Detection of Damaged DNA Bases by DNA Glycosylase Enzymes[†]

Joshua I. Friedman and James T. Stivers*

*Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, WBSB 314,
725 North Wolfe Street, Baltimore, Maryland 21205*

ABSTRACT: A fundamental and shared process in all forms of life is the use of DNA glycosylase enzymes to excise rare damaged bases from genomic DNA. Without such enzymes, the highly ordered primary sequences of genes would rapidly deteriorate. Recent structural and biophysical studies are beginning to reveal a fascinating multistep mechanism for damaged base detection that begins with short-range sliding of the glycosylase along the DNA chain in a distinct conformation we call the search complex (SC). Sliding is frequently punctuated by the formation of a transient "interrogation" complex (IC) where the enzyme extrahelically inspects both normal and damaged bases in an exosite pocket that is distant from the active site. When normal bases are presented in the exosite, the IC rapidly collapses back to the SC, while a damaged base will efficiently partition forward into the active site to form the catalytically competent excision complex (EC). Here we review the unique problems associated with enzymatic detection of rare damaged DNA bases in the genome and emphasize how each complex must have specific dynamic properties that are tuned to optimize the rate and efficiency of damage site location.

The problem of enzymatic detection of a single damaged base in the context of a vast genome of nearly isomorphous undamaged bases has intrigued the DNA repair community virtually since the discovery of the DNA base excision repair pathway (*1*). The initiating step in this pathway begins with the enzymatic hydrolysis of the glycosidic bond that attaches the damaged base to the deoxyribose phosphate DNA backbone, setting the stage for the multistep base excision repair process to begin (Figure 1). The cellular sentinels at this first step are the remarkable DNA glycosylase enzymes (*2*). Although these enzymes fall into different structural classes, and each is specialized for the detection and removal of different types of damaged bases (Table 1), with the sole exception of pyrimidine dimer DNA glycosylase (*3*), these enzymes have converged on a single mechanistic solution for damaged base recognition and excision: rotation of the damaged base from the DNA base stack into a sequestered active site pocket where chemistry occurs (Figure 2). This process has been called either base or nucleotide "flipping" by various investigators (*4, 5*) and connects the damage encounter event with the catalytic step of bond scission.

Base flipping involves one of the most extended and improbable reaction trajectories in biology. The overall reaction is driven forward solely by the use of enzyme binding energy for DNA, which is used to pay for the significant energetic costs of extracting a base from the DNA base stack (*11*). These costs include breaking of Watson−Crick hydrogen bonds, the disruption of aromatic stacking interactions with adjacent bases, and large perturbations in the phosphate torsion angles around the flipped base. Initial structural investigations into enzymatic base flipping focused on elucidating the final extrahelical state, where the damaged base was rotated fully into the enzyme active site and poised for glycosidic bond cleavage. Such structural efforts have
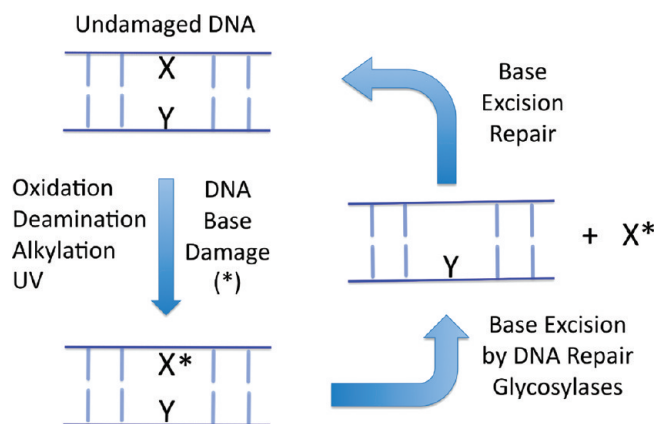
FIGURE 1: DNA base excision repair pathway that begins with the encounter of a rare genomic damaged base (X*) by a damage specific DNA glycosylase. These enzymes hydrolytically excise the damaged base and begin the extended process of replacing it with the original undamaged base (X).

been extraordinarily successful, and coordinates for more than 21 enzyme−DNA complexes have been deposited in the Protein Data Bank (PDB) (Table 1). Notwithstanding these successes, a mechanistic understanding of the extended reaction coordinate for base flipping has not been forthcoming via the documentation of interactions present in structural snapshots of the reaction end point. This realization has been the driving force for the development of new structural and biophysical methods capable of elucidating transient intermediates that occur very early in the pathway and for understanding the important mechanism of diffusional encounter with a damage site. These intriguing aspects of damage detection, and the mechanistic insights provided by these new approaches, are the primary focus of this review.

Here we highlight recent studies that are beginning to reveal the basic mechanistic elements in damaged base detection by several DNA glycosylases. We first discuss the fundamental physical problems associated with locating rare damaged bases in DNA so

Table 1: Summary of Substrate Specificities, Structural Characterizations with Bound DNA, and Kinetic Studies of Representative DNA Repair Glycosylases

enzyme	primary substrates[a]	enzyme fold family	IC structure[b]	EC structure[b]	transition state structure	rapid kinetic studies[c]	facilitated diffusion studies[c]
UNG	**U**/A, **U**/G, ss**U**	UNG	2OXM (6)	1EMH (7)	1Q3F (8, 9)	(10−13)	(14−17)
SMUG	**U**/G, ss**U**	UNG	1OE4[d] (18)	1OE5[d] (18)			
TDG	**T**/G, **U**/G	UNG	2RBA[e] (19)	2RBA[e] (19)			
hOGG1	°**G**/C	HhH	2I5W, 1YQK (20, 21)	1EBM (22)		23, 24	25
MutM	°**G**/C	MutM	2F5O, 3GO8, 3JR4 (26, 27)	1R2Y (28)		29, 30	25
MutY	**A**/°G	HhH		3G0Q (31)	3FSP (32)	33	34
AlkA	**m^{3}A**, **m^{2}T**, **m^{2}C**	HhH			3D4V (35)		
AAG	**hyX**, **m^{3}A**, **εA**	AAG		1F6O (36)	1BNK (37)		38
TAG	**m^{3}A**, **m^{3}G**	HhH		2OFI (39)			
EndoIII	**Tg**, **urea**, **ho^{5}C**, **hU**	HhH		1P59[f] (40)			
pyrimidine dimer DNA glycosylase	**T < > T** photodimers	T4 pyrimidine dimer glycosylase		1VAS, 2FCC			75

[a]The base that is excised is shown in bold, and the opposing base is also shown if this base is also important in recognition. [b]IC, interrogation complex; EC, excision complex. [c]Studies are listed that focus on the kinetics of base flipping and facilitated diffusion on DNA. [d]The IC is with partially extrahelical uracil, and the EC is approximated by a product complex with an abasic DNA mimic and uracil. [e]The EC and IC structures were observed simultaneously in the same crystal. The EC is approximated with an abasic product mimic. [f]Trapped Schiff base covalent intermediate.
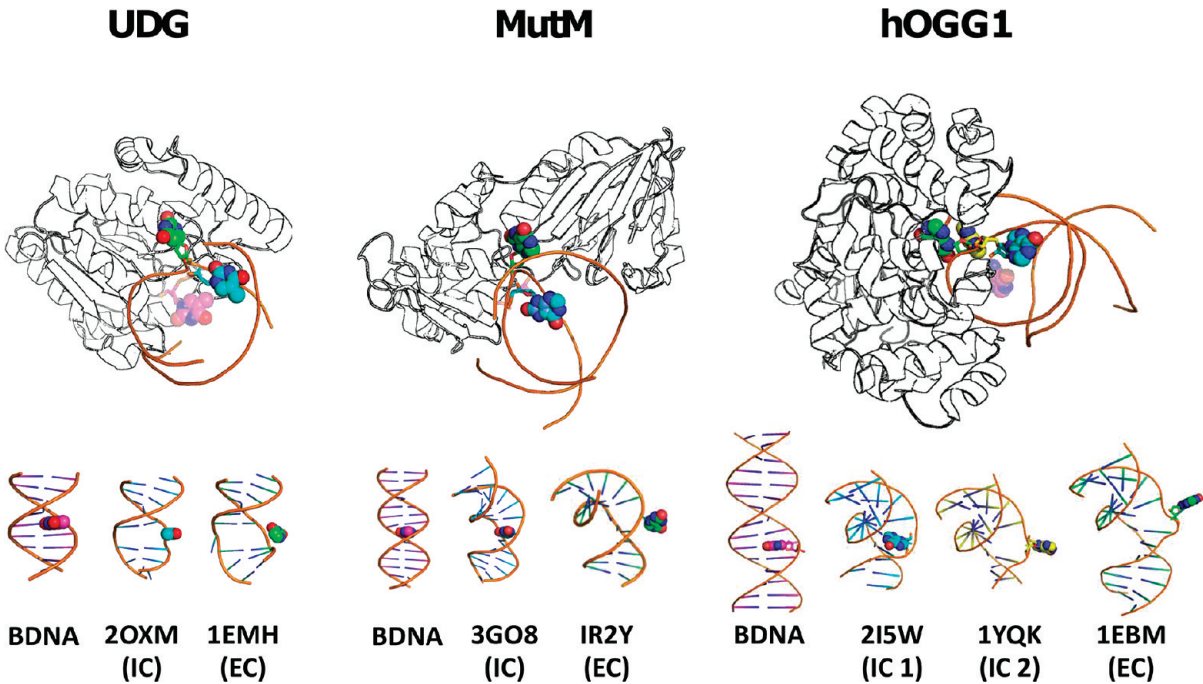

