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UV-induced mutagenesis of human *p53* in a vector replicated in *Saccharomyces cerevisiae*

(mutation spectrum/ultraviolet light/plasmid/shuttle vector/yeast)

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ABSTRACT **Mutation of the *p53* tumor suppressor gene is the most common genetic alteration identified to date in human cancers. Similarities of *p53* mutations found in human cancers with those induced in experimental systems have been interpreted as evidence supporting a causative role for environmental carcinogens in certain tumor types. We have developed and validated a method for generation of mutation spectra and measurement of mutation frequency directly on human *p53* cDNA in a vector following treatment with mutagens and replication in yeast. Mutants that had lost the DNA binding/transcription activation function of p53 were detected by yeast colony color, isolated, and sequenced. UV light was used to characterize and validate the system, and a dose-dependent increase in mutation frequency was seen following exposure of the plasmid to increasing doses of UV, resulting in an 18-fold increase over the spontaneous frequency (3.2×10^{-4}) at the highest level tested (300 J/m^2). Sequence analysis of *p53* in the mutants revealed that the types of mutations induced were similar to those obtained in previous studies of UV mutagenesis in other model systems, and the types and positions of mutations were also similar to those found in human skin tumors. This experimental system will be useful in further evaluation of the importance of environmental agents as risk factors for cancer.**

Epidemiologic evidence has demonstrated associations between exposure to certain environmental agents and the elevated risk of specific cancers. Well-documented examples include exposure to ultraviolet light as a major risk factor for skin cancers, cigarette smoke for lung cancers, and aflatoxin and hepatitis B virus for hepatocellular carcinoma. More recent investigations have identified specific genetic changes in tumors from exposed individuals that suggest, on a molecular level, mechanisms through which these agents may contribute to the carcinogenic process. A notable finding is that alterations in the *p53* tumor suppressor gene are common in a variety of human tumors believed to be caused by exposure to exogenous carcinogens (reviewed in ref. 1). The significance of this association lies in the fact that mutation of *p53* is thought to play a central role in the development of cancer by inactivating one or more of the growth suppression/genome maintenance functions of its protein, including cell cycle arrest and apoptosis following DNA damage (reviewed in refs. 2 and 3).

The *p53* mutation spectra present in various tumor types have been compared with specific carcinogen-induced spectra of selectable genes such as *supF* and *hprt* in experimental systems, and similarities have been interpreted as indicating that mutations detected in tumors could have been induced as a result of carcinogen–DNA adduction. For example, the consensus pattern of mutations induced by UV in various model systems, namely the predominance of GC to AT transitions at dipyrimidines and the existence of tandem CC to TT mutations, has led many to conclude that UV induces mutations in *p53* as an important step in the development of skin cancer (reviewed in ref. 4). However, since DNA sequence context is known to play a substantial role in the mutation spectrum generated in target genes (ref. 5 and references therein), the validity of the extrapolation of mutation data produced in selectable genes of experimental systems to those found in endogenous *p53* of human tumors may be compromised, especially in cases where an agent does not create a well-defined mutational pattern. We therefore sought to develop and validate an experimental system in which mutations induced by agents thought to be important as cancer risk factors could be characterized directly in the *p53* gene, thus circumventing concerns about sequence context.

With that objective, we adapted a previously described yeast functional assay, which distinguishes wild-type and mutant p53 (6–8), to generate a UV-induced mutation spectrum of the gene. UV light was used in these initial experiments to validate the experimental system because this agent has been extensively studied in numerous other models and the types of mutations induced correlate well with those found in skin tumors. The data are compared with those of previous studies and with mutations identified in human skin cancers to provide additional evidence concerning the possible role of UV in the induction of *p53* mutations of this tumor type.

MATERIALS AND METHODS

Plasmids and UV Treatment. The p53 expression vectors are 9.2-kb plasmids containing human *p53* cDNA under the yeast *ADH1* constitutive promoter, the *LEU2* gene for selection in yeast, centromeric and autonomously replicating sequences for stable low copy replication in yeast, and sequences necessary for propagation in bacteria. The vectors, pLS76 wt (wild-type p53) and pLS76 273H (mutant p53; arginine to histidine substitution at codon 273), were gifts from S. Friend (7). They were amplified in *Escherichia coli* strain DH5α, and large scale preparations were isolated using Qiagen plasmid purification columns as recommended (Chatsworth, CA).

