Recognition and Binding of Template-Primers Containing Defined Abasic Sites by Drosophila DNA Polymerase α Holoenzyme*

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Human DNA polymerase α holoenzyme follows an ordered sequential terreactant mechanism of substrate recognition and binding (Wong, S. W., Paborsky, L. R., Fisher, P. A., Wang, T. S.-F., and Korn, D. (1986) J. Biol. Chem. 261, 7958–7968). We confirmed this mechanism for the DNA polymerase α holoenzyme purified from Drosophila melanogaster embryos and studied the interaction of Drosophila pol α with synthetic oligonucleotide template-primers containing modified tetrahydrofuran moieties as model abasic lesions chemically engineered at a number of defined sites. Abasic lesions in the template had relatively little effect on the polymerase incorporation reaction at sites proximal to the lesion. However, incorporation opposite an abasic site was undetectable relative to that which occurred opposite a normal template nucleotide. Moreover, abasic residues in the primer region of the template-primer construct as far as 4 base pairs removed from the 3′-primer terminus prevented detectable nucleotide incorporation relative to that seen on an unmodified template-primer. Primer-region lesions had qualitatively similar effects whether they were located on the primer strand itself or on the complementary template strand. Data from polymerase incorporation experiments were corroborated by competitive binding assays performed under steady state reaction conditions. Results of these experiments suggested that polymerase binding to synthetic oligonucleotide template-primers was essentially unaffected by lesions located at sites that did not block incorporation. Lesions that did block incorporation apparently did so by abrogating template-primer binding. These observations have implications for understanding the mechanisms whereby DNA polymerase α recognizes noninformational template sites in vivo and prevents DNA synthesis from proceeding past these points.

Takeshita et al. (1987) recently reported the chemical synthesis of oligodeoxyribonucleotides containing modified tetrahydrofuran moieties inserted into the phosphodiester backbone at specific sites in place of normal nucleotides. They suggested that such sites were structurally analogous to naturally arising abasic sites in DNA. They found that synthetic abasic sites could be recognized and cleaved by endonuclease IV and exonuclease III and could serve as templates for a number of DNA polymerases including avian myeloblastosis virus reverse transcriptase, Escherichia coli DNA polymerase I, calf thymus DNA polymerase α, and Drosophila DNA polymerase α. Product analysis indicated that dAMP was the preferred nucleotide for incorporation opposite the abasic site although small amounts of the three other dNMPs could be detected as well. A detailed study of nucleotide insertion kinetics opposite abasic sites by Drosophila DNA polymerase α holoenzyme was carried out by Randall et al. (1987). Their findings were consistent with and extended those of Takeshita et al. (1987) in this specific area.

In analyzing the length distribution of products recovered after DNA polymerases were incubated with primed templates containing synthetic abasic sites, Takeshita et al. (1987) noted that two species predominated. These were first, the primer extended to the position opposite the template nucleotide immediately proximal to the abasic lesion and second, the primer extended one nucleotide further to the position opposite the abasic site. In the case of DNA polymerase α, small amounts of primer extended to one nucleotide beyond the abasic site were also detected as were small amounts of primer extended well beyond the site of the lesion.

Takeshita et al. (1987) suggested that the distribution of products observed following reaction of DNA polymerase α with primed templates containing synthetic abasic sites was consistent with the known mechanisms of substrate recognition and binding of human DNA polymerase α (Fisher and Korn, 1981, a and b; Wong et al., 1986). However, experiments by Takeshita et al. (1987) were performed in large enzyme excess in an effort to drive potential incorporation reactions. Under these conditions, it was not possible to study the mechanistic details of DNA synthesis on templates containing abasic sites nor was it possible to evaluate the relative efficiency of incorporation opposite an abasic site versus normal incorporation of a template-complementary nucleotide.

To obtain further insight into the capacity of DNA polymerase α to recognize and respond to templates containing abasic sites, we initiated a series of experiments performed under steady state reaction conditions, i.e. excess substrate. Our results are consistent with those of others (Randall et al., 1987; Takeshita et al., 1987). Moreover, they provide a mechanistic description of the interaction between DNA polymerase α and DNA template-primers containing abasic sites in template as well as primer strands. Current results both corroborate and extend previous models for the recognition and binding of nucleic acid substrates by DNA polymerase α. They also have implications for understanding the mechanisms whereby a high fidelity of DNA replication is maintained in vivo.