FIGURE 2: Extended reaction coordinate for extrahelical recognition of undamaged and damaged bases by uracil DNA glycosylase (UNG) and the bacterial and human 8-oxoguanine DNA glycosylases (MutM and hOGG1, respectively). Each of these enzymes excises its cognate damaged base through a multistep base flipping reaction coordinate beginning with one or more early interrogation complexes (ICs) where normal and damaged bases are inspected and ending with a fully extrahelical excision complex (EC) poised for glycosidic bond cleavage of the damaged base. The trajectory for base flipping is shown in two views: down the helical axis (top) and from an orthogonal perspective in which the enzyme has been removed for the sake of clarity (bottom).

that the mechanistic solutions taken by these enzymes can be placed in a contextual framework. The first and most fundamental problem is genome coverage. In other words, what mechanisms are used to ensure that these enzymes come in contact with 7 billion individual base pairs in the human genome such that an extremely rare ($\sim$1/10^6 bp) site of damage may be discovered before it permanently alters the genetic code? This question requires the consideration of various possible mechanisms for diffusional encounter and their contribution to both speed and coverage. We refer to this as the enzymatic "search" problem, and this work requires a special form of the enzyme we term the search complex [SC,[1] (Figure 3)]. A second problem is the initiating event that moves the enzyme from its search mode into an "interrogation" mode, where the enzyme can actually discern a structural or dynamic property of the damaged base that distinguishes it from a normal base. This key transition from the search complex to the interrogation complex [IC (Figure 3)] must be guided by a structural or dynamic property of the DNA or, alternatively, the enzyme. For many enzymes, this step is

---

[1]Abbreviations: SC, search complex; IC, interrogation complex; EC, excision complex.
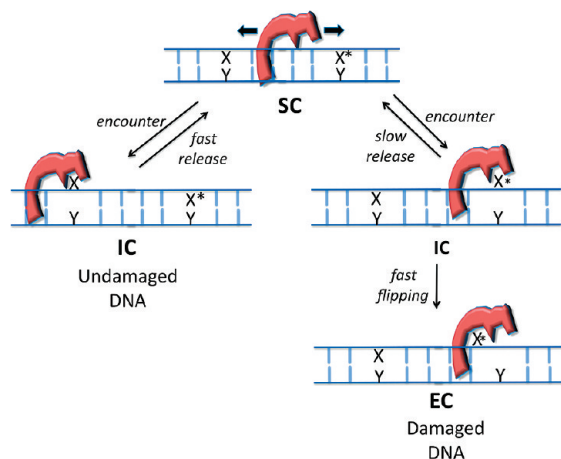
FIGURE 3: Damaged base recognition involves three discrete enzyme DNA complexes. The search complex (SC) is a loose nonspecific complex that is competent for hopping and sliding along the DNA chain. The interrogation complex (IC) is competent for extrahelical or intrahelical interrogation of damaged and undamaged basepairs. The excision complex (EC) is specific for binding of an extrahelical damaged base.

especially enigmatic because the target base lesion neither structurally perturbs the average structure of the DNA duplex nor provides a significant "handle" for detection while it is buried in the DNA base stack. Finally, the transition from an interrogation complex to a catalytically productive "excision" complex [EC (Figure 3)] involves a complex reaction coordinate for base flipping, which has been characterized in great detail for several enzymes. Our emphasis will be weighted toward three well-studied DNA glycosylases that recognize the pyrimidine base uracil [uracil DNA glycosylase (UNG)] or the oxidized purine base 8-oxoguanine [human and bacterial 8-oxoguanine DNA glycosylases (hOGG1 and MutM, respectively)]. Although we focus on three enzymes where the experimental light now shines the brightest, we will attempt to make connections to other related enzymes wherever possible.

MAGNITUDE OF THE SEARCH PROBLEM

It may be estimated that during each day of our lives $\sim 10^4$ base lesions arise in each cell of our bodies (41). Since mutations do not also arise with such alarming frequency, it must be concluded that base excision repair efficiently corrects these lesions, providing a lower limit to the efficiency by which DNA glycosylases locate these sites and initiate repair. In a genome size of $\sim 7 \times 10^9$ bp and given the relatively high abundance of DNA repair glycosylases ($\sim 10^5$ per nucleus) (42), it may be estimated that an individual glycosylase must survey ~ 70000 bp of DNA. Of course, this calculation assumes that all the base pairs of DNA are accessible to the glycosylase, which may not be true given the highly packed structure of chromatin, but it does give one measure of the magnitude of the problem.

It is of interest to ask how long the target search takes in a human nucleus. As a first approximation of the problem, one may use the Smoluchowski diffusion equation to calculate the average time for encounter of a single enzyme molecule with a target base pair in the absence of interactions with the rest of the DNA chain (eq 1) (43).

$$t_{\text{search,3D}} = V/D_3 r \qquad (1)$$

Equation 1 simply states that the search time ($t_{\text{search,3D}}$) for diffusional encounter with a damaged base pair increases in direct

proportion to the volume of solution that must be searched (in this case the nuclear volume) and decreases with increasing rates of three-dimensional diffusion (D_3) and as the target radius (r) increases (big targets are more likely to be encountered than small targets). Using reasonable values for D_3 of 10^8 nm^2/s for a typical enzyme with a diameter of 5 nm, a nuclear volume of $\sim 10^{11}$ nm^3, and a target radius of 0.34 nm for a single base pair, a search time of < 1 h may be calculated, which seems rapid enough compared to the average time for DNA replication in human cells. Although diffusion constants may be smaller in the nucleus, direct measurements indicate that this diminution is less than 10-fold (44), and the search time would still be smaller than the typical dividing time of a cell. Moreover, the time that is required for target location by simple diffusion is decreased in direct proportion to the number of enzyme molecules present in the nucleus ($\sim 10^5$), which when considered, pushes the calculated search time down to a very short time scale indeed.

However, this most simple of diffusional mechanisms becomes unsatisfactory when one considers that each enzyme molecule must locate a single damaged base pair that exists in a 70000-fold excess of nonspecific DNA binding sites. Thus, it is 70000-fold more probable that the enzyme will encounter undamaged DNA rather the target site. If no pathway existed for the transfer of the enzyme from a nonspecific site to a damage site, then undamaged DNA would serve as a potent competitive inhibitor of damage site repair. Fortunately, these enzymes have evolved a capacity to use nonspecific binding interactions with the DNA chain in a discrete search mode that allows efficient intramolecular transfer from an initial nonspecific binding site to the site of damage (45). As we further develop below, intramolecular transfer, even over short segment lengths, serves another important purpose beyond providing a pathway to the target site: the enzyme remains in contact with the DNA, which is a fundamental requirement if rare sites of damage are to be detected.

MOLECULAR SPEED LIMITS IN THE DAMAGE SEARCH

The discussion given above reveals that three-dimensional (3D) diffusion and the relative abundance of glycosylase enzymes in the nucleus are sufficeint to lead to rapid encounters with a DNA chain in the nucleus, and this is itself not a speed limit that would prevent timely repair. However, rapid encounter with single base pairs in DNA followed by dissociation to bulk solution provides exceedingly inefficient coverage because the enzyme must repeatedly diffuse to new landing sites, and it is exceedingly unlikely that a given association event will place the enzyme in perfect register with a damaged base if it is present. Thus, it would be advantageous if the enzyme could use the DNA chain as a conduit for one-dimensional (1D) sliding to the target site (Figure 4). The average length of the DNA that can be sampled in a single stochastic sliding event (l_{sl}) will depend on the 1D diffusion constant (D_1) and the lifetime ($t_{\text{bound}} = 1/k_{\text{off}}$) of the enzyme on the DNA ($l_{\text{sl}} \sim \sqrt{D_1 t_{\text{bound}}}$) (43). In the case of a search that uses sequential 3D and 1D steps, the total search time (t_{search}) to find a damage site embedded in a DNA chain of total length L will be equal to the sum of the 3D and 1D search times (eqs 2 and 3).

$$t_{\text{search}} = t_{\text{search, 3D}} + t_{\text{search, 1D}} \qquad (2)$$

$$t_{\text{search}} = V/D_3 l_{\text{sl}} + L l_{\text{sl}}/D_1 \qquad (3)$$

The implications of eq 3 may be appreciated in straightforward way. The first 3D term reflects the time needed to encounter a

$$t_{\text{search, 1D}} = Ll_{\text{sl}}/D_1$$

$$l_{\text{sl}}$$

$$t_{\text{search, 3D}} = V/D_3 l_{\text{sl}}$$

V (nm³) = volume
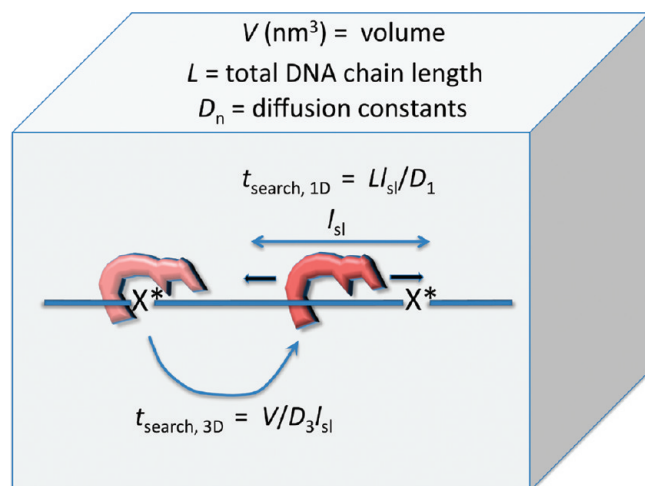L = total DNA chain length
D_n = diffusion constants

