Estimating the Effect of Human Base Excision Repair Protein Variants on the Repair of Oxidative DNA Base Damage

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Abstract

Epidemiologic studies have revealed a complex association between human genetic variance and cancer risk. Quantitative biological modeling based on experimental data can play a critical role in interpreting the effect of genetic variation on biochemical pathways relevant to cancer development and progression. Defects in human DNA base excision repair (BER) proteins can reduce cellular tolerance to oxidative DNA base damage caused by endogenous and exogenous sources, such as exposure to toxins and ionizing radiation. If not repaired, DNA base damage leads to cell dysfunction and mutagenesis, consequently leading to cancer, disease, and aging. Population screens have identified numerous single-nucleotide polymorphism variants in many BER proteins and some have been purified and found to exhibit mild kinetic defects. Epidemiologic studies have led to conflicting conclusions on the association between single-nucleotide polymorphism variants in BER proteins and cancer risk. Using experimental data for cellular concentration and the kinetics of normal and variant BER proteins, we apply a previously developed and tested human BER pathway model to (*i*) estimate the effect of mild variants on BER of abasic sites and 8-oxoguanine, a prominent oxidative DNA base modification, (*ii*) identify ranges of variation associated with substantial BER capacity loss, and (*iii*) reveal nonintuitive consequences of multiple simultaneous variants. Our findings support previous work suggesting that mild BER variants have a minimal effect on pathway capacity whereas more severe defects and simultaneous variation in several BER proteins can lead to inefficient repair and potentially deleterious consequences of cellular damage. (Cancer Epidemiol Biomarkers Prev 2006;15(5):1000–8)

Introduction

DNA is continuously being damaged by endogenous sources, such as oxidative base modification resulting from reactive oxygen species attack (reviewed in ref. 1). Reactive oxygen species are formed as metabolites during normal cellular respiration, with increased levels of reactive oxygen species, as well as increased DNA base damage, associated with ischemia (2, 3), hyperoxia in human lung cells (4), human artherosclerotic plaques (5), and chronic inflammation in ulcerative colitis patients (6). Reactive oxygen species are also formed as secondary damaging agents as a result of exposure to toxins and ionizing radiation (e.g., sunlight, environmental IR). DNA base damage can lead to genotoxicity, replication stalling, apoptosis, and mutagenesis, with physiologic consequences including disease, aging, and cancer, as reviewed in ref. 7. Modified bases not only affect transcriptional integrity and replication but also DNA-protein binding (8, 9). Consequently, oxidative DNA base damage is normally removed and repaired by the efficient base excision repair (BER) pathway system of prokaryotic and eukaryotic cells (reviewed in refs. 1, 10, 11).

Population screens have identified extensive genetic variation, in particular single-nucleotide polymorphisms, altering the primary amino acid sequence of DNA BER proteins (e.g., ref. 12). This genetic variation has been found in some instances to diminish the stability or efficiency of the encoded repair protein, reducing its kinetic effectiveness (13-15). Furthermore, sequence variants with potential kinetic effect have been predicted computationally (16). Splice variants of repair genes have also been identified in cancerous cells, some of which may lead to a dominant-negative protein form with reduced repair efficiency (17, 18).

Nonfunctional DNA repair alleles, such as BRCA1/2 for breast cancer (19) and MSH2 and MLH1 for hereditary nonpolyposis colorectal cancer (20), are ''cancer genes'' strongly linked to increased cancer risk. However, cancer genes account for only ~5% of known cancer cases (21, 22). Consequently, reduced function repair protein variants may be exposure-dependent susceptibility alleles responsible for the majority of observed increased cancer risk associated with family history (23) as well as sporadic cancer incidence. Epidemiologic evidence for a correlation of BER gene variants with cancer risk is abundant, albeit conflicting, as reviewed in refs. 24, 25. Nonetheless, extracts from cells of head and neck cancer patients (26) and smokers with lung cancer have been found to exhibit reduced DNA repair efficiency (27) associated with the presence of BER protein variants and defective BER protein activity, respectively.

The BER pathway system includes proteins with multiple enzymatic activities, cooperativity, and compensatory subpathways. This complexity makes it difficult to intuitively estimate how variation in a single repair protein will change the overall ability of a cell to tolerate DNA base damage. Moreover, there is a high likelihood that individuals will possess multiple variations, which in combination can further increase or reduce pathway capacity. Consequently, we apply a mathematical model of human BER that integrates biological knowledge of enzyme mechanisms and biochemical data on enzyme kinetics and protein concentration obtained from the literature. This model has been used to interpret published data from *in vitro* pathway reconstitution and cell extracts to evaluate mechanistic hypotheses of enzyme cooperativity and coordination and predict the relative significance of the BER subpathways (28). Notably, the model recently predicted that under normal conditions, background oxidative DNA base damage level is at the low end of the widely varying published

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measurements (i.e., up to hundreds of lesions per cell) and that the background level is relatively stable to small changes in enzyme kinetics (29). In the context of the normally robust operation of BER, we applied the model based on published experimental data to (*i*) estimate the potential effect if any of the previously characterized BER variant proteins, (*ii*) identify the magnitude of changes in individual protein kinetics and concentration required to observe a substantial difference in overall BER kinetics and capacity, and (*iii*) use the model to predict the effect of simultaneous variation in multiple repair proteins on total BER kinetics.

