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Bipartite substrate discrimination by human nucleotide excision repair

(xeroderma pigmentosum/DNA repair/molecular recognition/mismatches/carcinogenesis)

MARTIN T. HESS*, URS SCHWITTER†, MARIO PETRETTA†, BERND GIESE†, AND HANSPETER NAEGELI*‡

*Institute of Pharmacology and Toxicology, University of Zürich–Tierspital, Winterthurerstrasse 260, 8057 Zürich, Switzerland; and †Institute of Organic Chemistry, University of Basel, St.-Johanns-Ring 19, 4056 Basel, Switzerland

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ABSTRACT **Mammalian nucleotide excision repair (NER) eliminates carcinogen–DNA adducts by double endonucleolytic cleavage and subsequent release of 24–32 nucleotide-long single-stranded fragments. Here we manipulated the deoxyribose–phosphate backbone of DNA to analyze the mechanism by which damaged strands are discriminated as substrates for dual incision. We found that human NER is completely inactive on DNA duplexes containing single C4′-modified backbone residues. However, the same C4′ backbone variants, which by themselves do not perturb complementary hydrogen bonds, induced strong NER reactions when incorporated into short segments of mispaired bases. No oligonucleotide excision was detected when DNA contained abnormal base pairs without concomitant changes in deoxyribose–phosphate composition. Thus, neither C4′ backbone lesions nor improper base pairing stimulated human NER, but the combination of these two substrate alterations constituted an extremely potent signal for double DNA incision. In summary, we used C4′-modified backbone residues as molecular tools to dissect DNA damage recognition by human NER into separate components and identified a bipartite discrimination mechanism that requires changes in DNA chemistry with concurrent disruption of Watson–Crick base pairing.**

Nucleotide excision repair (NER) in mammals is carried out by the coordinated action of about 25 gene products (1, 2), many of which have been identified by characterization of the human cancer-prone disorder xeroderma pigmentosum (XP). Individuals afflicted by XP suffer from increased sensitivity to sunlight and exhibit a high incidence of skin cancer. Certain internal tumors also occur more frequently than in the general population (3, 4). XP patients are unable to repair ultraviolet (UV) radiation products and DNA adducts formed by chemical carcinogens because of a NER deficiency that results from mutations in one of seven genes, designated *XPA* through *XPG* (4–6).

Initiation of mammalian NER involves several multimeric factors, including a complex formed by XPA and the three subunits of replication protein A (7), the XPC–HHR23B heterodimer (8), and multiple subunits of transcription factor IIH, primarily XPB, XPD, p62, and p44 (9–11). Damage recognition and subsequent NER events mediated by these proteins are poorly understood but are thought to culminate in the formation of an unwound preincision complex flanked by single-stranded to double-stranded DNA junctions on either side of the targeted lesion (12, 13). Dual DNA incision at these junctions by structure-specific endonucleases (XPF–ERCC1 and XPG) generates excision products of 24–32 residues in length containing the damaged site (6, 14). DNA repair patches are then synthesized by DNA polymerase ε together with its accessory factors, and strand continuity is reestablished by DNA ligase I (2, 6).

DNA repair by oligonucleotide excision serves to eliminate a wide diversity of chemically dissimilar base adducts (4, 15). *In vitro* studies using human cell extracts have shown that the broad substrate range of NER extends to sites where a base is either lost (16) or replaced by synthetic organic derivatives such as a cholesterol moiety (17). This striking substrate versatility of mammalian NER prompted us to use DNA backbone modifications to dissect its damage recognition mechanism. As shown in Fig. 1, a series of novel backbone derivatives was constructed by linking a selenophenyl or a pivaloyl group to the C4′ position of single deoxyribose moieties or, alternatively, by inverting the stereochemistry at the C4′ position. This inverted deoxyribose residue may arise in cellular DNA as a product of C4′ radical attack following exposure to ionizing radiation and other sources of oxygen radicals (18), or by the action of bleomycin and related compounds (19).

Enzymatic probing with model DNA polymerases showed that oligomeric templates containing single C4′-modified backbone residues retain the correct hydrogen bonding pattern required for normal base pair complementarity (20). In this report, we show that such site-directed C4′ backbone modifications stimulate oligonucleotide excision in a NER-proficient human cell extract only when incorporated into short segments of mispaired bases. These results are consistent with a bipartite substrate discrimination mechanism in which disruption of complementary base pairing at sites of DNA damage constitutes an essential determinant of recognition. As a direct consequence of this damage recognition strategy, human NER is preferentially recruited to potentially mutagenic DNA lesions that destabilize hydrogen bonding interactions between complementary bases.