For UV treatment, pLS76 wt (20 μg dissolved in water at a concentration of 8 ng/μl) was spread in thin layers in 60-mm Petri dishes and exposed to 254 nm UV light generated from a germicidal lamp. Radiant flux was measured using a UVX Radiometer (UVP, San Gabriel, CA). Following exposure, DNA was concentrated with a Centricon 30 (Amicon), ethanol precipitated, and dissolved at a concentration of 100–200 ng/μl in TE buffer (10 mM Tris·Cl/1 mM EDTA) (pH 7.5).

Yeast Culture and Transformation. yIG397, the *Saccharomyces cerevisiae* strain suitable for p53 mutant selection,

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genotype *MAT***a** *ade2-1 leu2-3,112 trp1-1 his3-11,15 can1-100 ura3-1 URA3* 3XRGC::p*CYC1*::*ADE2*, was kindly provided by R. Iggo (8). Standard techniques for yeast culture were used as described (9). Cultures were routinely grown in yeast extract/peptone/dextrose (YPD) liquid or solid medium supplemented with 200 μg/ml adenine (YPD + Ade) to avoid selection of *ADE2*$^+$ revertants. Selection of pLS76 transformants and p53 mutants was performed on solid complete minimal medium (CM) containing 0.67% yeast nitrogen base, 2% dextrose, 2% agar, 4 μg/ml adenine, and all relevant amino acids except leucine (CM-Leu + low Ade). Media components were from Difco or Sigma.

Transformations were performed by the lithium acetate procedure as described (9), with minor revisions. Briefly, one colony of yIG397 was inoculated into 100 ml YPD + Ade liquid medium and shaken at 30°C until an OD_{600} of between 0.52 and 0.54 was attained (12–18 hr). Cells were washed with 10 ml of water and resuspended in 0.5 ml of LiOac solution (1× TE, pH 7.5/0.1 M LiOac). Treated or untreated pLS76-transforming DNA (a total of 1.5 μg for the reconstruction experiment or 0.5 μg for the mutagenesis studies) was added to 200 μl of yeast cell suspension along with 200 μg of denatured salmon sperm-carrier DNA. LiOac solution containing 40% polyethylene glycol 3350 (1.2 ml) was added and mixtures were shaken at 30°C for ≈30 min. Heat shock was performed at 42°C for 15 min. Cells were pelleted, resuspended in 1 ml TE (pH 7.5), and plated on selective medium. Mutants were scored after incubation at 35°C for at least 50 hr. Transformation of UV-irradiated DNA was performed in dark conditions and plating was done under yellow lights to avoid photoreactivation repair of the plasmid.

Plasmid Survival and Mutation Frequency. Survival was expressed as the ratio of the total number of colonies attained with UV-treated DNA to the number from untreated DNA in parallel transformations. Survival evaluations from distinct transformations were averaged for the final values. Cell densities were typically 1–2 thousand colonies per 150-mm plate.

Mutation frequency was expressed as the ratio of the number of red and pink colonies to the total number of colonies. For the reconstruction experiment, frequency calculations are based on at least 14 mutants (generally more than 20), and each value was obtained from a single transformation. For the spontaneous and induced mutation frequencies, multiple transformations (3–6) were carried out on the same batches of control or UV-treated plasmid to accumulate at least 50 spontaneous and 100 induced mutants to provide valid estimates of mutation frequencies. The same lot of DNA was used for each treatment to avoid variations in spontaneous mutation frequencies. Mean induced mutation frequency values were calculated from three independent treatments.

Statistical Analysis. Analysis of the differences in reconstruction experiment values and in spontaneous and UV-induced mutation frequency values was performed first by the *F* test to determine variances of the standard deviations, then by the appropriate two-tailed *t* test (for equal or unequal variances). Chi square analysis was used to determine whether numbers of mutants varied significantly between transformations.

Isolation and Characterization of Mutants. *Mutant prescreening.* As an initial screening method to identify yeast spontaneous mutants that were red due to loss of the p53-responsive *ADE2* gene as opposed to containing mutant pLS76, total yeast DNA was isolated by the glass bead lysis method as described (9), digested with *Bam*HI, and subjected to Southern blot analysis with a 29-mer probe identical in sequence to the p53 binding sites found in yIG397 (8, 10).