Model and Methods

BER Pathway Model Structure. The comprehensive BER pathway model is based on current biological knowledge, which is illustrated in Fig. 1. The BER pathway in the nucleus is initiated by a lesion-specific DNA glycosylase, which recognizes and removes damaged bases (30). For example, 8-oxoguanine (8-oxoG; y_1 in Fig. 1) is removed by 8-oxoG DNA glycosylase (Ogg1; e_1). Some DNA glycosylases (including Ogg1) also exhibit an apurinic/apyrimidinic (AP) lyase activity, incising 3′ to the abasic (AP) residue, leading to a subpathway (pathway A) in which the phosphodiesterase activity of human AP endonuclease (Ape1; e_2) removes the 3′ block (y_{10}; refs. 31, 32). In most cases, ''short-patch'' BER (pathway B) occurs (33), where Ape1 incises 5′ to the lesion (34). The resultant 5′-deoxyribose phosphate (y_4) group is removed by the deoxyribose phosphate lyase activity of DNA polymerase β (Polβ; e_3), which then executes single-base replacement at the gapped site (y_5 or y_{11}). In our model, the Polβ-catalyzed activities may occur in either order (thus the fork in pathway B). An alternative ''long-patch'' BER pathway (pathway C) exists in which gap-filling of two to seven nucleotides is done by Polδ (e_4) or possibly Polε (excluded from our model due to a lack of kinetic data), in complex with proliferating cell nuclear antigen, followed by flap excision by Fen1 (e_5) and subsequent ligation (35). The final step in all subpathways is done by DNA ligase 1 (Lig1; e_6) or Lig3 (e_7) stabilized in complex with Xrcc1 (36). Lig1 and Lig3/Xrcc1 activities seem to be largely interchangeable (37), although a few recent results indicate that Lig1 may be more significant for long-patch repair (38).

Model Assumptions. In general, reactions in the model are modeled using Michaelis-Menten enzyme kinetics, with k_{cat} and K_M variables defining a reaction velocity (v_i in Fig. 1). This implicitly assumes homogeneity and deterministic, continuous reaction kinetics, which are justified by the high concentration of repair proteins as discussed in ref. 28. If Michaelis-Menten assumptions hold for a biochemical system, then k_{cat} is interpreted as the catalytic turnover rate of the enzyme (its ''activity'') and K_M is a function of the affinity of the enzyme for the substrate.

To account for experimental measurement of 5- to 10-fold reduced diffusion velocities in the crowded cell nucleus (39), K_M of all reactions are increased 10-fold (due to the reduction in collision probability). The model includes experimentally measured protein cooperativity by k_{cat} increases as in previous modeling (28, 29), as well as including coordination of consecutive single enzyme activities (such as Polβ and the dual-function DNA glycosylases, e.g., Ogg1) by modeling the second step as a first-order reaction, as described in ref. 28. We also assume no product inhibition for individuals under normal conditions in the pathway, as suggested by observations (32, 40). We do not, however, include the potential role of Xrcc1 as a mediator of a multiprotein coordination complex (41), as the contribution of Xrcc1 to such a complex forming and any consequent enhancement of BER kinetics are unclear (42, 43). In addition, many of the potential coordinative effects are already in the model. Table 1 shows kinetic variables used in the model, including rate enhancements due to cooperativity.