FIGURE 4: Damaged base recognition involves both three-dimensional diffusion and one-dimensional DNA sliding (see the text and eqs 2 and 3 for definitions).

target region of the DNA chain of size l_{sl} in nanometers. Note that this target size will be much larger than that of a single base pair (eq 1) and reflects the fact that if the enzyme lands within l_{sl} nanometers of a damage site it will find it by sliding (Figure 4). The second 1D term reflects the time it takes to search an entire DNA chain of length L using a 1D search velocity of D_1/l_{sl} (this 1D search requires many repeated 3D encounter events). An important aspect of t_{search} is the fact that increasing values of l_{sl} decrease the $t_{\text{search,3D}}$ term and increase the $t_{\text{search,1D}}$ term. The decreasing efficiency of 1D intramolecular transfer with increasing sliding lengths reflects the intrinsic limitation of 1D stochastic motion. That is, the probability of taking a step toward a target site is exactly the same as that for moving away from the site, resulting in the statistical outcome that n^2 steps need to be taken to move n base pairs toward a site (46). Theoretical analyses have concluded that sliding lengths in the range of 10−100 bps (3.4− 34 nm) will optimize the total search time in the human nucleus as described by eq 3 (43). In conclusion, these considerations of 1D and 3D pathways suggest that efficient damage site location would involve frequent enzyme dissociation events. These dissociation events provide the opportunity for the enzyme to reassociate nearby to its site of dissociation or, alternatively, to a distant site in the linear chain, which would be a likely outcome in the compact DNA context of the human nucleus. These new landing sites could then be interrogated using 1 bp sliding steps over a 10−100 bp total sliding length. Such a mechanism encompasses the most efficient features of the 3D and 1D processes (Figure 4).

GENERAL MECHANISTIC CONSIDERATIONS

Kinetic Aspects of the Search Process. The theoretical considerations of diffusive pathways for damage site location described above, although highly instructive, treat the enzyme as a diffusing particle and thus do not provide any mechanistic or physical basis for how an enzyme might interact with nonspecific DNA in a sliding mode or what properties of the enzyme or DNA result in engagement of a damaged base when it is encountered. Although the process of DNA sliding is poorly understood both structurally and mechanistically (47, 48), it is possible to envision the central kinetic properties that would comprise a productive sliding mode. First, the enzyme must form sequence-independent binding interactions with the DNA that are neither too tight nor too loose. The simple rationale behind this statement is that very

tight interactions lead to a lack of mobility (small D_1) and an increase in t_{search} (eq 3). Alternatively, very loose interactions decrease the lifetime ($1/k_{\text{off}}$) of the enzyme on the DNA, resulting in very short sliding lengths (l_{sl}). Second, when the damaged site is encountered, it is essential that a property of the site, or the enzyme, must somehow efficiently lead to a halt in the sliding process and shift the enzyme into the interrogation conformation where it may begin the process of specifically engaging the site. If pausing at the site did not occur efficiently, leading to forward commitment along the base flipping pathway, then sliding would be an ineffective process. A closely related and essential question is whether pausing and interrogation of normal base pairs occur during the sliding process. If such interrogation events occur (and several lines of experimental evidence suggest that they do), then these must be extraordinarily dynamic events that do not result in time-consuming and unproductive pausing at undamaged base pairs. Consideration of these essential elements of sliding leads to the conclusion that each enzyme must have evolved a balance among its nonspecific binding lifetime on the DNA chain, its rate of sliding, and the rate of base pair interrogation. Although these individual rates may differ by orders of magnitude between different enzymes (see below), they must still be tuned within a given system to allow effective recognition. Thus, the relative magnitudes of these individual rates are key unknowns in developing an understanding of the search mode for each DNA glycosylase, and any experimental measurements of these individual rates (sliding, dissociation, and the rate of recognition) must be interpreted in the context of the other rates to achieve a satisfying mechanistic model for the process.

What Does a Sliding Enzyme Look Like? What interactions between the enzyme and DNA are important for sliding? Answering this question is paramount, yet little experimental data exist to answer it. In lieu of hard measurements, models have been proposed for phosphate backbone tracking, as well as minor and major groove tracking (49, 50). Indeed, it is possible that different enzymes may employ different tracking mechanisms depending on the individual features of the damaged base that must be detected. For instance, detection of a minor groove perturbing lesion such as N3-methyladenine may be enhanced by minor groove tracking, whereas it is difficult to envision how uracil would be detected by such a mechanism. Tracking mechanisms invariably bring forth visions of these enzymes spiraling around the DNA helix as they move from one site to the next (50), but the possibility that more complicated trajectories may be followed where the enzyme diffuses or tumbles on the DNA surface (51, 15), rapidly reorientating itself with respect to the DNA strand polarity (see examples below), cannot be excluded. The most likely interactive force that would allow rapid sliding on the DNA, irrespective of DNA sequence, would be the comparatively weak electrostatic force between the enzyme and the DNA phosphate backbone. The electrostatic force has the virtue of being active over comparatively large distances (the energy of interaction between two point charges is proportional to $1/r$, where r is their separation), which would serve to keep the enzyme in the vicinity of the DNA. In addition, the dense linear array of negatively charged phosphate atoms of the DNA backbone is ideally suited for intramolecular migration because the enzyme is always in the proximity of another set of phosphate interactions that would serve to pull it another step along the DNA chain. Electrostatically guided migration along the DNA backbone, perhaps transiently utilizing different regions of the protein surface distinct from the primary DNA binding mode,

provides a plausible mechanism for rapid intramolecular transfer and is consistent with the observation that the level of facilitated diffusion is dramatically reduced in the presence of physiological concentrations of salt in all systems that have been investigated (*52*).

Base Flipping. After the enzyme has contacted the damaged site, the reaction coordinate for base flipping begins (Figure 2). One of the most controversial aspects of base flipping is whether the enzyme uses binding energy to perturb the DNA structure and facilitate base pair opening or, alternatively, whether the dynamic properties of DNA base pairs themselves are sufficiently rapid, and along the correct trajectory, to initiate the process of base flipping (*53*). These two mechanisms have been historically labeled "active" and "passive" with respect to the role of the enzyme, but this terminology is mechanistically ambiguous because it fails to address the essential aspects of the problem. First, the term active is poorly descriptive because base flipping always involves active participation of these enzymes. This is obvious from the inspection of large structural distortions in the DNA (Figure 2), which requires the utilization of enzyme binding energy to drive the base along the flipping reaction coordinate. However, structural observations of an apparently active mechanism do not in any way address the elusive question of whether dynamic motions of the DNA served to initiate the process. The passive term is inscrutable because it also obscures the intrinsic dynamic nature of macromolecules, where thermally induced dynamic motions are often used to generate reactive conformers for a given biological process (*54*). Thus, a more useful way of framing the question is to ask whether damaged and undamaged DNA base pairs have kinetically competent motions along a coordinate that could serve to initiate the base flipping process and whether differences in the dynamics of damaged and normal base pairs give rise to specificity.

Base flipping can be separated into two reaction coordinates that are distinct yet coupled (Figure 5). The first describes the rotation of the base from the DNA base stack, which may be quantified in different ways but is usually described by a pseudo-dihedral angle defined by the base, sugar, and 3′- and 5′-phosphates of the flipped nucleotide (*55*). The second coordinate describes the extent of DNA bending, which can be quite severe (Figure 2). The act of DNA bending likely promotes the flipping process by disrupting the duplex structure and providing an unhindered path of the base out of the base stack. When intermediates during base flipping have been structurally characterized (Figure 2), various enzymes have shown different degrees of progress along each reaction coordinate, possibly reflecting different structural requirements for flipping purine and pyrimidine bases. For instance, the structure of the IC for human UNG shows little bending, yet base rotation has proceeded to 20% of the full trajectory (Figure 2). In contrast, the IC structures of hOGG1 and MutM show extreme amounts of bending before or during rotation of guanine or 8-oxoguanine from the duplex (see the discussion below).