EXPERIMENTAL PROCEDURES

Materials—Unlabeled deoxyribonucleoside and dideoxyribonucleoside triphosphates (dNTPs and ddNTPs, respectively) were from

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Boehringer. [α-32P]dTTPs and [γ-32P]dATP were from Amersham. T4 polynucleotide kinase was from Bethesda Research Laboratories. Terminal deoxynucleotidyltransferase was from Life Sciences, Inc. Phosphocellulose and DEAE-cellulose were from Whatman, hydroxyapatite was from Bio-Rad Laboratories, and DNA-cellulose was prepared in the laboratory from heat-denatured calf thymus DNA and Hi-Rac columns according to Carr and Herrick. Acrylamide and methylenebisacrylamide were from Eastman and were further purified by filtration of solutions through activated charcoal before use. Drosophila melanogaster, Oregon R P2 strain, were maintained in mass culture at 25 °C, and 0-12-h-old embryos were collected according to Allis et al. (1975). They were stored frozen at −70 °C until use. All other chemicals and reagents were obtained commercially and were used without further purification.

Polyacrylamide Gel Analysis of Synthetic Oligonucleotides and DNA Polymerase Reaction Products—Denaturing polyacrylamide gel analysis was according to Maxam and Gilbert (1980). Gels were run in the presence of 7 M urea. Before loading, samples were boiled in a standard denaturing gel loading buffer (Maniatis et al., 1982). Electrophoresis was performed at room temperature. Nondenaturing gels were formulated similarly except that urea was not included in the gel. All samples were not boiled before loading. Electrophoresis was performed at 4 °C.

Preparation of Synthetic Oligonucleotide Template-Primer Substrates and Inhibitors—Synthetic oligonucleotides containing tetrahydrofuran ("abasic") residues at specific sites were prepared and purified as described previously (Fisher and Korn, 1981a). The sequences of the various template-primer constructs that were synthesized are shown in Figs. 1A and 6. Positions of the abasic sites in the template region of the constructs were confirmed by primer-extension experiments performed with DNA polymerase α. Under the conditions used, pol α incorporates efficiently up to the abasic site. A small amount of incorporation opposite the lesion can also be detected. These results are shown in Fig. 1B. Synthetic oligonucleotide templates and primers were annealed by heating for 5 min at 75 °C in 5 mM Tris-HCl, pH 8.0, 100 mM KCl, followed by slow cooling.

The "hook" homopolymer, (dC)50(dT)5 shown in Fig. 3 was prepared using the enzyme terminal deoxynucleotidyl transferase exactly as previously described (Fisher et al., 1981). Terminal deoxynucleotidyl transferase was used to add dNTPs to the 3'-ends of synthetic oligonucleotide 12-mers shown in Fig. 3 under conditions previously described for the addition of dNMPs to the 3'-ends of hook homopolymers (Fisher and Korn, 1981a).

Purification of DNA Polymerase α Holoenzyme from D. melanogaster Embryos—Drosophila DNA polymerase α holoenzyme was purified from 0-12-h D. melanogaster embryos through chromatography on DNA-cellulose chromatography step according to Kaguni et al. (1983). Although not homogeneous, this polymerase fraction was of specific activity comparable to that reported by Kaguni et al. (1983) and was free of detectable nuclease activities; prolonged incubation of 5'-end-labeled synthetic oligonucleotides including those containing abasic residues with this polymerase fraction was without detectable effect on the polyacrylamide gel mobility of the oligonucleotides (not shown).

