# Influence of DNA Sequence on the Nature of Mispairing during DNA Synthesis†

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ABSTRACT: A series of synthetic oligonucleotide primers, annealed at various positions along the *lacZ*-$\alpha$ region of bacteriophage M13mp9 template, were elongated by purified DNA polymerases in the presence of only 3 of the 4 deoxynucleoside triphosphates to achieve misincorporation at a total of 49 different positions along the template. The newly synthesized strands (containing misincorporated bases) were isolated and sequenced to determine the identity of misincorporated deoxynucleoside monophosphates. The results indicate that the kind of mispairing that occurs during DNA synthesis is greatly influenced by the nucleotide sequence of the template. Transition-type base substitutions predominated overall, but at many template positions, transversion-type base substitutions occurred, most commonly via A·A mispairing. The results of parallel determinations made with *Escherichia coli* DNA polymerase I ("large fragment" form) and DNA polymerase of Maloney murine leukemia virus indicated that, overall, the identity of polymerase had only a small effect on the kind of misincorporation that occurred at different positions along the template. However, at certain template positions, the nature of mispairing during DNA synthesis was reproducibly affected by differing polymerase active-site environment.

The specificity of nucleotide incorporation during DNA synthesis, which is essential for accurate duplication of the genetic material, results from selective forces that occur at the active site of the enzyme. These may include not only formation of hydrogen-bonded Watson–Crick base pairs between template residue and incoming nucleotide but also base pair recognition by amino acid residues in the polymerase. Incorrect incorporation, though rare, may be specified to some degree by additional interactions, involving the nearest-neighbor sequences in the primer-template. To investigate this possibility, we have directly determined the identity of deoxynucleotides misincorporated in place of each of the four deoxynucleoside triphosphates (dNTPs)[1] at many positions along a natural DNA template during in vitro DNA synthesis.

The experimental approach used in this investigation is illustrated in Figure 1. A set of oligonucleotides was chemically synthesized to provide a series of primers complementary to the region of the bacteriophage M13mp9 genome encoding the $\alpha$ fragment of $\beta$-galactosidase (pictured on the top of Figure 1). These oligonucleotides were individually 5′-$^{32}$P labeled and annealed to the viral strand to form an overlapping series of primer-templates. Each primer was elongated by purified DNA polymerase in the presence of only three of the four dNTPs to permit misincorporation to occur at one or more template positions complementary to the missing dNTP (Hillebrand et al., 1984; Hillebrand & Beattie, 1984, 1985; Revich et al., 1984). The reaction products were then subjected to the procedure diagrammed in Figure 1 to determine the nucleotide sequence in the nascent strand containing misincorporated dNMPs at specific positions. This procedure was carried out with two different polymerases, the "Klenow fragment" form of *Escherichia coli* DNA polymerase I (Kf pol) and DNA polymerase of Maloney murine leukemia virus (MMLV pol). The results indicate that the nature of misincorporation during DNA synthesis is greatly influenced by the DNA sequence. Furthermore, at certain template positions, the identity of misincorporated nucleotide differed from one polymerase to another, suggesting that the polymerase active-site environment contributes to the specificity of mispairing during DNA synthesis.

## Materials and Methods

*DNA, Enzymes, and Chemicals.* Single-stranded template DNA from bacteriophage M13mp9 was prepared as described by Messing (1983). Oligonucleotide primers were synthesized by the solid-phase phosphoramidite procedure (Beaucage & Caruthers, 1981; McBride & Caruthers, 1983) using an Applied Biosystems Model 380A DNA synthesizer. DNA polymerase I of *E. coli* ("Klenow fragment" form, Kf pol) was purchased from Pharmacia/P-L and from Bethesda Research Laboratories. Some experiments were carried out with Kf pol purified in this laboratory from the overproducing *E. coli* strain CJ155 (kindly supplied by Drs. Joyce and Grindley), using the published procedure (Joyce & Grindley, 1983). Results did not significantly differ when experiments were performed with enzymes from these different sources. DNA polymerase of Maloney murine leukemia virus (MMLV pol) was purchased from Bethesda Research Laboratories. Restriction enzymes were purchased from New England Biolabs and Bethesda Research Laboratories. HPLC-purified dNTPs were obtained from Pharmacia/P-L and further purified by two additional rounds of HPLC, as described before (Revich et al., 1984). Primers were 5′-end-labeled and annealed to templates as described previously (Hillebrand et al., 1984).

*DNA Polymerase Reactions and Sequencing.* Primer elongation by Kf pol was carried out at 30 °C in a buffer containing 40 mM Tris-HCl (pH 7.4), 6 mM $MgCl_2$, and

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[1] Abbreviations: dNTP, 2′-deoxynucleoside 5′-triphosphate; dNMP, 2′-deoxynucleoside 5′-monophosphate; ddNTP, 2′,3′-dideoxynucleoside 5′-triphosphate; ddNMP, 2′,3′-dideoxynucleoside 5′-monophosphate; Tris, tris(hydroxymethyl)aminomethane; HPLC, high-performance liquid chromatography; Kf pol, DNA polymerase I (Klenow fragment) of *Escherichia coli*; MMLV pol, DNA polymerase of Maloney murine leukemia virus.