MATERIALS AND METHODS

Materials. Deoxynucleoside precursors containing C4′ selenophenyl or pivaloyl substitutions, or a C4′ residue with inverted configuration, were synthesized as described (20). All three C4′-modified deoxynucleosides were tritylated at their primary and phosphitylated at their secondary alcohol functions to produce building blocks for automated oligonucleotide synthesis. *N*-Acetoxy-2-acetylaminofluorene was purchased from the National Cancer Institute Chemical Carcinogen Repository. [γ-^{32}P]ATP (7,000 Ci/mmol; 1 Ci = 37 GBq) was from ICN. T4 polynucleotide kinase and T4 DNA ligase were from GIBCO/BRL.

Substrates. The 19-mer 5′-ACCACCCTT^{9}CG^{11}A^{12}ACCACAC-3′ was reacted with *N*-acetoxy-2-acetylaminofluorene to

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Abbreviations: AAF, acetylaminofluorene; NER, nucleotide excision repair; T_m, melting temperature; XP, xeroderma pigmentosum.

‡To whom reprint requests should be addressed.

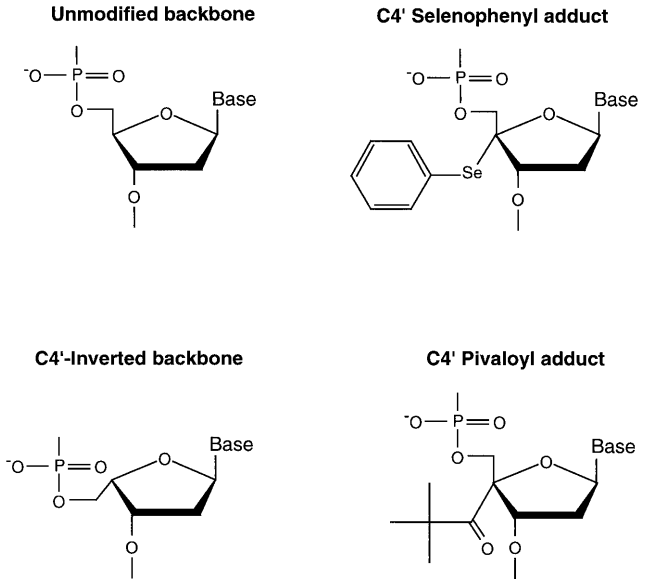

FIG. 1. Structure of C4′-modified backbone residues used in this study. A series of novel excision repair substrates was obtained by manipulating the C4′ position of a single deoxyribose moiety in the sugar–phosphate backbone.

form an acetylaminofluorene (AAF) adduct at the single guanine (G^{11}) residue (21). Oligonucleotides with the same sequence but containing either a C4′ selenophenyl adduct at position A^{12}, a C4′ inversion at the same position A^{12}, or a C4′ pivaloyl adduct at position T^9 were synthesized by the cyanoethyl phosphoramidite method using appropriate building blocks (20). Internally labeled double-stranded DNA fragments of 147 bp were constructed by ligating these 19-mer sequences with five other partially overlapping oligonucleotides as indicated in Fig. 2 (21). Sequence heterologies were constructed by appropriate base replacements in the unmodified strand.

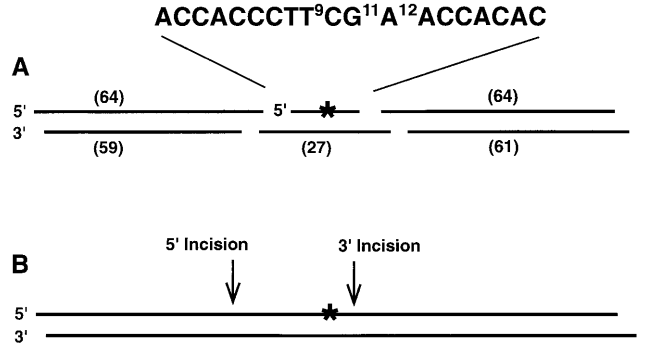

FIG. 2. Schematic representation of the oligonucleotide excision assay. (*A*) Internally labeled duplexes of 147 bp were assembled from six different oligonucleotides (their lengths are shown in parentheses). The central 19-mer contained one of the following modifications (indicated by the asterisk): an AAF–C^8–guanine adduct at position G^{11}, a C4′ selenophenyl or C4′ inversion in the backbone of position A^{12}, or a C4′ pivaloyl residue in the backbone of position T^9. Control substrate was constructed with unmodified 19-mers. Prior to ligation, the central 19-mer was 5′ end-labeled with ^{32}P[ATP], whereas the other five components were phosphorylated with nonradioactive ATP. Alternatively, the complementary 27-mer was radiolabeled to test strand selectivity of oligonucleotide excision. (*B*) After ligation and electrophoretic purification, the resulting 147-mer duplex substrate contained an internal radiolabel near the site-directed modification. The arrows indicate the expected major sites of dual DNA incision by human NER. Typically, this endonucleolytic cleavage reaction generates a ladder of radiolabeled excision products in the range of 24–32 nucleotides in length (7, 14, 16).