Plasmid recovery. The vector pLS76 was extracted from yIG397 by a modification of a previously described procedure for isolation of total yeast DNA (11). Red and pink colonies were grown in 5 ml of CM-Leu medium supplemented with adenine at 200 μg/ml (to promote optimal growth), harvested, and washed successively with 10 ml of TE and 10 ml of 1 M sorbitol. Cells were resuspended in 0.5 ml of buffer containing 1 M sorbitol, 0.1 M EDTA, 14 mM 2-mercaptoethanol, and 10 mM Tris (pH 8.0). Incubation with 1 mg of Zymolyase 20T (ICN) was then performed at 37°C for 45 min. After centrifugation, Wizard mini-prep plasmid purification columns (Promega) were used to isolate the plasmid from pelleted spheroplasts. To amplify recovered DNA, transformation into *E. coli* strain DH5α was performed with a BTX electroporator under recommended conditions, combined with the method for salt-free preparation of electro-competent bacteria described by Sharma and Schimke (12). The Perfect Prep DNA isolation system (5 prime → 3 prime) was used to isolate plasmid DNA from the bacteria for sequence analysis.

Sequencing. Sequencing was performed by the University of Georgia Molecular Genetics Instrumentation Facility with the primer 5′-GGCTTCTTGCATTCTGGGAC-3′ that flanks exon 5 of *p53*. Mutations were identified with the aid of Sequencher DNA sequence analysis software (Gene Codes, Ann Arbor, MI).

RESULTS

Reconstruction Experiment. The yeast strain yIG397 was previously shown to accurately distinguish wild-type from mutant p53 following transformation with pLS76 expression vectors; colonies harboring p53 mutants defective in DNA binding and transcription activation were red or pink due to lack of expression of a p53-responsive *ADE2* gene (8). However, it had not been experimentally established that the mutant frequency (i.e., ratio of red and pink colonies to total colonies) determined by this procedure accurately quantified the proportion of mutant pLS76 p53 expression vectors present in the transfected DNA. A reconstruction experiment was therefore carried out to validate estimation of mutation frequencies in the system, and to estimate the lower limit for detection of mutants. Known quantities of a vector expressing wild-type p53 (pLS76 wt) were mixed in varying proportions with that expressing a mutant shown to be inactive in this and similar yeast assays [pLS76 273H (6–8)]. After transformation into yIG397, ratios of red and pink colonies to the total number of colonies formed were determined in three separate experiments and compared with expected values. Observed mutant frequencies did not differ significantly from expected values when ratios of mutant to total plasmid were 1:500 or 1:3,000 (P = 0.21 and 0.31, respectively; data not shown). This indicates that the system can accurately detect a mutant frequency as low as 1/3,000 (3.3×10^{-4}). However, the average mutant frequencies obtained in these two cases were slightly lower than expected, and at a mutant to total plasmid ratio of 1:1,000, the observed mutant frequency was significantly lower than that expected [$(0.9 \pm 0.2) \times 10^{-3}$ versus $(1.3 \pm 0.1) \times 10^{-3}$; $P < 0.05$)]. This suggests that mutant frequencies determined by the procedure may slightly underestimate the actual number of mutant plasmids present. This experiment addresses two issues of importance in eliminating potential bias from data produced by the system. First, transformed yeast did not harbor more than one viable plasmid per cell; if they had, mutants would not have been detectable at the low frequency observed. Second, transformation efficiency of preparations containing wild-type *p53* is approximately the same as that of those containing mutant *p53*.

UV-Induced Mutations. Having established that this system is capable of providing valid estimates of mutation frequency, pLS76 wt was treated with UV at doses of 50, 150, and 300 J/m^2 and transformed into yIG397 to determine the frequency of inactivating *p53* mutations and the spectrum of mutations induced.

Plasmid survival and mutation frequency. Agarose gel electrophoresis indicated that the amount of strand breaks was not

increased by UV treatment (data not shown). However, a dose-dependent decrease in transformation efficiency was seen with plasmid DNA treated with increasing exposure to UV. The average transformation efficiency of untreated pLS76 wt was 7×10^4 colonies per μg of DNA; treatment with 50, 150, and 300 J/m^2 of UV light reduced this efficiency to approximately 95%, 50%, and 15% of values obtained with control plasmid, respectively.

Mutation frequency increased in a dose-dependent manner with increasing UV dose (Table 1). At the three treatment levels tested, mutation frequencies were 2.5-, 6.9-, and 18-fold higher than that of untreated vector (3.2×10^{-4}). Each of the values obtained was significantly higher than the spontaneous frequency ($P < 0.01$ for 50 and 150 J/m^2 and $P < 0.05$ for 300 J/m^2).

