Biochemistry

 Volume 36, Number 16 April 22, 1997

New Concepts in Biochemistry

DNA Polymerase β in Abasic Site Repair: A Structurally Conserved Helix−Hairpin−Helix Motif in Lesion Detection by Base Excision Repair Enzymes[†]

Gregory P. Mullen*[,‡] and Samuel H. Wilson[§,||]

Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06032, and Sealy Center for Molecular Science, The University of Texas Medical Branch, Galveston, Texas 77555

Received September 18, 1996; Revised Manuscript Received February 26, 1997

HhH Motif in DNA Lesion Detection and Catalysis

Mammalian DNA polymerase β (β-Pol)[1] has accessory activities for correction of abasic sites in DNA. By repairing or misrepairing DNA, β-Pol as well as other repair enzymes control genetic stability. The N-terminal (AP lyase)[2] domain of β-Pol contains a motif termed a helix−hairpin−helix (HhH) that contributes to recognition and excision of damaged nucleotides in DNA. Although the structure of the HhH motif in endonuclease III was determined some years back (Kuo et al., 1992), it was not until recently that this motif came to the forefront of structural biology (Seeberg et al., 1995; Thayer et al., 1995). The β-Pol N-terminal domain is the simplest of the HhH enzymes that recognize DNA damage. The structure is formed by a pair of antiparallel helices that cross to expose the connections between the pairs of helices for a bifurcated contact with DNA (Liu et al., 1994, 1996). Helix-3−turn−helix-4 forms the HhH motif. Comparative structure-function analysis of the β-Pol N-terminal domain and endonuclease III offers an explanation for HhH detection of DNA damage. We propose a mechanism for lesion detection and catalysis that encompasses Watson−Crick base pair checking and nucleophilic attack by the lysine-HhH at C1′ in the minor groove for damaged nucleotides.

Model for Base Pair Checking. Protection from Attack. In Watson−Crick base pair checking, contact by the protonated lysine-HhH with the correct conformation of the nucleotide results in protection from nucleophilic attack (Figure 1A). For this interaction, the lysine is positioned in the minor groove. The lysine ϵ-NH_3^+ forms an H-bond to O2 of a pyrimidine (or N3 in a purine) along one strand of the DNA duplex. The H-bond is formed for a nucleotide in a standard B-form conformation ($\chi \cong 80°$). After confirmation of the proper nucleotide conformation, the HhH lysine moves to the next nucleotide along the DNA strand. The interaction is analogous to an arginine side chain contact in the minor groove made by the thumb domain of β-Pol in polymerase catalysis (Beard et al., 1996). For a nucleotide in a B-like conformation, the van der Waals radii of the O2 and H1′ atoms protect C1′ from nucleophilic attack. The lysine ϵ-NH_3^+ when in an H-bond with the O2 atom is outside van der Waals contact with C1′. The protonated form

[†] This research was supported by Grants GM52738 (G.P.M.) and ES063839 (S.H.W.) from the National Institutes of Health.

* To whom correspondence should be addressed.
[‡] University of Connecticut Health Center.
[§] The University of Texas Medical Branch.
[||] Present address: National Institutes for Environmental Health Sciences, P.O. Box 12233, Research Triangle, NC 27709.

[1] Abbreviations: HhH, helix−hairpin−helix; dRPase, deoxyribose phosphodiesterase; β-Pol, DNA polymerase β; AP, apurinic/apyrimidinic; H-bond, hydrogen bond; rmsd, root mean square deviation.

[2] An AP lyase cleaves at an apurinic/apyrimidinic site and generates an $\alpha\beta$-unsaturated product. The β-elimination cleavage at a preincised AP site containing a cleaved 5′-phosphodiester bond, also termed deoxyribose 5′-phosphodiesterase activity, liberates the $\alpha\beta$-unsaturated deoxyribose 5′-phosphate group (4-hydroxy-2,3-pentenal 5-phosphate) and DNA containing a 5′-phosphate. The β-elimination cleavage at an intact AP site yields products with the $\alpha\beta$-unsaturated deoxyribose 5′-phosphate attached by a 5′-phosphodiester bond to the 3′-end of the DNA. A δ-elimination subsequent to β-elimination on a nonincised AP-containing DNA yields a 4-keto-2,3-pentenal and a gapped DNA containing phosphate groups at both the 3′- and 5′-ends.