Cell-Free Extracts and Excision Repair Assay. Soluble extracts were prepared as described (22) from the following sources: HeLa, human XP-A (GM2250), the NER-proficient mouse cell lines F20 (23) and wild-type MEF (24), or the XPA-deficient mouse cell line MEF (XPA −/−) (24). Oligonucleotide excision assays were performed by incubating cell extract (50 μg of proteins) with internally labeled DNA fragments of 147 bp (4–6 fmol, ≈75,000 dpm) at 30°C. Reaction conditions were as described (21). The resulting excision products were resolved on 10% polyacrylamide denaturing gels, visualized by autoradiography, and subsequently analyzed by laser scanning densitometry (Molecular Dynamics Computing Densitometer with IMAGEQUANT software). NER activity is expressed as the percentage of excised substrate molecules after reactions of 40 min. The radiolabeled 19-mer was used as a size marker.

Temperature-Dependent UV Spectroscopy. Melting temperatures (T_m) of DNA duplexes (1.6 μM) consisting of the central 19-mer annealed to the corresponding 27-mer sequences (Fig. 2) were determined on a Perkin–Elmer Lambda 2 UV/VIS Spectrometer. Buffer conditions were equivalent to those employed in the excision assay.

RESULTS

Oligonucleotide Excision Reaction. NER activity was measured *in vitro* using the excision assay devised by Huang *et al.* (14, 16), who exploited the dual DNA incision pattern of human NER for analytical purposes. Site-specifically modified DNA substrates of 147 bp were constructed as illustrated in Fig. 2. Prior to ligation, the central 19-mer was labeled with ^{32}P[ATP] at its 5′ end, such that the resulting 147-mer duplex contained an internal radiolabel in the vicinity of the site-directed modification (Fig. 2). After purification, the double-stranded fragments were incubated with a standard NER-competent HeLa cell extract (14, 22) in the presence of ATP and all four deoxynucleoside triphosphates. Damage-specific dual DNA incision by human NER generates radioactive products of 24–32 nucleotides in length (6, 14, 16), which were resolved by denaturing gel electrophoresis and visualized by autoradiography.

In vitro repair reactions performed with linear 147-mer substrate containing a site-directed AAF–C^8–guanine adduct yielded specific excision products that migrated in the polyacrylamide gel as an oligomeric ladder with major lengths ranging from 25 to about 30 nucleotides (Fig. 3, lane 2). Low amounts of smaller fragments result from degradation of the main excision products by nucleases that are present in the crude cell-free extract (14, 16). This characteristic oligonucleotide excision pattern was highly reproducible when independent preparations of AAF-modified substrate or HeLa cell extract were tested. In contrast, no excision products were released from undamaged control substrates (Fig. 3, lanes 1 and 3), although intact 147-mer substrate as well as radioactive bands generated by nonspecific nuclease activity can be observed at similar levels at the top of the gel.

Initiation of Human NER at Selenophenyl or Inverted C4′ Backbone Residues. The representative polyacrylamide gel of Fig. 3 shows the lack of oligonucleotide excision products after incubation of DNA substrates containing either a C4′ selenophenyl-adducted or a C4′-inverted backbone residue with HeLa cell extract (lanes 8 and 5, respectively). These reaction products with C4′-modified substrates were indistinguishable from those obtained with unmodified control substrate (lanes 1 and 3). Surprisingly, human NER was able to catalyze high levels of oligonucleotide excision when these single C4′-modified backbone residues were incorporated into 3-nucleotide-long segments of mispaired bases (Fig. 3, lanes 7 and 10). The resulting excision products were of a similar size range as those induced by the AAF–C^8–guanine adduct, and contained

