and amino acid. Crystallographic measurements at 3.1 Å resolution have shown the conformation of ATP soaked into native crystals of *T. thermophilus* seryl-tRNA synthetase and its analog AMPPCP soaked into crystals of the complex with $tRNA^{Ser}$ (*13*). In both cases the triphosphate moiety of ATP was in an extended conformation with the β-phosphate in a position incompatible with the simultaneous binding of the serine as observed in the adenylate complexes. This observation suggests that the initial conformation of the triphosphate in the presence of magnesium and the amino acid is different. This hypothesis is supported by comparison of the two independent active sites in the SerHx-AMP complex. In one active site, a SerHx-AMP molecule is observed as described above. In the other active site the electron density is less well defined but can be interpreted as being a superposition of ATP in a bent conformation and the adenylate product. Recent data at 2.4 Å resolution of a Mn^{2+}-ATP complex with the native synthetase shows the ATP in the bent conformation with the γ-phosphate interacting with the universally conserved Arg^{386} in motif 3 (*28*). The Mn^{2+} ion is coordinated by the ATP α- and β-phosphates as well as Glu^{345} and Ser^{348}, both residues being generally functionally conserved in class 2 synthetases (Fig. 5). This conformation of the ATP, when superimposed on that of the adenylate, is very suggestive of an in-line displacement mechanism for serine activation.

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Slow Repair of Pyrimidine Dimers at *p53* Mutation Hotspots in Skin Cancer

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Ultraviolet light has been linked with the development of human skin cancers. Such cancers often exhibit mutations in the *p53* tumor suppressor gene. Ligation-mediated polymerase chain reaction was used to analyze at nucleotide resolution the repair of cyclobutane pyrimidine dimers along the *p53* gene in ultraviolet-irradiated human fibroblasts. Repair rates at individual nucleotides were highly variable and sequence-dependent. Slow repair was seen at seven of eight positions frequently mutated in skin cancer, suggesting that repair efficiency may strongly contribute to the mutation spectrum in a cancer-associated gene.

Strong experimental and epidemiological evidence links ultraviolet (UV) irradiation to the development of human skin cancer (*1*, *2*). Mutations in the *p53* tumor suppressor gene have been found in a large percentage of such cancers (*3*), most being localized in exons 5 through 9, which contain conserved sequence blocks (*4*). The predominant mutations are C→T transitions and CC→TT double transitions at dipyrimidine sequences, base alterations specifically induced by UV light (*5*). These findings have implicated the *p53* gene as a critical target in UV-related malignancies.

One important step in the prevention of tumor formation is very likely the efficient removal of DNA lesions by cellular DNA repair enzymes. Repair of UV-induced lesions in mammalian cells has been studied at the level of the gene by Southern (DNA) blot techniques (*6*). It was found that repair is gene-specific (*6*) and is most efficient on the transcribed strand of active genes (*7*) because of transcription-repair coupling (*8*). Here we determine the repair rates of UV-induced cyclobu-

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Table 1. Repair of CPDs in the human *p53* gene. Repair rates were measured at mutation hotspots in human skin cancer (M) and at surrounding positions. Each position was analyzed three times; codons 278 and 289 were analyzed twice. Results are average values with variation between experiments being about 10 to 25%. The percentage of CPDs after 24 and 48 hours was calculated from the ratio of the band intensity at those times to the band intensity at 0 hour (*20*).

DNA strand	Codon	Sequence (5′→3′)	CPDs (%) remaining after 24 hours	CPDs (%) remaining after 48 hours
Nontranscribed	151 (M)	C^CC	58	37
	177 (M)	C^CC	95	94
	191	CC^T	26	4
	194	CT^T	36	25
	195	AT^C	13	5
	196 (M)	C^CGA	67	62
	278 (M)	T^CCT	100	12
	289	CT^C	10	5
Transcribed	243	C^CAT	11	5
	245 (M)	GC^C	56	26
	248 (M)	C^CG	30	21
	249	CCT^C	6	3
	285	CT^C	2	1
	286 (M)	TT^C	23	18
	287	CT^C	3	3
	288	ATT^C	3	3
	291	CT^T	29	18
	292	TT^T	4	5
	294 (M)	CT^C	30	28

tane pyrimidine dimers (CPDs) at single nucleotide resolution along human *p53* exons by ligation-mediated polymerase chain reaction (LMPCR) (*9*, *10*).

