Self-catalyzed site-specific depurination of guanine residues within gene sequences

Olga Amosova*, Richard Coulter, and Jacques R. Fresco*

Department of Molecular Biology, Princeton University, Princeton, NJ 08544

Edited by Jack W. Szostak, Massachusetts General Hospital, Boston, MA, and approved January 19, 2006 (received for review September 28, 2005)

A self-catalyzed, site-specific guanine-depurination activity has been found to occur in short gene sequences with a potential to form a stem-loop structure. The critical features of that catalytic intermediate are a 5′-G-T-G-G-3′ loop and an adjacent 5′-T-A-3′ base pair of a short duplex stem stable enough to fix the loop structure required for depurination of its 5′-G residue. That residue is uniquely depurinated with a rate some 5 orders of magnitude faster than that of random “spontaneous” depurination. In contrast, all other purine residues in the sequence depurate at the spontaneous background rate. The reaction requires no divalent cations or other cofactors and occurs under essentially physiological conditions. Such stem-loops can form in duplex DNA under superhelical stress, and their critical sequence features have been found at numerous sites in the human genome. Self-catalyzed stem-loop-mediated depurination leading to flexible apurinic sites may therefore serve some important biological role, e.g., in nucleosome positioning, genetic recombination, or chromosome supercoiling.

DNA self-catalysis | guanine depurination | stem-loop structure

Depurination in DNA has been recognized as a spontaneous form of intracellular DNA damage that affects both G and A residues in an essentially random way (1). In mammalian cells, such damage has been estimated to occur with a $k_{obs}$ of $3 \times 10^{-9}$ min$^{-1}$ (2). A complex and elaborate cellular DNA repair machinery has evolved to repair apurinic sites (3, 4). It has long been known that some G residues are more prone to depurination than others (5, 6), and such depurination hot spots have been associated with elevated mutation rates (6, 7). Notwithstanding these insights, notions to account for DNA depurination that go beyond recognition that hydrolysis of the purine–deoxyribose glycosyl bond is acid-catalyzed have not been forthcoming.

In the course of studies using a 29-residue single-stranded coding strand fragment encompassing the mutation site of the sickle cell beta-globin gene (8–10), we found that this 29-nt oligomer as well as its shortened 18-nt segment self-catalyze site-specific depurination of a singular G residue adjacent to the mutation site. In this report, we identify the precise location of the depurination site, the reaction mechanism and its immediate products, the structure of the catalytic intermediate, and some of the sequence requirements and their tolerance for variation within the intermediate. We also note the wide distribution of such putative self-depurinating sequences in the human genome.

Results

Specific Backbone Cleavage Is the Result of Site-Specific Depurination. It is as a consequence of slow spontaneous backbone cleavage at apurinic sites that the self-catalyzed site-specific depurination we report here was discovered. At the outset, it was observed that this cleavage occurs whether or not $10^{-3}$ M EDTA is present, indicating that divalent cations are not required for the cleavage. Incubation of the highly purified and homogeneous 29-nt oligomer D-1 at 22°C (to allow depurination to proceed) spontaneously yielded a 19-nt fragment and an $\sim$9- to 10-nt group of four fragments revealed by labeling their 5′ ends with $^{32}$PO$_4$ (Fig. 1). However, when 3′-labeling with terminal deoxynucleotransferase (11) was performed instead, only the $\sim$19-nt band was visible, whereas the shorter group of bands was barely visible (data not shown). The presence of the several bands refractory to terminal deoxynucleotransferase, with mobilities corresponding to $\sim$9–10 nt, is consistent with depurination that results in an equilibrium between C$′$ hemiacetal and C$′$ aldehyde sugars at the apurinic site, accounting for two of the bands. Subsequent loss of either the terminal phosphate or both the phosphate and sugar, each resulting in successively increasing fragment mobility, would account for the other two. Piperidine treatment at 75°C accelerates such backbone cleavage by base-catalyzed $\beta$-elimination (12, 13), thereby allowing detection and quantitation of the accumulated apurinic sites.

The initial backbone cleavage site was located by comparing the large cleavage product with a sequencing ladder on denaturing PAGE (Fig. 1). The large cleavage product band corresponded in mobility to a DNA polymerase product resulting from incorporation of 2′,3′-diol deoxadenosine opposite the sickle cell mutant T residue immediately downstream from the depurinated G. This band appeared equally intense in all four residue lanes because of significant presence of the 19-nt cleavage product in the D-1 template, as revealed by polyethylene glycol labeling. The next band was also equally intense in the

Conflict of interest statement: No conflicts declared.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: dG, deoxyguanosine; Exo III, exonuclease III.