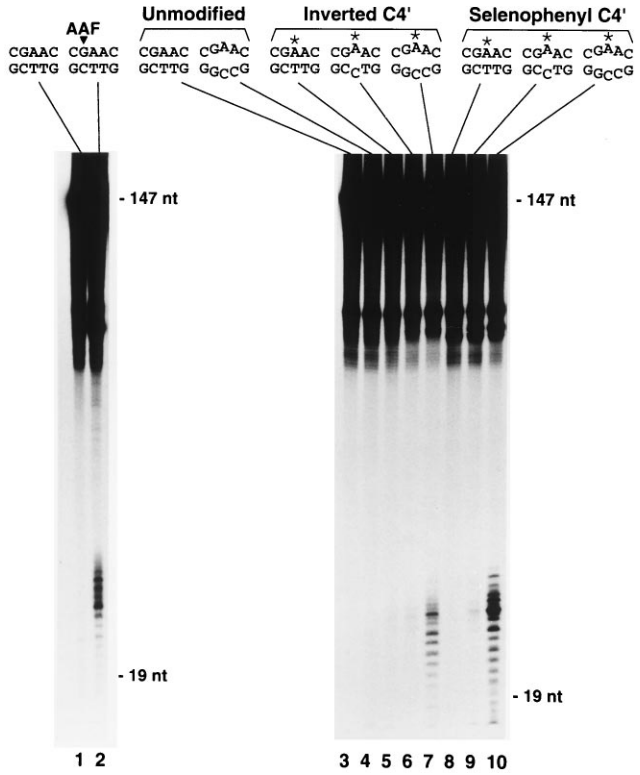

FIG. 3. Initiation of human NER at site-directed selenophenyl or inverted C4′ backbone modifications. Excision repair reactions in HeLa cell extract were performed for 40 min at 30°C. The tested lesions and their sequence context in the duplex substrate are indicated, with the asterisks denoting the site of C4′ backbone modification. The size of oligonucleotide excision products was estimated from a 19-mer marker that is identical to the unmodified 19-mer used to construct the substrates. Lane 2 shows a control reaction with a site-specific AAF–C^8–guanine lesion.

a major oligomeric component of 27–28 nucleotides in length. A weak but nevertheless detectable excision reaction was also observed when the same C4′-modified deoxyribose residues were combined with a single mismatch (Fig. 3, lanes 6 and 9). In contrast, 3-nucleotide-long sequence heterologies (lane 4) or single mispaired bases alone (Table 1) failed to induce detectable levels of NER activity.

Time course experiments in HeLa cell extract showed a progressive increase of oligonucleotide excision products when either a single C4′ selenophenyl adduct or a single C4′-inverted backbone residue were located within the 3-nucleotide-long mispaired sequence (Fig. 4, lanes 2, 5, and 8). Lower amounts of excision products accumulated in a time-dependent manner when the C4′ selenophenyl adduct or the C4′ inversion were combined with a single mismatch (Fig. 4, lanes 1, 4, and 7). In parallel control reactions, we consistently found that mispaired bases in the absence of C4′ substrate modifications failed to stimulate human NER (Fig. 4, lanes 3, 6, and 9).

Initiation of Human NER at a Single Pivaloyl C4′ Backbone Adduct. It appears that human NER is able to process C4′ modifications, but only when these backbone lesions are accompanied by concomitant disruption of base pairing. To support this conclusion, we tested another C4′ variant in a neighboring sequence context of our substrates. A fully complementary DNA duplex containing a site-specific C4′ pivaloyl adduct yielded no oligonucleotide excision in HeLa cell extract (Fig. 5, lane 3). However, NER was activated when the same C4′ pivaloyl adduct was combined with a 3-nucleotide-long sequence heterology (lanes 4 and 5). The resulting excision products were slightly shorter than those induced by AAF–C^8–guanine (lane 6) and consisted mainly of oligomers that ranged in size from 25 to 29 residues. In control reactions, we confirmed that DNA duplexes containing three mispaired bases alone (Fig. 5, lane 2) failed to stimulate oligonucleotide excision. NER activity in response to the C4′ pivaloyl modification within sites of improper base pairing was also determined in time course experiments (Fig. 6). The strand selectivity of NER in HeLa cell extract was established using DNA duplexes in which the unmodified partner strand was radiolabeled opposite to a base or backbone lesion. For that purpose, the central 27-mer oligonucleotide complementary to the modified 19-mer was 5′ end-labeled with ^{32}P[ATP] prior to annealing and ligation, as indicated in Fig. 2. Consistent with previous reports (25, 26), we observed that excision repair of substrates containing either an AAF–C^8–guanine adduct or a combination of C4′ pivaloyl adduct with three mismatches was restricted to the modified strand. In additional control experiments, we detected excision products of a nearly identical oligonucleotide pattern when the C4′ pivaloyl adduct was located within mispaired bases and incubated in NER-competent extracts from the mouse fibroblast cell lines F20 or wild-type MEF. Importantly, this lesion was not excised in extracts prepared from NER-deficient human XP-A or mouse MEF (XPA -/-) cells (gels not shown).

