Modulation of an ultraviolet mutational hotspot in a shuttle vector in Xeroderma cells

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ABSTRACT

Ultraviolet mutagenesis of the shuttle vector plasmid pZ189 in Xeroderma Pigmentosum cells yields a mutational pattern marked by hotspots at photoproduct sites on both strands of the supF marker gene. In order to test the influence of strand orientation on the appearance of hotspots the mutagenesis study was repeated on a vector with the supF gene in the inverted orientation. We recovered a pattern the same as that in the earlier work and conclude that the nature of the DNA polymerase involved in the replication of specific strands is not a primary determinant of hotspot occurrence in this system. One of the hotspots lies in an 8 base palindrome while the corresponding site on the other strand was not a hotspot. These results were obtained with calcium phosphate transfection of the UV treated vector. When DEAE dextran was used as a transfection agent both sites in the palindrome were hotspots. In a mixing experiment the calcium phosphate pattern was recovered. Our data suggest that the sequence determinants of mutational probability at these two sites lie outside the 8 bases of the palindrome and that mutagenesis at one, but not the other, site is sensitive to perturbation of cellular calcium levels.

INTRODUCTION

Mutagenesis of DNA modified by carcinogens and mutagens can occur during replication when inappropriate bases are incorporated in daughter strands across from damaged sites in the template strands. We have been studying this process in cells from a patient with the repair deficiency xeroderma pigmentosum using the pZ189 shuttle vector system [1]. We have characterized the kinds and location of mutations in the suppressor tRNA marker gene (supF) which appear after replication of UV radiated plasmid in the XP cells [2]. The dominant mutation in our experiments has been the G:C to A:T transition in agreement with much earlier work in prokaryotic systems [3,4,5]. These mutations are not distributed randomly but instead appear in a pattern of hotspots and cold spots. Although it might be expected that the hotspots and coldspots would occur as a simple function of the frequency of photoproducts at specific sites our analysis of the modification pattern indicates that this is not always true [6]. Instead we and others have found little correlation between mutation frequency and modification frequency at specific sites [7]. Presumably the sequence context of a photoproduct influences the probability of mutagenesis at particular modified sites (see 7 for the first observation and discussion of this problem). However at this time there is little insight into how or over what distance mutagenesis could be affected by sequence context. Recently we determined the uv mutational spectrum in the supF gene in several repair proficient and deficient cell lines [8]. Although comparison of the mutational patterns revealed many similarities we found that certain hotspots appeared with some lines but not others, suggesting that some cellular component(s) could also influence the probability of mutagenesis at particular modified sites.

In the last few years it has become apparent that different DNA polymerases are involved in the synthesis of the leading (polymerase δ) and lagging (polymerase α) daughter strands [9]. These observations raise the question of what contribution the individual polymerases might make to the specificity and probability of mutagenesis at particular sites in a gene. In a recent publication Vrieling et al [10] reported a strand bias in their study of ultraviolet mutagenesis in the HPRT gene in Syrian hamster cells and suggested that this was a reflection of the different polymerases involved in replication.

We are interested in the problem of mutational hotspots and wanted to assess the possible influence of specific DNA polymerases on them. In order to do this we constructed a variant of pZ189 in which the orientation of the marker gene relative to the origin of replication was inverted. In this report we describe the pattern of mutations recovered with this vector and the implications for mutagenesis at one particular site. Furthermore we find that the frequency of mutations at this site appears to be sensitive to calcium levels.

MATERIALS METHODS

Cells and Plasmids

The SV40 virus transformed xeroderma pigmentosum fibroblasts (XP12Be) were obtained from the Institute for Medical Research

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(Camden,NJ) and grown in medium supplemented with 10% fetal calf serum. The plasmids pMS189 and pMS981 were constructed by conventional techniques from the appropriate fragments from SV40 virus (the early region fragment containing the origin of replication and the T,t antigen genes), from pBR327 (the plasmid origin of replication and beta lactamase gene) as well as the supF marker gene [1]. The plasmid pZ189 has been described [1], and the critical organizational features of this plasmid were retained in the pMS vectors.

Plasmid treatment and Mutagenesis protocol

The plasmids were treated with 254 nm UV radiation from a germicidal lamp and cells were transfected by either calcium phosphate or DEAE dextran following standard procedures [11,12]. After 48 hours plasmids were harvested, purified, and the population screened for mutant plasmids as described previously [1]. Sequence analysis was with the Sequenase dideoxy protocol according to the supplier (USB, Cleveland, Ohio).