Human diploid skin fibroblasts were grown as contact-inhibited monolayers and were UV-irradiated at a dose of 12 J/m^2. After incubation to allow DNA repair, DNA was isolated and cleaved with T4 endonuclease V to generate single-strand breaks at CPDs and then digested with *Escherichia coli* photolyase to generate ligatable ends (*11*). Repair of CPDs in the total genome was analyzed by separation of T4 endonuclease V–cleaved DNA fragments on alkaline agarose gels. Repair was >80% complete after 48 hours (*12*). Northern (RNA) blot analysis showed that the *p53* gene was expressed in fibroblasts; a slight (<15%) decrease of mRNA amounts was observed after UV irradiation (*12*).

We used LMPCR (*13*) to map the repair of UV-induced CPDs in the *p53* gene (*14*). In our analysis of repair of CPDs along exon 9 and flanking intron sequences (Fig. 1), the initial distribution of CPDs at adjacent pyrimidines is seen in the 0 hour lanes. Repair of CPDs at a particular site is indicated by a progressive decrease in the intensity of the corresponding gel band. The average repair rate in *p53* exons 5 through 9 was comparable to repair rates for total genomic DNA (*12*) but considerably slower than repair of the transcribed strand of the housekeeping gene *PGK1*, encoding phosphoglycerate kinase 1 (*13*). The transcribed strand (Fig. 1A) of the *p53* gene was more rapidly repaired than the nontranscribed strand (Fig. 1B), in agreement with results obtained by Southern blotting (*15*). On the transcribed strand (Fig. 1A), repair at many sites became apparent after 6 hours, whereas on the nontranscribed strand (Fig. 1B), repair at most CPDs was seen only after 24 hours. Unexpectedly, however, repair efficiency varied among dipyrimidine sites, and these differences were reproducible when we used a different UV dose (20 J/m^2) (*12*). At some positions, CPDs were 70 to 95% repaired after 24 hours (Fig. 1A, bands 1 to 3). At other dipyrimidine sites, however, repair was

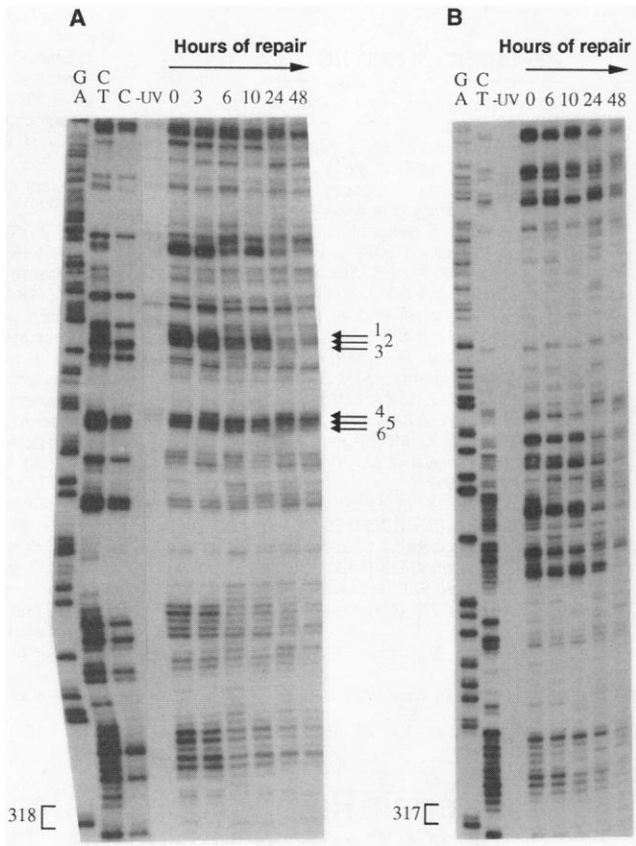

Fig. 1. Repair of UV-induced CPDs along exon 9 and flanking intron sequences of the *p53* gene. (**A**) Transcribed strand and (**B**) nontranscribed strand. Lanes labeled G+A, C+T, and C contain the products of Maxam-Gilbert sequencing reactions (*19*) done on HeLa cell DNA. The arrows indicate different repair rates at positions 1 to 3 versus 4 to 6. Codon numbers are given at the bottom.