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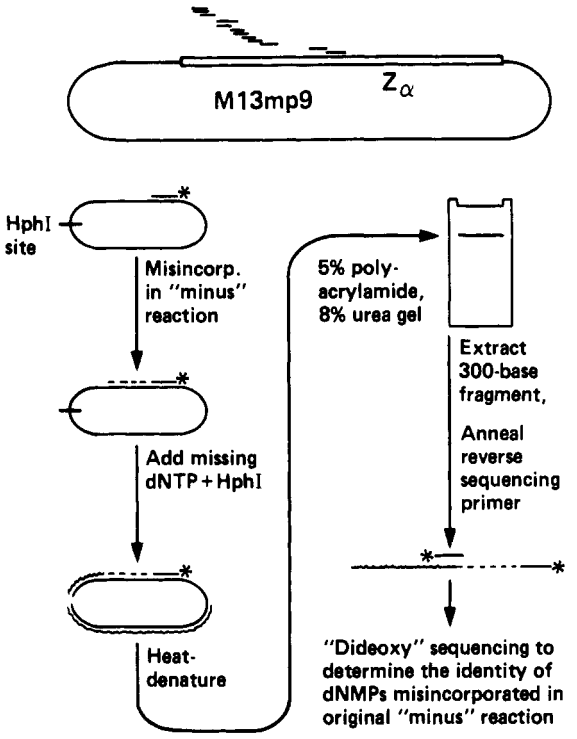


FIGURE 1: Experimental procedure for identification of dNMPs misincorporated during DNA synthesis. Primer-templates were incubated in "minus" reactions (containing only three of the four dNTPs) to permit misincorporation to occur at one or more template positions (nascent strands containing misincorporated dNMPs are represented by dashed lines). After misincorporation occurred, the missing dNTP was added, along with restriction endonuclease *Hph*I, to permit further elongation and generate a discrete fragment containing dNMPs misincorporated at one or more positions. The mixture was then heat-denatured and electrophoresed on a 5% polyacrylamide–8 M urea gel. After autoradiography, the 300-base fragment (containing misincorporated dNMPs) was extracted from the gel, precipitated with ethanol, and annealed with a 5′-$^{32}$P-labeled reverse sequencing primer. Dideoxy sequencing was carried out to determine the identity of dNMPs misincorporated during the initial minus reaction.

75–150 μM dNTPs. MMLV pol catalyzed chain elongation was carried out at 37 °C in 60 mM Tris-HCl (pH 8.3), 6 mM $MgCl_2$, and 100 μM dNTPs. Reactions generally contained 1–2 pmol of primer-template and 10–50 units of polymerase, in a total volume of 50–100 μL. After misincorporation occurred for several hours in the "minus reaction" containing three dNTPs, the missing dNTP was added, together with 10–20 units of restriction endonuclease *Hph*I. Mixtures were then incubated for an additional 2 h, heat-denatured, and subjected to electrophoresis in a 5% polyacrylamide–7 M urea gel. The 300-base nascent strand was visualized by autoradiography, extracted from the gel, and then sequenced by the "dideoxy" method (Sanger et al., 1977; Sanger & Coulson, 1978) to identify the dNMPs misincorporated in the minus reaction.

## RESULTS

The success of the experimental approach illustrated in Figure 1 for characterizing mispairing during DNA synthesis depends on the purity of dNTPs used in the in vitro misincorporation reactions. We have found that unless dNTPs (including commercially HPLC-purified dNTPs) are further purified by HPLC, primer elongation in the minus reactions can sometimes be due to incorporation of contaminating dNTPs (data not shown). The conditions we use to purify dNTPs are carefully chosen to eliminate cross-contamination of dNTPs, including the deamination products dUTP and dITP (Revich et al., 1984). The latter would have the same effect as contaminating dTTP and dGTP, respectively, since dUTP base pairs like dTTP and dITP base pairs only as dGTP during DNA synthesis (Lai & Beattie, 1988). It is highly unlikely that any other chemically modified dNTPs could generate artifactual results in our assay, since the modified dNTP would have to (i) escape removal from dNTP preparations during HPLC purification, (ii) efficiently compete with unmodified dNTPs for incorporation, and (iii) exhibit highly ambiguous base pairing during DNA synthesis. Although it is conceivable that the first criterion could be met, in our work with numerous dNTP analogues we have not found a modified dNTP that simultaneously meets the second and third criteria. Therefore, we are confident that the data presented here represent the specificity of misincorporation of the four canonical dNTPs during DNA synthesis.