Mutation spectrum. To characterize *p53* mutations, plasmids that had been exposed to UV at a dose of 300 J/m^2 were recovered from red and pink yIG397 colonies. Plasmids were first subjected to agarose gel electrophoresis to identify those containing gross insertions or deletions in the *p53* region. One mutant containing a large deletion was detected in the total of 112 induced by UV treatment. Further analysis by *Sac*I/*Bsp*120I digestion, which excises a 3.2-kb fragment containing *p53* (plus promoter and terminator) in the wild-type plasmid, confirmed that the deletion occurred in the region of interest. Analyses of four additional preparations of this mutant plasmid from distinct bacterial colonies confirmed that the deletion had occurred in plasmid replicated in yeast, not as a result of bacterial transformation.

A total of 111 mutants not containing deletions were analyzed by sequencing exons 5–8 of the *p53* cDNA. Of these, 64% (71) contained detectable noncryptic mutations; the remainder presumably harbored mutations in regions of *p53* not analyzed, in the promoter region, or in the p53 responsive element of yIG397. Ten induced mutants contained mutations identical to those of other plasmids from the same transformation, and were thus presumed to have been siblings; sequence data from such plasmids was not included in the analysis.

A summary of the types of mutations identified is shown in Table 2. GC to AT transitions predominated in the induced spectrum, representing 61% of the total mutations; an additional 11% were GC to TA transversions, and 5% were GC to CG base changes. Only 5% of the induced mutations occurred at AT sites; all of these were AT to GC transitions. Four CC to TT (GG to AA) mutations, characteristic base changes induced by UV, were found, representing 6% of total mutations. Additional minor contributions to the overall mutation spectrum included AC to TT (TG to AA), CCC to ACT (GGG to TGA) base changes, a small insertion, and two single GC base pair deletions in runs of GCs. The types of *p53* mutations that have been identified in human skin tumors are also summarized in Table 2 for comparative purposes.

Table 1. Mutation frequency of pLS76 wt following treatment with UV light and replication in yIG397

UV dose, J/m^2	Trial	Red/total colonies*	Mutation frequency†
0	1	67/230,660	
	2	80/206,125	
	3	62/226,435	$(3.2 \pm 0.6) \times 10^{-4}$
50	1	141/170,325	
	2	105/128,400	
	3	120/157,715	$(8.0 \pm 0.4) \times 10^{-4}$
150	1	130/60,835	
	2	136/59,520	
	3	166/76,670	$(2.2 \pm 0.1) \times 10^{-3}$
300	1	120/22,480	
	2	127/16,545	
	3	158/37,730	$(5.7 \pm 1.8) \times 10^{-3}$

*Each value represents the sum of multiple transformations. In 8 of the 9 sets of transformations of treated DNA, the number of mutants did not vary significantly between distinct transformations.
†Number of red colonies divided by the total number of colonies: average of the three trials ± SD.

Table 2. Types of mutations in spontaneous and UV-induced (300 J/m^2) pLS76 and in human cells

Sequence alteration	No. of mutations (% of total)		
	Untreated*	UV-induced*	Human skin *p53*†
Transitions			
GC to AT	5 (21)	38 (61)	105 (45)
AT to GC	1 (4)	3 (5)	18 (8)
Transversions			
GC to TA	4 (17)	7 (11)	22 (9)
GC to CG	0 (0)	3 (5)	14 (6)
AT to TA	0 (0)	0 (0)	9 (4)
AT to CG	2 (8)	0 (0)	10 (4)
CC to TT			
(or GG to AA)	0 (0)	4 (6)	32 (14)
Other double mutants	0 (0)	2 (3)	5 (2)
Deletion (<10 bp)	3 (12)	3 (5)	12 (5)
Deletion (>10 bp)	5 (21)	1 (2)	2 (1)
Insertion (<10 bp)	0 (0)	1 (2)	4 (2)
Insertion (>10 bp)	4 (17)	0 (0)	0 (0)
Total mutations	24	62	233

*Mutations in exons 5–8 of *p53* in pLS76 (this study).
†Noncryptic mutations in the coding region of *p53* in human skin tumors, precancerous lesions, and cell lines (13).