SPECIFIC EXAMPLES

There are a handful of DNA glycosylases for which mechanistic and structural studies have reached a high level of detail. In these cases, sufficient experimental observations have been made to allow the construction of reasonable models for the search, interrogation, and excision stages of damaged base repair. These enzymes serve as paradigms for other glycosylases for which fewer experimental data are currently available. The first enzyme that will be considered below is uracil DNA glycosylase (UNG),
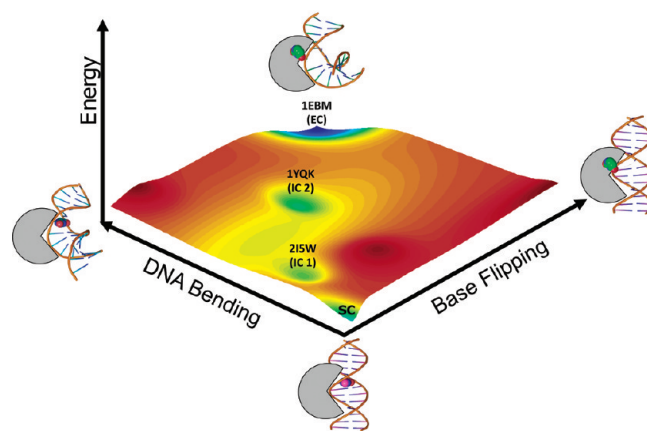

FIGURE 5: Free energy surface for enzymatic base flipping that involves a DNA bending and base rotation reaction coordinate. Different enzymes show different degrees of progression along each axis during the process of flipping (Figure 2). The progression of hOGG1 along both coordinates is qualitatively depicted in the free energy surface shown. The trajectory followed by UNG involves a greater progression along the rotation coordinate as compared to bending, while that of MutM may involve full bending before any rotation occurs (Figure 2).

which locates and excises the pyrimidine base uracil from U/G and U/A base pairs, and also from single-stranded DNA (ssDNA). The enzyme falls in its own structural class (Table 1) and is the most catalytically powerful of all the DNA repair glycosylases (*2, 56*). Unlike the other enzymes considered here, UNG acts equally well on double-stranded and single-stranded DNA substrates, and for this reason, the search and interrogation mechanisms of UNG may differ from those of other glycosylases listed in Table 1 that require duplex DNA for maximal activity. The two remaining enzymes that we consider recognize the oxidized guanine base, 8-oxoguanine (^{o}G), in the context of ^{o}G/C base pairs (*57*). Human 8-oxoguanine DNA glycosylase (hOGG1) is a representative member of the largest structural superfamily of DNA glycosylases. These enzymes are characterized by a "helix−hairpin−helix" (HhH) structural motif that is associated with their DNA binding mode, making hOGG1 an important reference point for understanding the recognition mechanism of this entire superfamily. Bacterial 8-oxoguanine DNA glycosylase (MutM) has a protein fold entirely different from that of hOGG1, providing a nice comparative example for ^{o}G detection. In each case, we try to link the experimental observations with the intrinsic problems associated with searching the genome, pausing to interrogate base pairs, and moving damaged bases along the base flipping reaction coordinate.

Case of Uracil and Uracil DNA Glycosylase (*UNG*). (*i*) *Search for Uracil*. Uracil in DNA is generated from the deamination of cytosine or through the incorporation of dUTP by DNA polymerases, resulting in U/G or U/A base pairs that are substrates for UNG, as well as uracil in ssDNA (*2*). The DNA damage search mechanism of UNG has been investigated independently by several groups (*15, 16, 58*). The most extensive study employed an approach in which two uracil sites were incorporated into a single linear DNA chain, and spacing between sites was varied over the range of 20−800 bp (*15*). Under appropriate initial rate conditions, it is possible to quantify the fraction of single-site excision events that are then followed by intramolecular translocation and excision of the second uracil site in the same DNA chain, without dissociation of the enzyme to bulk solution. The efficiency of second-site excision is termed the intramolecular transfer efficiency ($f_{intra} = T \times E$) and is determined

by the probability that intramolecular transfer between sites occurs successfully (T), and the probability that once the second site is reached, the uracil is excised before the enzyme falls off the DNA (E). By varying the spacing between the two sites, we are able to evaluate if T diminishes with an n^2 dependence on base pair (n) separation, as expected for a pure sliding mechanism (see above), or a $1/r$ dependence as expected from a hopping mechanism (where r is the distance in nanometers between the two sites) (46). Such data for *Escherichia coli* UNG at low salt concentrations clearly followed a $1/r$ dependence (15), indicating that intramolecular transfer involved predominantly hopping, rather than sliding. A key finding was that only ∼40% of UNG molecules that cleave one site ever make it to a second site located 20 bp away. The landing site size that could be extracted from these data was about one turn of the DNA helix, suggesting that when the enzyme lands within ∼10 bp of a uracil site it remains in contact with the DNA and uses short-range sliding as the final step of the search process ($l_{sl} \sim 10$ bp). By expanding the target region by a factor of 10 over that for a single base pair, UNG has greatly increased its coverage of the genome per binding event. These findings are entirely consistent with the expected elements of a highly efficient search process as discussed above (eq 3).

A very interesting finding that arose from the two-site intramolecular transfer studies of UNG was that f_{intra} was the same regardless of whether the two uracils were on the same or opposite strands of the DNA, even for site spacings as small as 20 bp (15). As originally noted by Halford and co-workers (51), when two target sites are located on opposite DNA strands, successful intramolecular transfer must require at least one microscopic "dissociation" event that allows the enzyme to reorient its binding site with respect to the DNA strand polarity. Such a dissociation event does not result in the loss of the enzyme from the vicinity of the DNA chain but is sufficient to allow rapid rotation of the enzyme molecule with respect to the DNA axis. The importance of this observation with respect to the mechanism of intramolecular transfer by UNG cannot be overstated: UNG cannot simply track along a DNA groove or the DNA backbone. If such 1D tracking occurs, it must be frequently punctuated by rotation events.

Much is known about the kinetic properties of UNG, allowing a rough reconstruction of the temporal events involved in DNA association and scanning. UNG associates with short DNA oligonucleotides with a diffusion-controlled rate constant in the range of $2-4 \times 10^8 \, M^{-1} \, s^{-1}$ ($D_3 \sim 10^8 \, nm^2 \, s^{-1}$) as determined in stopped-flow association measurements at room temperature in the presence of 10 mM NaCl (11). Thus, UNG has optimized $t_{search,3D}$ (eq 2). With respect to the 1D search time via sliding ($t_{search,1D}$, eq 2), an estimated sliding length (l_{sl}) of ∼10 bp can be obtained from the target size measured in intramolecular transfer studies of UNG discussed above. Combining this estimate with measurements of the lifetime of binding of UNG to undamaged DNA ($t_{bound} \sim 5$ ms under the same conditions) (15) and the assumption that each sliding event results in stochastic sampling of each base pair over a sliding distance of l_{sl}, we can calculate a sliding diffusion constant (D_1) of ∼$10^4 \, bp^2 \, s^{-1}$ ($D_1 = l_{sl}^2/2t_{bound}$) (43). Although these are only rough magnitude estimates, they are instructive: over its relatively short 10 bp (n) sliding distance UNG will make $n^2 = 100$ random walk steps, providing great redundancy for the search process without the time-consuming oversampling of longer DNA stretches.

(*ii*) *Dynamic Properties of the UNG Search Complex.* Human UNG is amenable to modern heteronuclear NMR studies both in its free form and while bound to DNA (6, 59).

Recent NMR studies focused on the nanosecond to millisecond time scale dynamic motions of the peptide backbone UNG and revealed that the free enzyme has very little measurable dynamics over this broad time scale range (59). In contrast, when UNG binds to an undamaged 10-mer DNA duplex, dynamic motions were induced on a millisecond and microsecond time scale, suggesting that nonspecific DNA binding loosens the structure of UNG. The regions of the enzyme that displayed enhanced dynamics mapped to the known DNA binding surface that includes a minor groove intercalating residue (Leu272) and important serines that interact with the phosphate backbone around the flipped base (60). A normal mode analysis was performed to provide a structural interpretation for these dynamic motions. These computations revealed that the lowest-energy motions available to the enzyme involved an open-to-closed structural transition that resembled the structural changes that occur when UNG binds to DNA (Figure 6). A rapid oscillation of UNG between an open form that interacts weakly with DNA and is competent for sliding and a closed form that interrogates base pairs is consistent with the dynamics of the sliding step and also the dynamics of base pair opening (see below). Thus, such dynamics may serve as the bridge that links the open search conformation to the closed interrogation conformation, allowing the enzyme to pause and inspect individual base pairs.

(*iii*) *Spontaneous Uracil Dynamics Initiates Formation of the Interrogation Complex* (*IC*). Duplex DNA is a very stable molecule held together by the synergistic effects of nucleobase π-stacking, hydrogen bonding, solvent exclusion, and steric interactions. However, on a per residue basis, those forces favoring the canonical B-form structure are relatively weak, and bases are surprisingly free to sample conformations outside the DNA helix driven solely by thermal background energy (61). These spontaneous opening events are of significant interest as they seem to mirror the early stages of the base flipping pathway utilized by DNA glycosylases and suggest that the ubiquity of the base flipping strategy may stem from exploitation of the intrinsic dynamic properties of the DNA molecule itself. It has been stated that spontaneous base pair opening events cannot serve as a viable mechanism for the initiation of the base flipping pathway because the concentration of the extrahelical conformation is vanishingly small ($K_{op} = $ [out]/[in] $< 10^{-5}$) (62). However, for a sliding enzyme, the problem is not the unfavorable equilibrium thermodynamics; the issue is entirely kinetic (63). Thus, two conditions must be satisfied for a sliding enzyme to capture a spontaneously emerging base. First, the spontaneous breathing motions must be fast enough for the bound enzyme to sample, on average, at least one opening event during its lifetime on each base pair, and second, the motions must move the base into a pose that is recognized by the interrogation conformation of the enzyme (i.e., the motion must place the base on the flipping pathway).