*To whom correspondence may be addressed. E-mail: amosova@princeton.edu or jrfresco@princeton.edu.

© 2006 by The National Academy of Sciences of the USA
Because the synthetic 10-nt oligomer had a 5' phosphate on the 19-nt product of spontaneous cleavage were confirmed by successful ligation of a synthetic 10-nt oligomer bearing a 3'-OH and a 5'-phosphate, and the gel-purified 19-nt spontaneous cleavage product after their annealing to the complementary 29-nt anti-D-1 strand (see Table 1 and Fig. 2). Ligation can succeed only when the two fragments annealed with the sequence requirements immediately adjacent. Because the synthetic 10-nt oligomer had a 3'-OH, the 19-nt fragment must bear a 5'-phosphate for ligation. In fact, ligation produced a full-size D-1 strand, as well as a D-1 dimer, a product of blunt-end ligation of two duplexes (Fig. 2, lane 1). Alkaline phosphatase treatment of the 19-nt fragment before annealing resulted in only a trace of full-size D-1 (Fig. 2, lane 3). Because phosphate treatment (data not shown), it must be the removal of its 5'-phosphate that prevents ligation.

**A Guanine Residue Is Required 5' to the Cleavage Site.** To determine the sequence requirements immediately adjacent to the cleavage site that are essential for the depurination, several 29-nt sequence variants (Table 1) were compared. The site-specific depurination occurred only when the residue 5' to the cleavage site was G (Fig. 3). Its replacement by a pyrimidine eliminated cleavage, and only a trace of the cleavage product was visible for the A replacement. Cleavage was significantly less for the wild-type oligomer DH-1, indicating that the nature of the immediate downstream residue is also important. It is worth noting that all of these 29-nt oligomers had at least six other G residues in their sequences (seven in case of D-1 and DH-1, the sickle cell and wild-type β-globin gene fragments, respectively); yet none of them displayed any detectable depurination. Taken together, the results in Figs. 1 and 3 show that the depurination is very site-specific.

**Free Guanine Is One Initial Product of the Catalysis.** With depurination presumed to be the primary event leading to backbone cleavage, direct evidence was sought for free guanine and the resultant apurinic site. Fig. 4 compares HPLC profiles for incubated D-1, D1-T [which does not undergo piperidine-induced cleavage and therefore does not lose its base (Fig. 3)], and a mixture of D1-T and deoxyguanosine (dG) as a marker (neither dG nor guanine binds to the C18 column), with the molar ratio of dG:D1-T similar to that expected from the catalytic depurination of oligomer D-1. The small, immediately

**Fig. 2.** Ligation of the 5'-32P-labeled 19-nt spontaneous cleavage product and the 10-nt synthetic fragment on the complementary anti-D-1 template. Lane 1, ligation without prior phosphatase treatment. This denaturing gel indicates that the unlabeled 19-nt fragment has a 5'-phosphate and so is successfully ligated with the 10-nt oligomer on the complementary template. Lane 2, 32P-labeled 10-nt oligomer. Lane 3, ligation after alkaline phosphatase treatment of the purified 19-nt fragment yields almost no ligated D-1. Lane 4, 32P-labeled fresh D-1 stock.

**Table 1. Some deoxyoligonucleotides used in the study**

<table>
<thead>
<tr>
<th>Deoxyoligonucleotide</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-D-1</td>
<td>3'-GACTGAGGACCTCTTGGTAGCAATG-5'</td>
</tr>
<tr>
<td>D-1</td>
<td>5'-CTGACTTCTGCTGGAAAGCTGGTTTAC-3'</td>
</tr>
<tr>
<td>DH-1</td>
<td>5'-CTGACTTCTGCTGGAAAGCTGGTTTAC-3'</td>
</tr>
<tr>
<td>D1-T</td>
<td>5'-CTGACTTCTGCTGGAAAGCTGGTTTAC-3'</td>
</tr>
<tr>
<td>D1-A</td>
<td>5'-CTGACTTCTGCTGGAAAGCTGGTTTAC-3'</td>
</tr>
<tr>
<td>D1-C</td>
<td>5'-CTGACTTCTGCTGGAAAGCTGGTTTAC-3'</td>
</tr>
</tbody>
</table>

**Fig. 3.** Piperidine cleavage of 29-nt sequence variants of the D-1 fragment of the sickle cell β-globin gene coding strand after 48-h incubation at 22°C (pH 5.0). No cleavage was evident when a pyrimidine residue replaced the specifically depurinated G residue. Only a trace of cleavage product was evident with an A substitution.