RESULTS

We constructed two variants of pZ189. In pMS981 the orientation of the suppressor tRNA marker was inverted relative to the orientation in the original pZ189 plasmid. In pMS189 the orientation was the same as in pZ189 [1]. The plasmids were treated with 254 nm radiation at a dose of 150 Joules/m2 and introduced into XP12BE cells via calcium phosphate transfection. Progeny plasmids were harvested, and those plasmids with mutations in the marker gene were identified in a microbiological screen. In agreement with our previous results we recovered mutant plasmids at frequencies about 100 fold over the background (about 0.5%) [2]. The sequence of the supF gene was determined in 55 independently derived mutant plasmids from the pMS981 transfection. There were 44 plasmids with single base substitutions and 11 with tandem mutations. The sequences of 18 mutant pMS189 plasmids were also determined. There were 17 plasmids with single base substitutions and 1 with tandem changes. Most of the mutations in both groups were G:C to A:T transitions, as in our earlier work [2]. These data indicated that the change in orientation of the marker had no effect on the nature of the mutations recovered.

The patterns of mutations with both vectors are shown in Fig. 1. The gene is shown in the orientation of our previous publications so as to facilitate comparison. However the base changes are indicated as read from the sequencing films. Consequently a G:C to A:T transition at a particular site will appear as an A with one orientation and a T with the other. We found strong hotspots at positions 156 and 168 with both vectors. There were no mutations at position 169 which was represented in the all the pZ189 spectra reported by us in previous publications [2,8,13,14,]. With the exception of the events at position 169 the pattern of mutations with the pMS981 vector was essentially the same as with the earlier work with pZ189. In contrast to position 156 (19 mutations) there were only two mutations at position 155, a site which was infrequently mutagenized in our earlier study with this cell line. In the pMS 189 experiment there were 8 mutations at 156 and none at 155. These two sites were of particular interest because they are the corresponding positions on opposite strands in an 8 bp palindrome (Fig.2). The results of this experiment indicated that the differences in mutation frequency at these two sites were not a function of the DNA polymerases involved in replication.

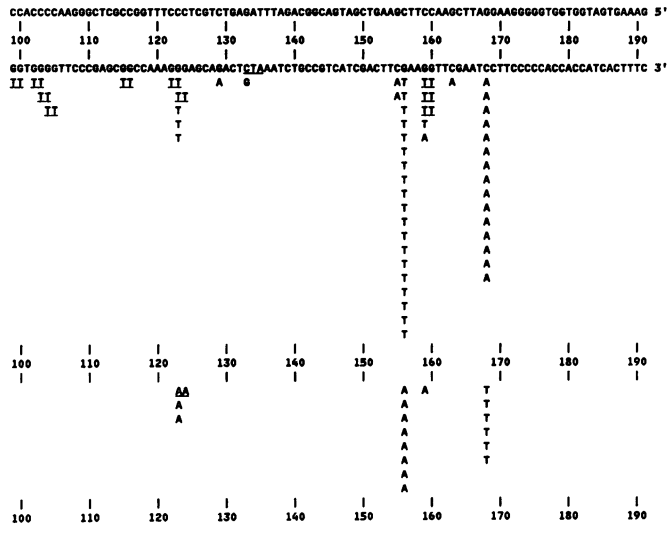

Fig. 1. The UV mutational spectrum with pMS981 (upper) and pMS189 (lower) in XP12BE(SV4O) fibroblasts. The plasmids were introduced into the cells by calcium phosphate transfection. Mutations were recovered only in the region presented.

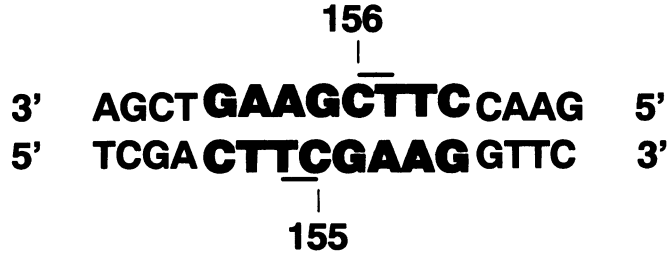

Fig. 2. The sequence around the hotspot sites 155 and 156. The pyrimidines involved in the relevant photoproducts are indicated.

Table I. Kinds of mutations in pZ189 after replication of the UV treated plasmid in XP12BE(SV4O) cells, DEAE dextran transfection.

