DNA Repair in the MYC and FMS Proto-oncogenes in Ultraviolet Light-irradiated Human HL60 Promyelocytic Cells during Differentiation

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ABSTRACT

In order to better understand the role of transcription in cellular processing of damage in specific DNA sequences, we have used an in vitro differentiation system to modulate the activity of the MYC gene. When human HL60 promyelocytic cells differentiate in vitro, the transcriptional activity of the MYC gene is down-regulated. We have shown that in the expressed MYC gene, 56% of UV-induced cyclobutane pyrimidine dimers (CPDs) are removed within 18 h and the transcribed strand is selectively repaired. However, late in differentiation, when the MYC gene is maximally down-regulated, only 15% of the CPDs are removed within the same period. During early differentiation, the MYC gene is regulated by a block to transcription elongation at the 5' end of the first intron. Our results reveal no significant difference in the rate of CPD removal between the restriction fragments upstream and downstream of this elongation block. Furthermore, both strands of each fragment exhibit similar repair characteristics. In contrast, the constitutively expressed FMS gene exhibits its proficient removal of CPD in both the differentiated and undifferentiated cells. Furthermore, the repair appears to be more proficient at the 5' end (exon 1) than in the 3' end of the gene about 35 kilobases downstream from exon 1. Since efficient repair of the active FMS gene is maintained in the differentiated cells the loss of repair competence seen in MYC is more likely associated with its reduced transcriptional activity than with a decrease in the overall repair capacity of the terminally differentiated cells.

INTRODUCTION

A number of studies have convincingly demonstrated differences in the cellular processing of various types of DNA lesions within different specific DNA sequences in the genome (1). Such heterogeneity of excision repair was first reported for psoralen adducts in nontranscribed α DNA sequences in cultured African green monkey cells (2); removal of psoralen adducts was much less efficient in the highly repetitive, tandemly arrayed α DNA sequences than in the overall genome. Bohr et al. (3) showed preferential removal of UV light-induced CPD from the expressed DHFR gene in Chinese hamster ovary cells. Mellon et al. (4) demonstrated that most or all of the selective repair in the Chinese hamster ovary DHFR gene was due to the removal of CPD from the transcribed strand of the gene; little repair was seen in the nontranscribed strand. Similar results were obtained for human cells, in which the repair of the transcribed strand occurred at a faster rate than that in the nontranscribed strand. However, in human cells, both strands ultimately exhibited the same extent of repair. The phenomenon of strand-specific repair has now been reported in a number of organisms including Escherichia coli, yeast, and cultured mammalian cells (5). Furthermore, preferential repair of actively transcribed sequences has been shown to occur for a number of different types of lesions including (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene diol epoxide adducts, psoralen photoadducts, and nitrogen mustard adducts (6–8). However, it should also be noted that there are several types of lesions that do not appear to be preferentially repaired, most notably alkylation damage induced by such agents as dimethyl sulfate (9), and even bulky N-(deoxyguanosine-C8-yl)-2-aminofluorene lesions induced by N-acetoxy-acetylaminofluorene do not show preferential repair in the actively transcribed DHFR gene as compared to the rest of the genome in Chinese hamster ovary cells (10). Furthermore, it was reported that lesions induced by 4-nitroquinoline-1-oxide do not exhibit preferential repair in actively transcribed sequences (11).

What factors influence the preferential repair of CPD in expressed genes? In general, the fact that preferential repair can be accounted for by the more proficient repair of CPD on the transcribed strand of actively expressed genes has naturally led to the hypothesis that transcription plays a key role in directing or signaling the cellular nucleotide excision repair mechanism to these lesions (4, 12). A transcription-repair coupling factor from E. coli evidently facilitates the removal of an RNA polymerase molecule stalled at the site of a CPD on the DNA (13–15). However, the actual mechanism of how the factor recruits the repair machinery remains to be elucidated. In human cells, the ERCC6 gene, deficient in Cockayne’s syndrome, has been implicated in transcription-coupled repair and models for the process have been suggested (5, 16, 17). The process may require an actively elongating RNA polymerase II complex.