Figure 2 shows representative data from experiments in which primer elongation was carried out in the presence of three of the four dNTPs until misincorporation occurred at one or more sites; then (after addition of the missing dNTP) the elongated primer was isolated, annealed to a "reverse sequencing primer", and sequenced by the dideoxy chain termination method (Sanger et al., 1977; Sanger & Coulson, 1978). The nucleotide sequence of the original template is displayed in the center, flanked by the nucleotide sequence of the newly synthesized strand (listed 5′ → 3′, top to bottom). The upper right side of Figure 2 (panel B) shows results of the control experiment, in which the first stage of synthesis occurred in the presence of all four dNTPs. The expected pattern of dideoxy bands was produced [from incorporation of chain-terminating 2′,3′-dideoxynucleoside monophosphates (ddNMPs) only at positions in the nascent strand complementary to the dideoxy analogue]. It is essential to carry out such a control experiment with each "forward primer", to eliminate the contribution of occasional artifactual bands produced by nonspecific chain termination at certain template positions.

The upper left side of Figure 2 (panel A) shows the dideoxy sequencing results obtained with a primer elongated in the absence of dGTP. In this "–G" reaction, essentially all primers had bypassed the first C residue in the template, and at least half had undergone misincorporation at the second C in the template (data not shown). At the position in the autoradiograph (panel A) corresponding to the first site of misincorporation, only a "ddT" band (marked "1") was produced, indicating that only dAMP was misincorporated opposite this C residue in the template. At the position in the dideoxy sequencing pattern corresponding to the second C in the template, some chain termination occurred with ddCTP, corresponding to incorporation of dGMP after addition of dGTP to the "–G" reaction (in primers that had not yet undergone two misincorporations). The only other chain termination at this position occurred with ddTTP (band marked "2"), indicating that dAMP was the only detectable nucleotide misincorporated at this position, as well.

At certain positions along the M13mp9 template, more than one type of misincorporation occurred. The lower part of Figure 2 shows an example of such an occurrence. Panel D shows the result of the control reaction, wherein elongation in the presence of all four dNTPs produced only the "correct" dideoxy bands. Panel C shows the outcome of the "–T" reaction carried out in this region of the template. At the first template A residue examined with this primer, only one kind of misincorporation was detected (C·A mispair), as shown by the "ddG" band (marked "3"), along with the "ddA" band