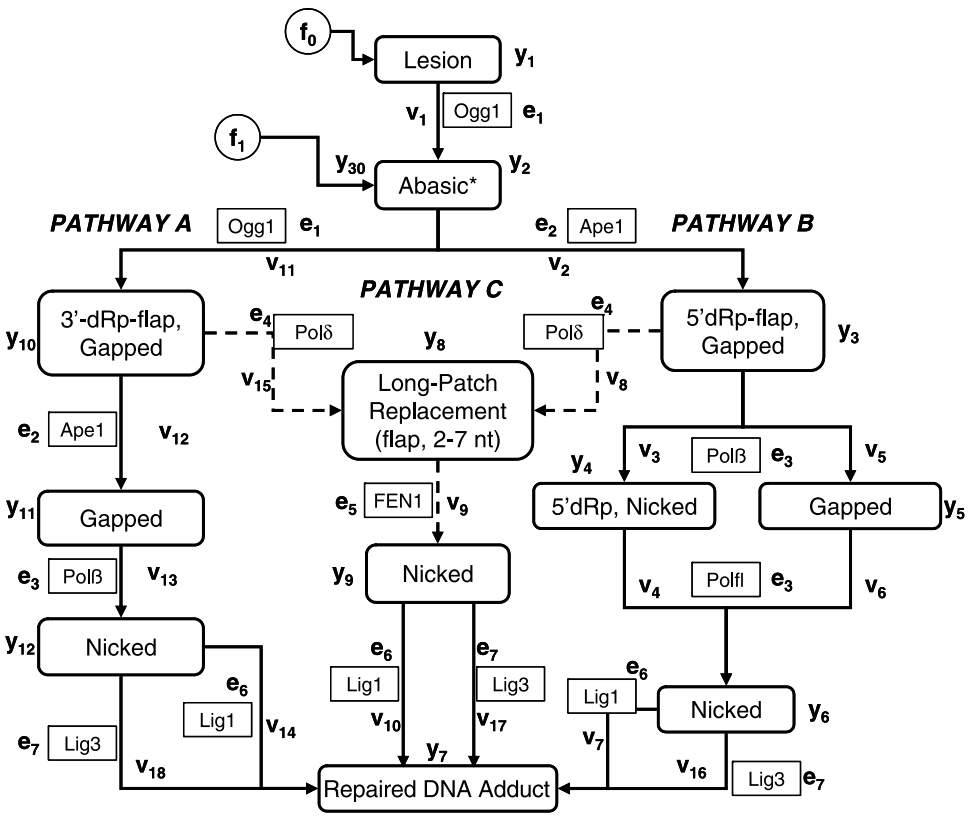

Figure 1. Schematic of the human BER pathway model, described in the main text, showing substrates and intermediates (*y*), enzymes (*e*), and reactions (*v*). (*, abasic sites can be formed directly, y_{30}, or as intermediates of a bifunctional DNA glycosylase, e.g. Ogg1, y_2.).

Table 1. Normal BER protein kinetics

Reactions (v_i)	k_{cat} (s^{-1})*	K_M (nmol/L)*	References	Rate enhancements	References
1	0.0052	121.5	(14, 15, 32, 109-111)	10× decreased K_M (Ape1); 8× increased k_{cat} (Ape1; posttranslational modifications)	(32, 112)
2	3.2028	34.7	(32, 113-120)	—	—
3, 6 †	0.817	210	(121-124)	—	—
4 †, 5	0.075	500	(125)	6× increased k_{cat} (Ape1)	(126, 127)
7, 10, 14, 16-18 ‡	0.0213	56.7	(121, 128, 129)	4× increased k_{cat} (PCNA, RpA)	(129, 130)
8, 15	0.6	100	(131-133)	Rate in presence of PCNA	—
9	0.1335	39	(124, 134)	2× increased k_{cat} (Ape1)	(135)
11	0.000887	7.2	(32)	2.5× increased k_{cat} (Ape1; posttranslational modification)	(112, 136)
12	0.0608	169	(114, 137, 138)	—	—

Abbreviations: PCNA, proliferating cell nuclear antigen; RpA, replication protein A.
*Where multiple sources are used in the literature, the median is used to avoid excessive influence by outliers.
† Assuming pathway coordination, this reaction is first order with a rate of k_{cat}.
‡ Lig3 kinetics assumed equal to those for Lig1 kinetics (37).

Model Protein Concentrations. Table 2 shows BER protein concentrations determined from published data calculated by assuming the number of proteins measured per cell is homogeneously distributed in a nucleus with a diameter of 5 μm, as described in refs. 28, 29. Because of the limited data currently available, these protein levels were drawn from various cell lines. Where multiple measurements were available, a conservative assumption was used because some are from tumor cell lines, which have been measured to have higher protein expression levels than normal cells (44). Furthermore, Ape1 (45, 46) and DNA glycosylases, such as Ogg1 (47), may be distributed in both the nucleus and other cell compartments (i.e., cytosol and mitochondria). In our analysis, we neglect the contribution to overall cellular DNA damage of the oxidative damage in mitochondria, which is repaired equally or more efficiently than nuclear DNA and does not occur at levels measurable in whole-cell assays (48-50). Consequently, protein concentrations in Table 2 do not include Ogg1 and Ape1 localized to mitochondria (47, 51, 52). We have found in our analysis of the sensitivity of our results to protein concentration that it closely follows that of sensitivity to k_{cat}, which is published here (results not shown; similar results were also found in our previous work on estimating steady-state BER capacity; ref. 29). This is expected from the mathematical form of the Michaelis-Menten equations used to model most of the BER pathway.

Solution of Model Equations. The mathematical structure of the model is identical to that in ref. 29. Differential equations were solved using the ''ode15s'' stiff solver of MATLAB R14 using variables from Tables 1 and 2, adjusted by the analysis described below. Steady-state reactant levels were the equilibrium point found by simulating the differential equations on a time scale of 24 hours and then setting equations for repair intermediate concentrations equal to zero and using the nonlinear least squares MATLAB routine ''fsolve'' to solve the resulting algebraic system (results not shown).

Table 2. BER protein concentrations

Protein	Concentration (nmol/L)	References
Ogg1	406	(51, 111)
Ape1	2,000	(51, 137, 139)
Polβ	419	(44, 140)
Polδ	600	*
Fen1	450	*
Lig1	254	(141)
Lig3	254	†

*In absence of data, assumed based on concentration of other nuclear proteins.
† Assumed equal to Lig1 (37).