In an attempt to determine how repair and transcription are related, we chose to study the removal of CPD from the MYC gene in human HL60 promyelocytic leukemia cells as a model system. The well characterized transcriptional regulation of MYC in HL60 cells provides an excellent opportunity to modulate the transcriptional activity of the gene and then assess the effects on the efficiency of CPD removal. The MYC gene in HL60 cells was the first example of a growing class of eukaryotic genes that is regulated at the level of transcription elongation, in a manner reminiscent of bacterial attenuation (18, 19). When HL60 cells are differentiated by a variety of agents such as TPA, DMSO, or retinoic acid, the MYC gene is down-regulated from its maximally transcribed state in the undifferentiated cell type to a state in which transcription elongation is blocked at the 5' end of the first intron. The block to transcription elongation appears between 45 min and 8 h after treatment; after 24 h, the level of transcription initiation from the MYC promoter declines; this is reflected by the reduction in DNase I hypersensitive sites near the promoter region (20). Recent evidence suggests that much of the elongation block can be accounted for by promoter proximal pausing of RNA polymerases within 30 nucleotides of the major promoter (21–23). We have taken advantage of these variable levels of expression of the MYC gene to determine whether there are differences in the repair characteristics of the fully transcribed gene in the undifferentiated cell type as compared to the stage late in differentiation when the gene is maximally down-regulated. Also, when HL60 cells have partially differentiated to the point at which the level of MYC regulation is primarily due to the block of transcription elongation, we have examined the removal of CPD in restriction fragments both upstream and downstream of the elongation block. We find that,
consistent with the notion that preferential repair is dependent on transcription, repair in the actively transcribed MYC gene is proficient and strand specific. In contrast, when the gene is maximally down-regulated late in differentiation, the rate and extent of CPD removal is much reduced. However, at a time during differentiation when the gene is regulated primarily at the level of transcription elongation, we see little difference between the removal of CPD upstream and downstream of the elongation block, suggesting that although there are dramatically different rates of transcription in the two fragments, the repair efficiency is roughly the same. As a positive control we have measured the repair characteristics of the FMS gene, which is constitutively expressed during differentiation. We found rapid removal of CPD from the FMS gene both in differentiated and undifferentiated cell types, suggesting that differentiation per se does not affect preferential repair in the HL60 system.

MATERIALS AND METHODS

Materials. The MYC probes were constructed as follows. pBSCE, an exon 3 probe, is a 1.5-kilobase Clal/EcorI fragment that contains the entire third exon and part of the 3’ end of the second intron cloned into the polylinker of pBS+ from Stratagene (San Diego, CA). This allowed us to generate strand-specific riboprobes for the transcribed (using the T7 promoter) and nontranscribed (using the T3 promoter) strands. A probe for the first exon, pSKPX, was generated in a two-step subcloning; a 594-base pair SstI/XhoI fragment that spans the site of the block of transcription elongation was cloned into the polylinker of pSK+ (Stratagene, San Diego, CA). A 446-base pair PstI/XhoI fragment contained entirely within the first exon was then subcloned into pSK+ to generate pSKPX. The FMS probe, exon 1a probe that spans the entire region of the first exon, was a generous gift of Inder Verma (Salk Institute) (24). The FMS probe spanning the 3’ end of the gene was purchased from Oncogene Science, Inc. (Uniondale, NY). All probes were generated by either nick-translation or by generating strand-specific RNA probes using [32P]dCTP (3000 Ci/mmol) or [32P]dCTP (3000 Ci/mmol) from Amersham (Arlington Heights, IL).

Culture and Differentiation Conditions for HL60 Cells. HL60 human promyelocytic leukemia cells were obtained from American Type Culture Collection and cultured in 75- or 175-cm² tissue culture flasks in RPMI 1640 supplemented with 10% fetal bovine serum (heat inactivated at 56°C for 30 min) and 5 mM glutamine. For those experiments using differentiated HL60 cells, we induced the cells to differentiate using TPA at a concentration of 32 nM (Sigma Chemical Co., St. Louis, MO) in 15-cm tissue culture dishes (Falcon). TPA differentiates the cells along the monocyte/macrophage pathway (25). The cells begin to attach to the plastic dishes giving a good morphological indicator for estimating the extent of cellular differentiation. TPA also regulates the MYC gene at the transcriptional level as well as at the translational level (26–28).

UV Irradiation and Repair Conditions. Cells were irradiated with 20 J/m² (254 nm), which yielded approximately 5% survival as estimated by trypan blue exclusion. For the undifferentiated cell type, the cells were pelleted and resuspended in RPMI 1640 (without fetal bovine serum) containing 20 mM HEPES (pH 7.3) and 2 mM sodium pyruvate as a free radical scavenging agent. The cells were then plated on 15-cm tissue culture dishes and irradiated with a GE germicidal lamp at 0.167 J/m² s⁻¹ for 2 min (20 J/m²). For the differentiated cells, the media were aspirated off and the media described above were used for the irradiations. All cells were plated at a density of between 8.0 × 10⁵ and 1.0 × 10⁶ cells/ml, which minimized shielding due to stacking of cells. Furthermore, the cells were shaken on a platform shaker during the irradiation to ensure uniform dosage. The cells were either lysed immediately after irradiation with a lysis solution (10 mM Tris-Cl, pH 8.0-1 mM EDTA-0.5% SDS-200 μg/ml proteinase K) or incubated in media containing 10 μM bromodeoxyuridine and 1 μM fluorodeoxyuridine to density label any newly replicated DNA over the period of repair prior to lysis.