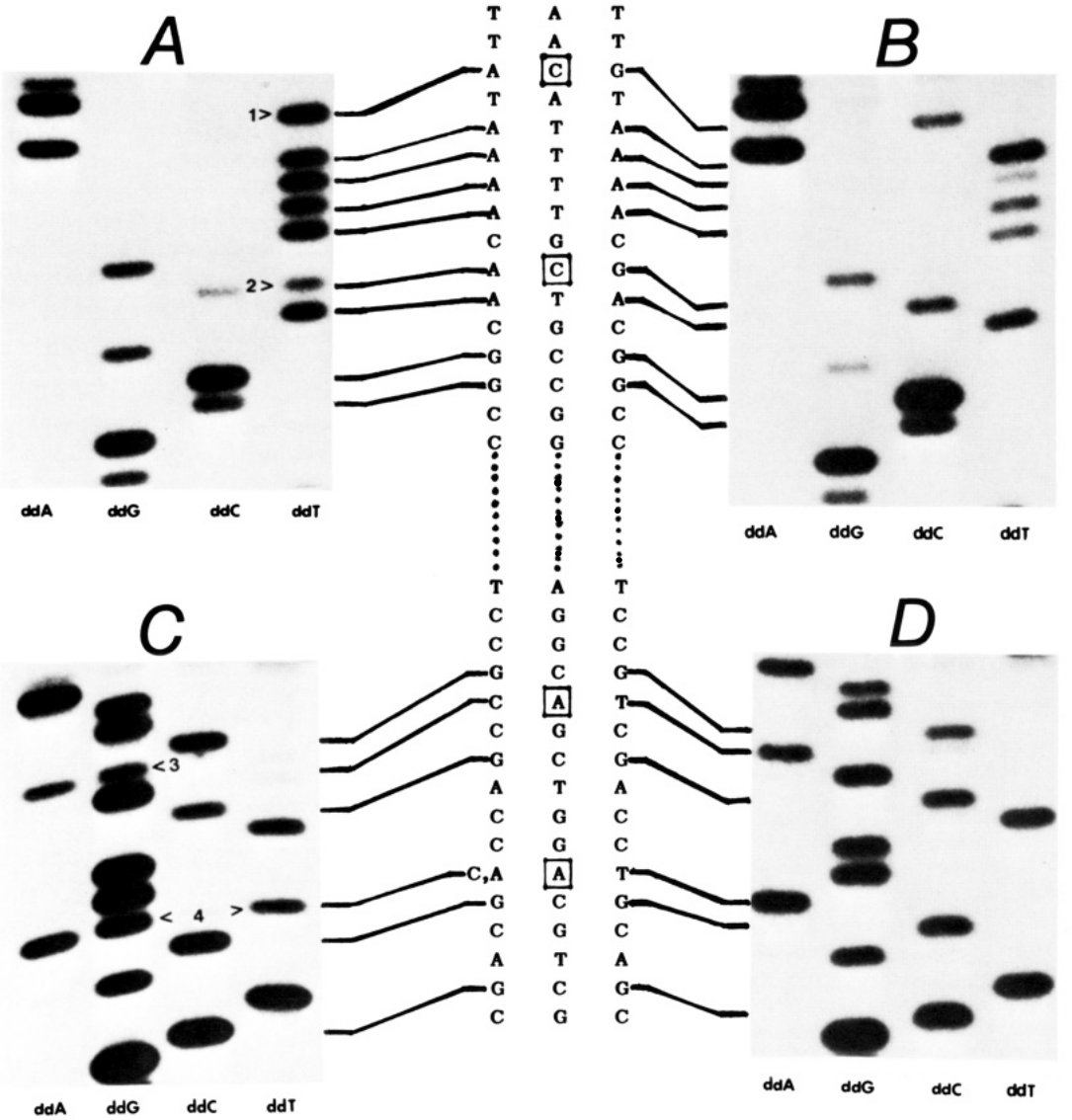


FIGURE 2: Dideoxy sequencing of strands synthesized in the presence of three dNTPs. These representative data were obtained over two regions of the M13mp9 template (sequence of viral strand is displayed in the center, and template positions at which misincorporation occurred are boxed). To the right (panels B and D) is displayed the sequence of dNMPs incorporated into the primer during elongation (in the direction top to bottom) in the presence of all four dNTPs, as determined by dideoxy sequencing reactions carried out after annealing the "reverse sequencing" primer to the isolated nascent strand. Lines connect positions in the autoradiograph with the corresponding residues in the nascent strand. Panels A and C display dideoxy sequencing data obtained from strands synthesized in the absence of dGTP and dTTP, respectively. At the positions where misincorporation was observed (boxed residues in original template), only the identity of misincorporated dNMPs is indicated in the sequence of the nascent strand (additional dideoxy bands, corresponding to "correct" incorporation, resulted at sites that were not completely bypassed in the minus reaction, after addition of the missing dNTP). The autoradiographic bands labeled 1 and 2 correspond to dNMPs misincorporated in the absence of dGTP, and those labeled 3 and 4 correspond to dNMPs misincorporated in the absence of dTTP. At the positions where more than one kind of misincorporation occurred, the relative occurrence of the different mispairs was determined by quantitative densitometry.

produced from molecules that had not yet undergone misincorporation at this site when dTTP was added. However, at the position on the autoradiograph corresponding to the second A residue in the template, the existence of both "ddG" and "ddT" bands (marked "4") indicates that both dCMP and dAMP were misincorporated opposite this A residue in the template.

Figure 3 displays the identity of dNMPs misincorporated by Kf pol at 33 different positions along the M13mp9 template, and Figure 4 displays the data obtained at 39 sites with MMLV pol. Transition-type base substitutions predominated. The base substitutions were exclusively or predominantly transitions at 76% of the sites examined with Kf pol and at 74% of the sites with MMLV pol. Transversion-type base substitutions were detected at 36% of the sites examined with both Kf pol and MMLV pol. Pu·Pu mispairing was detected at 9 of 10 template A residues examined, but at none of the 12 template G residues examined (data for Kf pol and MMLV pol combined). A·A mispairing accounted for nearly all of the transversion-type base substitutions seen at A residues in the template (misincorporation of dGMP was detected at only one template A). Transversion-type base substitutions at C residues in the template (observed at four sites) resulted almost exclusively from C-T mispairing (small amounts of C·C were formed at one site), whereas transversions observed at T residues occurred by T·T mispairing (two sites), T·C mispairing (three sites), or a mixture of T·T and T·C mispairing (two sites).

The type of mispairing that occurred during DNA synthesis was strongly influenced by the nucleotide sequence of the template. For example, at the 10 template A residues examined, a mixture of A·A and A·C mispairing occurred at 5 sites,