S0006-2960(96)02363-X CCC: $14.00

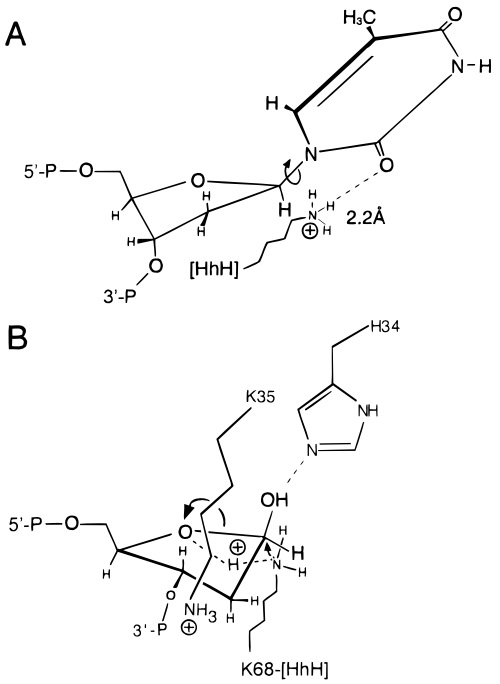

FIGURE 1: Mechanism for detection of a correct nucleotide conformation by a lysine-HhH motif and catalysis by the N-terminal domain. (A) The base checking mechanism in which the protonated lysine forms an H-bond to O2 (or N3 for purines) and is stabilized in the non-nucleophilic form. The curled arrow shows the direction of rotation about the χ torsion that could result from a modified base. (B) The arrangement of the lysine-HhH and other catalytic residues in the N-terminal domain while bound to an abasic site showing the mechanism of protonation of O4′ by the lysine concomitant with breaking of the O4′−C1′ bond. In a second step, the carbonium ion transition state species is attacked by the deprotonated lysine.

of the lysine is stabilized by the H-bond to the weakly basic carbonyl oxygen and is rendered non-nucleophilic.

Nucleophilic Attack at C1′. Figure 1B depicts the nucleophilic form of the lysine-HhH for the β-Pol N-terminal domain in the absence of an H-bond acceptor. Nucleophilic attack by the lysine-HhH is a consequence of the missing H-bond acceptor and lysine deprotonation. The absence of a base explains the accessibility of C1′ to nucleophilic attack and its reactivity. The reason for nucleophilic attack at a modified base is less obvious but can be explained by an altered conformation about the χ torsion angle. A nucleotide with a low anti conformation (e.g., $\chi \cong 10°$) will not form an H-bond with the similarly positioned lysine side chain. The direction of ring rotation for a low anti conformation is represented by the curled arrow about the glycosidic bond and results in O2 moving toward the viewer and away from the lysine (Figure 1B). This conformation would allow the lysine ϵ-NH_2 to contact the exposed C1′ in a nucleophilic attack. Deprotonation of the lysine can occur through transfer of the proton to O4′ prior to nucleophilic attack (Figure 1B). Protonation of O4′ allows development of a carbonium ion transition state at C1′ stabilized by electron donation from the 1′-OH (i.e., a protonated aldehyde species). The same type of transition state is possible when the 1′-OH is a damaged base. Alternatively, for S_N2 displacement of the base, either general base or bound water abstraction of the lysine proton could be operative.

Structural docking of the β-Pol HhH with DNA indicates that the H34 and K35 side chains can contribute to catalysis