The distribution of mutations within the *p53* coding regions sequenced is summarized in Fig. 1. A hotspot, representing 13% of the total induced mutations, occurred at the first base of codon 213. Seven of eight mutations at this site were C to T transitions, resulting in a nonsense mutation in the gene product, and one was a C to G transversion, resulting in an arginine to glycine substitution. The second most prevalent modified site (8% of total mutations) was at the second base of the same codon. Three of five mutations at this site were G to A transitions, resulting in an arginine to glutamine substitution, and the remaining two mutants had G to T transversions, which caused an arginine to leucine substitution. Also, indicated in Fig. 1 (arrows) are positions at which base changes identified in this study were identical to those that have been detected in human skin tumors. Eleven such mutations were found out of a total of 28 distinct sites with single base changes.

Spontaneous Mutations. *Mutation frequency.* The spontaneous mutation frequency of pLS76, obtained by determining the number of red and pink colonies resulting from transformation of untreated plasmid, was 3.2×10^{-4}. However, in this experimental system, red colonies could also have resulted from loss of the p53-responsive element in yIG397, as opposed to mutation of *p53* in the pLS76 plasmid. A previous study reported the rate of loss of yeast vectors integrated by standard homologous recombination techniques (analogous to the manner in which the p53-responsive *ADE2* gene was integrated into yIG397) to be relatively high (≈1% after 15 generations) during growth in nonselective conditions similar to those used in this study (14). Therefore, to identify and screen out such mutants before sequencing of the vectors, genomic DNA from red and pink colonies was subjected to Southern blot analysis with a probe for the p53 binding sites. Of 118 spontaneous mutants analyzed in this manner, 71 (60%) had lost the p53 binding sites and were thus not subjected to sequence analysis of pLS76. Agarose gel analysis of the 47 spontaneous mutants retaining the p53 binding sites detected 4 insertions and 5 deletions. As was the case for the large deletion in the UV-induced mutant, these size changes occurred in the *p53*-