DNA Polymerase Assays—All DNA polymerase assays were performed in a standard reaction mixture which contained 50 mM Tris-HCl, pH 8.0, 10 mM MgCl₂, 5 mM dithiothreitol, 20 mM (NH₄)₂SO₄, and 200 μg/ml gelatin. Additional details including dNTP and template-primer concentrations are provided in the individual figure legends. One unit of DNA polymerase activity is defined according to Kaguni et al. (1983) as the amount of enzyme that catalyzes the incorporation of 1 nmol of acid-soluble dNTP into acid-insoluble DNA product in 60 min at 37 °C under optimal assay conditions on an activated DNA substrate. For most assays of DNA polymerase activity in which synthetic oligonucleotide template-primers with abasic residues in the template region of the construct (30-mer residues 9, 12, 15, and 18) were present, incubations were performed at 25 °C. With 12-mers residues were localized in the primer region of the construct (30-mer residues 20, 22, 24, and 26; 12-mers residues 11, 9, 7, and 5), incubations were at 18 °C. This was done to compensate for any destabilizing effect that an abasic residue located in the primer region might have on the template-primer construct. Native polyacrylamide gel electrophoresis indicated that the presence of a single abasic residue in the primer region of the template-primer construct did not significantly destabilize it (not shown).

Two different types of assays were performed. In the first, a direct assay, the synthetic template-primer constructs containing the abasic sites served as the substrates for DNA synthesis by the polymerase. In these instances, [α-32P]dGTP was the only dNTP present, and the products of dGMP incorporation were analyzed by polyacrylamide gel electrophoresis. The products were identified by autoradiography, and the regions of the gel containing the radioisotopes were excised and counted directly.

In the second, an indirect assay, the template-primer constructs containing the abasic sites were used as competitors for the polymerase with the substrate (dC)₅₀(dG)₅₀. As in the direct assay, [α-32P]dCTP was provided as the substrate for dGMP incorporation. In this assay, incorporation was quantitated by trichloroacetic acid precipitation and filtration on GF/C filters (see e.g. Fisher et al., 1981). This assay system using the hook homopolymer as a substrate for incorporation allowed us to include unlabeled dNTPs other than the one being incorporated, for purposes of mechanistic analysis regarding the binding of DNA polymerase α to template-primer constructs containing abasic sites.

RESULTS
Effect of Abasic Lesions on the Incorporation of Nucleotide by Drosophila DNA Polymerase α Holoenzyme—To study the impact of abasic lesions in a DNA template on relative rates of incorporation by DNA polymerase α holoenzyme, a synthetic 30-nucleotide template was designed. Eight different versions of this 30-mer were prepared, each with an abasic site at a different position. A ninth 30-mer, lacking abasic sites, was also synthesized as a control. A 12-nucleotide primer, complementary to nucleotides 19-30 of the 30-mer templates was also synthesized. The structure of the 30-12-mer template-primers prepared by annealing complementary oligonucleotides is shown in Fig. 1A. The different positions at which abasic sites were engineered are designated by asterisks.

To monitor nucleotide incorporation, incubations were set up to include only [α-32P]dGTP, the dNTP complementary to template nucleotide 18 as shown in Fig. 1. Results of these incorporation experiments are shown in Fig. 2. Fig. 24 demonstrates the impact of abasic lesions at positions 9, 12, 15, and 18, the "template" portion of the 30-mer shown in Fig. 1. Fig. 25 shows the effect of abasic lesions at positions 20, 22, 24, and 26, the "primer" portion of the 30-mer shown in Fig. 1. The results of a summary experiment on the effects of abasic site position on the activity of DNA polymerase α are presented in a bar graph in Fig. 2C.

From these incorporation experiments, it seemed clear that abasic sites in the single-stranded template region of the 30-mer at positions 9, 12, and 15 were at least permissive for incorporation of dGMP opposite the dCMP residue at position 18; in fact, dGMP incorporation opposite position 18 was apparently stimulated severalfold by the presence of an abasic site at template position 15. In contrast, incorporation was not detectable when the abasic site was at position 18, the position of the template nucleotide required to direct incorporation, nor was incorporation evident when abasic lesions were placed at positions 20 and 22, both sites in the primer region of the synthetic 30-mer. When lesions were placed at positions 24 or 26, significant incorporation of dGMP opposite template residue 18 was restored. When the abasic site was at position 18, incorporation of dAMP, the preferred nucleotide for incorporation opposite the abasic site under conditions of enzyme excess (Takeshita et al., 1987) was similarly undetectable under the conditions used in this assay (data not shown).