Results

Simulation of 8-OxoG Levels as an Estimate for Oxidative DNA Base Repair Capacity. We focus on the repair of 8-oxoG initiated by Ogg1, which includes the full set of pathway steps in Fig. 1. This is motivated by the large amount of data from experimental sources for the kinetics of wild-type and polymorphic forms of Ogg1 (28), as well as cellular and physiologic levels of 8-oxoG (e.g., ref. 53); also reviewed in ref. 29. 8-OxoG is frequently used as a marker for oxidative DNA damage associated with disease consequences (54). As further data accumulate for the kinetics and cellular abundance of other DNA glycosylases (55), we can extend our analysis in the future using the approach described herein. In our studies, we assume that the contribution of NEIL1/2 enzymes recently found to have 8-oxoG DNA glycosylase activity (56) is quantitatively insignificant as compared with Ogg1; recent studies have suggested that their biological role may be primarily for specialized cases, such as damage within bubble DNA structures (57, 58).

We account for the production of other base lesions in our modeling by including an additional damage load of abasic (AP) sites, comprising the products of other DNA glycosylases, as well as spontaneous hydrolysis and base loss, the majority of which result in apurinic sites (59). Overall, we predict the combined numbers of original lesions and repair intermediates, which are a significant component of observed lesions (60) and may be highly genotoxic and mutagenic themselves (61, 62).

Estimated Effect of Mild Protein Variants Found in the Population on BER Kinetics and Capacity. If a protein variant is found to affect the kinetics of a BER reaction, then its effect on the overall pathway is estimated by modifying the appropriate k_{cat} and K_M variables for that reaction in the comprehensive BER system model. Published data are available for the kinetics of some BER proteins coded by sequence variants identified in population screens. Ape1 variants were characterized with AP endonuclease efficiencies from 35% to 110% of wild type (13). These are calculated based on the amount of repair of an initial lesion concentration over a fixed period of time, and they may be interpreted as being equivalent to the catalytic rate (k_{cat}) of Ape1. The commonly found Ogg1 polymorphism $Ser^{326}Cys$ has a reported k_{cat} 63% of wild type (15) and other Ogg1 variants were found to have a k_{cat} 44% ($Arg^{154}His$) and 85% ($Arg^{36}Gln$) of wild type (14). In

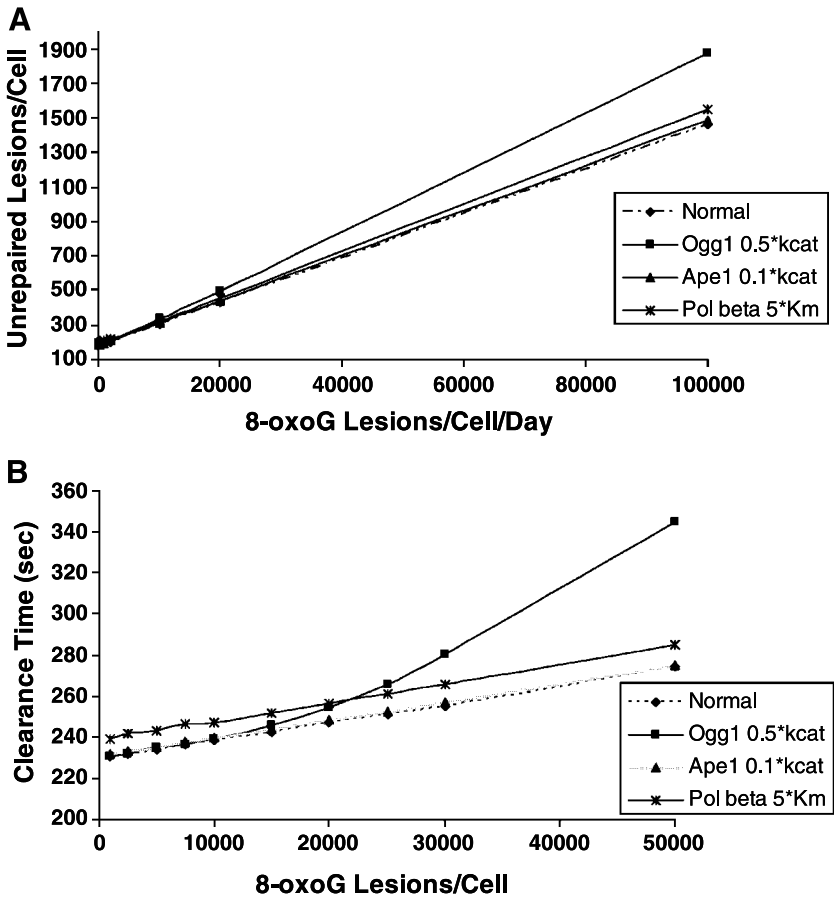

Figure 2. Sensitivity of 8-oxoG BER capacity and kinetics to mild kinetic defects in Ogg1 (50% of normal k_{cat}), Ape1 (10% of k_{cat}), and Polβ (5-fold increased K_M). **A.** Total number of 8-oxoG, abasic, and repair intermediate bases per cell given increasing 8-oxoG rates of formation (assuming simultaneous production of an additional 20,000 AP sites/cell/d). **B.** Time required to clear an instantaneous pulse of 100,000 AP sites/cell and an increasing number of 8-oxoG lesions/cell and restore damage to the background steady-state level for continuous formation of 2,000 8-oxoG/cell/d and 20,000 AP sites/cell/d. Simulated data for the pathway with normal kinetics and with a 10% k_{cat} Ape1 variant are nearly overlapping.

the case of Polβ, one quantitatively characterized allelic variant is a dominant-negative inhibitor of wild-type activity (17). Based on the measured equilibrium binding affinity (K_D) of the variant with the Polβ substrate and potential cell concentration, the effective K_M for Polβ gap-filling reaction is increased 3- to 5-fold (calculated based on competitive inhibition in the Michaelis-Menten model). The estimated effect of protein variants in these ranges of variation are shown in Fig. 2, where we have simulated the effect of Ape1 with 10% of normal k_{cat} (the highest amount at which any difference could be observed), Ogg1 with 50% of normal k_{cat}, and Polβ with a 5-fold increase in K_M.