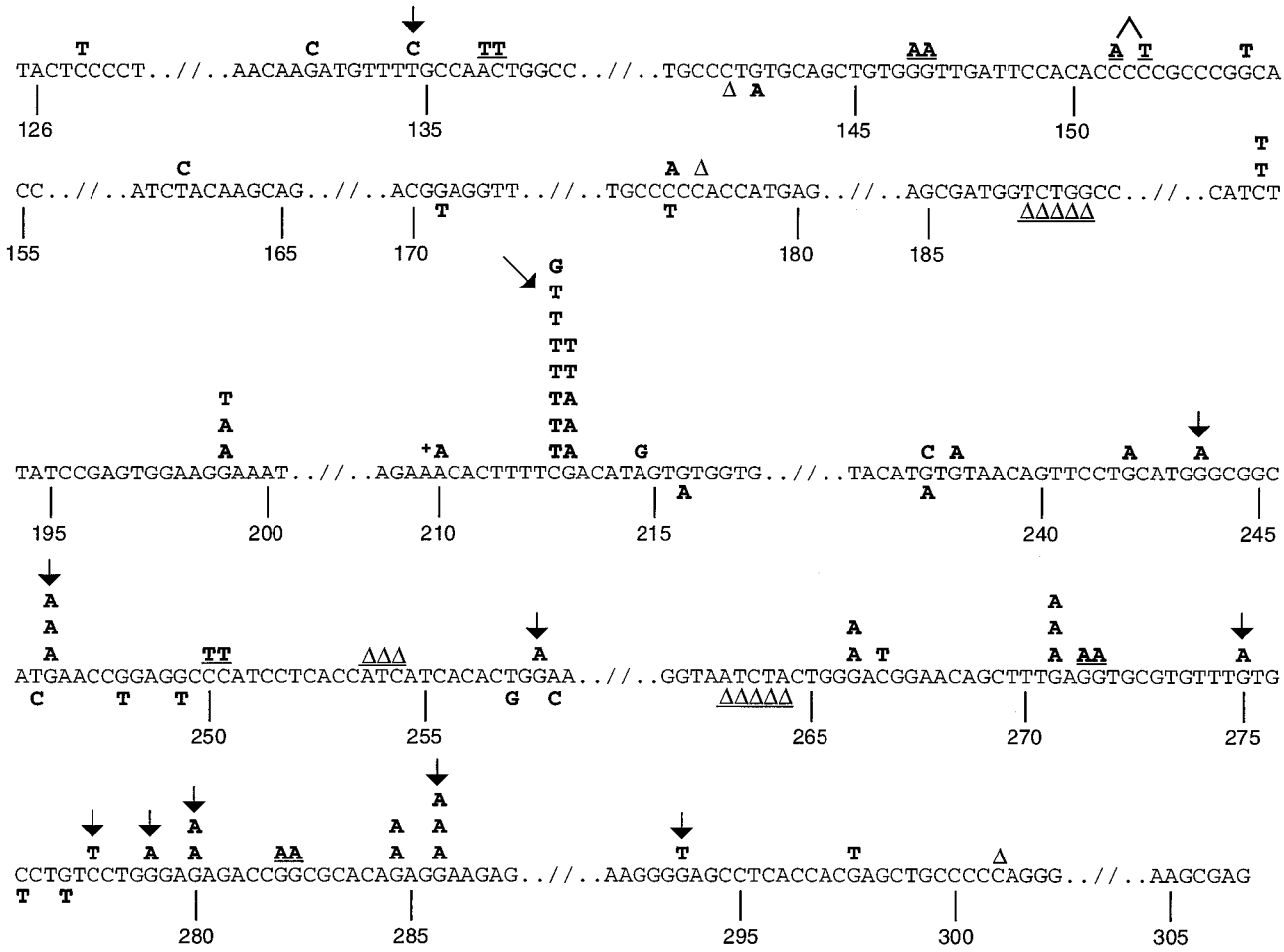

FIG. 1. Distribution of spontaneous and UV-induced (300 J/m^2) mutations in exons 5–8 of *p53* in pLS76. Spontaneous mutations are listed below the sequence and induced mutations above. Underlined bases are multiple mutations from the same plasmid, and Δ signifies a 1-bp deletion. Arrows point to identical mutations seen in human skin tumors, precancerous lesions, and cell lines (13). Numbers correspond to codons.

containing region of pLS76 and were induced in yeast, not as a result of bacterial transformation.

Mutation spectrum. The 38 spontaneous mutants that were of normal size and from colonies that retained p53 binding sites were analyzed by sequencing exons 5–8 of *p53*. A total of 12 base-substitution mutations and 3 small deletions were found, whereas 23 mutants contained no sequence changes in this region. As shown in Table 2, one-half of all spontaneous mutants contained small or large deletions (33%) or insertions (17%); the remainder being base-substitutions. The majority (75%) of the base substitutions occurred at GC base pairs, with GC to AT and GC to TA changes nearly equally represented. Base changes also occurred at AT sites, but these represented only 12% of total mutations. As is seen in the spectrum of spontaneous mutations presented in Fig. 1, base changes were distributed randomly throughout the sequenced region, and none occurred more than once.

Site and Strand Specificity of Mutations. Many previous UV mutagenesis studies have examined the bases flanking mutation sites in attempting to identify premutagenic DNA lesions and the basis for localization of mutational hotspots (reviewed in ref. 15). Table 3 summarizes the site and strand specificity of spontaneous and induced mutations identified in our experiments. Of the induced single base changes, 90% (46/51) occurred at dipyrimidine sites, whereas only 58% (7/12) of the spontaneous point mutations occurred at these sequences. Additionally, there was a slight bias (67%) toward mutations caused by lesions in the transcribed (antisense) strand. Spontaneous mutations occurred with approximately equal frequency when the dipyrimidine was located on the sense or antisense strand.

In the induced spectrum, within sites where only one pyrimidine dimer could form (i.e., where the mutated pyrimidine was flanked by a purine and a pyrimidine), the majority (83%) of mutations occurred at the 3′ C of TC sequences (Table 3). Additionally, two mutations were located at the 3′ T of CT, and one each occurred at the 5′ C of CC and the 3′ T of TT. In cases where the mutated pyrimidine was flanked by two pyrimidines it was not possible to attribute the mutation to one putative dimer or the other; however, if it is assumed that the 3′ base is the mutated site, as was the case in 96% of the mutations where only one dimer could form, then 19 of 22 would have occurred at TC sites and the remainder at CC. Also of note is the fact that the frequencies of transitions (80%) and transversions (20%) were the same whether pyrimidine dimers could form at the mutated site or not.