Do spontaneous DNA base pair dynamic motions meet these two criteria? The fastest forward kinetic step that has been detected during rapid kinetic studies of multistep enzymatic base flipping by UNG has a rate k of ∼700 s^{-1} at room temperature, and this step has been assigned to exit of the uracil base from the base stack (64, 65). Therefore, if spontaneous base pair breathing motions are involved in the initiation of the whole process, these motions must be at least as fast as this reference rate to be kinetically competent. The spontaneous opening rates (k_{op}) of T/A (30 s^{-1}) and U/A (200 s^{-1})[2] DNA base pairs in matched duplexes
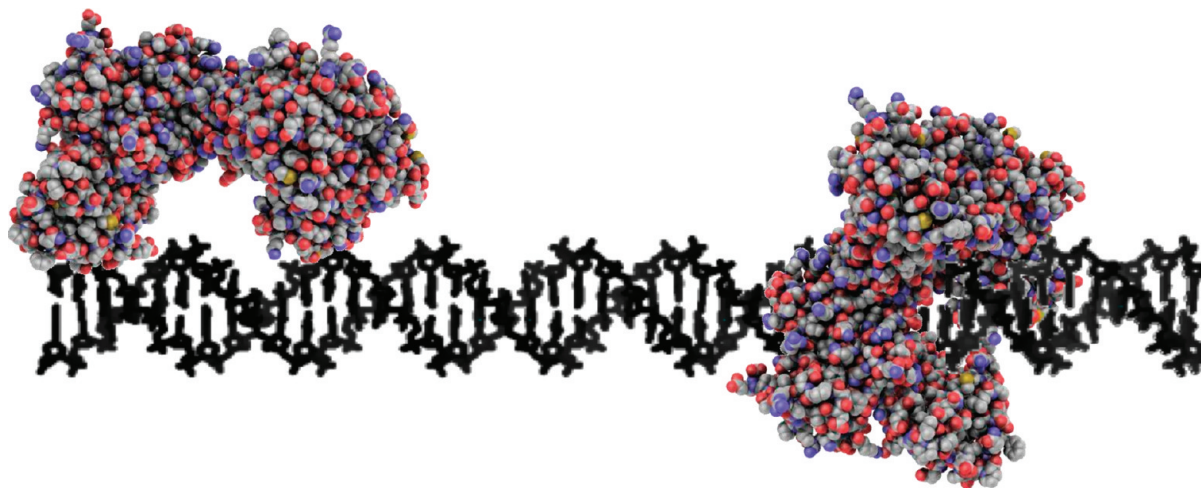

FIGURE 6: Normal mode analysis and NMR dynamic data suggest an open (left) to closed (right) transition in UNG during the interrogation of DNA for damage (see the text). The open state is envisioned as being competent for DNA sliding, and the closed conformation [PDB entry 2OXM (Figure 2)] is competent for base interrogation.

have been directly measured by NMR imino proton solvent exchange methods at 10 °C (*63*). After normalization to room temperature to match the kinetic conditions (*66*), the rates of U/A opening exceed several thousand per second and easily meet the kinetic competence requirement. The 7-fold faster rates of U/A opening compared to the rate of T/A opening also suggest that the intrinsic dynamic differences between these base pairs may facilitate specific damage recognition (U/G base pairs are expected to be even more dynamic) (*66*). Finally, the evidence that spontaneous breathing motions generate a conformer that is on the enzymatic base flipping pathway is compelling. Computer simulations indicate that imino exchange requires a ∼50° rotation of the base from the DNA stack (*67*), which results in breaking of the interstrand hydrogen bonds but only partial unstacking of the flipped base. This computational view of extrahelical DNA bases is very similar to the actual crystal structure of extrahelical T captured in the exosite of UNG (Figure 7) (*6*). We have concluded from these comparisons that spontaneous opening events also meet the criterion of generating on-pathway conformers.

Finally, if spontaneous base pair opening motions are the initiating event in recognition, it follows that the rate of these motions should not be altered by the enzyme. Indeed, comprehensive NMR studies of the opening rate of T·X base pairs in the presence and absence of UNG have shown that the enzyme does not significantly alter the opening rate (k_{op}) (*6, 63, 68*). Instead, UNG only acts to increase the open lifetime of T and U by transiently holding these bases in the exosite. The lifetime of thymine in the exosite is vanishingly small (∼0.1 ms), which is consistent with the expectation that an efficient enzyme should not spend an undue length of time interrogating normal base pairs. In this regard, UNG does not appear to spend time interrogating normal G/C base pairs because the intrinsic stability of this pair does not allow sufficiently frequent opening events for capture by the enzyme, and the exosite is not compatible with binding of C, G, or A bases (*6, 63*).

(*iv*) *Late Events on the Reaction Coordinate for Uracil Flipping*. Once base pair dynamic motions have ejected uracil or thymine, placing the Watson−Crick edge of these bases in the U and T specific exosite, the binding energy of the enzyme is used to selectively guide uracil the rest of the way into the active site. Rapid kinetic studies have revealed the presence of at least one more kinetic intermediate between the initial exosite complex and
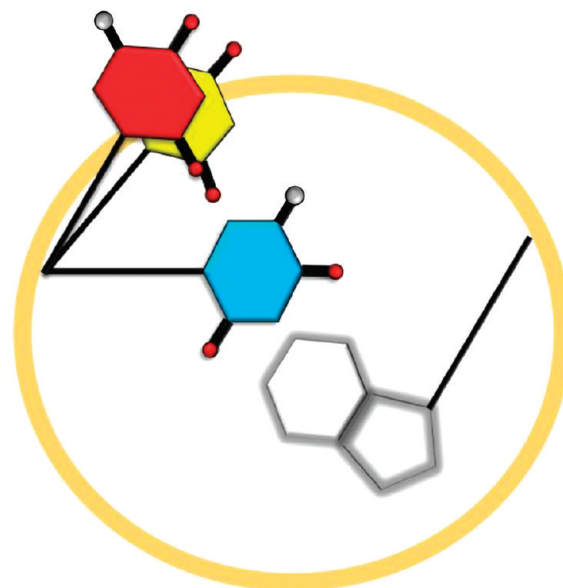

FIGURE 7: Thermally induced flipping of a thymidine base results in an open state that closely resembles the conformation of thymine when bound in the exosite of UNG (see the text). The figure is a view down the DNA helical axis and depicts the conformation of intrahelical thymine in B DNA (blue), the minimum extrahelical state of thymine that is required for imino proton exchange (yellow), and the conformation of thymine when bound to the exosite of UNG (red). To facilitate visual comparisons, the bases have been projected onto a common plane, and the syn conformation of thymine in the exosite structure is shown in the anti form for ease of comparison with the other two states. For reference, the circle depicts the circumference described by the DNA sugar phosphate backbone.

the final catalytically competent Michaelis complex (*64*). Although a structure of this intermediate has not been obtained, indirect evidence suggests that the DNA is more severely bent than in the exosite structure (*11*). The immense specificity for uracil over thymine may be achieved at this intermediate or, alternatively, at the subsequent Michaelis complex. The net effect is that thymine is excised 10^8 times slower than uracil because of the combined kinetic and thermodynamic barriers that select against this normal base. The enzymatic strategy during the late stages of base flipping is to break the process into discrete motional segments, punctuated by one or more base docking events that specifically

guide uracil down a thermodynamic gradient into the active site pocket.

Case of 8-Oxoguanine Detection. (*i*) *Search for 8-Oxo-guanine.* Oxidized guanine bases are a prevalent form of DNA damage caused by reactive oxygen species and are removed by two structurally distinct enzymes, MutM in bacteria and hOGG1 in humans (*57*). Similar to UNG, MutM and hOGG1 have diffusion-controlled association rates with duplex DNA oligonucleotides ($\sim 3 \times 10^8$ M^{-1} s^{-1}) (*23, 30*), and therefore, both enzymes have optimized $t_{search,3D}$ values. The ability of both enzymes to track along the DNA chain has been investigated using DNA constructs with two substrate sites incorporated into a single chain (see above for UNG) (*25*) and, also, by direct observation using high-speed imaging of single molecules (*50, 69*). The efficiency with which hOGG1 and MutM translocate and cleave a second 8-oxo-G·C site spaced 19 bp from another site was determined to be $\sim 0.65-0.8$ in the presence of $0-10$ mM KCl. However, these efficiencies fell to around 0.2 at a physiological concentration of salt (150 mM KCl). Although both enzymes have higher transfer efficiencies than UNG under similar conditions, the distance that is being interrogated is quite short, and it is important to realize that $\sim 80\%$ of the molecules dissociate before translocating even 19 bp at physiological salt concentrations. Thus, the picture that emerges from these findings with MutM and hOGG1 is one of rapid and efficient three-dimensional diffusion to the DNA chain, and relatively short-range sliding. These features are mechanistically similar to those of UNG.