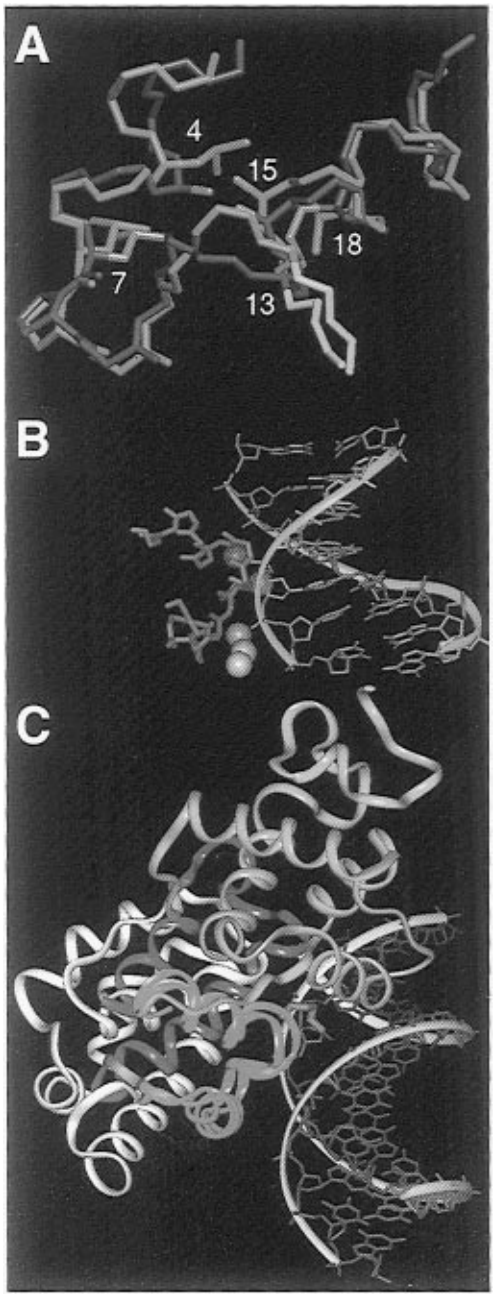

FIGURE 2: (A) Superimposition of the HhH structures in endonuclease III (green) and in the N-terminal domain (red) shown with the hydrophobic side chains (magenta) at positions 4, 15, and 18 (making contacts between the helices) and the leucine side chain at position 7 packing outside of the motif. The carbonyl oxygen at position 7 H-bonds in the type II (hairpin) turn. Shown is the conserved lysine side chain at position 13 (blue). (B) The nonspecific fingers HhH−DNA interaction in the β-Pol ternary complex showing water (or metal ions) at the DNA fingers interface (Pelletier et al., 1994). (C) A modeled interaction of the superimposed HhH motifs (green) of endonuclease III and the N-terminal domain with B-form DNA (orange) shown with an abasic site (yellow) and the lysine (blue) at position 13 approaching C1′. Overlaid ribbons for residues 14−52 (red) of the N-terminal domain and 23−104 (gray) of the HhH domain of endonuclease III are shown with 1−22 and 131−211 of the Fe_4S_4 domain in endonuclease III in blue.

as shown in Figure 1B. Interaction of H34 in the major groove is feasible with the imidazole ring fitting to the abasic site hole in the base stack of the DNA. The imidazole ring thereby stabilizes the carbonium ion intermediate formed on cleavage of the hemiacetal through H-bonding to the 1′-OH.

In displacement of the 1′-OH group to form the Schiff base, H34 would act as a general acid catalyst. During deprotonation of C2′ in the β-elimination, H34 would act as a general base. The D138 group in endonuclease III likely provides similar roles. In the β-Pol N-terminal domain, K35 is optimally positioned for stabilization and protonation of the leaving phosphate group from C3′ during the β-elimination.