Binding of Drosophila DNA Polymerase α Holoenzyme to Synthetic Template-Primers Containing Abasic Lesions at Various Defined Sites—Because DNA polymerase incorporation reactions depend on the catalytic activity of the polym-
Interaction of DNA Polymerase α with Abasic Sites

Fig. 1. A, synthetic 30-12-mer template-primer constructs used for DNA polymerase incorporation studies with abasic lesions on the 30-mer. Numbering is from the 5'-3' direction on the template strand (30-mer). Positions of the modified tetrahydrofuran moiety on the template strand are indicated by the asterisks. A given 30-mer contained only one such site. B, primer extension by Drosophila DNA polymerase α holoenzyme on several of the synthetic template-primer constructs shown in A. Incubations were for 10 min at 25 °C in a final volume of 10 μl. Reaction mixtures were formulated as described (see "Experimental Procedures") and included all four dNTPs, each at a final concentration of 0.0625 μM and 0.07 unit of Drosophila DNA polymerase α holoenzyme. Each incubation contained 0.0625 μM (usable 3'-OH termini) of 30-12-mer template-primer constructs as follows. Lanes a and e, unmodified 30-12-mer; lanes b and f, 30-12-mer with an abasic lesion at position 9 of the 30-mer; lanes c and g, 30-12-mer with an abasic lesion at position 12 of the 30-mer; lanes d and h, 30-12-mer with an abasic lesion at position 15 of the 30-mer. After incubation, samples were subjected to urea-polyacrylamide gel electrophoresis. Gels were dried, and autoradiography was for 14 h at room temperature. Lanes a-d, incubations were performed in the presence of [α-32P]dGTP; lanes e-h, incubations were performed in the presence of [α-32P]dCTP. Positions of 5'-end-labeled 30-mer and 30-12-mer are indicated to the right of the autoradiogram; 30-mer positions as designated in A are indicated to the left.

Fig. 2. Incorporation of dGMP by Drosophila DNA polymerase α holoenzyme on 30-12-mer template primer constructs containing abasic lesions at specific sites on the 30-mer. Incubations were performed in a final volume of 10 μl and contained 30 μM [α-32P]dGTP (5300 cpm/pmol), 0.07 unit of Drosophila DNA polymerase α, and various concentrations of the different template-primers as indicated on the abecissa. Template-primer concentrations are expressed in terms of moles of 30-12-mer construct. A, abasic sites were present in the 30-mer at either the 9-position (A), 12-position (B), 15-position (C), or 18-position (D). A control 30-mer lacking abasic sites was also included (E). Incubations were for 10 min at 25 °C, and product analysis was by electrophoresis, autoradiography, and band excision as described (see "Experimental Procedures"). B, abasic sites were present in the 30-mer at either the 20-position (A), 22-position (B), 24-position (C), or 26-position (D). A control lacking abasic sites was also included (E). Incubations were for 10 min at 18 °C; product analysis was as in A. C, assays were for 10 min at 18 °C as in B at 1 μM template-primer. All activity values are expressed relative to that determined on a control 30-mer lacking abasic residues (−). Product analysis was as in A and B.

Fig. 3. Hook homopolymer and 30-12-mer-ddNMP constructs used for competition studies. The hook homopolymer substrate, (dC)30, (dG)30 was synthesized as previously described using terminal deoxynucleotidyltransferase (Fisher et al., 1981). The 30-12-mer template-primer construct was as depicted in Fig. 1. Terminal deoxynucleotidyltransferase was used to add either a ddGMP or a ddAMP to the synthetic 12-mer primer to generate either 12-mer ddGMP or 12-mer ddAMP as shown.
30-13-mers in which the 3′-terminal position on the 30-mer primer was either 2′,3′-ddGMP, complementary to the dCMP residue at template position 18 on the 30-mer; or 2′,3′-ddAMP, a template-noncomplementary nucleotide. We tested the capacity of each of these competitors to promote induced substrate (dNTP) inhibition diagnostic for the ordered sequential mechanism of substrate recognition and binding (Fisher and Korn, 1981a; Cleland, 1979). The results in Fig. 4A demonstrate that when the fully base-paired deoxy-terminated 13-mer is used to prime the 30-mer template, induced dNTP inhibition indeed occurs and does so only with dTTP, the nucleoside triphosphate complementary to dAMP, the template nucleotide at position 17. In contrast, no such inhibition is detected when the 3′-terminally mismatched deoxy-terminated 13-mer is used to prime the 30-mer template (Fig. 4B). These results are identical with those obtained for human DNA polymerase α (Fisher and Korn, 1981, a and b; Wong et al., 1986).