Figure 2 illustrates two possible kinds of effect on the BER pathway: the capacity of BER to tolerate a constant rate of base lesion formation (8-oxoG and AP site formation combined) and the kinetics of BER repairing an instantaneous pulse of oxidative DNA damage (also consisting of both 8-oxoG and AP site lesions). Such a pulse simulates acute exposure (e.g., a dose of radiation therapy, increased metabolism during intense exercise, or acute inflammation during an infection). We consider repair of transient damage pulses in the context of continuing background base lesion formation. Base lesion formation rates (2,000 8-oxoG/cell/day and 20,000 AP sites/cell/day) are based on experimental data for normal background rates of formation (59, 63, 64). They are adjusted upwards to reflect (*i*) increased 8-oxoG corresponding to exercise and other variations in metabolism (65) and (*ii*) AP sites produced by the action of additional DNA glycosylases processing other base lesions. Damage ''clearance'' is defined as the time at which the background steady-state level of lesions corresponding to the constant damage formation rate is restored. Notably, as Fig. 2 shows, 8-oxoG repair rate is more sensitive to small changes in protein kinetics than the steady-state repair capacity.

Table 3 summarizes the effect on BER capacity (steady-state number of lesions per cell on exposure to the formation of 2,000 8-oxoG/cell/d and 20,000 AP sites/cell/d) and BER kinetics (time to clear 10,000 8-oxoG and 100,000 AP sites) of each protein in the pathway with 10% and 50% of their normal (Table 1) k_{cat}. Results are given for each of the activities of multiple-function proteins separately. Data are provided as a percentage increase in steady-state damage and clearance time with respect to normal protein kinetics. Table entries of ''0%'' represent a change <0.01% (results not shown). Notably, because of the dominance of pathway B for our model of 8-oxoG repair, assuming normal Polβ function, pathway C proteins, Polδ and Fen1, will have negligible effect on kinetics, as was found in our previous modeling analysis (28).

Sensitivity Analysis of BER Pathway Capacity to Hypothetical Protein Variants. In Fig. 2, curves showing an increased lesion burden and clearance time as a result of increased damaged load show a quantitatively small difference for mild kinetic variants of BER proteins. This suggests that the BER pathway is apparently robust to mild kinetic variation. Accordingly, we explored a wider range of protein kinetic variables to identify levels at which variants may have a qualitatively observable effect on BER pathway capacity, as shown in Fig. 3 for k_{cat} and K_M (modified relative to the normal values in Table 1; not shown are variants for BER reactions that were found to have no effect on steady-state damaged base levels, e.g., Fen1 k_{cat} and K_M). Whereas the analysis in Fig. 3 is specifically for steady-state damage levels given physiologically relevant levels of 8-oxoG and AP site formation, it qualitatively reflects the general sensitivity of the BER pathway to its individual components. Regions on the plots in Fig. 3 that show a rapidly changing curve represent variable values for which BER capacity is highly sensitive and less

Table 3. Predicted effect of protein kinetic variants on background damage levels and time to clear lesions (% of wild type)

Protein	Activity (v_i)		10% k_{cat}		50% k_{cat}	
			Damage	Time	Damage	Time
Ogg1	1	Base excision	29%	341%	3%	9%
Ape1	2	5′-incision	2%	1%	0%	0%
Polβ	3, 6	Gap-filling	13%	7%	4%	2%
Polβ	4, 5	5′-Deoxyribose phosphate lyase	32%	56%	4%	1%
Lig1*	7, 10, 14, 16-18		53%	90%	21%	34%
Polδ	8, 15		1%	0%	0%	0%
Fen1	9		0%	0%	0%	0%
Ogg1	11	AP lyase	0%	0%	0%	0%
Ape1	12	3′-Deoxyribose phosphate lyase	0%	0%	0%	0%

NOTE: Steady-state number of lesions per cell is based on 2,000 8-oxoG and 20,000 AP sites/cell/d; time to clear is calculated for 10,000 8-oxoG and 100,000 AP sites/cell.