DISCUSSION

In this study, we used an experimental system in which a p53 yeast expression vector was exposed to UV light *in vitro* and then introduced into *S. cerevisiae* for replication, repair, and mutant selection. A reconstruction experiment showed the lower limit of detection of the system to be at least 3.3×10^{-4}, and demonstrated that it provided accurate estimates of mutation frequency. When the vector was treated with increasing amounts of UV light, a dose-dependent decrease in survival and increase in mutation frequency were seen, with

Table 3. Site and strand specificity of spontaneous and UV-induced mutations

Mutation site*	No. of spontaneous events (sense or antisense strand)‡	Spontaneous base change†‡	No. of induced events (sense or antisense strand)†‡	Induced base change‡
T<u>C</u>A/G	0	—	20 (8s, 12a)	C to :T(16) :A(3) :G(1)
C<u>T</u>A/G	1 (s)	T to G	2 (1s, 1a)	T to :C(2)
G<u>C</u>C	2 (1s, 1a)	C to :T(1) :A(1)	1 (a)	C to A
T<u>T</u>G	0	—	1 (s)	T to C
C<u>C</u>G	1 (a)	C to A	0	—
Total at dipyrimidines	4		24	
T<u>C</u>C	1 (a)	C to A	12 (2s, 10a)	C to :T(10) :A(2)
T<u>C</u>T	0	—	7 (2s, 5a)	C to :T(6) :G(1)
C<u>C</u>C	1 (s)	C to T	3 (1s, 2a)	C to :T(2) :A(1)
T<u>T</u>C	1 (a)	T to G	0	—
Total at overlapping dipyrimidines	3		22	
A<u>C</u>A/G	4	C to :T(3) :A(1)	4	C to :T(3) :G(1)
G<u>C</u>A	0	—	1	C to T
A<u>T</u>G	1	T to C	0	—
Total at nondipyrimidines	5		5	

*Mutated bases are underlined.
†Mutations resulting from UV photoproducts on the (s)ense or (a)ntisense strand.
‡Number of events is in parentheses.

the highest dose tested (300 J/m^2) resulting in 15% survival and an 18-fold induction of mutants over the spontaneous frequency of 3.2×10^{-4}.

Sequence analysis of *p53* in spontaneous mutants and in those induced by UV light at 300 J/m^2 revealed that the two spectra differed significantly. One-half of the spontaneous events were insertions or deletions, whereas fewer than 10% of the induced mutants contained these alterations. Additionally, transitions and transversions were present in equal proportions in the spontaneous spectrum, but transitions clearly predominated in the induced. It is also noteworthy that tandem CC to TT base changes, hallmark mutations of UV mutagenesis, were detected only in the induced spectrum.

The most prevalent induced mutation was the GC to AT transition, which occurred in 61% of the UV-induced mutants. The predominance of this mutation is in agreement with previous yeast studies of UV mutagenesis in the endogenous *ADE2* gene (16) or the *sup4*-o gene carried on a centromeric plasmid (17) after treatment of whole cells, but differs from results of a similar study on an integrated *URA3* gene (18). The latter investigators found that the most common base alterations in their system were AT to GC transitions, and that the frequency of transitions was equal to that of transversions. Indeed, more than 10% of mutations in both the *ADE2* (16) and *sup4*-o (17) systems also were AT to GC transitions. Interestingly, the paucity of transitions at AT sites in the system used here and the predominance of GC to AT base changes more closely resembles reported effects of UV irradiation in a variety of bacterial and mammalian experimental models (19–27) than it does with the data generated in yeast systems (16–18). However, in all of the above studies, transversions at AT sites were detected, whereas none were identified in our study. Reasons for these discrepancies are not known, but the small sample size examined here makes it difficult to assess their significance.

In accord with many previous UV studies in both prokaryotic and eukaryotic systems (reviewed in ref. 15) the majority of mutations (90%) occurred at dipyrimidine sites, most notably the 3′C of TC. Recent studies involving the kinetics of C deamination in TC dimers, delayed photoreactivation of UV-irradiated phage (ref. 28 and references therein), and mutagenesis of a site-specifically placed T-U dimer (29) in a vector in SOS-induced *E. coli* have led to the proposal that C to T mutations can result from the incorporation of A opposite either U (the deamination product of C) or the (E)-imino tautomer of cytosine in the context of a cyclobutane pyrimidine dimer. Since these dimers have been shown to be the primary lesions responsible for UV mutagenesis in yeast (30), this mechanism of mutation may have been operative in the present study. The induction of nearly 70% of detected mutations by lesions in the transcribed strand is surprising in light of the fact that the opposite strand bias has been observed in *E. coli* and mammalian cells. This has been attributed to the preferential removal of lesions on the strand of genes transcribed by RNA polymerase II, a phenomenon seen in these cell types and yeast (reviewed in ref. 31). However, our results were similar to those of a study of mutagenesis in an integrated *URA3* gene of yeast, in which 64% (16/25) of the mutations resulted from lesions on the transcribed strand (18).