We next used this approach to evaluate each of the abasic site-containing 30-mers shown in Fig. 1. Representative results with three of these 30-mers are shown in Fig. 5A; results from all of these analyses are summarized in a bar graph in Fig. 5B. In Fig. 5A, induced dTTP inhibition was demonstrable when abasic sites were placed either at the 15-position (template portion) or the 24-position (primer portion) of the 30-mer. Both of these template-primers supported incorporation although at much different levels (Fig. 2), yet the inhibitory potency of each was similar as were the kinetics of induced inhibition by dTTP. When the abasic site was placed at the 20-position (primer portion), no induced dTTP inhibition was demonstrable; this template-primer was unable to support incorporation when assayed directly (Fig. 2B).

In considering the summary data presented in Fig. 5B, some inhibition is apparent with the template-primer that contains an abasic lesion at the 22-position even though this template-primer failed to support incorporation when assayed directly (Fig. 2). However, it should be noted that when assayed directly, the 30-mer containing the abasic site at the 22-position was annealed to a 12-mer primer, thus placing the abasic site at the 22-position of the template-primer construct containing abasic lesions at specific sites. Similar experiments were performed with each of the abasic lesion-containing 30-mers depicted in Fig. 1. Only representative examples of the data from these experiments are shown in A; a complete data set is shown in B. All incubations were performed in a final volume of 50 μl and contained 1 μM (nucleotide) (dC)25, (dG)20, 18 μM (nucleotide) 30-12-mer ddGMP, and 40 μM [α-32P]dGTP (300 cpm/pmol). A, the 30-12-mer ddGMP contained abasic lesions on the 30-mer at either the 15-position (○), the 20-position (▲), or the 24-position (●). Unlabeled dTTP was added as indicated on the abscissa. B, incubation conditions were as in A and contained, in addition, 640 μM dTTP. All inhibitory potencies are expressed relative to reactions performed in the absence of 30-12-mer ddGMP. The inhibitory potency of a 30-12-mer ddGMP lacking an abasic site is shown for comparison (−).

**Fig. 5.** Substrate (dTTP) inhibition of *Drosophila* DNA polymerase α holoenzyme induced by 30-12-mer ddGMP template-primer constructs containing abasic lesions at specific sites. Similar experiments were performed with each of the abasic lesion-containing 30-mers depicted in Fig. 1. Only representative examples of the data from these experiments are shown in A; a complete data set is shown in B. All incubations were performed in a final volume of 50 μl and contained 1 μM (nucleotide) (dC)25, (dG)20, 18 μM (nucleotide) 30-12-mer ddGMP, and 40 μM [α-32P]dGTP (300 cpm/pmol). A, the 30-12-mer ddGMP contained abasic lesions on the 30-mer at either the 15-position (○), the 20-position (▲), or the 24-position (●). Unlabeled dTTP was added as indicated on the abscissa. B, incubation conditions were as in A and contained, in addition, 640 μM dTTP. All inhibitory potencies are expressed relative to reactions performed in the absence of 30-12-mer ddGMP. The inhibitory potency of a 30-12-mer ddGMP lacking an abasic site is shown for comparison (−).

**Fig. 6.** Synthetic 30-12-mer template-primer constructs used for DNA polymerase incorporation studies with abasic lesions on the 12-mer. Constructs were as described in Fig. 1, except that abasic lesions, indicated by asterisks, were placed on the primer strand. The 12-mer is numbered in the 5′-3′ direction. Hence, primer nucleotide 1 is opposite template nucleotide 30; primer nucleotide 12 is opposite template nucleotide 19.

