Sequence-specific and Domain-specific DNA Repair in Xeroderma Pigmentosum and Cockayne Syndrome Cells*

Yuqing Tu, Steven Bates, and Gerd P. Pfeifer‡
From the Beckman Research Institute of the City of Hope, Department of Biology, Duarte, California 91010

Xeroderma pigmentosum (XP) and Cockayne syndrome (CS) cells have specific DNA repair defects. We had previously analyzed repair rates of cyclobutane pyrimidine dimers at nucleotide resolution along the human JUN gene in normal fibroblasts and found very efficient repair of sequences near the transcription initiation site but slow repair along the promoter. To investigate sequence-specific repair rate patterns in XP and CS cells, we conducted a similar analysis in XPA, XBP, XPC, XPD, and CSB fibroblasts. XPA cells were almost completely repair-deficient at all sequences analyzed. XPC cells repaired only the transcribed DNA strand beginning at position +20 relative to the transcription start site. Both XBP and XPD cells were deficient in repair of nontranscribed DNA strand and also very inefficiently repaired the transcribed strand including sequences near the transcription start site. CSB cells exhibited rapid repair near the transcription initiation site but were deficient in repair of sequences encountered by RNA polymerase during elongation (beginning at position +20). Since transcription of the JUN gene was UV-induced in all fibroblast strains, including CSB, the defective repair of the transcribed strand in CSB cannot be explained by a lack of transcription; rather, it appears to be a true DNA repair defect.

UV irradiation induces two major types of photoproducts in DNA, the cyclobutane pyrimidine dimers (CPDs) and the pyrimidine (6–4)-pyrimidone photoproducts, as well as much lower amounts of purine dimers, pyrimidine monoaducts, and a photoproduct between adjacent A and T bases (for reviews see Refs. 1–4). UV mutagenesis is characterized by a high frequency of transition mutations at dipyrimidine sequences containing cytosine (1–4). Based on its much slower repair rate relative to the pyrimidine (6–4)-pyrimidone photoproduct (2, 5) and based on a number of mutagenesis studies using photoreactivation of CPDs, the CPD is thought to be the major mutagenic UV-induced lesion in mammalian cells (1, 3).

Repair rates of both CPDs and pyrimidine (6–4)-pyrimidone photoproducts are heterogeneous along the mammalian genome. This heterogeneity could result in selective mutation of specific DNA sequences. There is a preferential repair of active genes relative to inactive genes, and the transcribed strand of active genes is repaired faster than the nontranscribed strand (5–8). The specific chromatin environment of the lesion is also considered to be an important factor that may affect recognition of lesions and repair rates (4, 9–11). Furthermore, the repair rates of UV-induced CPDs can vary along the same gene sequence, even between adjacent base positions (12–17).

Both CPDs and pyrimidine (6–4)-pyrimidone photoproducts are repaired by nucleotide excision repair (NER; for reviews, see Refs. 18–25). In vitro studies have shown that the reconstituted NER reaction involves about 30 polypeptides in mammalian cells (26, 27). Defects in several of these proteins are associated with rare inherited genetic disorders, including xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (20, 28–31). There are seven complementation groups for XP (XP-A–G) and two for CS (CSA and CSB). Many of these genes and their gene products have been cloned and are characterized by their ability to correct the defects in XP or CS cells. The XPA protein binds preferentially to damaged DNA and is believed to be a major factor in the DNA damage recognition step of the NER process (32). Both XBP and XPD are components of the basal transcription factor TFIIH (33–35), both have DNA helicase activity, and both may be involved in the DNA unwinding step of NER. XPC, complexed with HHR23B (the human homologue of the yeast protein RAD23B), is involved in the global genome repair pathway and appears to be dispensable for transcription-coupled repair of active genes (36, 37). The precise function of the XPC protein is unknown. The Cockayne syndrome complementation group B gene product is essential for transcription-coupled repair of active genes (38, 39) and may act as the transcription-repair coupling factor (8, 40) or, as suggested more recently, as a repair-transcription uncoupling factor (41).

Exposure of mammalian cells to UV irradiation triggers the so-called UV response, a transcriptional response that may serve a protective function and provide a mechanism for the cells to replace damaged components (42–44). The JUN gene, which codes for a component of the AP-1 transcription factor, is one of the UV response genes in mammalian cells (45, 46). Previously, we established a detailed map of DNA repair rates along the JUN gene and its upstream promoter region using ligation-mediated polymerase chain reaction (15). We reported slow repair rates along the promoter sequences, very fast repair rates surrounding the transcription initiation site, and a repair gradient along the transcribed DNA strand with faster repair within the 5’-end and diminished repair toward the 3’-end of the gene (15).