*Based on model assumptions, Lig3 will have the same results.

robust to increases in damage level. Regions for which no curve is plotted at all represent variable ranges of BER pathway kinetics that preclude a steady-state equilibrium point. For these hypothetical kinetic variants, the BER pathway breaks down, with lesions and repair intermediates accumulating uncontrollably. For normal BER reaction kinetics, this breaking point is ~125,000 8-oxoG/cell/d (29), a level that seems to be physiologically unrealistic. By comparison, in cell culture, 8-oxoG formation was measured at $0.01/10^6$ bases/Gy (66), and 8-oxoG levels on the order of $100/10^6$ have been measured in naked DNA exposed to highly oxidative agents or >5 Gy radiation (67). However, for some modeled changes in the k_{cat} and K_M of Ogg1, Ape1, Polβ, and Lig1, it can be on the order of 10^4 8-oxoG/cell/d (data not shown) or less (e.g., where the curves are not shown in Fig. 3). Such variants will result in a BER pathway incapable of tolerating even normal damage formation rates (i.e., 10^3 8-oxoG/cell/d and 10^4 AP sites/cell/d) without accumulating unrepaired lesions.

Figure 3 shows the sensitivity of repair capacity to changes in only Lig1 kinetic efficiency. The concentration and kinetics of Lig3 were assumed to be equivalent to those of Lig1. Consequently, simulated predictions for Lig3 are identical to those for Lig1, showing redundancy in the DNA ligation step of the repair pathway. This agrees partially with experimental results in ref. 37, which do suggest that in some cases one DNA ligase may be preferred to another. Due to the redundant role of Lig1 and Lig3 in our model, simulated data predict that BER capacity is sensitive to reduction in Lig1 kinetics to a certain

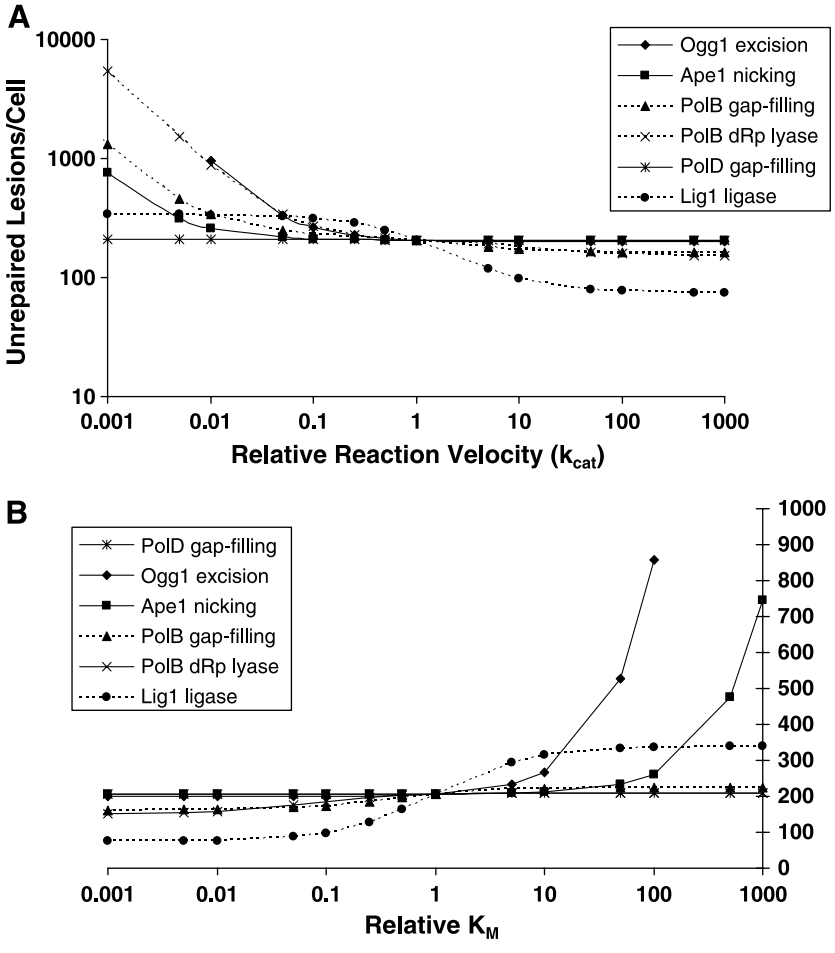

Figure 3. Sensitivity over a broad range of k_{cat} (**A**) and K_M (**B**) of enzymatic activities in BER (Ogg1 excision of 8-oxoG, Ape1 nicking at the 5′ end, Polβ gap-filling and 5′-deoxyribose phosphate (*dRp*) lyase reactions, Polδ gap-filling, and the ligase activity of Lig1. Changes in kinetic variables are fractions and multiples of the values for those reactions in Table 1 (with all K_M increased 10-fold as described in the text). The steady-state total number of 8-oxoG, AP sites, and repair intermediates is calculated based on a constant total damage formation rate of 2,000 8-oxoG lesions/cell/d and 20,000 AP sites/cell/d. The plot is truncated at the point where a continued K_M increase results in no physically possible steady state.