![Graph](https://via.placeholder.com/150)

**Fig. 4.** Induced substrate (dNTP) inhibition of *Drosophila* DNA polymerase α holoenzyme. All incubations were performed in a final volume of 50 μl and contained 40 μM [α-32P]dGTP (320 cpm/pmol), 1 μM (nucleotide) (dC)25, (dG)20, and 0.1 unit of *Drosophila* DNA polymerase α. Incubations were for 10 min at 25 °C, and product analysis was by trichloroacetic acid precipitation and filtration. A, to each incubation was added 16 μM ddGMP and varying amounts of unlabeled dNTPs as indicated on the abscissa; dTTP, ▲, dCTP, ■, dATP, ▲. A control series of incubations included dTTP but no 30-12-mer ddGMP (▼). B, incubations were as in A except that 30-12-mer ddAMP (16 μM nucleotide) replaced 30-12-mer ddGMP. dTTP, ▲, dCTP, ■, dATP, ▲. A control minus the 30-12-mer ddAMP was not included in the experiment shown in panel B.
primer duplex are similarly important in binding interactions those shown in Fig. 2B. Template-primers with abasic lesions the polymerase.

oligonucleotide template-primers containing chemically engineered abasic sites. To fully exploit the power of such substrate recognition and binding by DNA polymerase α with Abasic Sites

taining the primer. To evaluate whether there were any differences depending on which side of the duplex the lesions were on, the constructs shown in Fig. 6 were designed. For these template-primers, 12-mers containing abasic lesions at positions 11, 9, 7, and 5, corresponding to 30-mer positions 20, 22, 24, and 26, respectively, were annealed to unmodified 30-mer. The template-primers so formed were then used in a direct incorporation assay comparable to that shown in Fig. 2B. The results of this experiment (Fig. 7), although quantitatively somewhat different, were qualitatively similar to those shown in Fig. 2B. Template-primers with abasic lesions at 12-mer positions 7 and 5 (i.e. 30-mer positions 24 and 26) supported detectable levels of dGMP incorporation opposite the template dCMP at position 18. Template-primers with abasic lesions at 12-mer positions 11 and 9 (i.e. 30-mer positions 20 and 22) were unable to support incorporation. These data suggest that both sides of the double-stranded primer region are similarly involved in recognition and binding by the polymerase.

Induced substrate (dNTP) inhibition experiments were performed with the template-primer constructs shown in Fig. 6. As with the direct incorporation experiments shown in Fig. 7, results qualitatively similar to those obtained with the constructs shown in Fig. 1 (Fig. 5) were obtained (not shown), thus further supporting the notion that both strands of the primer duplex are similarly important in binding interactions with polymerase.

DISCUSSION

In the experiments reported in this paper, we used steady state enzymologic analyses to study the interaction of D. melanogaster DNA polymerase α holoenzyme with synthetic oligonucleotide template-primers containing chemically engineered abasic sites. To fully exploit the power of such analyses, it was necessary to establish that the mechanism of substrate recognition and binding by Drosophila DNA polymerase α was fundamentally similar to that established for the homologous enzyme from human KB cells (Fisher and Korn, 1979, a and b, 1981, a and b; Wong et al., 1986). That such indeed was the case was suggested by the preliminary results of others (Diffley, 1985). This impression was explicitly supported by the results of induced substrate inhibition experiments shown in Fig. 4 and implicitly corroborated by all of the experimental data reported herein.

It is DNA in vivo whether DNA polymerase α encounters abasic template sites during DNA replication in vivo. Such sites arise with considerable frequency due to spontaneous depurination and enzymatic hydrolysis of N-glycosidic bonds (Lindahl, 1982), but are probably repaired efficiently under most circumstances. Nevertheless, the abasic site has been proposed as a common intermediate through which many pathways of chemical mutagenesis may pass (Loeb, 1985; Loeb and Preston, 1987). It thus seems likely that at least some such sites escape the vigilance of cellular repair mechanisms, particularly when the damage occurs during the S-phase of the cell cycle.

Previous in vitro studies on the impact of synthetic abasic residues on DNA polymerases focused largely on the potential of such residues to direct nucleotide incorporation at a base-paired primer terminus (Randall et al., 1987; Takeshita et al., 1987). By focusing on incorporation, however, these previous experiments (Randall et al., 1987; Takeshita et al., 1987) did not address the question of what typically happens when a DNA polymerase encounters an abasic site in the template being replicated. Results reported in this paper help to answer this question.