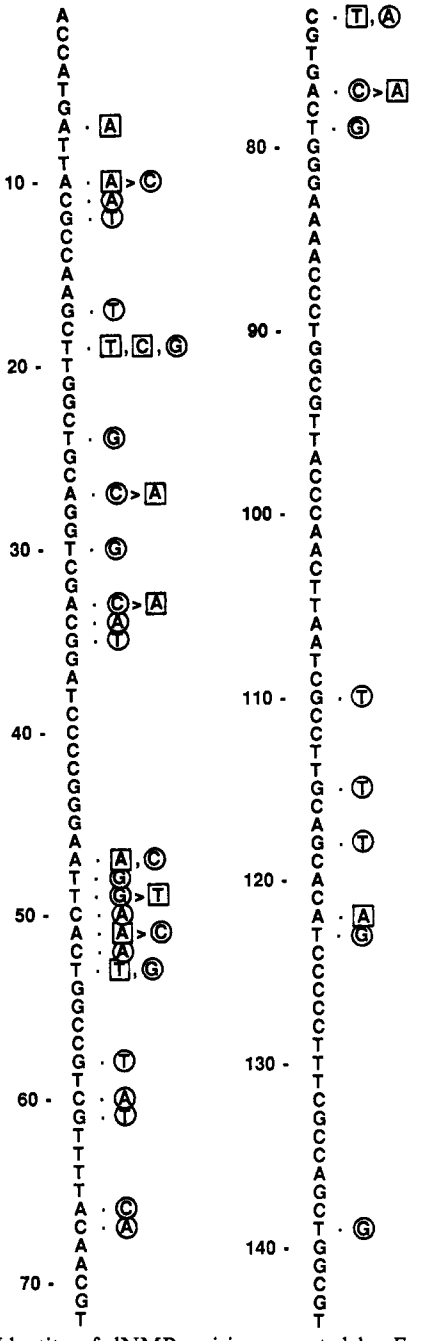

FIGURE 3: Identity of dNMPs misincorporated by *E. coli* DNA polymerase I ("Klenow fragment"). The sequence of the M13mp9 template is listed 5′ → 3′, top to bottom. The residues are numbered with reference to the sequence encoding the Z-$\alpha$ polypeptide (position 1 corresponds to the first position of codon 1). The polylinker region (containing multiple restriction endonuclease recognition sequences) corresponds to positions 15–50. The remaining sequences are identical with those of all M13mp phages. The mispairs formed during DNA synthesis are indicated by a central dot between template and incorporated dNMPs. Transition-type base substitutions are circled, while transversions are boxed. At positions where more than one kind of misincorporation was detected, a ">" appears where one type of mispair occurred at 60–90% and the other type at 10–40% frequency. When approximately equal occurrence of different mispairs was detected, misincorporated dNMPs are separated by commas. The absence of base mispairing at a given site does not indicate that misincorporation is infrequent at this site. We have not synthesized all of the primers that would be necessary to collect data at all positions along the template (in each minus reaation, misincorporation occurs at only one to three sites).

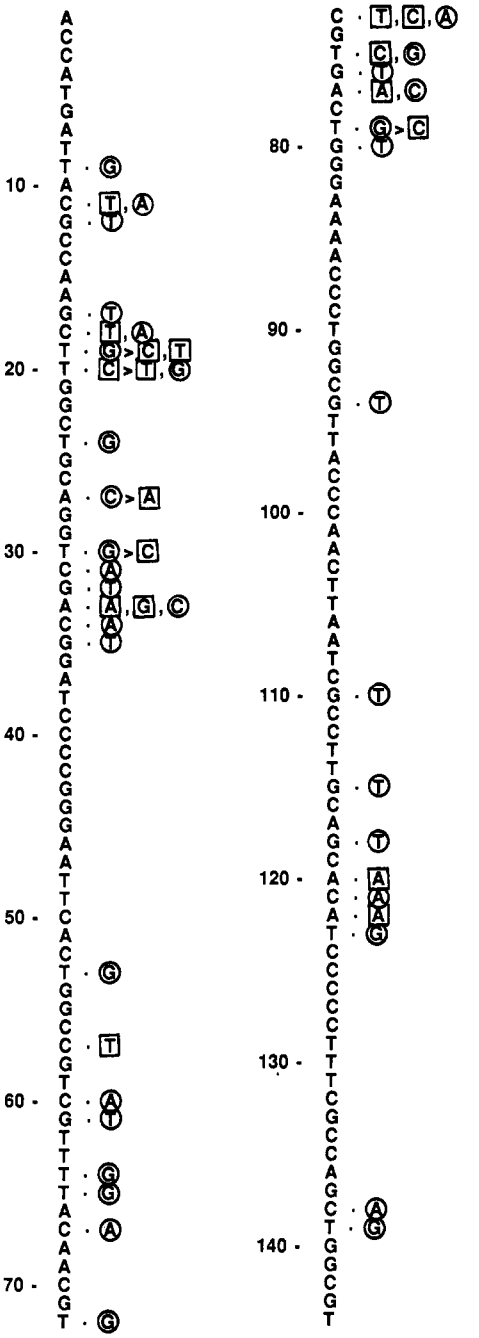

FIGURE 4: Identity of dNMPs misincorporated by DNA polymerase of Maloney murine leukemia virus. See legend to Figure 3 for explanation.

only A·C mispairing was observed at 1 site, and only A·A mispairing was seen at 3 sites. Of the 12 template C residues, 8 exhibited only C·A mispairing, 2 showed both C·A and C·T mispairing, 1 gave only C·T mispairing, and 1 yielded all 3 possible mispairs. Finally, among the 13 template T residues examined, only T·G mispairing was observed at 6 sites, a mixture of T·G and T·C mispairing occurred at 3 sites, T·G and T·T mispairing was seen at 2 sites, and all 3 possible mispairs occurred at 2 sites. A basic characteristic of mispairing spectra revealed by these results is that the occurrence of a given type of base mispair during DNA synthesis varies greatly at different positions along the template.