**Fig. 4.** HPLC profiles of incubated D1-T, a mixture of D1-T/dG, and incubated D-1 from a C18 reverse-phase column eluted with a 10–60% acetonitrile gradient (diagonal dotted line), started at 3 min, and ended at 13 min of elution in 25 mM sodium phosphate (pH 7.0). Strnad concentrations were 0.05 mM, and free dG concentration was 0.01 mM. Neither dG nor guanine binds to the column and is eluted within the first 4 min. The slight difference between the elution profiles of incubated D-1 and of the D1-T/dG mixture is due to the deoxyribose moiety of the dG (deoxynucleoside) marker present in the “artificial” mixture and absent in the D-1 sample, because it is free guanine that is accumulated during the incubation.
eluted peak in the D1-T/dG mixture was absent in the elution profile of the D1-T sample alone, but such a peak was present in the elution profile of incubated D-1. When HPLC was conducted under highly acidic conditions (10% trichloroacetic acid), this small peak displayed, in addition to UV absorption, strong blue fluorescence characteristic of free guanine. Thus, one primary product of self-catalytic depurination was defined.

**Accumulation of Apurinic Sites Revealed by Piperidine Treatment Is Paralleled by Apurinic Endonuclease Cleavage.** Fig. 5 illustrates accumulation of apurinic sites monitored both by piperidine cleavage (Fig. 5A) and by cleavage with an apurinic endonuclease (Fig. 5B). The apurinic endonuclease activity of exonuclease III (Exo III) was used for this purpose. When an 18-nt subfragment of the D-1 strand, D(-2), which is depurinated at the same rate as D-1 (Table 2), was incubated at pH 5 and 22°C for various times and then digested with Exo III, a 10-nt band, the equivalent of the 19-nt 3’-end digestion product of D-1, appeared on the gel. As in the case of piperidine cleavage, the digestion site was unique; but, unlike piperidine cleavage, which produces several short fragments, the Exo III 7-nt digestion product at the 5′ side of the 18-nt D1(-2) was also unique (data not shown). The slope of the initial kinetics of cleavage was the same whether by piperidine or Exo III (Fig. 5C), indicating that they both reflect the rate of accumulation of apurinic sites. Hence, the self-catalyzed depurination gave rise to the apurinic sites that are cleavable by either piperidine treatment or apurinic endonuclease.

It is also noteworthy that the kinetics of depurination, as measured by the yield of piperidine cleavage products, was independent of strand concentration over 2 orders of magnitude (Fig. 5D), with \( k_{obs} \approx 2 \times 10^{-6} \text{ min}^{-1} \). This result is consistent with first-order kinetics and indicates that the catalytic intermediate is not a multistranded structure; rather, an intramolecular structure is critical for the self-catalytic depurination.

**Table 2. Reduction in the rate of self-catalytic depurination when D-1 sequence is shortened**

<table>
<thead>
<tr>
<th>Oligomer</th>
<th>Sequence</th>
<th>( k_{obs} ) min(^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-1</td>
<td>5′-CTGACTGCTCTCAGAGATTCGTTATC-3′</td>
<td>2 \times 10^{-4}</td>
</tr>
<tr>
<td>D-20</td>
<td>5′-CTGACTGCTCTCAGAGATTCAGATC-3′</td>
<td>2 \times 10^{-4}</td>
</tr>
<tr>
<td>D(-2)</td>
<td>5′-CTGACTGCTCTCAGAGATTCGTTATC-3′</td>
<td>2 \times 10^{-4}</td>
</tr>
<tr>
<td>D-19</td>
<td>5′-CTGACTGCTCTCAGAGATTCGTTATC-3′</td>
<td>4 \times 10^{-5}</td>
</tr>
<tr>
<td>D-3</td>
<td>5′-CTGACTGCTCTCAGAGATTCGTTATC-3′</td>
<td>3.6 \times 10^{-5}</td>
</tr>
<tr>
<td>D-18</td>
<td>5′-CTGACTGCTCTCAGAGATTCGTTATC-3′</td>
<td>1 \times 10^{-5}</td>
</tr>
<tr>
<td>D-17</td>
<td>5′-CTGACTGCTCTCAGAGATTCGTTATC-3′</td>
<td>2 \times 10^{-6}</td>
</tr>
<tr>
<td>D-14</td>
<td>5′-CTGACTGCTCTCAGAGATTCGTTATC-3′</td>
<td>&lt;10^{-6}</td>
</tr>
<tr>
<td>D-6</td>
<td>5′-CTGACTGCTCTCAGAGATTCGTTATC-3′</td>
<td>&lt;10^{-6}</td>
</tr>
</tbody>
</table>