	No. of Changes
Transitions	65
G:C-A:T	65 (82%)
A:T-T:A	0
Transversions	14
G:C-T:A	6 (8%)
G:C-C:G	7 (9%)
A:T-T:A	1 (1%)
A:T-C:G	0

Mutagenesis after transfection with DEAE dextran

In the study with the pMS vectors and our previous experiments with pZ189 we introduced the modified plasmid into the XP cells by calcium phosphate transfection. During the course of our experiments we determined the UV mutational spectrum with pZ189 in these cells using DEAE dextran as the transfection agent. While the majority of the mutations were again G:C-A:T transitions, there were also transversion mutations not commonly found in previous experiments with calcium phosphate transfections of these cells (Table I)[2]. In Fig. 3A is shown the

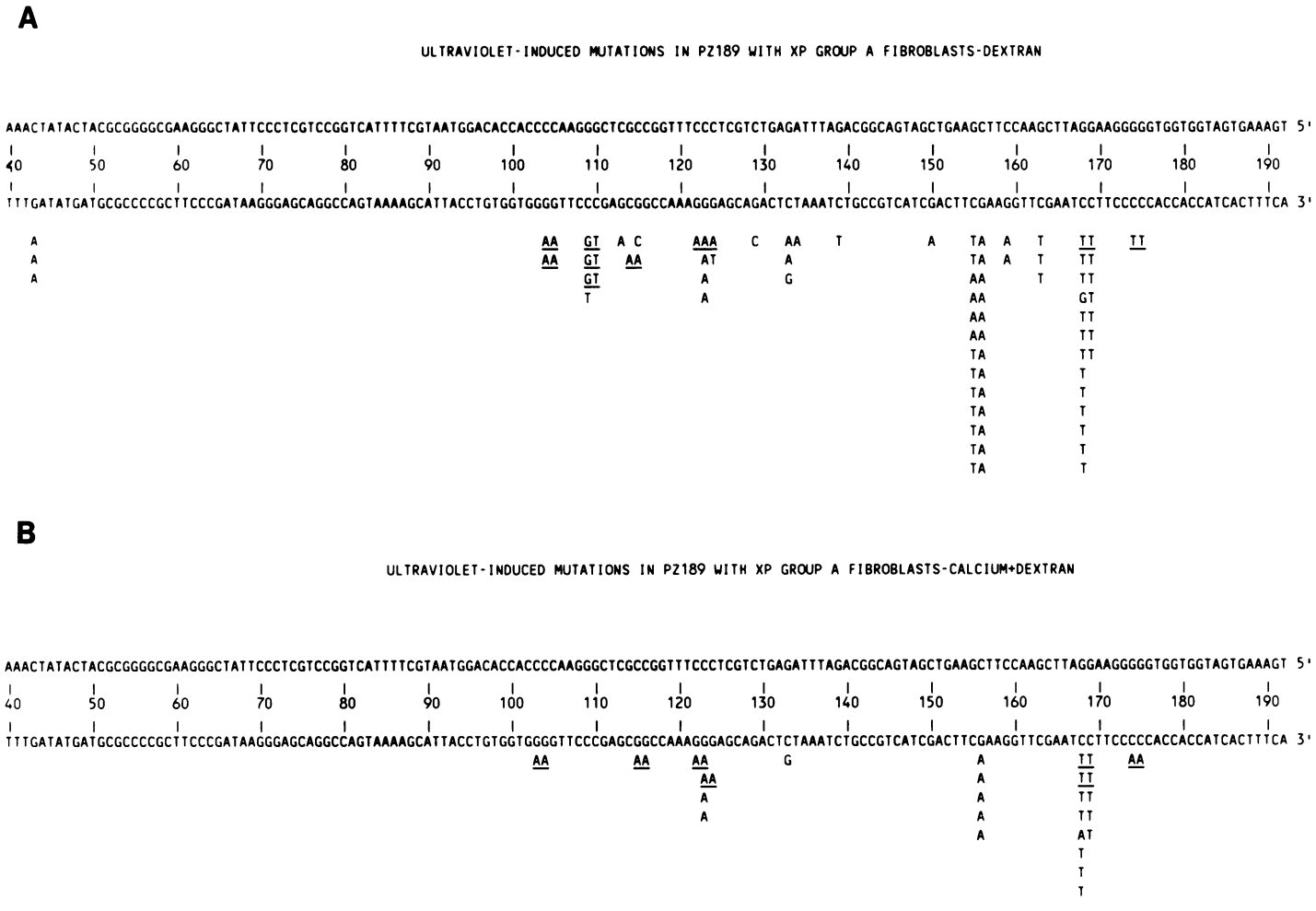

Fig. 3. The UV mutational spectrum with pZ189 in XP12BE(SV4O) fibroblasts. The plasmid was introduced by DEAE dextran transfection (A) or a mixture of DEAE dextran and calcium phosphate (B).

mutational pattern. In addition to the familiar hotspots at 156 and 168 we observed a strong hotspot at 155, consisting of both transitions (G:C to A:T) and transversions (G:C to T:A). In order to test which pattern would dominate in a mixing experiment, we repeated the experiment and combined DEAE dextran and calcium phosphate in the transfection of the modified DNA. The pattern obtained was that seen in all our previous work with calcium phosphate transfections (Fig. 3B). There were hotspots at 156 and 168 but not at 155.