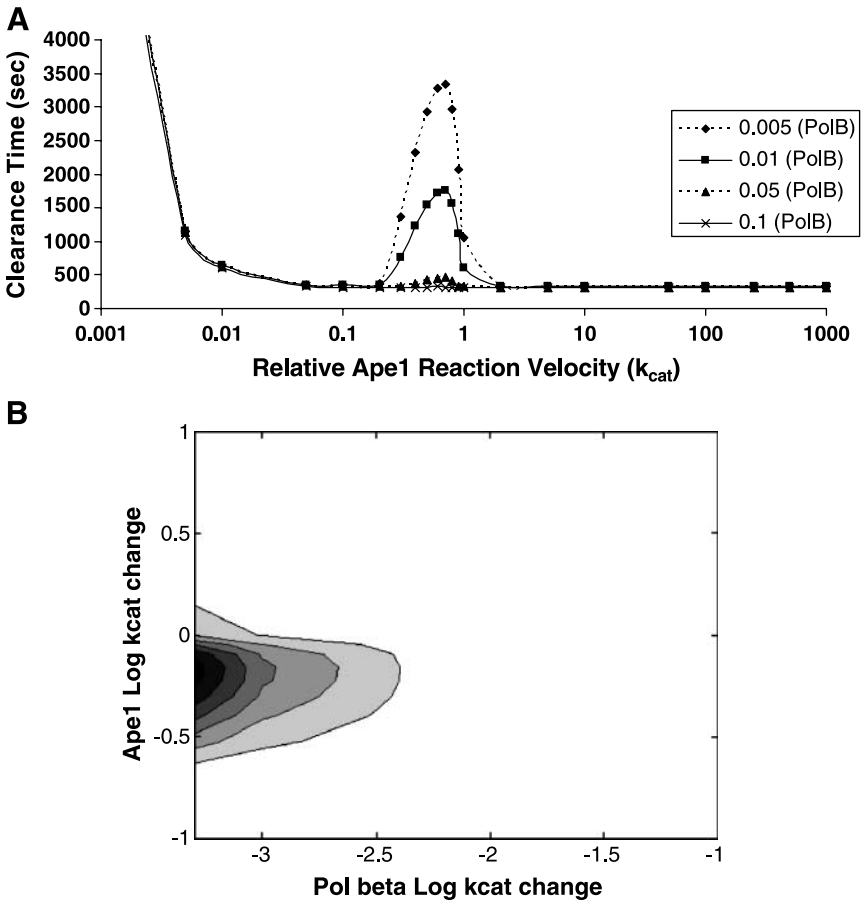

Figure 4. Simulation of the effect of varying Ape1 and Polβ k_{cat} simultaneously on the time required to clear 10,000 8-oxoG sites and 100,000 AP sites, with a constant background damage formation rate of 2,000 8-oxoG/cell/d and 20,000 AP sites/cell/d. **A.** Clearance time for varying Ape1 k_{cat} (multiple of normal variable value in Table 1) for fractions of normal Polβ k_{cat}. **B.** Contour plot of clearance time for simultaneous changes (log ratio to normal k_{cat}) in Ape1 and Polβ; darker regions correspond to higher clearance times.

point; after which, further reduction no longer affects the pathway, as shown in Fig. 3.

Observation of Unexpected Synergies for Hypothetical Multiple Protein Variants in the BER Pathway. In some cases, the complexity of individual protein kinetics within the context of the whole BER pathway leads to unexpected synergistic variation. To illustrate this, we estimate the consequence of simultaneous variation of Ape1 5′-incision activity and Polβ gap-filling and 5′-deoxyribose phosphate lyase catalytic activities (k_{cat}) on overall BER dynamics for the repair of a transient pulse of DNA damage, assuming that the Polβ rate enhancement by Ape1 is unaffected. In the steady-state analysis (not shown), there is only a 3.75 to 4 lesions/cell difference (including repair intermediates) between Polβ variants with 200-fold reduced k_{cat} and 10-fold reduced k_{cat} (assuming constant rates of formation of 2,000 8-oxoG/cell/d and 20,000 AP sites/cell/d), with the exception of the situation with a 1,000-fold Ape1 k_{cat} reduction, in which case the number of steady-state lesions differs by 27 per cell. This contrasts with the findings shown in Fig. 4 for transient repair dynamics. The time to fully clear a pulse of 8-oxoG and abasic lesions and repair intermediates for the Polβ-inefficient pathway is at its maximum when Ape1 k_{cat} is 70% of normal. As Fig. 4B shows, this point of respective Ape1 and Polβ activity modification is a peak (darkest region). Repair efficiency improves (more lightly shaded regions of Fig. 4B) as Ape1 kinetics either increase or decrease, and the overall system shows less sensitivity to reduced Polβ kinetics.

Based on a study of the estimated levels of BER intermediates during the simulation, it seems that at that particular critical level of Ape1, there is a shifting of repair to pathways A and C—which are slower than pathway B—and sites being repaired through pathway A are particularly sensitive to Polβ kinetics following the first steps. Consequently, there is a persistence of repair intermediates and prolongation of the time to reach the normal steady-state background. As this model analysis shows, the complexity of multiple subpathways in the BER pathway can be a mechanism for unexpected synergies between protein variants, which are not merely additive results of reduced kinetic rates.

