich, M. (1983). *Biochim. Biophys. Acta* 741, 237-243.