Biochemical and Structural Results That Support the Mechanism

Structural Aspects of Activity and DNA Binding. The N-terminal domain functions as a dRPase catalyzing a β-elimination reaction at AP sites in single-stranded DNA and at preincised AP sites in double-stranded DNA (Matsumoto & Kim, 1995). The N-terminal domain also functions as an AP lyase catalyzing β-elimination on intact AP sites in double-stranded DNA. Experiments are necessary to determine whether β-Pol or another enzyme processes the $\alpha\beta$-unsaturated product at the 3′-end of the DNA. Together with its well-characterized nucleotidyl transferase activity (Abbotts et al., 1988), β-Pol functions in repairing an abasic site in DNA to a corresponding nicked site. Structural conservation of the lysine-HhH in two different enzymes sharing activity is an important finding, since structure can correlate with function. In fact, the HhH in the N-terminal domain is nearly identical to the HhH in endonuclease III (Figure 2A), an enzyme with *N*-glycosylase activity for damaged bases and AP lyase activity at intact AP sites. Structures of DNA complexes of β-Pol that utilize the HhH motif have been determined (Figure 2B) (Pelletier et al., 1994, 1996). These β-Pol structures represent the only presently determined HhH−DNA interactions. Nonspecific contacts were found at the phosphate backbone for the HhH in the β-Pol fingers domain (Pelletier et al., 1994) and for the HhH in the β-Pol N-terminal domain (Pelletier et al., 1996). In the DNA complex, there were phosphate contacts with amides at the amino terminus of the second HhH helix and with carbonyl−metal or carbonyl−water molecules at the C terminus of the first HhH helix (Figure 2B). The N-terminal domain did not make contacts like those we propose here. The N-terminal domain−DNA interaction in the crystal is nonphysiological and results from two β-Pol protein molecules contacting one DNA duplex. The Ω-loop did not interact with the DNA due to the nature of the complex and the short duplex. NMR analysis has identified the HhH and an Ω-loop adjacent to helix-2 in the N-terminal domain as the major structural determinants in DNA interaction. Cross-linking studies (Prasad et al., 1993) indicate that the Ω-loop of the N-terminal domain makes contacts with single-stranded DNA with cross-links found to S30 and H34. Endonuclease III protects the DNA backbone from DNAse cleavage across the minor groove at positions adjacent to a reduced abasic site but does not protect bases from methylation in the major groove (O'Handley et al., 1995).

Schiff Base Formation. Endonuclease III and the β-Pol N-terminal domain form a Schiff base with AP containing DNA sites (Hilbert et al., 1996; Piersen et al., 1996). Site-directed mutagenesis of the conserved HhH-lysine (K120) in endonuclease III, corresponding to K68 in the β-Pol N-terminal domain, results in loss of catalytic activity and a reduction in k_{cat} from 10^5 to 10^1 s^{-1}, while the K_M was affected only 4-fold (Thayer et al., 1995). A mammalian homologue of endonuclease III (Hilbert et al., 1996) and a UV endonuclease (also containing a lysine-HhH sequence) (Piersen et al., 1995) form an imino intermediate (Schiff base) with abasic site DNA. A K72A mutant of the N-terminal domain of β-Pol forms a Schiff base, thereby ruling out K72 as the nucleophilic residue (Piersen et al., 1996). The conservation of the lysine-HhH and the loss in activity for mutation of K120 in the HhH of endonuclease III are strongly supportive of this residue forming the Schiff base intermediate.

Flexibility in Catalytic Residues. There are major differences between the NMR solution structure and the X-ray crystal structure of the N-terminal domain of β-Pol in regions that are important for DNA binding and catalysis. The catalytic residues (K68, H34, and K35) are in flexible loops so that they can contact an abasic site in DNA optimally. Excluding flexible segments, the rmsd between the solution and crystal structure is 1.6 Å for the backbone atoms of the four helices (defined by NMR) and the 62−65 reverse turn. T67 and K68 in the helix−hairpin−helix are not helical in solution (unlike in the crystal) and can readily penetrate into the minor groove. The Ω-loop (27−35) found between helix-1 and helix-2 in the NMR structure is absent from the X-ray structure with portions of it being fit as helical in the X-ray model. The backbone shows significant dynamic flexibility (M. W. Maciejewski, D.-J. Liu, and G. P. Mullen, unpublished) in the segment connecting the helices in the HhH and in residues near the connection of the Ω-loop with a somewhat bent helix-2. Similarly, in endonuclease III, flexibility in the HhH is suggested by higher temperature factors in the turn in comparison to the adjoining helices in a crystal structure refined at 1.85 Å (Thayer et al., 1995). A common feature among DNA binding proteins that utilize basic residues in the minor groove is flexibility in the backbone for the residue involved.

Structural Details of Our Model. We docked the HhH and the Ω-loop of the β-Pol N-terminal domain and the superimposed HhH of endonuclease III against B-form DNA (Figure 2C). The superimposed protein structures reduce dramatically the types of DNA contacts that could be accommodated. Since the HhH and the Ω-loop are spaced for minor/major groove contact, two docking models were initially considered. Only the model presented with the HhH at the minor groove and the Ω-loop in the major groove was consistent with the superimposed structures and the structural requirements for catalysis. This complex is distinct from the nonspecific complex determined by crystallography. The model had no bad contacts and could be substantially energy minimized and more tightly docked by changes in flexible side chains in the proteins. Since this interaction is different from that of the nonspecific complex, it is important to establish precedence for more than one mode of DNA interaction with a DNA duplex by a helical motif in a DNA polymerase. Indeed, this is found in a Taq DNA polymerase−DNA complex and a Klenow fragment−DNA complex, where a homologous helix was positioned on the DNA dramatically differently in the complexes corresponding to the editing mode or the polymerase mode of binding (Eom et al., 1996).