The boldface G residue is depurinated, and residues that form stem base pairs are underlined.
tected for D1(-6) or D-17, which have a potential to form only 3 or 4 bp in the stem (one of them mismatched). Thus, the reduction in depurination rate correlated with the destabilization of the hairpin structure.

Formamide destabilizes base pair hydrogen bonds, reducing the $T_m$ value of duplex DNA by $\sim 0.65^\circ C$ for every 1% in the solvent (16). Because the $T_m$ value for the D-1 stem-loop is 42$^\circ C$ and a formamide concentration of 50% reduces $T_m$ by $\sim 30^\circ C$, it is not surprising that the self-catalytic depurination activity is completely lost at 22$^\circ C$.

The effect of temperature on the depurination rate is also informative. The rate of spontaneous acid-catalyzed depurination should follow the usual temperature dependence, doubling with every 10$^\circ C$ rise; the background depurination rate at 22$^\circ C$, 70% of D-1 was cleaved after 45 h of incubation, the temperature-dependence on temperature (Fig. 6). Thus, after 45 h of incubation at 22$^\circ C$, 70% of D-1 was cleaved at the specific depurination site by piperidine treatment, but only 40% was cleaved after incubation at 43$^\circ C$ (just above $T_m$, where half the molecules would be in the hairpin form), and only 16% was cleaved at 65$^\circ C$. This inverse dependence must reflect the thermal denaturation of the stem-loop structure required for the self-catalysis.

The spontaneous depurination leading to nonspecific cleavage at other sites within the same molecule is also illustrated in Fig. 6. That process accelerated with increasing temperature, so that depurination was the same (14–16%) for all of the G residues at 65$^\circ C$. Taken together, these results are consistent with a stem-loop being the catalytic intermediate for the self-catalytic depurination reaction.

The Stem-Loop Structure Is Confirmed by the Mung Bean Endonuclease Digestion Pattern. A diagram of the putative stem-loop is shown along with the 18-nt subfragment cleavage pattern obtained with single-strand-specific mung bean endonuclease in Fig. 7. Three major cutting sites are indicated in Fig. 7A, and their fragmentation pattern is shown in Fig. 7B, lanes 2 and 3. These cleavage sites surround the apurinic site localized by apurinic endonuclease (Fig. 7B, lane 4). The reason for much weaker digestion at the 3’ end of the loop, where there are two other G residues, may be their strong tendency to stack on each other and on the following 3’-base-paired A residue. Regardless, the mung bean endonuclease digestion pattern clearly differentiates between the putative loop and stem residues, thereby confirming the stem-loop structure.

Effects of Sequence Variation Within the Stem-Loop Define Requirements for the Self-Catalytic Depurination. Several 18-nt sequence variants of the stem closed by the 5’-G-T-G-G-3’ loop were investigated: an “inverted” variant [D(-2)inv] in which the stem strands were exchanged; a “perfect” variant [D(-2)p] in which the A-C mismatch was replaced by a G-C base pair; a “changed” variant [D(-2)ch] with an arbitrary stem duplex sequence; and a “disrupted” variant [D(-2)d] in which the number of potential mismatches in the stem should prevent duplex formation at room temperature. As shown in Table 3, $k_{obs}$ for the perfect stem sequence was approximately twice that of the natural sickle cell $\beta$-globin-derived sequence D(-2), suggesting that a more stable stem enhances the catalytic activity. The inverted variant showed a substantial loss of activity even though a complementary base-paired duplex structure was maintained. However, the changed oligomer, in which only the first base pair adjacent to the loop (5’-T-A-3’) and the A-C mismatch were conserved, retained full activity. Thus, this T-A base pair appears to be essential for the self-catalytic activity. Moreover, when the stem was fully complementary, just 5 bp were sufficient for full catalytic activity (D14p). Not surprisingly, the disrupted variant