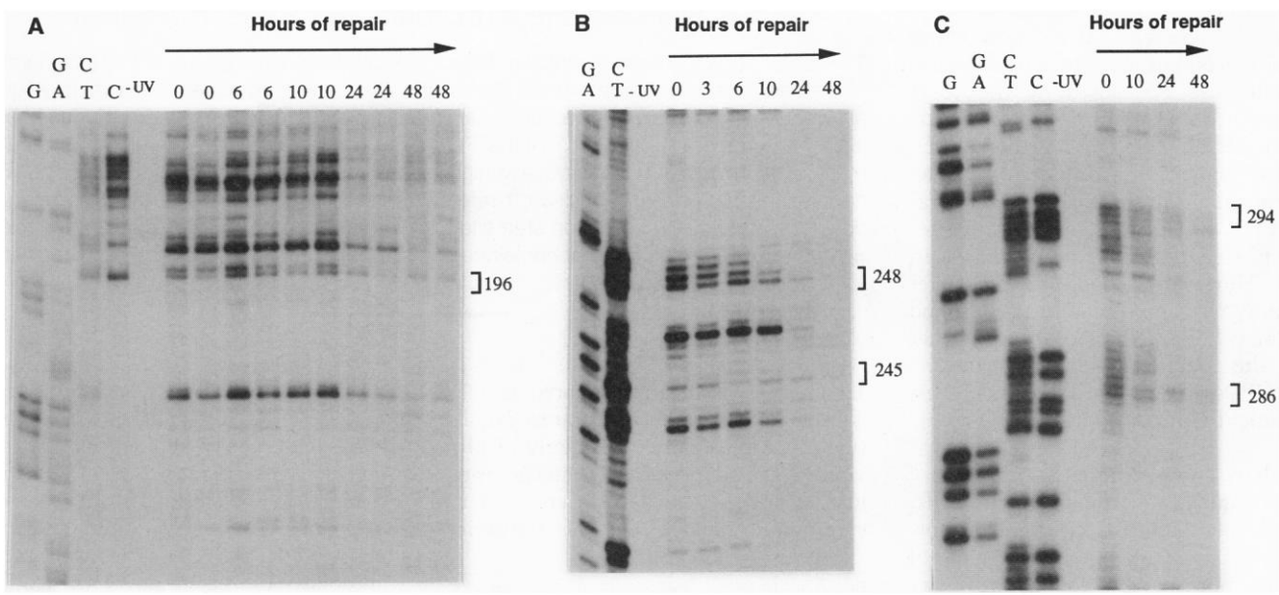

Fig. 2. Repair of CPDs at skin cancer mutation hotspots in the *p53* gene. (**A**) Exon 6, nontranscribed strand; (**B**) exon 7, transcribed strand; and (**C**) exon 8, transcribed strand. Codons that contain mutation hotspots are shown to the right.

almost absent, even after 48 hours (Fig. 1A, bands 4 to 6).

Previously, we analyzed the initial damage distribution after high-dose UV irradiation at *p53* codons frequently mutated in skin malignancies (*16*). Mutations are produced when DNA lesions are encountered by DNA polymerase during DNA replication. Thus, the mutation frequency depends not only on initial damage frequency, but also on the repair rate for each individual lesion. To study the relation between repair and mutation hotspots, we examined the repair efficiency at *p53* codons 151, 177, 196, 245, 248, 278, 286, and 294—positions that are frequently mutated in human skin cancers (*3*). The CPD signal at these positions was sufficient to allow repair rates to be analyzed at 12 J/m^2. We found that, with the possible exception of position 151, which is repaired at a similar rate as most neighboring positions, all CPDs at mutation hotspots were repaired more slowly than those at surrounding positions on the same strand (Fig. 2 and Table 1). Densitometric quantitation (Table 1) revealed that CPDs on the nontranscribed strand at codons 177, 196, and 278 were only 0 to 33% repaired after 24 hours, whereas other CPDs that are not at mutation hotspots were 70 to 90% repaired at that time. The CPDs at codons 177 and 196 were still less than 40% repaired after 48 hours (Fig. 2A and Table 1). On the transcribed strand, between 23 and 56% of the CPDs remained at codons 245, 248, 286, and 294 after 24 hours, whereas adjacent positions on the same strand in the same exon were almost completely repaired (Fig. 2, B and C, and Table 1).