Bipartite Substrate Discrimination. NER activity was quantified by monitoring the percentage of excised substrates using laser scanning densitometry (Table 1). In parallel, the substrates were characterized by assessing their T_ms to confirm the extent of base pair destabilization (Table 1). These evaluations demonstrate that neither C4′ backbone variants nor sites of mispaired bases stimulated oligonucleotide excision to levels that exceeded background radioactivity resulting from non-specific degradation (on the average ≈0.05% of total substrate). Huang *et al.* (16) reported low levels (0.05–0.2%) of excision when duplexes containing purine–purine mismatches were incubated with HeLa cell extract and, in this study, we found no detectable excision of mismatches involving mixed purine–pyrimidine bases or multiple bases. In contrast, high levels of excision repair activity (up to 12.53% of excised substrate molecules in 40 min) were observed when C4′ backbone modifications were combined with improper base pairing, indicating a dual requirement for covalent modifications of DNA chemistry with concomitant disruption of its normal base pair configuration.

DISCUSSION

Efficient substrates of mammalian NER such as AAF–C^8–guanine adducts have been found to consist of covalent base modifications that destabilize hydrogen bonding between complementary bases, for example by extrahelical displacement of the adducted base (27, 28). Additionally, we observed that excision of AAF lesions is stimulated about 2.5-fold by insertion of a single site of abnormal Watson–Crick hydrogen bonding near the modification (M.T.H. and H.N., unpublished results). This finding was confirmed by recent studies showing that NER activity in response to UV radiation products or cisplatin adducts is increased when these lesions are combined with mismatches (26, 29). In this report, we used C4′ backbone alterations as model lesions to uncouple covalent DNA modifications from their destabilizing effects on DNA secondary structure and demonstrate an absolute requirement for improper base pairing during the damage recognition process of human NER.

Crystal and solution structure analysis of C4′-modified nucleosides or their respective nucleotides revealed that these residues adopt essentially the same conformation as unmodified nucleotides in double-stranded DNA (30, 31). Also, C4′ modifications appear to exert minimal effects on the hydrogen bonding geometry that mediates complementary base pairing,

Table 1. Summary of substrates and oligonucleotide excision reactions

Sequence context†	Covalent C4′ modification	NER activity‡	ΔT_m of 19-mer duplexes§
* CTTCGAAC GAAGCTTG	C4′ selenophenyl adduct	≤0.05%	−0.9°C
* CTTCG^{A}AC GAAGC$_{C}$TG	C4′ selenophenyl adduct	1.01 ± 0.11%	−9.0°C
* CTTC^{G}A$_{A}$C GAAG$_{G}$C$_{C}$G	C4′ selenophenyl adduct	7.34 ± 0.88%	−15.6°C
* CTTCGAAC GAAGCTTG	C4′ inversion	≤0.05%	−1.2°C
* CTTCG^{A}AC GAAGC$_{C}$TG	C4′ inversion	0.46 ± 0.08%	−7.5°C
* CTTC^{G}A$_{A}$C GAAG$_{G}$C$_{C}$G	C4′ inversion	4.55 ± 0.37%	−15.3°C
CTTCG^{A}AC GAAGC$_{C}$TG	None	≤0.05%	−8.3°C
CTTC^{G}A$_{A}$C GAAG$_{G}$C$_{C}$G	None	≤0.05%	−15.8°C
* CTTCGAAC GAAGCTTG	C4′ pivaloyl adduct	≤0.05%	−1.0°C
* C^{T}T$_{C}$GAAC G$_{T}$T$_{T}$CTTG	C4′ pivaloyl adduct	12.53 ± 2.93%	−14.2°C
C^{T}T$_{C}$GAAC G$_{T}$T$_{T}$CTTG	None	≤0.05%	−14.4°C

†Asterisks indicate the sites of C4′ backbone modification
‡Fractions of excised fragments after 40 min at 30°C. Mean values of 4–7 determinations ±SD. The AAF–C^{8}–guanine adduct in the same sequence yielded 3.99 ± 1.20% excised duplexes.
§Changes in T_m of the central 19-mer duplex were determined as indicated in *Materials and Methods*. T_m of the unmodified duplex was 66.5°C.

presumably because of their particular localization diametrically opposite to the α-glycosylic bond that links DNA bases to the corresponding deoxyribose moieties (Fig. 2). In fact, enzymatic probing with Klenow fragment or a 3′–5′ exonuclease-deficient mutant showed that the coding ability of template bases is not disturbed by C4′ deoxyribose manipulations, indicating intact base pairing fidelity opposite such backbone lesions (20). This conclusion is supported by the observation that single C4′ modifications induce only marginal changes in the T_m of 19-mer DNA duplexes. Similarly, C4′ variants do not further depress the T_m of duplexes containing mispaired bases (Table 1).