![Fig. 7. The stem-loop formed by the D-2 subfragment, and its digestion with mung bean endonuclease. (A) The minimal fully active self-depurinating oligomer forms a stem-loop structure with one A-C mismatch in the stem. The dotted arrow indicates the site of G-depurination, which is then cleaved by Exo III. The solid arrows indicate phosphodiester bonds digested by the single-strand-specific mung bean endonuclease. (B) PAGE analysis of mung bean endonuclease and Exo III digests (pH 5.5, 22°C) of the minimal fully active oligomer D(-2) after labeling at the 3’ end and a 24-h incubation at 22°C to allow depurination to proceed. Lane 1, control (no added enzyme); lanes 2 and 3, mung bean nuclease digests after 1 and 5 min of incubation, respectively; lane 4, Exo III digestion at the apurinic site.](image-url)
exhibited no activity. These results define the required consensus sequence and structural elements.

**Self-Catalytic Depurination Under Physiological Conditions.** Although the results presented above were obtained at pH 5 and low salt concentration, the catalytic activity, albeit slightly reduced, was retained under essentially physiological conditions. Fig. 8 shows site-specific depurination kinetics revealed by piperidine cleavage of D(-2)ch after incubation at 37°C in 0.1 M NaCl/5 mM MgCl$_2$/20 mM Tris-HCl, pH 6.9/20% PEG 1000 to mimic intranuclear macromolecular crowding (17). Both the gel and its quantitation clearly demonstrate the occurrence of the site-specific self-catalytic depurination; its $k_{obs}$ is $7 \times 10^{-6}$ min$^{-1}$, 30 times slower than at pH 5, but still $\approx 3 \times 10^5$ faster than spontaneous background depurination. It is important to note that for the D(-2)p strand, in which the A-C$^+$ mismatch in the stem is replaced with a G-C base pair, the reduction in the depurination rate under physiological conditions is only $\approx 15$ times (data not shown), indicating that the acidic milieu required for maximum activity of the original sequence is due partially to the stabilization of the A-C$^+$ mismatch.

**Potential Depurination Sites Are Abundant in the Human Genome.** Preliminary data mining was undertaken by using a Perl search engine to find sequences in the human genome with a potential to undergo self-catalytic G-depurination. We have already identified >50,000 sites, distributed among all human chromosomes, as having a central 5'-G-T-G-G-3' (loop) sequence, an adjacent 5'-T-A-3', and a capacity to form a stem of at least four additional complementary base pairs. Two of these sequences, both 14 nt in length, have been experimentally tested so far, and each was found to possess self-depurinating activity with a $k_{obs}$ of $5 \times 10^{-4}$ min$^{-1}$ at pH 5.

**Discussion**

We have demonstrated a site-specific, self-catalytic, G-depurinating activity in a short DNA fragment of the sickle cell β-globin gene that is $\approx3 \times 10^3$ faster under physiological conditions ($\approx10^5$ faster at pH 5) than the spontaneous depurination rate (2). In this connection, the pH optimum shifts toward neutrality when the A-C$^+$ base pair of the stem is replaced by a G-C base pair.

Spontaneous depurination of DNA, a major type of endogenous DNA damage, is thought to be mediated by protonation of a G residue at N-7, with an intrinsic pKa of $\approx2.3$. Presumably, such protonation is also implicated in the self-catalyzed depurination, probably facilitated in part by intracellular macromolecular crowding known to alter acid-base equilibria (17). Because only one of the three G residues in the “catalytic loop” is depurinated, it is likely that some subtle aspect of the stem-loop structure contributes as well.

We have shown that the self-catalytic depurination rate correlates with the ability of a single-stranded DNA fragment to form a stable hairpin with a 5'-G-T-G-G-3' loop and the adjacent 5'-T-A-3' base pair of the stem. The remaining sequence requirement is only for an additional number of complementary base pairs to stabilize the loop residues stacked on the T-A base pair. With increasing stability of the stem, helix breathing must be reduced, increasing the time average over which the catalytic conformation is maintained, hence a faster rate of depurination.

The critical sequence features for this G-depurinating activity are present in numerous natural gene sequences. The activity is self-catalytic and requires no cofactors, and the consensus stem-loop structure is far smaller than the only reported site-specific G-depurinating deoxyribonuclease obtained by *in vitro* selection (18). The latter activity is contained within a complex three-stem-loop structure, requires divalent cations, and acts on a singular G residue in the three-residue loop of a stem-loops that is bound to the deoxyribonuclease by complementary base pairing. Thus, this catalyst depurinates a G residue of a deoxyribonuclease substrate, acting like a true enzyme with turnover, unlike our activity, which does not represent catalysis in the enzymatic sense because turnover is not possible.