Single-molecule imaging of hOGG1 and MutM sliding on undamaged, flow-stretched λ DNA presents a somewhat different picture compared with the ensemble measurements (*69*). First, since flow methods efficiently wash away dissociated enzyme molecules, only sliding events can be measured using this approach. [However, single-molecule hopping has been observed using nonflow conditions, as recently demonstrated with nucleotide excision repair enzymes (*70*).] At a nearly physiological salt concentration (100 mM NaCl), a mean sliding distance of 440 bp was reported for hOGG1. This mean sliding distance occurred during a mean binding lifetime of 25 ms and thus required an extremely rapid 1D diffusion constant (D_1) of 5×10^6 bp^2 s^{-1}, corresponding to a near barrierless activation energy for sliding of 0.5 kcal/mol. Similar studies with MutM revealed a 10-fold slower diffusion constant than hOGG1 ($D_1 = 4 \times 10^5$ bp^2 s^{-1}), which was rationalized with respect to the weakened requirement for scanning a bacterial genome that has 1/1000th the number of base pairs. Since 8-oxo-G-damaged sites were not present in the λ DNA, it is not known how efficiently such rapid sliding events may be paused to interrogate base pairs for damage.

A second aspect of the search mechanism of hOGG1 and MutM has been indirectly revealed by single-molecule imaging: these proteins move by spinning around the DNA helix (*50*). This interesting study varied each protein's radius (r) through conjugation of strepavidin and then measured the 1D diffusion constants (D_1) of the native and conjugated forms of the enzymes. Although the limited spatial resolution of single-molecule imaging cannot directly detect spinning of individual enzyme molecules on DNA, it can test the expected effect of a given change in molecular radius on D_1. If only linear motion along the DNA chain axis is occurring, then D_1 should show a $1/r$ dependence; if coupled rotation is also occurring, a dependence that is proportional to $1/r^3$ would be expected. The data for both hOGG1 and MutM were best fitted with the $1/r^3$ dependence on protein

radius, leading the authors to conclude that these proteins track along the major or minor groove, or the backbone of the DNA chain. It will be of interest to uncover whether this apparent spinning behavior also involves rapid molecular rotation of the enzymes with respect to the strand polarity, as required in strand transfer by UNG (see above).

(*ii*) *Transitioning to the Interrogation Complex.* A key question raised by the rapid sliding rates of both MutM and hOGG1 is how these enzymes know when to stop moving like race cars down the track. A further enigma is the fact that hOGG1 is known to dramatically kink DNA both in the absence and in the presence of an 8-oxo-G/C lesion, and the kinking appears to be required to present both G and 8-oxo-G in the exosite (*21, 22, 71*). Since it seems implausible that the kinked complex is competent for the observed rapid sliding, let alone spinning, there must be an essential dynamic transition between a loose sliding conformation of hOGG1 and the conformation in which the DNA is bent. Achievement of the MutM or hOGG1 complexes in which the DNA is bent and destabilized occurs on a time frame of < 10 ms as judged by rapid kinetic measurements (*23, 30*), and the initial nonspecific encounter complexes dissociate rapidly (bound lifetimes in the range of $1-5$ ms at 50 mM KCl) (*23, 30*). The shorter lifetimes of the encounter complexes in these ensemble kinetic measurements, as compared to those from the single-molecule studies described above, may arise from the short DNA sequences that were used (12-mers) or may reflect the fact that the single-molecule studies observe a small fraction of the binding events due to the time resolution limitations of the imaging and flow methods. Regardless, the similarities among the interrogation mechanisms of MutM, hOGG1, and UNG are abundant, with rapid dynamic motions leading to efficient interrogation of base pairs without undue pausing at undamaged sites. The most significant mechanistic difference between these 8-oxo-G detecting enzymes and UNG is that they severely bend DNA, even in the absence of damage. Thus, on the basis of these limited examples, there appears to be a divergence in the enzymatic strategy for extrahelical inspection of small pyrimidine bases such as thymine and uracil, and bulky purine bases such as G and 8-oxo-G. It is possible that the kinetic barriers for spontaneous exposure of G and 8-oxo-G are not kinetically competent for efficient recognition. [Spontaneous G/C base pair opening is typically much slower than T/A opening (*62*), but 8-oxo-G has been suggested by computation to have a lower barrier to base extrusion than G (*72*).] Alternatively, some other structural requirement for recognition may not be satisfied by a spontaneous base pair opening process, forcing these enzymes to evolve a bend and flip strategy for extrahelical recognition of G and 8-oxo-G. If this is true, it may be generally found that extrahelical recognition of purine bases follows such a strategy.

(*iii*) *Events on the Reaction Coordinate for 8-oxo-G Flipping by MutM and hOGG1.* The strategy of bending both damaged and undamaged DNA (at least in the case of hOGG1) likely comprises the earliest event in G and 8-oxo-G flipping by these enzymes. Once DNA is bent, its dynamic properties are no longer those of naked B DNA but, instead, those of the new enzyme-induced conformation. Thus, if spontaneous thermal motions of G and 8-oxo-G are important in moving these bases onto the flipping reaction coordinate, enzyme binding energy may be used to strain the DNA and lower the kinetic barrier to base flipping.

The structures of early, unstable extrahelical intermediates involving G and 8-oxo-G have been investigated by trapping enzyme–DNA complexes using the disulfide cross-linking (DXL)

method developed in the Verdine lab (*20−22*, *26−28*). In the case of MutM, the beneficial effect of early DNA bending has been recently investigated in the context of a loop mutant that is deficient in stabilizing an extrahelical base (*27*). In this context, DNA containing G or 8-oxo-G was trapped using DXL, and in both cases, the base was found to still reside within the DNA stack, even in the presence of nearly complete DNA bending. The salient structural features of the intrahelical complex with this loop mutant that may lead to facilitated base extrusion are (i) insertion of a destabilizing Phe residue into the base stack adjacent to the target base pair and (ii) conformational distortions in the DNA phosphate backbone and sugar pucker that introduce ground state strain and thereby lower the barrier to flipping out of the minor groove, which differs from the major groove pathway of UNG and hOGG (*7*). It is not clear whether this structure should be interpreted as an extremely early intermediate during the flipping process or simply the lowest-energy conformation to which the system relaxes in the absence of loop interactions that stabilize extrahelical G and 8-oxo-G. What can be inferred is that bending alone is not sufficient to drive these bases into a stable extrahelical state. As with any crystal structure, it is never possible to discern the order of events that lead to an observed conformation. Thus, even in the case of DNA bending and destabilization by MutM, it is possible that intrinsic base pair dynamics initiates the process by allowing insertion of the destabilizing Phe residue, and damage discrimination could then arise from the enhanced dynamic behavior of 8-oxo-G·C base pairs compared to that of G·C pairs (*72*).

Like UNG, both MutM and hOGG1 have taken a strategy of docking the extrahelical base in one or more transient binding sites along the reaction coordinate, and some of these intermediates have been trapped using DXL strategies (Figure 2). Kinetic studies of the 8-oxo-G flipping pathway establish that the interconversion of these intermediates is 1 or 2 orders of magnitude slower than the interconversion rates for late intermediates during UNG-facilitated base flipping (*10*, *23*, *30*). However, in all three cases, a theme emerges in which one or more discrete extrahelical intermediates form rapidly, and the last step before glycosidic bond cleavage involves a conformational change in each enzyme that results in the final docking interactions within the active site. This final step appears to involve the insertion of an amino acid plug into the DNA stack to fill the void left by the now fully extrahelical base. It is of interest to note that each intermediate that forms along the pathway to the active site can kinetically partition forward or backward because of the similar activation barriers in both directions. In the case of hOGG1 and MutM, many of these steps are quite slow ($\sim$0.01$-$1 s^{-1}), suggesting that normal bases never make it to these late intermediates, or else these enzymes would spend far too much time lingering on undamaged DNA. The slow rates for interconverting the late extrahelical intermediates for MutM and hOGG1 are appropriately tuned with the slow rate of glycosidic bond cleavage by these enzymes ($\sim$0.03 s^{-1} compared to 110 s^{-1} for UNG): if these intermediates formed and decayed much more quickly than the rate of chemistry, then the process would be extremely inefficient and unproductive.

DAMAGE REMOVAL IN THE CONTEXT OF CHROMATIN

A reasonable person can wonder what the study of enzymes interacting with short duplex DNAs in vitro has to do with DNA repair in the cell. Certainly, the work described above has relevance, but only so far as these enzymes execute repair on a naked DNA molecule. The vast majority of DNA in the human genome does not exist in this form on average, which begs the question of how efficiently these DNA glycosylases operate on DNA wrapped in nucleosomes or in the context of chromatin. Several investigations of the activity of UNG against uracil sites in the context of nucleosome-bound DNA have been reported; uracil is found to be efficiently excised in a position-dependent manner that is far from well-understood (*73*, *74*). The observation of DNA glycosylase activity in the context of nucleosomes pushes the study of DNA repair into a new realm where enzymatic damage detection may be closely coupled with the local and global dynamics of histone-bound DNA. The largely 3D hopping strategies used by these enzymes are perfectly suited for bypassing nucleosome-bound DNA when it is encountered, and for short-range scanning of transient stretches of naked DNA. However, it is now clear that when nucleosomes are encountered, the remarkable DNA glycosylases have adapted mechanisms for detecting and repairing damage in the context of this very different substrate.