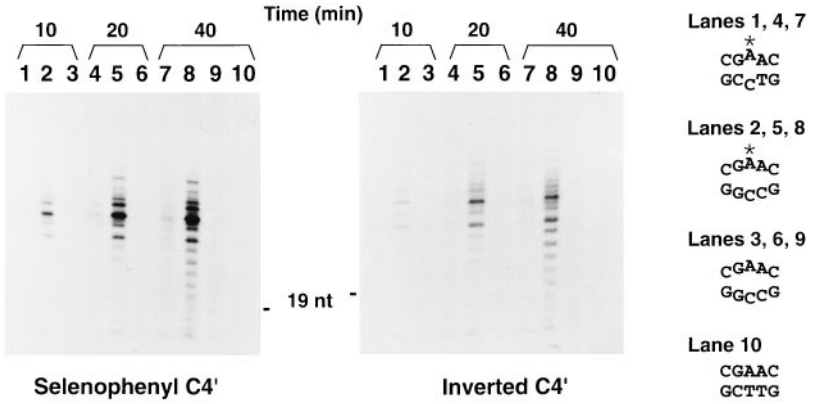

FIG. 4. Time course of oligonucleotide excision observed when a site-directed selenophenyl or inverted C4′ backbone modification were incorporated within sites of one (lanes 1, 4, and 7) or three mispaired bases (lanes 2, 5, and 8). Control reactions contained substrates with the three mispaired bases but no C4′ backbone lesion (lanes 3, 6, and 9). Only the bottom part of the gel is shown.

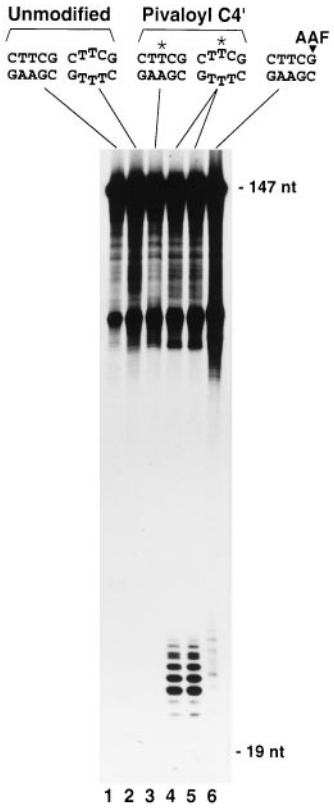

FIG. 5. Initiation of human NER at a site-directed pivaloyl C4′ adduct. Excision repair reactions in HeLa cell extract were performed for 40 min at 30°C. The sequence context is indicated, with the asterisks denoting the site of C4′ backbone modification. Lanes 4 and 5 show results obtained with two independent substrate preparations. The size of oligonucleotide excision products was estimated from the same 19-mer marker used in the experiments of Fig. 3. For comparison, lane 6 represents a control reaction with the site-directed AAF–C^8–guanine adduct.

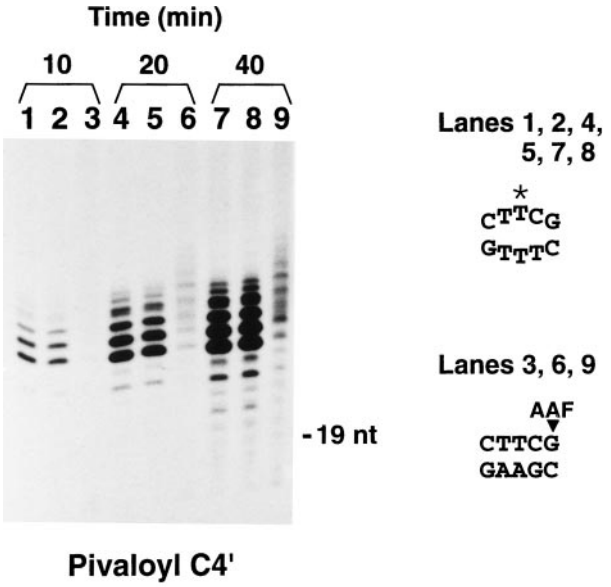

FIG. 6. Time course of NER activity in response to a pivaloyl C4′ backbone adduct situated within sites of three mispaired bases (duplicates of independent substrate preparations). Lanes 3, 6, and 9 show a time course performed with AAF-damaged substrate. Only the bottom part of the gel containing the excision products is shown.