The following considerations were used in arriving at the catalytically relevant model complex. The C1′ of the abasic nucleotide should be near the conserved lysine. In endonuclease III, the strand to be cleaved in the β-elimination should also be near D138. D138 is a catalytic residue (likely

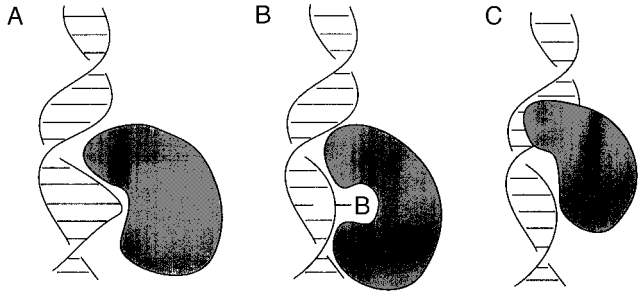

FIGURE 3: Schematic depiction of the types of interactions that could be used by DNA repair enzymes. (A) Recognition of a phosphate backbone distortion. (B) Base flipping. (C) Minor groove interaction.

a general base in the deprotonation of C2′) as determined by mutation (Thayer et al., 1995). H34 in the β-Pol N-terminal domain is in a spatial position similar to that of D138 on superimposition of the HhH motifs. For endonuclease III, nucleophilic attack by the lysine ϵ-NH_2 at C1′ is sterically accessible from the H1′ side of the deoxyribose ring, which is exposed in the minor groove. C1′ is covered by the base in the major groove and is least accessible to nucleophilic attack from the major groove. For endonuclease III, displacement can occur with the damaged base leaving from the major groove.

Comparison to MutY and the 3-MeA DNA Glycosylase II. The activity of MutY can be explained with the Ser-OH performing the nucleophilic displacement of the unmodified adenine paired to 8-oxo-G, G, or C (Michaels et al., 1992). In an 8-oxo-G·A mismatch, A is in a low anti conformation ($\chi = 30°$), while the 8-oxo-G is syn with the C1′ position protected from nucleophilic attack by the 8-oxo group of the syn G conformation (Kouchakdjian et al., 1991; McAuley-Hecht et al., 1994). For the 3-MeA DNA glycosylase II, the Trp (position 13) indole NH could perform the H-bonding function. For this HhH motif, water is used as the nucleophile (since Trp replaces Lys) and the enzyme shows no AP lyase activity (Labahn et al., 1996).

Alternative Mechanisms for Lesion Detection and Damage Removal

Phosphate Backbone Distortion, Base Flipping, and Minor Groove Binding. DNA repair enzymes have been shown to recognize DNA damage through interaction with distortions in the phosphate backbone, through base flipping, and through interaction in the minor groove (Figure 3). Unlike the depiction in Figure 3, these types of DNA interactions are not necessarily mutually exclusive, and two or all three of these interactions may act cooperatively in damage recognition. The structure of the uracil DNA glycosylase complexed to DNA containing a uracil-cleaved G·U mismatch (Slupphaug et al., 1996) and the structure of the T4 endonuclease V complexed to DNA containing a thymine dimer (Vassylyev et al., 1995) show that base flipping and interaction with a distortion in the phosphate backbone are important in damage recognition. In the base flipping mechanism, a damaged base is directly detected by interaction in a specificity pocket on the enzyme (Figure 3B). Others have recognized that base flipping alone is not an adequate initial recognition mechanism (Cheng & Blumenthal, 1996). It is known that, for DNA structures containing a base modification or mismatch (Kalnick et al., 1989a,b; Fagan et al., 1996; Kouchakdjian et al., 1991), the modified bases are generally not extrahelical (or "flipped out"). This has led to the conclusion that initial lesion detection might occur through recognition of a distortion in the phosphate backbone (Cheng & Blumenthal, 1996). For the uracil DNA glycosylase, a non-HhH enzyme, the base was pushed out of the duplex by interaction of an arginine side chain in the minor groove (Slupphaug et al., 1996).