REFERENCES

1. Lindahl, T. (1974) An N-Glycosidase from *Escherichia coli* that Releases Free Uracil from DNA Containing Deaminated Cytosine Residues. *Proc. Natl. Acad. Sci. U.S.A. 71*, 3649–3653.
2. Stivers, J. T., and Jiang, Y. L. (2003) A Mechanistic Perspective on the Chemistry of DNA Repair Glycosylases. *Chem. Rev. 103*, 2729–2759.
3. Vassylyev, D. G., Kashiwagi, T., Mikami, Y., Ariyoshi, M., Iwai, S., Ohtsuka, E., and Morikawa, K. (1995) Atomic Model of a Pyrimidine Dimer Excision Repair Enzyme Complexed with a DNA Substrate: Structural Basis for Damaged DNA Recognition. *Cell 83*, 773–782.
4. Slupphaug, G., Mol, C. D., Kavli, B., Arvai, A. S., Krokan, H. E., and Tainer, J. A. (1996) A Nucleotide-Flipping Mechanism from the Structure of Human Uracil-DNA Glycosylase Bound to DNA. *Nature 384*, 87–92.
5. Roberts, R. J., and Cheng, X. (1998) Base Flipping. *Annu. Rev. Biochem. 67*, 181–198.
6. Parker, J. B., Bianchet, M. A., Krosky, D. J., Friedman, J. I., Amzel, L. M., and Stivers, J. T. (2007) Enzymatic Capture of an Extrahelical Thymine in the Search for Uracil in DNA. *Nature 449*, 433–438.
7. Parikh, S. S., Walcher, G., Jones, G. D., Slupphaug, G., Krokan, H. E., Blackburn, G. M., and Tainer, J. A. (2000) Uracil-DNA Glycosylase-DNA Substrate and Product Structures: Conformational Strain Promotes Catalytic Efficiency by Coupled Stereoelectronic Effects. *Proc. Natl. Acad. Sci. U.S.A. 97*, 5083–5088.
8. Bianchet, M. A., Seiple, L. A., Jiang, Y. L., Ichikawa, Y., Amzel, L. M., and Stivers, J. T. (2003) Electrostatic Guidance of Glycosyl Cation Migration Along the Reaction Coordinate of Uracil DNA Glycosylase. *Biochemistry 42*, 12455–12460.
9. Werner, R. M., and Stivers, J. T. (2000) Kinetic Isotope Effect Studies of the Reaction Catalyzed by Uracil DNA Glycosylase: Evidence for an Oxocarbonium Ion-Uracil Anion Intermediate. *Biochemistry 39*, 14054–14064.
10. Jiang, Y. L., Song, F., and Stivers, J. T. (2002) Base Flipping Mutations of Uracil DNA Glycosylase: Substrate Rescue using a Pyrene Nucleotide Wedge. *Biochemistry 41*, 11248–11254.
11. Krosky, D. J., Song, F., and Stivers, J. T. (2005) The Origins of High-Affinity Enzyme Binding to an Extrahelical DNA Base. *Biochemistry 44*, 5949–5959.
12. Bellamy, S. R., Krusong, K., and Baldwin, G. S. (2007) A Rapid Reaction Analysis of Uracil DNA Glycosylase Indicates an Active Mechanism of Base Flipping. *Nucleic Acids Res. 35*, 1478–1487.
13. Wong, I., Lundquist, A. J., Bernards, A. S., and Mosbaugh, D. W. (2002) Presteady-State Analysis of a Single Catalytic Turnover by *Escherichia coli* Uracil-DNA Glycosylase Reveals a "Pinch-Pull-Push" Mechanism. *J. Biol. Chem. 277*, 19424–19432.
14. Bennett, S. E., Sanderson, R. J., and Mosbaugh, D. W. (1995) Processivity of *Escherichia coli* and Rat Liver Mitochondrial Uracil-DNA Glycosylase is Affected by NaCl Concentration. *Biochemistry 34*, 6109–6119.
15. Porecha, R. H., and Stivers, J. T. (2008) Uracil DNA Glycosylase Uses DNA Hopping and Short-Range Sliding to Trap Extrahelical Uracils. *Proc. Natl. Acad. Sci. U.S.A. 105*, 10791–10796.

16. Sidorenko, V. S., Mechetin, G. V., Nevinsky, G. A., and Zharkov, D. O. (2008) Correlated Cleavage of Single- and Double-Stranded Substrates by Uracil-DNA Glycosylase. *FEBS Lett. 582*, 410–414.

17. Higley, M., and Lloyd, R. S. (1993) Processivity of Uracil DNA Glycosylase. *Mutat. Res. 294*, 109–116.

18. Wibley, J. E., Waters, T. R., Haushalter, K., Verdine, G. L., and Pearl, L. H. (2003) Structure and Specificity of the Vertebrate Anti-Mutator Uracil-DNA Glycosylase SMUG1. *Mol. Cell 11*, 1647–1659.

19. Maiti, A., Morgan, M. T., Pozharski, E., and Drohat, A. C. (2008) Crystal Structure of Human Thymine DNA Glycosylase Bound to DNA Elucidates Sequence-Specific Mismatch Recognition. *Proc. Natl. Acad. Sci. U.S.A. 105*, 8890–8895.

20. Banerjee, A., and Verdine, G. L. (2006) A Nucleobase Lesion Re-models the Interaction of its Normal Neighbor in a DNA Glycosylase Complex. *Proc. Natl. Acad. Sci. U.S.A. 103*, 15020–15025.

21. Banerjee, A., Yang, W., Karplus, M., and Verdine, G. L. (2005) Structure of a Repair Enzyme Interrogating Undamaged DNA Elucidates Recognition of Damaged DNA. *Nature 434*, 612–618.

22. Bruner, S. D., Norman, D. P., and Verdine, G. L. (2000) Structural Basis for Recognition and Repair of the Endogenous Mutagen 8-Oxoguanine in DNA. *Nature 403*, 859–866.

23. Kuznetsov, N. A., Koval, V. V., Nevinsky, G. A., Douglas, K. T., Zharkov, D. O., and Fedorova, O. S. (2007) Kinetic Conformational Analysis of Human 8-Oxoguanine-DNA Glycosylase. *J. Biol. Chem. 282*, 1029–1038.

24. Kuznetsov, N. A., Koval, V. V., Zharkov, D. O., Nevinsky, G. A., Douglas, K. T., and Fedorova, O. S. (2005) Kinetics of Substrate Recognition and Cleavage by Human 8-Oxoguanine-DNA Glycosylase. *Nucleic Acids Res. 33*, 3919–3931.

25. Sidorenko, V. S., and Zharkov, D. O. (2008) Correlated Cleavage of Damaged DNA by Bacterial and Human 8-Oxoguanine-DNA Glycosylases. *Biochemistry 47*, 8970–8976.

26. Banerjee, A., Santos, W. L., and Verdine, G. L. (2006) Structure of a DNA Glycosylase Searching for Lesions. *Science 311*, 1153–1157.

27. Qi, Y., Spong, M. C., Nam, K., Banerjee, A., Jiralerspong, S., Karplus, M., and Verdine, G. L. (2009) Encounter and Extrusion of an Intra-helical Lesion by a DNA Repair Enzyme. *Nature 462*, 762–766.

28. Fromme, J. C., and Verdine, G. L. (2003) DNA Lesion Recognition by the Bacterial Repair Enzyme MutM. *J. Biol. Chem. 278*, 51543–51548.

29. Koval, V. V., Kuznetsov, N. A., Zharkov, D. O., Ishchenko, A. A., Douglas, K. T., Nevinsky, G. A., and Fedorova, O. S. (2004) Pre-Steady-State Kinetics shows Differences in Processing of various DNA Lesions by *Escherichia coli* Formamidopyrimidine-DNA Glycosylase. *Nucleic Acids Res. 32*, 926–935.

30. Koval, V. V., Kuznetsov, N. A., Ishchenko, A. A., Saparbaev, M. K., and Fedorova, O. S. (2010) Real-Time Studies of Conformational Dynamics of the Repair Enzyme *E. coli* Formamidopyrimidine-DNA Glycosylase and its DNA Complexes during Catalytic Cycle. *Mutat. Res. 685*, 3–10.

31. Fromme, J. C., Banerjee, A., Huang, S. J., and Verdine, G. L. (2004) Structural Basis for Removal of Adenine Mispaired with 8-Oxoguanine by MutY Adenine DNA Glycosylase. *Nature 427*, 652–656.

32. McCann, J. A., and Berti, P. J. (2008) Transition-State Analysis of the DNA Repair Enzyme MutY. *J. Am. Chem. Soc. 130*, 5789–5797.

33. Wong, I., Bernards, A. S., Miller, J. K., and Wirz, J. A. (2003) A Dimeric Mechanism for Contextual Target Recognition by MutY Glycosylase. *J. Biol. Chem. 278*, 2411–2418.

34. Francis, A. W., and David, S. S. (2003) *Escherichia coli* MutY and Fpg Utilize a Processive Mechanism for Target Location. *Biochemistry 42*, 801–810.

35. Hollis, T., Ichikawa, Y., and Ellenberger, T. (2000) DNA Bending and a Flip-Out Mechanism for Base Excision by the Helix-Hairpin-Helix DNA Glycosylase, *Escherichia coli* AlkA. *EMBO J. 19*, 758–766.

36. Lau, A. Y., Wyatt, M. D., Glassner, B. J., Samson, L. D., and Ellenberger, T. (2000) Molecular Basis for Discriminating between Normal and Damaged Bases by the Human Alkyladenine Glycosylase, AAG. *Proc. Natl. Acad. Sci. U.S.A. 97*, 13573–13578.