This report identifies two separate determinants for effective DNA incision, as neither the tested C4′ variants nor short segments of mispaired bases were processed by human NER *in vitro*. In combination, however, C4′ backbone lesions situated within mispaired bases triggered strong NER reactions, thereby generating standard oligonucleotide excision patterns. Thus, endonucleolytic cleavage by human NER depends on a bipartite discrimination mechanism that requires altered deoxyribonucleotide chemistry along with concurrent conformational changes that derange normal base pairing configurations. The identification of these specific determinants of DNA damage recognition has multiple implications. First, it provides a rational basis for educated predictions on the repairability of carcinogen–DNA adducts, at least in those cases where their effects on base pairing interactions have been established by conformational analysis. Second, the bipartite mode of substrate discrimination accounts for the failure of intrinsically thermolabile sequences such as poly(dT) tracts or base mismatches to induce significant NER responses (16). Third, the bipartite mode of substrate discrimination is different from the mechanism of DNA damage recognition in base excision repair, where DNA glycosylases recognize damaged bases without a strict requirement for duplex destabilization (32), or mismatch repair, which is active at sites of mispaired bases in the absence of covalent alterations of DNA constituents (33). Finally, the identification of two separate determinants of damage recognition indicates that two distinct sensors of DNA damage may coexist in mammalian NER. A subset of known nucleotide excision repair factors consisting of XPA and replication protein A have been shown to bind preferentially to damaged DNA (7, 34) and are potential candidates for probing the stability of Watson–Crick base pairs. These proteins may exploit their single-stranded DNA binding activity (7, 35) to promote the assembly of preincision complexes preferentially at sites of ruptured base pairing. The paradigm of NER in *Escherichia coli* suggests, on the other hand, that enzymes possessing DNA-dependent ATPase activity (possibly XPB and XPD) may serve to probe the chemical integrity of DNA substrates (36, 37). A hypothetical scenario includes stalling of a DNA helicase component of transcription factor IIH at sites of covalent damage. This mechanism is suggested by experiments on Rad3 protein (the conserved *Saccharomyces cerevisiae* homolog of XPD), showing that this particular DNA helicase is extraordinarily sensitive to covalent modifications affecting the bases (38) or the DNA backbone (39). After arresting its DNA helicase activity, Rad3 protein interacts stably with damaged strands (38) and, therefore, provides a possible molecular basis for the formation of damage- and strand-specific preincision complexes.

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1. Mu, D., Park, C.-H., Matsunaga, T., Hsu, D. S., Reardon, J. T. & Sancar, A. (1995) *J. Biol. Chem.* **270,** 2415–2418.
2. Aboussekhra, A., Biggerstaff, M., Shivji, M. K. K., Vilpo, J. A., Moncollin, V., Podust, V. A., Protic, M., Hübscher, U., Egly, J.-M. & Wood, R. D. (1995) *Cell* **80,** 859–868.
3. Kraemer, K. H., Lee, M.-M., Andrews, A. D. & Lambert, W. C. (1994) *Arch. Dermatol.* **130,** 1018–1021.
4. Friedberg, E. C., Walker, G. C. & Siede, W. (1995) *DNA Repair and Mutagenesis* (Am. Soc. Microbiol., Washington, DC), pp. 283–365.
5. Hoeijmakers, J. H. J. (1994) *Eur. J. Cancer* **30,** 1912–1921.
6. Sancar, A. (1996) *Annu. Rev. Biochem.* **65,** 43–81.
7. He, Z., Henricksen, L. A., Wold, M. S. & Ingles, C. J. (1995) *Nature (London)* **374,** 566–569.
8. Masutani, C., Sugasawa, K., Yanagisawa, J., Sonoyama, T., Ui, M., Enomoto, T., Takio, K., Tanaka, K., van der Spek, P. J.,