Nonspecific Contacts by an HhH Are Not Sufficient for Catalysis. Doherty et al. (1996) describe the HhH as a non-sequence-specific DNA recognition motif that binds to the phosphate backbone. Nonspecific contacts alone are not sufficient for lesion detection. However, recognition of a phosphate distortion could be important in lesion detection in an initial step of HhH−DNA interaction. It has been suggested that the grouping of three lysines (K35, K68, and K72) at the nonspecific HhH−DNA interface explains catalysis at abasic sites by the N-terminal domain (Pelletier et al., 1996). The lysines that bind the 5′-phosphate terminus of the duplex were thought to mimic the abasic site binding to 5′-deoxyribose (or alternatively mimics the binding to the 5′-phosphate−DNA product). In this mechanism, K68 is described as interacting with the phosphodiester bond to be cleaved and as forming the Schiff base. There are issues with the structural mechanism. First, and most importantly, in the nonspecific complex, K68 is not positioned for catalysis. Second, the dual roles seem unlikely since K68 would contact the leaving phosphate group in the β-elimination at the same time that it would be covalently attached to C1′ via a double bond.

Proposals for Base Flipping by HhH Enzymes. Base flipping has been proposed for endonuclease III and for the HhH-containing 3-MeA DNA glycosylase. A hydrophilic pocket at the interface of the HhH domain and the Fe_4S_4 domain in endonuclease III was proposed to accommodate a flipped out base (Thayer et al., 1995). A hydrophobic pocket at the interface of the HhH domain and a second domain in the 3-MeA DNA glycosylase II is lined by multiple aromatic residues and was suggested to be well-suited to binding a positively charged aromatic ring (Labahn et al., 1996).

Base Pair Checking, a More Efficient Mechanism. Base pair checking is a reasonable mechanism because the bases and the base modification are within the DNA duplex. The repair enzyme−DNA interaction should efficiently detect DNA damage. A facile method for checking for damage is scanning the stacked base pairs in either the major or the minor groove. The minor groove is the most repetitious in terms of H-bond acceptors along the base pair stack and therefore offers the repair protein a pattern to read. Wobble base pairs, as found in the X-ray structures of DNA duplexes containing mismatches, are examples of asymmetry that is recognized by repair proteins. The more asymmetric mismatches are recognized more readily (Hunter et al., 1986). Base flipping is an inefficient process and would not be a likely first detection mechanism. Base flipping requires substantial binding energy and distortion of the duplex. For endonuclease III, the mechanism that we propose does not require that each base be flipped but does not exclude base flipping at a later step. Base pair checking allows the repair protein to readily diffuse along the minor groove of the duplex, detect and bypass correct nucleotides, and proceed with steps leading to catalysis in the case of DNA damage.

For the N-terminal domain, this could entail insertion of the H34 ring into the abasic slot in the duplex. For endonuclease III, this may involve base flipping for damage verification.

Future Experimental Approaches

We have proposed a mechanism whereby a protonated lysine that is conserved in the HhH motif is inserted into the minor groove and is stabilized in the protonated form when detecting the correct conformation of a nucleotide through an H-bond to O2 or N3 of the base in a Watson−Crick base pair. Structures of DNA complexes containing a Schiff base intermediate, while difficult to obtain for lysine-HhH enzymes, would provide valuable information on the catalytic mechanism. Structures of HhH repair proteins complexed to undamaged or base-altered DNA, and DNA with an abasic-like site will help answer the question of lysine interaction and H-bonding to bases in the minor groove. Experiments with DNA containing multiple sites of damage could provide clues into sliding versus distributive repair for a repair enzyme or a repair enzyme complex. We hope that the concept of minor groove Watson−Crick base pair checking will stimulate experiments and scientific debate on the mechanisms of DNA repair.

REFERENCES

Abbotts, J., SenGupta, D. N., Zmudzka, B., Widen, S. G., Notario, V., & Wilson, S. H. (1988) *Biochemistry 27*, 901−909.

Beard, W. A., Osheroff, W. P., Prasad, R., Sawaya, M. R., Jaju, M., Wood, T. G., Kraut, J., Kunkel, T. A., & Wilson, S. H. (1996) *J. Biol. Chem. 271*, 12141−12144.