DISCUSSION

In a prior study we demonstrated that the frequency of photoproduct formation was not the primary determinant of the probability of mutagenesis at modified sites [6]. In an effort to explain our pattern of base substitution mutations we proposed a pass/fail model of mutagenesis. In our scenario we described three classes of modified sites. There would be those where the probability of replicative bypass would be relatively high such that sites with a relatively low frequency of modification would be frequently mutagenized. Sites of this class were termed 'mutation prone' by Fuchs in his discussion of frameshift mutagenesis in E. coli [7]. An example of such a site in pZ189 is 168 which has been a hot spot in all of our experiments with the XP12BE cells. The second class of sites are those which are frequently modified yet are infrequently mutagenized. Position 43 is in this class. Finally there are those sites which are frequently modified and frequently mutagenized. Position 156 which has been a hotspot in all our experiments with all cell lines is a good example. It is generally assumed that the basis for hotspots and coldspots lies in the sequence context of the site in question [7,5]. However recent understanding of the asymmetry of repair and the strand specificity of the DNA polymerases would seem to allow additional factors to be considered. Our results with the pMS981 and 189 vectors permit the simple conclusion that strand orientation and thus the specific DNA polymerase involved in replication are not the primary determinants of the pass/fail ratio at modified sites in this system. Since the experiments were performed in repair deficient cells variability in repair of different sites was not an issue. Consequently it appears that the molecular rationale for hotspots and cold spots in these cells does indeed lie in the sequence context of the site.

In the light of this conclusion mutagenesis at positions 155 and 156 is of particular interest. As noted above, position 156 has been a hotspot in all our previous experiments. We interpret mutations at this site as the consequence of lesions at the 5' TC at 157,156. Inspection of the sequence around 156 reveals that

the TC lies in an eight base sequence which is also found in the same region of the gene on the opposite strand (Fig.2). The cytosine of the corresponding TC is at position 155. The results with the pMS981 and 189 vectors, introduced via calcium phosphate, and all our pZ189 data in which calcium phosphate was used in the transfection, indicate that mutations at position 155 appeared at only about 12% of the frequency of those at 156 (174 total mutations; 49 at 156, 6 at 155, $p < .0001$). After adjustment for the relatively small difference in modification frequency at these sites it is apparent that under these experimental conditions the pass/fail ratio at 155 is approximately 6 fold lower than at 156.

An even more intriguing observation is that the pass/fail ratio at 155 is sensitive to the conditions of the experiment. There was a significant change in the frequency of mutations at 155, from 3% (6/174) in the calcium phosphate transfections to 15% (13/85) in the DEAE dextran experiments ($p = .001$). Calcium phosphate particles persist in cells for several hours after uptake [15] and it seems likely that the calcium balance of the cells is perturbed during the time of the replication of the modified templates. We suggest that the simplest interpretation of our data is that as a result of increased calcium levels the probability of mutagenesis at position 155 is reduced in these cells. It would seem that following DEAE dextran transfection the pass/fail ratio at 155 is similar to that at 156 and thus the probability of mutagenesis at this site is similar to that at 156. Sequence context must again play a role in this calcium sensitivity as mutagenesis at other sites was not obviously affected (see position 168).

The possibility that cellular calcium levels might influence the pass/fail ratio at selected sites is reminiscent of the well known relaxation of the fidelity of DNA polymerases by Mn++ [16]. Perhaps modulation of cellular calcium could have a direct effect on the probability of mutagenesis at specific sites. It should also be noted that Ca++ is an important second messenger in cells and perturbation of calcium levels can influence gene expression [17]. Consequently it is possible that the variable appearance of the hotspot at 155 might reflect some variability in the complement of enzymes and protein factors,or the modification of these factors, involved in the replication of damaged DNA. These are not exclusive explanations.

Although progress has been made towards an understanding of the influence of sequence context on hotspots in frame-shift mutagenesis [18,19], no such insight is available for the problem of base substitution hotspots. Many discussions of mutational hotspots include a nearest neighbor analysis. Such an analysis would appear to be unproductive in so far as 155 and 156 are concerned. In a recent study on the interaction between a DNA polymerase and a primer template complex it was shown that contacts between the two extend over as much as 19 nucleotides [20]. It seems possible that the sequence determinants of a hotspot might lie several nucleotides away from the site of the mutation.

We have argued that hotspots could be categorized as a function of their relationship to modification (see above). The data presented here indicate that calcium sensitivity may define another specific category. At this time we have too few examples in each category to allow the sequence comparisons which would permit the recognition of common features and yield genuine insight into the sequence context of mutational hotspots and coldspots. Additional studies with other marker genes will be necessary to develop the appropriate data base before useful models can be developed.

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