To further elucidate mechanisms of sequence-specific and domain-specific NER, we have characterized the repair rate patterns along the JUN promoter and sequences surrounding the transcription initiation site in several repair-deficient fibroblast strains including xeroderma pigmentosum complementation groups A, B, C, and D and Cockayne syndrome complementation group B.
EXPERIMENTAL PROCEDURES

Cell Culture and UV Irradiation—The following repair-deficient human fibroblast strains were obtained from the NIGMS (National Institutes of Health) human genetic mutant cell repository: GM00710B (XPA), GM13025 (XPB), GM00676 (XPC), GM10428 (XPD), and GM01098B (CSB). The repair-deficient fibroblast strains were grown as contact-inhibited monolayers in Dulbecco’s modified Eagle’s medium with 15% fetal calf serum. Normal human foreskin fibroblasts (strain HF-35) were used as repair-proficient cells. Before UV irradiation (254 nm), the medium was removed, and the cells were washed in phosphate-buffered saline. The UV dose was 10 J/m² as determined with a UVX radiometer (Ultraviolet Products, San Gabriel, CA). For DNA repair experiments, the original medium was returned to the cells, and the cells were incubated for various periods of time before lysis and DNA analysis. Nonirradiated cells and cells collected immediately after irradiation served as negative controls and positive controls (no repair), respectively.

RNA Analysis—Total cellular RNA from nonirradiated fibroblasts and from fibroblasts at various times after UV irradiation was isolated by the guanidinium isothiocyanate method (47). RNA was separated on formaldehyde-agarose gels and transferred to nylon membranes. The membranes were sequentially hybridized with probes specific for the human JUN and GAPDH genes. The probes were made by repeated run-off polymerization from PCR products (48).

DNA Isolation and Cleavage at CPDs—After incubation to allow DNA repair, cells were lysed, and DNA was isolated and purified as described previously (49). DNA was dissolved in TE buffer (10 mM Tris-HCl, pH 7.6, 1 mM EDTA) to a concentration of 0.2 μg/μl. The UV-irradiated DNA was cleaved to completion with T4 endonuclease V for 1 h at 37°C to generate single-strand breaks at CPDs and then incubated with Escherichia coli photolyase to generate ligatable ends (50). After enzyme treatment, the DNA was purified by phenol-chloroform extraction and ethanol precipitation and was dissolved in TE buffer to a concentration of 1 μg/μl.

Analysis of Total Repair by Alkaline Agarose Gel Electrophoresis—Damage and repair of CPDs in the total genome was estimated by separation of T4 endonuclease V cleavage products on 0.6% alkaline agarose gels using published procedures (51).

RESULTS

Expression of the JUN Gene after UV Irradiation—The expression of the JUN gene is induced severalfold following UV
irradiation in normal human fibroblasts (15). To investigate the effects of UV irradiation on transcription of the JUN gene in repair-deficient fibroblasts, we UV-irradiated several repair-deficient fibroblast strains including xeroderma pigmentosum complementation groups A, B, C, and D, and Cockayne syndrome complementation group B and determined the mRNA levels at various time points after irradiation (Fig. 1). For all repair-deficient fibroblasts analyzed, as well as for the normal fibroblasts, the JUN mRNA levels were induced significantly between 1 and 2 h following irradiation. The degree of induction varied somewhat between the strains. Induction was approximately 2-fold for normal cells and XPA, XPB, XPD, and CSB cells but was higher for the XPC strain (5-fold). Maximum mRNA levels were generally attained after 2 h with the exception of XPB, which showed a peak level of expression at 1 h following UV irradiation. The results show that induction of the JUN gene is not abolished in repair-deficient cells, i.e. there is active transcription of the gene in all cell types after UV damage.

Global DNA Repair in Normal and Repair-deficient Fibroblasts—To estimate repair of total genomic DNA in the various fibroblast strains, we digested DNA from UV-irradiated cells with T4 endonuclease V and separated the cleavage products in alkaline agarose gels. Fig. 2 shows that in normal fibroblasts and CSB cells, there is an increase in molecular weight of T4 endonuclease-digested DNA beginning 6–10 h after irradiation that is most pronounced at the 24-h time point. Repair was approximately 70% complete after 24 h, which is consistent with data on genomic repair of CPDs as determined by others (53). In contrast, XPA, XPB, XPC, and XPD cells showed almost no repair of total genomic DNA (<15%) after 24 h (Fig. 2).