The two most highly mutated bases were in codon 213, within the sequence 5′-ACTTTT<u>CG</u>AC-3′ (mutated bases underlined), with a hotspot at the C. This result is consistent with the observation that some mutation hotspots occur at sites 3′ to a run of pyrimidines in *E. coli* (32). Interestingly, in numerous studies of UV-induced mutagenesis involving the bacterial *supF* gene replicated in mammalian cells (see ref. 15), a hotspot was consistently found within a similar sequence, 5′-CTTC<u>G</u>AAG-3′, where the C adjacent to the mutated G was a hotspot in some systems but less frequently mutated in others. The existence of a similar hotspot in these two very different settings suggests that local sequence context may play a significant role in determining the mutability of certain sites. These findings are supported by the fact that when the 8-bp *supF* region noted above was transferred to a different region of the gene, it remained a hotspot following replication in repair-deficient human cells (33). However, in that system, a new hotspot was created approximately 70 bp from the site of the 8 base insertion, suggesting that local sequence contexts also have long range effects on mutability.

When the mutation spectrum obtained in this study is compared with that reported for *p53* in human skin tumors, precancerous lesions, and cell lines from normal and repair-deficient individuals, it can be seen that 11 of the single base changes detected in pLS76 at 28 distinct sites were identical to those found in human skin (arrows in Fig. 1). Additionally, the types of mutations identified in the *p53* cDNA of UV-treated pLS76 are similar to those observed *in vivo* (Table 2). The fact

that 92% of the mutations found in human skin cancers are located within dipyrimidine sites further reinforces the similarities of the results of the two systems, inasmuch as 90% of the mutations occurred at these sites in this study.

Of the two most frequently reported mutations in skin cancer (occurring in codons 248 and 278), one was detected in this study in a single mutant (a codon 278 CCT to TCT mutation), but the other was not detected (a codon 248 CGG to TGG). The fact that the codon 248 arginine to tryptophan mutation can be detected in this and similar yeast assays (6–8) raises the possibility that the codon 248 mutations in tumors (also a hotspot for internal tumors) may not have been induced by UV. This would be in agreement with a study that examined codons 247–250 of *p53* for mutations following UV treatment of human skin fibroblasts and concluded that the results obtained did not correlate with mutations in skin tumors (34). However, caution must be used in drawing firm conclusions about the *in vivo* situation from the data generated here. Reasons why discrepancies may exist between the results of the two systems include: differences in repair in yeast and human cells, differential mutability of DNA sites *in vitro* compared with *in vivo*, inconsistencies in transcription activation in the two cell types, and selective growth advantage of p53 mutants *in vivo*.

The experimental system used in this work has several advantages over previous model systems used for mutation analysis. Because the vector employed contains the intact human *p53* cDNA, the spectra of mutations induced by *in vitro* exposure to mutagens can be directly compared with the spectra of the same gene in tumors without concerns about sequence-dependent mutation effects. Additionally, repair of the damaged gene takes place in a eukaryotic setting (yeast) in this system, which should more closely mimic the situation in human cells than does bacterial repair. Finally, this method can be used with any mutagen and can thus contribute to the ongoing effort to determine the *p53* mutation pattern of agents important in the development of cancer.

The described system also has limitations. Most notably, the *p53* mutations detected here are those which inactivate the DNA binding and transcription activation function of the protein, specifically for the RGC (ribosomal gene cluster) binding site (10) present in the p53-responsive element in the yeast strain used (8). Previous studies have indicated that transactivation by p53 correlates well with its growth suppression of cells (35–37), but not tumor suppression (36, 37), which may require transcription activation dependent and independent activities of the protein (reviewed in refs. 2, 3, and 38). Thus, it is likely that not all of the p53 mutants found in human cancer can be detected using this system. Also, a current limitation of this system is that not all of the inactivating mutations in *p53* have been characterized. Thus, definitive conclusions cannot yet be drawn about mutagen site and strand specificity.

We have introduced a model system for the generation of a *p53* mutation spectrum following treatment with UV and have demonstrated that the results have similarities to the mutation pattern of human skin tumors. It is our intention to use this system for the future characterization of other mutation patterns within *p53* as a result of modification by other mutagens, especially those that fail to produce as distinct a "fingerprint" on the DNA as does UV, because the method may provide the means to determine whether other agents are involved in mutating *p53 in vivo* as a step in the carcinogenic process.

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