We tested whether the effect of template sequence on the identity of misincorporated dNMPs is always mediated through interactions involving the immediate nearest neighbors. Among the data collected in this study are three examples of different mispairs formed during DNA synthesis, at template positions having identical nearest neighbors. The sequences displayed in Figure 5 represent the newly synthesized strands, containing misincorporated dNMPs (indicated within brack-

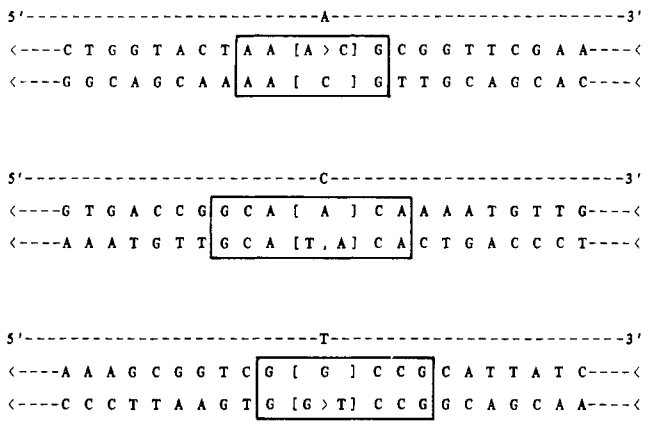


FIGURE 5: Different mispairs formed within identical sequence contexts during DNA synthesis. In each group, the first line represents the template (5′ → 3′) used to identify misincorporated dNMPs at two different A (top), C (middle), or T (bottom) residues. Below each template is displayed the nucleotide sequence of two different elongated primers, with the identity of mispair(s) enclosed within brackets and the direction of chain elongation indicated by arrows. Identical nearest neighbors flanking the misincorporation sites are enclosed within boxes. Data were taken from Figure 3 (Kf pol), although results obtained with MMLV pol revealed the same phenomenon.

ets) opposite different A, C, and T residues in the template. The identical nearest neighbors are boxed. At one of the two template A residues shown, A·A mispairing predominated, and a lesser amount of A·C mispairing occurred. At the other site, only A·C mispairing was detected, and yet the misincorporated dNMPs were imbedded within identical nearest-neighbor sequences (one identical residue preceding misincorporation and two following misincorporation). A similar phenomenon was exhibited at two different C residues and at two different T residues in the template. Thus, in some cases, the effect of DNA sequence on the nature of mispairing during DNA synthesis involves nucleotide residues other than the immediate nearest neighbors.

The influence of DNA sequence on the specificity of misincorporation was remarkably similar for the two DNA polymerases used in this work. The similarity is depicted in the "mispairing profile" shown in Figure 6, in which transition-type mispairs are represented by hatched bars and transversions by open bars (above the template sequence for Kf pol and below the sequence for MMLV pol). The type of misincorporation is indicated only at those template positions where determinations were made with both polymerases. The mispairing profile was largely independent of the polymerase used. Even those positions at which unusual types of misincorporation occurred generally gave identical results for both polymerases, such as template T at position 19, at which all possible mispairs were seen, and template A at position 122, at which only A·A mispairing was detected (see also Figures 3 and 4). However, at some positions along the template, different dNMPs were misincorporated by Kf pol and MMLV pol (and at position 33 a third result was seen with polymerase $\alpha$). The boxed residues in the template sequence (positions 11, 33, 53, and 79) mark positions at which the identity of the polymerase affected in a major way the kind of mispairing that occurred during DNA synthesis. A smaller (but reproducible) difference in mispairing was seen between Kf pol and MMLV pol at positions 30 and 77. These differences contribute to an apparent overall greater tendency of MMLV pol to make