Because the self-depurinating activity is associated with a single-stranded deoxyribonuclease, it might be viewed as biologically irrelevant. However, superhelical stress in vivo induces formation of stem-loops in duplex DNA at inverted repeats (19, 20), and stems as short as 5–7 bp easily extrude under physiological superhelical stress (21).

Many sites with a potential to form stem-loops are known to be hypermutagenic (22, 23). The ability of some stem-loop structures to depurinate a specific loop residue could explain such mutagenicity. But if the result of depurination were merely to mutate or otherwise damage DNA, then we would expect that evolution would have found a way to reduce the number of such sites (by making use of alternative codons that would not disturb encoded proteins but would disrupt stem base pairing). Because this is clearly not the case, formation of apurinic sites may serve some functional role. Thus, apurinic sites also slightly destabilize DNA duplexes (24) and increase local DNA flexibility (25), which may, for example, facilitate formation of open DNA–protein complexes and/or play a role in DNA packaging in viruses, in nucleosome formation or positioning, or in facilitating genetic recombination.

With the discovery of a site-specific, self-catalytic, G-depurinating activity intrinsic to numerous DNA sequences, we are alerted to the possibility of yet other self-catalytic activities contained within genomic DNA.

**Materials and Methods**

**Deoxyoligonucleotides.** Deoxyoligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA), purified to
homogeneity by denaturing PAGE, and recovered from gel slices by soaking overnight in 0.1 M sodium phosphate (pH 7.0). Final purification was accomplished by 100% acetonitrile elution from a C18 Sep-Pak reverse phase column (Waters) followed by spin evaporation. Oligomer concentration was then adjusted spectrophotometrically in Aldrich ACS reagent-grade water.

3'-End Labeling. 3'-End labeling was performed by using terminal deoxynucleotransferase (USB Corp.) (5 units) on ~5 pmol of purified oligomer with ~5 pmol of [α-32P]dideoxyATP (Amersham Pharmacia Biotech) (13). The labeled oligomers were purified by denaturing PAGE before use.

Kinetics of Depurination. Oligomers were incubated at 22°C in 10 mM sodium cacodylate (pH 5.0) (unless otherwise noted), and aliquots were frozen on dry ice at appropriate intervals. To reveal apurinic sites, aliquots were treated with 0.1 M piperidine for 30 min at 75°C to induce base-catalyzed backbone cleavage (12, 13). Either a trace amount of end-labeled oligomer was mixed with an unlabeled stock of appropriate concentration (12, 13). Either a trace amount of end-labeled oligomer was mixed with an unlabeled stock of appropriate concentration before incubation or the aliquots were labeled after the piperidine treatment (both methods yield the same depurination rates). Radioactivity of intact oligomer, its 3'-end cleavage product, and nonspecific cleavage at other G residues were quantitated by using a Molecular Dynamics PhosphorImager, and these data were used for the kinetic analysis. Each experiment was performed at least three times, and the kinetic data were averaged.

Electrophoresis. Denaturing PAGE analysis and oligomer purification were performed by using 16% slab gels 25 × 45 cm in 8 M urea/TBE at 1.5 kV for ~2 h.

Sequencing. To determine the depurination/cleavage position, the oligomer was sequenced by using a Sequenase 2.0 kit (USB Corp.) and 32P-5'-end-labeled 8-nt primers complementary to the 3' end of the 29-nt sickle cell β-globin gene fragment D-1.

Alkaline Phosphatase Treatment, Ligation, and 5'-End Labeling. Standard protocols were followed.

This paper is dedicated to Marianne Grunberg-Manago on the occasion of her 85th birthday. We thank those who have graciously helped us with their expertise: Moshe Pritsker and Damian Glumcher with data mining, Nicole Ford with determining the concentration dependence of depurination kinetics, and Peter Wolanin with the HPLC analyses. We are also grateful to Lucille Fresco-Cohen for a critical reading of the manuscript, to Ronald Breaker for valuable advice at the outset of this work, and to Steven Broitman for directing R.C. to the project. This work was supported by National Institutes of Health Grants HL 63888 and CA 088547.