Repair of the JUN Gene in Normal Fibroblasts—We had previously derived a map of nucleotide excision repair rates along the JUN gene in normal fibroblasts using a UV dose of 20 J/m² (15). Since the NER-deficient fibroblast strains are much more sensitive to UV irradiation than normal cells, all experiments described here were carried out with a UV dose of 10 J/m². To determine if a similar repair rate pattern is seen in normal cells at 10 and 20 J/m² and to determine whether the ligation-mediated polymerase chain reaction assay is sensitive enough to be used at the lower UV dose, we mapped the repair of CPDs along the JUN promoter and transcribed sequences at 10 J/m² (Fig. 3). The promoter of the JUN gene is covered by a number of sequence-specific transcription factors, which we had mapped previously using in vivo footprinting techniques (54, 55). CPDs along the promoter region were repaired slowly with little repair evident after 10 h and some remaining signals after 24 h (Fig. 3; data summarized in Fig. 9). In accordance with the previous study (15), we found that repair rates were significantly increased beginning around nt -40 upstream of the major transcription initiation site near the binding site of the transcription factor RSRF (related to serum response fac-
Repair rates were seen at sequences surrounding the transcription start site, where most of the repair events took place between 1 and 2 h after irradiation. In general, the repair rate patterns were very similar between the UV doses of 10 and 20 J/m² (this study; Ref. 15). At the lower dose, repair rates were about 1.5-fold faster, perhaps because the repair system is less saturated.

**Repair in XPA Cells**—We have analyzed the same sequences of the JUN gene and promoter in a series of repair-deficient fibroblast strains. The first repair-deficient cells analyzed were fibroblasts from a patient belonging to complementation group A (Fig. 4). These XPA fibroblasts showed very little repair when total genomic DNA was analyzed on alkaline agarose gels after T4 endonuclease V cleavage (Fig. 2). The data for repair in the JUN gene and promoter show that repair was almost completely absent along all sequences analyzed. Even sequences near the transcription initiation site and along the transcribed strand, which were repaired very efficiently in normal fibroblasts, were repaired extremely poorly in XPA cells (Fig. 4).

**Repair in XPD Cells**—A fibroblast strain from an XPD patient was analyzed in Fig. 5. Similar to XPA, these cells showed a strong deficiency of repair at all sequences analyzed including the sequences near the transcription initiation site and the transcribed strand. However, a comparison between the 0- and 24-h lanes indicates that there is some residual repair activity along the transcribed strand (although less than 50% after 24 h).

**Repair in XPC Cells**—XPC cells are characterized by a DNA repair defect in transcriptionally silent regions of the genome (36). We have analyzed repair of CPDs along the promoter and transcription start site of the JUN gene in XPC cells (Fig. 7). There was a lack of repair along the nontranscribed upstream promoter sequences, and fast repair rates were seen along the transcribed strand downstream of the transcription start site. It appears that repair of the transcribed strand is even more efficient in XPC (Fig. 7) than in normal cells (Fig. 3), perhaps because the gene is most efficiently transcribed in XPC cells (see Fig. 1). In contrast to normal cells, where the domain of fast repair extended upstream to nt −40, fast repair was seen only up to nt −20 in XPC cells. The results suggest that the XPC protein is specifically involved in repair of promoter DNA including the sequence positions spanning nt −20 to −40 upstream of the transcription initiation site but is not required for repair of sequences near the start site.

**Repair in CSB Cells**—Cells from Cockayne syndrome patients are characterized by a DNA repair defect that selectively
affects repair of the transcribed strand of active genes (38, 39). The CSB cells used in our study showed efficient repair of total genomic DNA, which approached similar levels as seen in normal fibroblasts (Fig. 2). Fig. 8 shows the repair rate pattern in CSB cells along the JUN gene. There was inefficient repair of the promoter from nt 240 to 2105, and there was a diminished repair of sequences along the transcribed strand beginning approximately at nt position 120 and extending downstream. However, a clear window of remaining repair activity was seen at sequences surrounding the transcription initiation site, from nt −40 to +20 (Figs. 8 and 9).

**Repair Differences between the Transcribed and Nontranscribed DNA Strands**—To determine repair rates on the two DNA strands within the same area of the gene, we analyzed repair rates between nt 1250 and 1450. The sequences near the transcription start site contain only very few dipyrimidines on the nontranscribed strand that would make comparisons in this area more difficult. We first analyzed repair in normal fibroblasts and obtained faster repair rates for the transcribed strand relative to the nontranscribed strand as previously reported (15). The transcribed strand was repaired about 1.5–2 times faster at 10 J/m² compared with 20 J/m² (data not shown); i.e., the strand difference was more pronounced at the lower levels of genomic damage, as similarly observed by others (5).

XPC cells showed the most pronounced strand-specific repair (Fig. 10). Repair of the transcribed strand was virtually completed after 4 h, similar to normal cells (Fig. 3 and data not shown), while there was a complete lack of repair of the nontranscribed strand. XPB was repair-deficient on both DNA strands, although there was some remaining repair activity at some sites, in particular on the transcribed strand at the 24-h time point (Fig. 10; quantitated by PhosphorImager analysis). CSB cells showed a clear deficiency of repair in the transcribed strand (Fig. 10). Repair of the nontranscribed strand was similar to that in normal fibroblasts (Ref. 15 and data not shown).