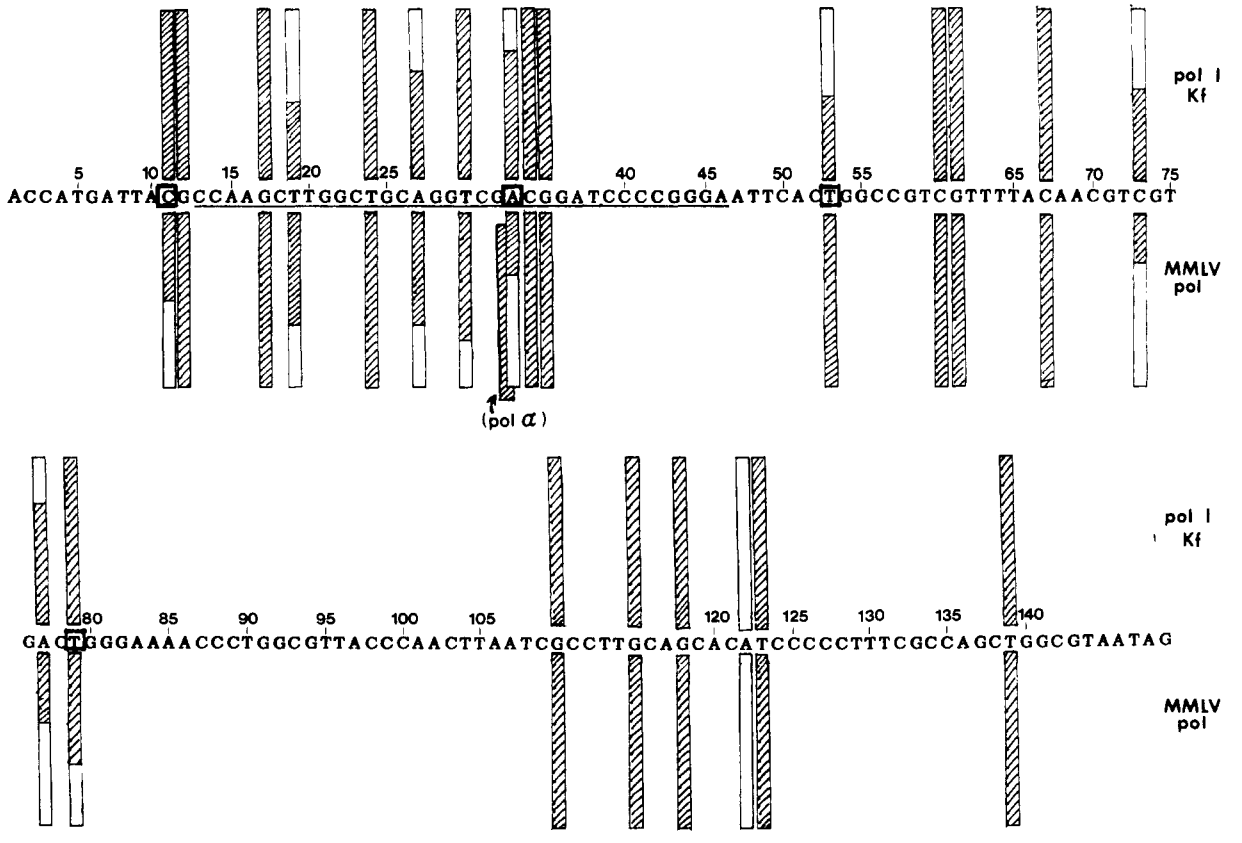


FIGURE 6: Influence of the identity of DNA polymerase on the nature of mispairing during DNA synthesis. Open bars represent transversion-type base substitutions (resulting from Pu·Pu or Py·Py mispairing). Hatched bars represent transition-type base substitutions. Position 1 in the template sequence corresponds to the first coding residue in the Z-$\alpha$ sequence of M13mp9. These data, taken from Figures 3 and 4, represent misincorporation only at sites examined with both polymerases. The boxed template residues (positions 11, 33, 53, and 79) exhibited major differences in the identity of misincorporated dNMPs between Kf pol and MMLV pol (position 33 gave a third result with DNA polymerase $\alpha$ from calf thymus).

transversion-type base substitutions, compared with Kf pol. These results suggest that at certain template positions the active-site environment of the polymerase contributes to the specificity of misincorporation.

## Discussion

Kunkel (1985a,b; Kunkel & Alexander, 1986) recently employed a combination of biochemical and genetic techniques to characterize the base changes associated with mutations arising during in vitro DNA synthesis. In vitro DNA synthesis was carried out in the presence of all four dNTPs, across a 250-residue target sequence within bacteriophage M13mp2, consisting of the regulatory region and coding sequence for the Z-$\alpha$ portion of $\beta$-galactosidase (including much of the template sequence used in our studies). The products of in vitro DNA synthesis were used to transfect recipient cells, and the DNA from mutant plaques was sequenced to determine the base change associated with each mutation.

Although the experimental approach used in those studies was very different from that used in our work, the results were similar in several respects. The data of Kunkel and co-workers suggested a striking dependence of DNA sequence on the nature of mispairing during DNA synthesis catalyzed by purified polymerases. Both the frequency and type of base changes that occurred during in vitro polymerization varied along the template, in agreement with the results reported here on the specificity of misincorporation and our previous findings on the relative frequency of misincorporation at different positions along the template (Hillebrand et al., 1984; Hillebrand & Beattie, 1984, 1985; Revich et al., 1985). Furthermore, at certain positions along the template, the specificity of misincorporation appeared to depend on the identity of the polymerase, as shown by both our work and that of Kunkel. Results of both experimental approaches indicated that transition-type base substitutions represented the major type of misincorporation. Nevertheless, a surprisingly high occurrence of transversion-type base substitutions was observed in both studies. The above similarities applied even though the results were obtained with different DNA polymerases (*E. coli* DNA polymerase I and MMLV DNA polymerase in this work and eucaryotic polymerases $\alpha$, $\beta$, and $\gamma$ in Kunkel's work).

The major difference between Kunkel's results and ours was the nature of mispairing observed at G residues in the template. The G·T mispair was formed most frequently as shown by our data, whereas transversions were most frequently observed at template G residues from Kunkel's data. This difference could be due to either (i) specific removal of G·T mispairs by the *E. coli* mismatch repair system after transfection in Kunkel's assay or (ii) expression of "cryptic lesions" in the template (e.g., small amount of depurination) during in vitro DNA synthesis, significantly contributing to a portion of the mutations seen at template G residues in Kunkel's system. In fact, it has been shown that dA is preferentially misincorporated opposite apurinic sites (Loeb, 1985), which might explain the frequent occurrence of G·A mispairing apparent from Kunkel's experiments, as opposed to ours.