Cheng, X., & Blumenthal, R. M. (1996) *Structure 4*, 639−645.

Doherty, A. J., Serpell, L. C., & Ponting, C. P. (1996) *Nucleic Acids Res. 24*, 2488−2497.

Eom, S. H., Wang, J., & Steitz, T. A. (1996) *Nature 382*, 278−281.

Fagan, P. A., Fàbrega, C., Eritja, R., Goodman, M. F., & Wemmer, D. E. (1996) *Biochemistry 35*, 4026−4033.

Hilbert, T. P., Boorstein, R. J., Kung, H. C., Bolton, P. H., Xing, D., Cunningham, R. P., & Teebor, G. W. (1996) *Biochemistry 35*, 2505−2511.

Hunter, W. N., Brown, T., Anand, N. N., & Kennard, O. (1986) *Nature 320*, 552−555.

Kalnick, M. W., Li, B. F. L., Swann, P. F., & Patel, D. J. (1989a) *Biochemistry 28*, 6170−6181.

Kalnick, M. W., Li, B. F. L., Swann, P. F., & Patel, D. J. (1989b) *Biochemistry 28*, 6182−6192.

Kouchakdjian, M., Bodepudi, V., Shibutani, S., Eisenberg, M., Johnson, F., Grollman, A. P., & Patel, D. J. (1991) *Biochemistry 30*, 1403−1412.

Kuo, C.-F., McRee, D. E., Fisher, C. L., O'Handley, S. F., Cunningham, R. P., & Tainer, J. A. (1992) *Science 258*, 434−440.

Labahn, J., Schärer, O. D., Long, A., Ezaz-Nikpay, K., Verdine, G. L., & Ellenberger, T. E. (1996) *Cell 86*, 321−329.

Liu, D.-J., DeRose, E. F., Prasad, R., Wilson, S. H., & Mullen, G. P. (1994) *Biochemistry 33*, 9537−9545.

Liu, D.-J., DeRose, E. F., Prasad, R., Wilson, S. H., & Mullen, G. P. (1996) *Biochemistry 35*, 6188−6200.

Matsumoto, Y., & Kim, K. (1995) *Science 269*, 699−702.

McAuley-Hecht, K. E., Leonard, G. A., Gibson, N. J., Thomson, J. B., Watson, W. P., Hunter, W. N., & Brown, T. (1994) *Biochemistry 33*, 10266−10270.

Michaels, M. L., Tchou, J., & Grollman, A. P. (1992) *Biochemistry 31*, 10965−10968.

O'Handley, S., Scholes, C. P., & Cunningham, R. P. (1995) *Biochemistry 34*, 2528−2536.

Pelletier, H., Sawaya, M. R., Kumar, A., Wilson, S. H., & Kraut, J. (1994) *Science 264*, 1891−1903.

Pelletier, H., Sawaya, M. R., Wolfle, W., Wilson, S. H., & Kraut, J. (1996) *Biochemistry 35*, 12742−12761.

Piersen, C. E., Prince, M. A., Augustine, M. L., Dodson, M. L., & Lloyd, R. S. (1995) *J. Biol. Chem. 270*, 23475−23484.

Piersen, C. E., Prasad, R., Wilson, S. H., & Lloyd, R. S. (1996) *J. Biol. Chem. 271*, 17811−17815.

Prasad, R., Kumar, A., Widen, S. G., Casas-Finet, J. R., & Wilson, S. H. (1993) *J. Biol. Chem. 268*, 22746−22755.

Seeberg, E., Eide, L., & Bjørås, M. (1995) *Trends Biochem. Sci. 20*, 391−397.

Slupphaug, G., Mol, C. D., Kavli, B., Arvai, A. S., Krokan, H. E., & Tainer, J. A. (1996) *Nature 384*, 87−92.

Thayer, M. M., Ahern, H., Xing, D., Cunningham, R. P., & Tainer, J. A. (1995) *EMBO J. 14*, 4108−4120.

Vassylyev, D. G., Kashiwagi, T., Mikami, Y., Ariyoshi, M., Iwai, S., Ohtsuka, E., & Morikawa, K. (1995) *Cell 83*, 773−782.

BI962363A