37. Lau, A. Y., Scharer, O. D., Samson, L., Verdine, G. L., and Ellenberger, T. (1998) Crystal Structure of a Human Alkylbase-DNA Repair Enzyme Complexed to DNA: Mechanisms for Nucleotide Flipping and Base Excision. *Cell 95*, 249–258.

38. Hedglin, M., and O'Brien, P. J. (2008) Human Alkyladenine DNA Glycosylase Employs a Processive Search for DNA Damage. *Biochemistry 47*, 11434–11445.

39. Metz, A. H., Hollis, T., and Eichman, B. F. (2007) DNA Damage Recognition and Repair by 3-Methyladenine DNA Glycosylase I (TAG). *EMBO J. 26*, 2411–2420.

40. Fromme, J. C., and Verdine, G. L. (2003) Structure of a Trapped Endonuclease III-DNA Covalent Intermediate. *EMBO J. 22*, 3461–3471.

41. Lindahl, T. (1993) Instability and Decay of the Primary Structure of DNA. *Nature 362*, 709–715.

42. Cappelli, E., Hazra, T., Hill, J. W., Slupphaug, G., Bogliolo, M., and Frosina, G. (2001) Rates of Base Excision Repair are Not Solely Dependent on Levels of Initiating Enzymes. *Carcinogenesis 22*, 387–393.

43. Halford, S. E., and Marko, J. F. (2004) How do Site-Specific DNA-Binding Proteins Find their Targets? *Nucleic Acids Res. 32*, 3040–3052.

44. Carmo-Fonseca, M., Platani, M., and Swedlow, J. R. (2002) Macromolecular Mobility Inside the Cell Nucleus. *Trends Cell Biol. 12*, 491–495.

45. Lloyd, R. S., Hanawalt, P. C., and Dodson, M. L. (1980) Processive Action of T4 Endonuclease V on Ultraviolet-Irradiated DNA. *Nucleic Acids Res. 8*, 5113–5127.

46. Berg, H. C. (1993) Random Walks in Biology, Princeton University Press, Princeton, NJ.

47. Kampmann, M. (2005) Facilitated Diffusion in Chromatin Lattices: Mechanistic Diversity and Regulatory Potential. *Mol. Microbiol. 57*, 889–899.

48. Zharkov, D. O., and Grollman, A. P. (2005) The DNA Trackwalkers: Principles of Lesion Search and Recognition by DNA Glycosylases. *Mutat. Res. 577*, 24–54.

49. Givaty, O., and Levy, Y. (2009) Protein Sliding Along DNA: Dynamics and Structural Characterization. *J. Mol. Biol. 385*, 1087–1097.

50. Blainey, P. C., Luo, G., Kou, S. C., Mangel, W. F., Verdine, G. L., Bagchi, B., and Xie, X. S. (2009) Nonspecifically Bound Proteins Spin while Diffusing Along DNA. *Nat. Struct. Mol. Biol. 16*, 1224–1229.

51. Gowers, D. M., Wilson, G. G., and Halford, S. E. (2005) Measurement of the Contributions of 1D and 3D Pathways to the Translocation of a Protein Along DNA. *Proc. Natl. Acad. Sci. U.S.A. 102*, 15883–15888.

52. Halford, S. E. (2009) An End to 40 Years of Mistakes in DNA-Protein Association Kinetics? *Biochem. Soc. Trans. 37*, 343–348.

53. Stivers, J. T. (2008) Extrahelical Damaged Base Recognition by DNA Glycosylase Enzymes. *Chemistry 14*, 786–793.

54. Hammes-Schiffer, S., and Benkovic, S. J. (2006) Relating Protein Motion to Catalysis. *Annu. Rev. Biochem. 75*, 519–541.

55. Priyakumar, U. D., and MacKerell, A. D., Jr. (2006) Computational Approaches for Investigating Base Flipping in Oligonucleotides. *Chem. Rev. 106*, 489–505.

56. Zharkov, D. O., Mechetin, G. V., and Nevinsky, G. A. (2010) Uracil-DNA Glycosylase: Structural, Thermodynamic and Kinetic Aspects of Lesion Search and Recognition. *Mutat. Res. 685*, 11–20.

57. David, S. S., O'Shea, V. L., and Kundu, S. (2007) Base-Excision Repair of Oxidative DNA Damage. *Nature 447*, 941–950.

58. Purmal, A. A., Lampman, G. W., Pourmal, E. I., Melamede, R. J., Wallace, S. S., and Kow, Y. W. (1994) Uracil DNA N-Glycosylase Distributively Interacts with Duplex Polynucleotides Containing Repeating Units of either TGGCCAAGCU or TGGCCAAGCTT-GGCCAAGCU. *J. Biol. Chem. 269*, 22046–22053.

59. Friedman, J. I., Majumdar, A., and Stivers, J. T. (2009) Nontarget DNA Binding Shapes the Dynamic Landscape for Enzymatic Recognition of DNA Damage. *Nucleic Acids Res. 37*, 3493–3500.

60. Werner, R. M., Jiang, Y. L., Gordley, R. G., Jagadeesh, G. J., Ladner, J. E., Xiao, G., Tordova, M., Gilliland, G. L., and Stivers, J. T. (2000) Stressing-Out DNA? The Contribution of Serine-Phosphodiester Interactions in Catalysis by Uracil DNA Glycosylase. *Biochemistry 39*, 12585–12594.

61. Banavali, N. K., and MacKerell, A. D. (2001) Re-Examination of the Intrinsic, Dynamic and Hydration Properties of Phosphoramidate DNA. *Nucleic Acids Res. 29*, 3219–3230.

62. Gueron, M., Charretier, E., Hagerhorst, J., Kochoyan, M., Leroy, J. L., Moraillon, A., Charretier, E., Hagerhorst, J., Kochoyan, M., Leroy, J. L., and Moraillon, A. (1990) Structure Methods: Applications of Imino Proton Exchange to Nucleic Acid Kinetics and Structures (Gueron, M., Ed.) Adenine Press, Guilderland, NY.

63. Cao, C., Jiang, Y. L., Stivers, J. T., and Song, F. (2004) Dynamic Opening of DNA during the Enzymatic Search for a Damaged Base. *Nat. Struct. Mol. Biol. 11*, 1230–1236.

64. Jiang, Y. L., and Stivers, J. T. (2002) Mutational Analysis of the Base Flipping Mechanism of Uracil DNA Glycosylase. *Biochemistry 41*, 11236–11247.

65. Wong, A. W., He, S., Grubb, J. H., Sly, W. S., and Withers, S. G. (1998) Identification of Glu-540 as the Catalytic Nucleophile of Human β-Glucuronidase using Electrospray Mass Spectrometry. *J. Biol. Chem. 273*, 34057–34062.

66. Moe, J. G., and Russu, I. M. (1992) Kinetics and Energetics of Base-Pair Opening in 5′-d(CGCGAATTCGCG)-3′ and a Substituted

Dodecamer Containing G·T Mismatches. *Biochemistry 31*, 8421–8428.

67. Giudice, E., Varnai, P., and Lavery, R. (2003) Base Pair Opening within B-DNA: Free Energy Pathways for GC and AT Pairs from Umbrella Sampling Simulations. *Nucleic Acids Res. 31*, 1434–1443.

68. Cao, C., Jiang, Y. L., Krosky, D. J., and Stivers, J. T. (2006) The Catalytic Power of Uracil DNA Glycosylase in the Opening of Thymine Base Pairs. *J. Am. Chem. Soc. 128*, 13034–13035.

69. Blainey, P. C., van Oijen, A. M., Banerjee, A., Verdine, G. L., and Xie, X. S. (2006) A Base-Excision DNA-Repair Protein Finds Intrahelical Lesion Bases by Fast Sliding in Contact with DNA. *Proc. Natl. Acad. Sci. U.S.A. 103*, 5752–5757.

70. Kad, N. M., Wang, H., Kennedy, G. G., Warshaw, D. M., and Van Houten, B. (2010) Collaborative Dynamic DNA Scanning by Nucleotide Excision Repair Proteins Investigated by Single-Molecule Imaging of Quantum-Dot-Labeled Proteins. *Mol. Cell 37*, 702–713.

71. Chen, L., Haushalter, K. A., Lieber, C. M., and Verdine, G. L. (2002) Direct Visualization of a DNA Glycosylase Searching for Damage. *Chem. Biol. 9*, 345–350.

72. Cheng, X., Kelso, C., Hornak, V., de los Santos, C., Grollman, A. P., and Simmerling, C. (2005) Dynamic Behavior of DNA Base Pairs Containing 8-Oxoguanine. *J. Am. Chem. Soc. 127*, 13906–13918.

73. Beard, B. C., Wilson, S. H., and Smerdon, M. J. (2003) Suppressed Catalytic Activity of Base Excision Repair Enzymes on Rotationally Positioned Uracil in Nucleosomes. *Proc. Natl. Acad. Sci. U.S.A. 100*, 7465–7470.

74. Cole, H. A., Tabor-Godwin, J. M., and Hayes, J. J. (2010) Uracil DNA Glycosylase Activity on Nucleosomal DNA Depends on Rotational Orientation of Targets. *J. Biol. Chem. 285*, 2876–2885.

75. Lloyd, R. S., Hanawalt, P. C., and Dodson, M. L. (1980) Processive action of T4 endonuclease V on ultraviolet-irradiated DNA. *Nucleic Acids Res. 8*, 5113–5127.