Discussion

There have been numerous investigations of potential correlations between BER gene variants (e.g., single-nucleotide polymorphisms, truncations, and frameshifts) with levels of biomarkers, including DNA damage and mitotic delay, prevalence in tumor cells, and increased cancer susceptibility. To date, many of these studies have been inconclusive or contradictory. For instance, the commonly found $Ser^{326}Cys$ variant of Ogg1 has been found to be correlated epidemiologically with increased cancer risk, increased measurements of cellular DNA base damage, and reduced base repair kinetics in several studies and assays (68-75), whereas null or statistically insignificant correlations have been found in others (refs. 69, 76-79); also a recent extensive review in ref. 80). Applying a previously developed and tested comprehensive BER pathway model integrating reaction mechanisms and variables obtained from published experimental data, we estimate that Ogg1 base excision k_{cat} reduced by 50%, comparable to that of the Ogg1 $Ser^{326}Cys$ polymorphism (15), results in a decrease of ~5% to 10% in BER capacity, as defined by the steady-state number of lesions per cell for a wide range of constant damage formation rates (as shown in Fig. 2). The transient kinetics of repairing a pulse of damage are somewhat more sensitive to mild kinetic variants but the effects are still minimal.

Overall, our modeling supports a complex role for genetics in tolerating exposure to genotoxic stresses in determining

DNA base damage tolerance and cancer risk. Under normal and increased damage loads and rates (simulated based on experimental measurements), results favor the null or marginal experimental findings for the effect of Ogg1 $Ser^{326}Cys$ on cellular tolerance of oxidative damage and cancer susceptibility. Furthermore, Ape1 population variants characterized in ref. 13 are estimated by our modeling to have a negligible effect on BER kinetics and capacity. These results are supported by a null finding for cancer susceptibility with the Ape1 variant $Asp^{146}Glu$ in pooled studies (75).

Our analysis (Fig. 3) reveals that further increases in individual repair protein kinetics have a minor effect on overall BER pathway kinetics, as suggested by the long evolutionary history of BER, conserved to a relatively high degree from *E. coli* through human cells (7). Of all its steps, the BER pathway is most sensitive to decreases in Ogg1 base excision, Ape1 5′-incision of the abasic site, and Polβ gap-filling and 5′-deoxyribose phosphate lyase reaction kinetics, with limited compensated sensitivity to DNA ligase activity. The high sensitivity to Ogg1 kinetics is supported by evidence that allelic loss (which reduces Ogg1 concentration and reaction velocity) is prevalent in tumor cells with reduced 8-oxoG repair activity (69, 76, 81, 82). Furthermore, whereas there is little evidence for Polβ variants in cells of normal tissues in the human population, several variants with diminished activity have been found in tumor cell lines and are associated with reduced overall BER pathway capacity and a Polβ mutator phenotype, in accord with estimates presented here (17, 18, 83-88). This suggests a potentially significant role for inherited Polβ variants in increasing cellular susceptibility to DNA base damage and cancer risk, suggesting targets for further epidemiologic studies.

Whereas the assumption of redundant Lig1 and Lig3 activities results in a modeling prediction of some compensation for the case of variants, there is still notable sensitivity to kinetics that would be exacerbated if activities are not completely overlapping. A Lig1 $Arg^{771}Trp$ variant has been characterized in an immunodeficient patient, with cell cultures showing hypersensitivity to DNA damaging agents and impaired BER activity with 3% to 5% Lig1 kinetics (89), and a noncoding variant of Lig3 has been associated with increased esophageal cancer risk (90). In addition, Lig3 activity requires Xrcc1, and there are reports associating Xrcc1 variants with increased cancer susceptibility (70, 90-94). However, other studies indicate null or statistically marginal results for Xrcc1 variants, highlighting the complexity of variant protein roles in the BER pathway (95-97).

Whereas the association of most proteins involved in oxidative DNA base damage repair with cancer remains a controversial question, there is recent evidence of a direct link between protein sequence of MYH (the DNA glycosylase that removes a mismatched adenine across from the 8-oxoG lesion) and colorectal cancer (refs. 98-100; reviewed in ref. 101). There is also some evidence that MYH variants associated with increased cancer risk and polyposis have reduced catalytic efficiency but published studies have been on human MYH sequence variants generated by site-directed mutagenesis not found in the population (102) or mutants of the *E. coli* homologue corresponding to the population variants (103). As further enzymatic data are obtained for MYH activity, our comprehensive BER system model can be extended to include MYH along with Ogg1 and other DNA glycosylases, allowing for estimation of variant effect on the whole pathway.

In the post-genome era, there has been tremendous investment in the search for genetic variants in the population related to increased cancer risk and chemoresistance, particularly single-nucleotide polymorphisms (104). The relative prevalence of single-nucleotide polymorphisms in the population suggests that many are low-penetrance alleles, which will reduce the efficiency of biological pathways without eliminating their function entirely. As this study shows, biochemically modeling the effect of variants reveals complex behavior and pathway sensitivity and can provide insight to interpret incomplete and contradictory results obtained from both epidemiology and laboratory experiments. Progress in understanding how complex genotypes can predict the response to endogenous background and exogenous environmental exposure to DNA damaging stresses will allow an individualized, predictive approach in the interpretation of the medical significance of DNA damage quantification (105), identification of chemoprevention strategies for individuals with known susceptibility factors (106, 107), and unveiling of mechanisms of chemoresistance (108) to therapeutics. In general, the analysis presented here underscores the critical role quantitative biological system modeling and experiments will play in the long-term goal of the post-genomic era, effectively interpreting complex genetic studies and translating their findings to benefit human health.

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