**DISCUSSION**

**Gene-specific and Nucleotide-specific DNA Repair Defects in XP and CS Cells**—Cells from patients having the DNA repair-deficient diseases xeroderma pigmentosum and Cockayne syndrome are characterized by specific defects in one of the proteins that function in nucleotide excision repair. Depending on the complementation group and the nature of the specific defect, the general DNA repair activity or only a specific subpathway of NER may be affected.

NER in mammalian cells can be subdivided into at least two major subpathways. Based on present knowledge, this hierarchy of repair activity involves (i) the genome overall repair pathway thought to be involved in the repair of all DNA sequences including nontranscribed DNA and (ii) the transcription-coupled repair pathway that efficiently removes lesions from transcriptionally active DNA sequences and has a prefer-
Fig. 9. Quantitation of DNA repair rates along the promoter and transcription initiation site. Sequence data are from Hattori et al. (65). Transcription factor binding sites identified by genomic footprinting (54, 55) are indicated by ellipsoid boxes. The major transcription initiation site is indicated by the arrow. Repair rates, determined as the time at which 50% of the CPD signal was removed, were estimated for each CPD position with a significant signal above background and are represented by vertical columns. Positions that were repaired less than 50% after 24 h are indicated by a longer column of darker shading.
FIG. 10. **Repair of the transcribed and nontranscribed DNA strands.** An area between nt +250 and +450 of the JUN gene was analyzed along the nontranscribed and transcribed DNA strands in XPC cells (top, left), XPB cells (top, right), and CSB cells (bottom).
repair, which is consistent with data obtained with yeast mutants defective in TFIH subunits (61, 62). The XBP and XPD fibroblast strains used here displayed some residual repair activity of the transcribed DNA strand. In addition, there was a clear reduction in repair rates near the transcription initiation site (Figs. 5 and 6). The results obtained with XPD and XBP cells are thus consistent with a model in which TFIH is somehow functionally involved in the rapid repair of sequences near this site.

Cells representing Cockayne syndrome (CSB) were clearly deficient in repair of the transcribed DNA strand but still efficiently repaired sequences near the transcription initiation site. This implies that the CSB gene product is not required for repair of these sites but functions specifically in repair of sequences that are encountered by RNA polymerase when it is in an elongation mode (downstream of nt +20). This is strikingly similar to the situation in E. coli, where, under conditions of in vitro transcription, stimulation of repair by transcription-repair coupling factor in the transcribed strand starts only at position +15 (63). These similarities suggest that the CSB protein is the mammalian homologue of the E. coli transcription-repair coupling factor.

CSB Is a True DNA Repair Defect—It may be argued that the defective repair of the transcribed DNA strand in Cockayne syndrome cells is not due to a DNA repair defect but is rather a transcription defect in which a gene is not repaired because it is not transcribed (64). The data presented here argue against this possibility. The expression of the JUN gene was actively induced by UV irradiation in CSB cells as indicated by an increase in mRNA levels (Fig. 1). A block of transcription initiation by UV light would instead have resulted in a decrease of mRNA levels given the relatively short half-life of JUN mRNA. The size of the JUN gene is approximately 3 kilobases, and it is likely that a significant proportion of the genes were without dimers at the UV dose employed. Thus, formally we are measuring transcription and repair in two populations of the molecules. However, there is no reason to assume that the presence of a dimer in the transcribed DNA strand would prevent transcription initiation further upstream, i.e. in cis (if this were the case, transcription-coupled repair as we understand it according to current concepts would not exist, even in normal cells). In conclusion, we observed a lack of repair of the transcribed DNA strand despite ongoing transcription. From this argument it would appear that the CSB defect is not merely a transcription defect (with lack of transcription-coupled repair being only a secondary consequence of lack of transcription), but is rather a true DNA repair defect, with the CSB gene product being the transcription-repair coupling factor.

Repair Domains along the JUN Gene—The data on repair along the JUN gene in normal and repair-deficient fibroblasts can be incorporated into a hypothetical model as follows (Fig. 11). Repair of the promoter from nt –105 to nt –20 requires the XPC gene product as well as functional XPA, XBP, and XPD (interestingly, repair of this promoter region was also reduced in the CSB fibroblast strain despite rather efficient overall genome repair); repair of sequences near the transcription initiation site (nt –40 to +20) depends on functional XBP, XPD, and XPA proteins but does not require XPC or CSB proteins; repair of the transcribed strand beginning from nt +20 is dependent on the CSB gene product and also requires XPA, XPD, and XBP. Repair of the nontranscribed strand requires XPA, XPD, and XPC. Further studies will show if this model can be generalized and applied also to the repair of other genes.

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