Two types of enzymologic assay, direct nucleotide incorporation and indirect competitive induced dNTP inhibition, were performed. Both gave complementary and mutually consistent data. From results of incorporation experiments shown in Fig. 2, we conclude that nucleotide incorporation opposite an abasic template residue is indeed an unfavorable event. Based on the lack of detectable incorporation of either dAMP or dGMP opposite the abasic residue, we can define an exclusion limit. Assuming that we would have been able to detect 100 cpm above background in the electrophoresis/autoradiography/gel excision assays shown in Fig. 2, we estimate that nucleotide incorporation opposite abasic residues occurs at less than 1% the rate of incorporation opposite a normal template nucleotide. This is consistent with the conclusion of Randall et al. (1987) that incorporation opposite an abasic site is about 4000-fold less efficient than incorporation opposite a normal template nucleotide. Efficiency was defined in their study in terms of kinetic constants as $V_{max}/K_m$.

We also found from the experiments shown in Fig. 2 that abasic residues in template-primer constructs at sites other than the one required to direct nucleotide incorporation nevertheless had significant effects on that incorporation reaction. Both positive and negative effects were recorded ranging from more than 3-fold stimulation of incorporation to complete inhibition. For purposes of subsequent discussion, these lesions may be grouped into two categories; those which affected the maximal rate of nucleotide incorporation either positively or negatively but for which at least some incorporation was detectable, and those which resulted in complete loss of detectable incorporation. The first group included abasic lesions in the template region of the 30-mer at positions 12 (50% inhibition) and 15 (350% stimulation), in the primer region of the 30-mer at positions 24 (55% inhibition) and 26 (70% inhibition), and in the 12-mer at positions 7 (90% inhibition) and 5 (55% inhibition). The second group (complete, i.e. >99% inhibition of incorporation) included abasic lesions in the primer region of the 30-mer at positions 20 and 22 and in the 12-mer at corresponding positions 11 and 9.

At this time, we can offer no certain explanation for the first group of effects, i.e. partial inhibition or stimulation.
Previous studies of human DNA polymerase α have suggested that this enzyme interacts with between 5 and 10 template nucleotides and 8 primer nucleotides at any given time during catalysis; the rate of replication through a particular region is highly dependent on template-primer base composition (Fisher et al., 1981; Fisher and Korn, 1981b). Others have noted the existence of polymerase pause sites (DePamphilis et al., 1980). These are presumably regions of template-primer throughput which replication is particularly slow. A likely explanation, therefore, for partial inhibition or stimulation by abasic residues at sites other than the one necessary to direct incorporation is that, in modifying template-primers at these sites, we have effectively altered the base composition through the region of polymerase binding and thus altered the rate of nucleotide incorporation ($k_{\text{cat}}$). A direct and systematic analysis of this phenomenon would be required to test this hypothesis.

That such may indeed be the case is supported by results of competitive induced substrate inhibition studies (Fig. 5), the second type of assay used to evaluate the interaction of DNA polymerase α with synthetic oligonucleotides containing abasic sites. In these experiments, because of the dideoxy block at the 3'-end of the primer, incorporation does not occur on the abasic site-containing template primers. It is therefore possible to analyze binding to these synthetic oligonucleotides without the complications brought about by $k_{\text{cat}}$ effects. For this reason, this type of assay is preferred over direct incorporation analyses.

From the inhibition experiments shown in Fig. 5, we conclude that binding to those template-primers which support detectable incorporation is relatively unaffected by the presence of abasic sites in either the template region or the primer region. This is consistent with the notion that the rate effects shown in Fig. 2 result from effects on rates of catalysis ($k_{\text{cat}}$ effects) that are manifest subsequent to binding. In contrast, those template-primer constructs which do not support detectable nucleotide incorporation as analyzed in Fig. 2 are significantly limited in their ability to bind polymerase and to bring about induced dNTP inhibition.

These data obtained with *Drosophila* DNA polymerase α holoenzyme regarding its interactions with template-primer constructs containing abasic sites in the primer region of the construct are highly reminiscent of those obtained in studies of the primer requirements of human DNA polymerase α (Fisher and Korn, 1979b, 1981b). These current results, along with those obtained previously, have implications for understanding the mechanisms whereby the fidelity of DNA synthesis is maintained *in vivo*. In previous studies (Fisher and Korn, 1981b), we noted that human DNA polymerase α apparently required an octanucleotide to prime DNA synthesis. However, based on investigations of snap-back synthesis on single-stranded DNA restriction fragments of known sequence, we concluded that for replication to occur, only the three or four terminal nucleotides of the primer strand needed to be complementary to corresponding nucleotides on the template strand (Fisher and Korn, 1979b). Regardless of the degree of distal complementarity, a single base mismatch at the 3'-terminus of the primer was sufficient to abrogate primer binding and subsequent dNTP recognition and DNA synthesis completely (Fisher and Korn, 1981b).