Mutations induced by UV are generally targeted to dipyrimidine sequences (*1, 5, 17*). CPDs represent 70 to 80% of all photoproducts, but mutations in skin cancer could also be caused by the (6-4) photoproducts. The (6-4) photoproducts are generally repaired faster than CPDs (*18*) and appear less likely to be the principal mutagenic lesion after UV irradiation (*1*). Some of the slow repair sites in the *p53* gene are not mutated in skin cancer. However, not all base changes necessarily result in a mutant protein and there may be a selection pressure that affects the mutation spectrum. Furthermore, CPDs containing T are much less mutagenic than those containing C (*1, 17*).

We have shown that excision repair of CPDs is sequence-specific in human cells. The molecular basis of excision repair slow spots is not known. These slow spots of repair do not share a common primary sequence. Excision repair in human cells is initiated on chromatin but not on naked DNA. Gao and co-workers (*13*) have shown that some slowly repaired sites are within transcription factor binding sites. Transcription factors are not expected to bind to *p53* exons, as these sequences are more likely to be associated with nucleosomes. Chromatin folding may impede repair efficiency at certain sequence positions while allowing higher repair rates at others.

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14. After sequential treatment with T4 endonuclease V and photolyase (*11*), the DNA fragments [1.5 μg, quantified by DAPI (4′,6-diamidino-2-phenylindole dihydrochloride) fluorimetry] were subjected to LM-PCR. Synthetic primers specific for the human *p53* gene were as in (*16*). Primer extension, ligation of the linker, PCR (21 cycles), and gel electrophoresis were carried out as in (*10, 16*).
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20. Quantitation was done by video densitometry or phosphoimaging. Most reported mutations (*3*) are at the 3′ pyrimidine of the listed dimer position. Mutations within a run of pyrimidines could, in theory, be caused by two different dimers. However, in most cases mutations were found at the 3′ base of a dipyrimidine sequence [H. C. Hsia, J. S. Lebkowski, P.-M. Leong, M. P. Calos, J. H. Miller, *J. Mol. Biol.* **205**, 103 (1989); J. D. Armstrong and B. A. Kunz, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 9005 (1990); (*17*)]. This observation was taken into account when listing individual dimers in Table 1.

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DNA Repair Rates Mapped Along the Human *PGK1* Gene at Nucleotide Resolution

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The repair of cyclobutane pyrimidine dimers (CPDs), DNA lesions induced by ultraviolet light, was studied at nucleotide resolution. Human fibroblasts were irradiated with ultraviolet light and allowed to repair. The DNA was enzymatically cleaved at the CPDs, and the induced breaks along the promoter and exon 1 of the *PGK1* gene were mapped by ligation-mediated polymerase chain reaction. Repair rates within the nontranscribed strand varied as much as 15-fold, depending on nucleotide position. Preferential repair of the transcribed strand began just downstream of the transcription start site but was most pronounced beginning at nucleotide +140 in exon 1. The promoter contained two slowly repaired regions that coincided with two transcription factor binding sites.

In both prokaryotes and eukaryotes, DNA is repaired at nonuniform rates. Nucleotide excision repair, particularly of ultraviolet (UV)-induced DNA damage, is rapid in domains near active genes (*1*) and even more rapid along the transcribed strand of active genes (*2*). We have been studying the incidence and repair of CPDs, the most frequent class of UV-induced DNA damage. In previous work, we mapped CPD lesions in the 5′ region of the human *PGK1* (phosphoglycerate kinase 1) gene. Specifically, we irradiated fibroblasts or naked DNA with a lethal dose (1000 J/m^2) of UV light (254 nm) and determined the CPD frequency at

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