Bootsma, D., Hoeijmakers, J. H. J. & Hanaoka, F. (1994) *EMBO J.* **13,** 1831–1843.
9. Schaeffer, L., Roy, R., Humbert, S., Moncollin, V., Vermeulen, W., Hoeijmakers, J. H. J., Chambon, P. & Egly, J.-M. (1993) *Science* **260,** 58–63.
10. Drapkin, R., Reardon, J. T., Ansari, A., Huang, J.-C., Zawel, L., Ahn, K. J., Sancar, A. & Reinberg, D. (1994) *Nature (London)* **368,** 769–772.
11. Sung, P., Guzder, S. N., Prakash, L. & Prakash, S. (1996) *J. Biol. Chem.* **271,** 10821–10826.
12. O'Donovan, A., Davies, A. A., Moggs, J. G., West, S. C. & Wood, R. D. (1994) *Nature (London)* **371,** 432–435.
13. Matsunaga, T., Park, C.-H., Bessho, T., Mu, D. & Sancar, A. (1996) *J. Biol. Chem.* **271,** 11047–11050.
14. Huang, J.-C., Svoboda, D. L., Reardon, J. T. & Sancar, A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 3664–3668.
15. Hanawalt, P. C. (1994) *Environ. Mol. Mutagen.* **23,** Suppl. 24, 78–85.
16. Huang, J.-C., Hsu, D. S., Kazantsev, A. & Sancar, A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 12213–12217.
17. Mu, D., Hsu, D. S. & Sancar, A. (1996) *J. Biol. Chem.* **271,** 8285–8294.
18. Akhlaq, M. S., Schuchmann, H.-P. & von Sonntag, C. (1987) *Int. J. Radiat. Biol.* **51,** 91–102.
19. Stubbe, J., Kozarich, J. W., Wu., W. & Vanderwall, D. E. (1996) *Acc. Chem. Res.* **29,** 322–330.
20. Hess, M. T., Schwitter, U., Petretta, M., Giese, B. & Naegeli, H. (1997) *Biochemistry* **36,** 2332–2337.
21. Hess, M. T., Schwitter, U., Petretta, M., Giese, B. & Naegeli, H. (1996) *Chem. Biol.* **3,** 121–128.
22. Wood, R. D., Robins, P. & Lindahl, T. (1988) *Cell* **53,** 97–106.
23. Wang, Z.-Q., Auer, B., Singl, L., Berghammer, H., Haidacher, D., Schweiger, M. & Wagner, E. F. (1995) *Genes Dev.* **9,** 509–520.
24. de Vries, A., van Oostrom, C. T. M., Hofhuis, F. M. A., Dortant, P. M., Berg, R. J. W., de Gruijl, F. R., Wester, P. W., van Kreijl, C. F., Capel, P. J. A., van Steeg, H. & Verbeek, S. J. (1995) *Nature (London)* **377,** 169–172.
25. Sancar, A. (1995) *J. Biol. Chem.* **270,** 15915–15918.
26. Moggs, J. G., Szymkovski, D. E., Yamada, M., Karran, P. & Wood, R. D. (1997) *Nucleic Acids Res.* **25,** 480–490.
27. Gunz, D., Hess, M. T. & Naegeli, H. (1996) *J. Biol. Chem.* **271,** 25089–25098.
28. O'Handley, S. F., Sanford, D. G., Xu, R., Lester, C. C., Hingerty, B. E., Broyde, S. & Krugh, T. R. (1993) *Biochemistry* **32,** 2481–2497.
29. Mu, D., Tursun, M., Duckett, D. R., Drummond, J. T., Modrich, P. & Sancar, A. (1997) *Mol. Cell. Biol.* **17,** 760–769.
30. Marx, A., Erdmann, P., Senn, M., Körner, S., Jungo, T., Petretta, M., Imwinkelried, P., Dussy, A., Kulicke, K. J., Macko, L., Zehnder, M. & Giese, B. (1996) *Helv. Chim. Acta* **79,** 1980–1994.
31. Marx, A., MacWilliams, M. P., Bickle, T. A., Schwitter, U. & Giese, B. (1997) *J. Am. Chem. Soc.* **119,** 1131–1132.
32. Slupphaug, G., Eftedal, I., Kavli, B., Bharati, S., Helle, N. M., Haug, T., Levin, D. W. & Krokan, H. E. (1995) *Biochemistry* **34,** 128–138.
33. Modrich, P. (1991) *Annu. Rev. Genet.* **25,** 229–253.
34. Li, L., Lu, X., Peterson, C. A. & Legerski, R. J. (1995) *Mol. Cell. Biol.* **15,** 5396–5402.
35. Jones, C. J. & Wood, R. D. (1993) *Biochemistry* **32,** 12096–12104.
36. Grossman, L. & Thiagalingam, S. (1993) *J. Biol. Chem.* **268,** 16871–16874.
37. Lin, J.-J. & Sancar, A. (1992) *Mol. Microbiol.* **6,** 2219–2224.
38. Naegeli, H., Bardwell, L. & Friedberg, E. C. (1992) *J. Biol. Chem.* **267,** 392–398.
39. Naegeli, H., Bardwell, L., Harosh, I. & Friedberg, E. C. (1992) *J. Biol. Chem.* **267,** 7839–7844.