Current results with *Drosophila* DNA polymerase α holoenzyme are consistent with the notion that the abasic site is mechanistically equivalent to a template deoxyribonucleotide for which no complement can be found. This conclusion is supported by the fact that Randall et al. (1987) found the efficiency of incorporation opposite the abasic site to be similar to the efficiency of misincorporation opposite a normal template nucleotide. When the abasic site is encountered, replication initially stops immediately proximal to the lesion as the polymerase searches for a complementary dNTP. In the unlikely instance that incorporation does take place, it is similar to misincorporation, also a low frequency event. In either case, once "misincorporation" occurs, the polymerase now recognizes the product of its mistake as a mismatched primer and synthesis is halted at this point.

The fact that each of the first four primer-terminal nucleotides must be base-paired for normal primer function suggests that there are in fact five block points to prevent mismatched or unmatched nucleotides from being incorporated and immortalized to yield mutations. The first point of blockage is that of initial misincorporation or incorporation opposite an abasic site. This is obviously highly unfavorable. However, the subsequent addition of a template-directed nucleotide to the unpaired primer terminus may be as unfavorable as initial misincorporation. The suitability of the primer is apparently not fully restored until four such unlikely additions to the mismatched terminus take place. Each of the four base-pairing interactions that a mismatched or un-matched nucleotide once it has been incorporated represents an opportunity for simple exonucleolytic repair of the damage before replication resumes at a normal pace.

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REFERENCES

Alberts, B. M., and Herrick, G. (1971) Methods Enzymol. 21, 198-217

Allia, C. D., Waring, G. L., and Mahowald, A. P. (1977) Dev. Biol. 56, 372-381

Cleland, W. W. (1979) Methods Enzymol. 63A, 500–513

DePamphilis, M. L., Anderson, S., Cusick, M., Hay, R., Herman, T., Krokan, H., Shelton, E., Tack, L., Tapper, D., Weaver, D., and Wassarman, P. (1980) JCN-UCLA Symp. Mol. Cell. Biol. 29, 55–78

Difflay, J. F. X. (1985) Structural and Mechanistic Investigations into a DNA Polymerase from Drosophila melanogaster Embryos. PhD. thesis, New York University

Fisher, P. A., and Korn, D. (1979a) J. Biol. Chem. 254, 11033–11039

Fisher, P. A., and Korn, D. (1979b) J. Biol. Chem. 254, 11040–11046

Fisher, P. A., and Korn, D. (1981a) Biochemistry 20, 4560–4569

Fisher, P. A., and Korn, D. (1981b) Biochemistry 20, 4570–4578

Fisher, P. A., Chen, J. T., and Korn, D. (1981) J. Biol. Chem. 256, 133–141

Kaguni, L. S., Rossignol, J.-M., Conway, R. C., and Lehman, I. R. (1983) Proc. Natl. Acad. Sci. U.S. A. 80, 2221–2225

Lindahl, T. (1982) Annu. Rev. Biochem. 51, 61–87

Loeb, L. A. (1988) Cell 40, 483–484

Loeb, L. A., and Preston, B. D. (1987) Annu. Rev. Genet. 20, 201–209

Madiatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Meller, A. M., and Gilbert, W. (1980) Methods Enzymol. 65, 499–500

Randall, S. K., Eritja, R., Kaplan, B. E., Petrushka, J., and Goodman, M. F. (1987) J. Biol. Chem. 262, 6864–6870

Takeshita, M., Chang, C.-N., Johnson, F., Will, S., and Grollman, A. P. (1987) J. Biol. Chem. 262, 10171–10183

Wong, S. W., Paborsky, L. R., Fisher, P. A., Wang, T. S.-F., and Korn, D. (1986) J. Biol. Chem. 261, 7958–7968