The experimental approach employed by Kunkel has obviously yielded a great deal of important information about mispairing events during DNA synthesis. However, the method that we have used to characterize the mispairs formed during in vitro DNA synthesis offers several potential advantages over the genetic system: (1) The data are not complicated by *E. coli* mismatch repair systems, which may alter the ratio of different mispairs after the misincorporation products are transfected into *E. coli* to produce phage progeny. Some misincorporation events would be underestimated if those mispairs were removed preferentially by the mismatch repair system. In fact, it is known that methylation-directed mismatch repair can specifically act on certain mispair types (Kramer et al., 1984). (2) The statistical problem inherent in the genetic system (due to analysis of small numbers of mutants at most sites) is avoided in our system, since the population of DNA molecules analyzed is very large. (3) Since a large proportion of DNA substrates are converted to misincorporation products in our assay, the mispairing spectra obtained by this method could not be affected by small amounts of preexisting damage to the template substrate. Such "cryptic lesions" could give rise to a portion of the mutations analyzed in Kunkel's system. (4) Although data are difficult to obtain at sites of very low misincorporation frequency in our method (a large excess of enzyme may be required to bypass "cold spots" for misincorporation), the mispairing spectra obtained in our work represent a more random sample of template positions than in the genetic assay used by Kunkel, which quantitates only phenotypically observable mutations and is inevitably biased toward collection of data at "hotspots" for misincorporation.

A discussion of how the DNA sequence might influence the identity of misincorporated dNMPs must take into account the multiple interactions that occur at the primer terminus during polymerization:

Sequence-dependent base stacking interactions at the primer terminus might stabilize non-Watson–Crick base pairs to different extents at different positions along the template. This effect could operate either prior to or after phosphodiester bond formation (in the latter case, stabilizing the mispair against the 3′-exonuclease "editing" activity associated with the polymerase or replication complex). From spectroscopic and theoretical studies of duplex polynucleotides containing internal mismatches, Fresco and co-workers (Topal & Fresco, 1976; Fresco et al., 1980) predicted that the identity of nearest neighbors should specify to some degree the type of mispair that would be formed during DNA synthesis, via sequence-dependent base stacking interactions. During DNA synthesis, a mispair (involving a dNTP bound adjacent to the primer terminus, or a newly incorporated dNMP) could be stabilized by a stacking interaction on only one side of the misincorporated base (as opposed to an internal mismatch in duplex DNA) but on both sides of the mispaired template residue. Although the base stacking forces operative at the primer terminus during DNA synthesis would be diminished, compared with that in duplex DNA, stacking interactions might provide sufficient stabilization energy to influence (in a sequence-dependent manner) such a rare event as misincorporation.

Effects of DNA sequence on the identity of misincorporated dNMPs might also be indirectly mediated through sequence-dependent protein–nucleic acid interactions that alter the stereochemical properties of the active site of the polymerase as it progresses along the template. For example, slight conformational changes in the polymerase, induced by sequence-dependent variations in its interaction with the primer-template, might alter the dielectric, hydrophobic, or steric properties of the active site and thereby influence events necessary for the occurrence of specific mispairs, including (i) formation of rare tautomers, (ii) rotation of *N*-glycosyl bonds, (iii) formation of rare ionic forms, and (iv) wobbling. The phenomenon shown in Figure 5 (occurrence of different misincorporations at template positions with identical nearest neighbors) could have arisen through protein–DNA interac-

tions at some distance from the mispair.

The results shown in Figure 6 and those of Kunkel (1985a,b; Kunkel & Alexander, 1986) indicated that at certain template positions the specificity of base substitutions arising during in vitro DNA synthesis depends on the identity of the DNA polymerase. It is unlikely that these differences are attributable to sequence-dependent exonucleolytic proofreading activity, since demonstration of 3′-exonuclease activity associated with the DNA polymerases used by Kunkel and coworkers has proven elusive. Furthermore, the largest effect of the identity of the polymerase on the type of mispairing that we observed was at template position 33 (Figure 6), between MMLV pol and DNA polymerase $\alpha$, both which reportedly lack 3′-exonuclease activity. These results suggest that mispairs formed at certain template positions may be stabilized (or destabilized) by interactions that are unique for different polymerases.

The "mispairing profiles" obtained with Kf pol (possessing 3′-exonuclease activity) and MMLV pol (reportedly lacking this activity) were similar (Figure 6). This results suggests that exonucleolytic editing, which may affect the frequency of mispairing during DNA synthesis, does not play a major role in the specificity of misincorporation. Further enzymological and physical studies are needed to determine the importance of base stacking interactions, protein–nucleic acid interactions, and exonucleolytic proofreading in determining the sequence dependence of misincorporation during DNA synthesis.

**Registry No.** dAMP, 653-63-4; dCMP, 1032-65-1; dGMP, 902-04-5; dTMP, 365-07-1; DNA polymerase, 9012-90-2.

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