Repair Assay. DNA from the cellular lysates was prepared using standard phenol extraction procedures. The ethanol precipitated DNA was then resuspended in TE (10 mM Tris-Cl, pH 8.0-1 mM EDTA) and incubated with the appropriate restriction enzyme. The restricted DNA samples were then run on CsCl isopyknic gradients to remove the bromodeoxyuridine-labeled replicated DNA. The CsCl gradients were fractionated and the parental unreplicated fractions were pooled and dialyzed extensively against TE. We used T4 endo V to nick the DNA at sites of CPD as described previously (3). Briefly, each DNA sample was split into two equal parts. One sample was incubated with T4 endo V at 37°C for 15 min, while the other served as an untreated control, not exposed to T4 endo V. The samples were then run on a 1% alkaline agarose gel containing 30 mM NaOH and 1 mM EDTA. The gels were transferred onto Hybond N+ (Amersham) nylon membrane and hybridized with the appropriate radioactively labeled probe. T4 endo V will nick the DNA at the site of the CPD, resulting in the reduction in the intensity of the full length band as compared to the untreated control band. The enzyme is used to measure the number of CPD remaining in the DNA as a function of time yielding a relative repair value (29).

RESULTS

The Transcriptional State of the MYC Gene. In an attempt to measure the transcriptional activity of the MYC gene, total cellular RNA was isolated. The RNA was slot blotted and probed for fragments containing exon 1 and exon 3 MYC RNA (Fig. 1a). Also, nuclear run-on analysis was conducted on undifferentiated cells or cells differentiated for 16 or 48 h to measure transcription in different regions of the MYC gene at the various stages of differentiation (Fig. 1b) (30). See Fig. 2 for map.

The Transcriptional State of the MYC Gene. In an attempt to measure the transcriptional state of the MYC gene, as well as to measure the onset of the block of transcription elongation, we isolated total cellular RNA at various times during differentiation and slot blotted the samples onto nitrocellulose filters. Since the elongation block occurs no further than the 5’ end of the first intron (31–34), using probes specific for exons 1 and 3 allowed us to quickly measure the elongation block. The exon 1 signal gradually decreases as a function of time (Fig. 1a, Lane A). However, the signal for exon 3 decreases dramatically at about 8 h after TPA treatment (Fig. 1a, Lane B). It should be noted that the half-life of full length MYC RNA is very short, between 15–30 min; hence, the measure of steady-state RNA levels gives a rough estimate of the rate of transcription of the gene. But to more accurately determine the transcriptional state of MYC, we carried out nuclear run-on analysis of cells either undifferentiated or differentiated for 16 or 48 h (Fig. 1b). Transcripts are clearly produced in the undifferentiated cell type. However, after 48 h of differentiation, few or no transcripts were detected.

Repair of the MYC Gene. We found rapid removal of CPD from the 8.5-kilobase EcoRI/HindIII maximally transcribed MYC gene fragment in undifferentiated HL60 cells at early time points (Fig. 3), leveling off at 16 h postirradiation. Furthermore, the rate of removal of CPD from the transcribed strand was more rapid than that from the nontranscribed strand, especially at early time points (Fig. 4). When the gene was maximally down-regulated, that is, when the cells were differentiated for a long enough time that the regulation of the MYC gene was not only at the level of transcription elongation but also at the level of transcription initiation, then the repair characteristics of the MYC gene changed dramatically (Fig. 3). Few if any CPD were removed within the first 2 h after irradiation. However, the extent of CPD removal by 48 h reached that of the maximally transcribed MYC gene as expected for generally repair-proficient human cells.

Removal of CPD from the FMS Gene in Differentiated and Undifferentiated Cells. The relatively poor efficiency of CPD removal in the MYC gene when the cells were differentiated may have been due to the absence of transcription in the gene or it might have resulted from a more global repression of all selective repair when the cells were differentiating. To test this latter possibility, we measured the removal of CPD from the actively transcribed FMS gene as a positive control to test for preferential repair. The FMS gene encodes the CSF-1 receptor and is actively transcribed in both the differenti-
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Gene was repaired either at a faster rate or to a better extent in both the differentiated and undifferentiated cell types; and (c) the repair of the FMS gene was somewhat more proficient in differentiated cells than in the undifferentiated cells.

Repair Upstream and Downstream of the Block of Transcription Elongation. When the cells were differentiated for 16 h, a point at which regulation of the MYC gene was primarily due to the block of transcription elongation, we saw little difference in the repair of CPD upstream versus downstream of the block (Fig. 7). Both the transcribed and nontranscribed strands downstream of the block of elongation showed similar rates of CPD removal (Fig. 8). Both strands of the restriction fragment upstream of the block of elongation are transcribed, due to transcription in exon 1 of MYC and transcription of the divergent gene, the expression of which is not affected by the MYC elongation block (34). The strand encoding the divergent upstream transcript shows slightly faster repair than that encoding the first MYC exon (Fig. 8).

DISCUSSION

Transcription has been shown to play an important role in directing the nucleotide excision repair mechanism to remove DNA damage preferentially from actively expressed genes. To test this further, we modulated the expression of the MYC gene in HL60 cells and correspondingly measured the removal of UV light-induced CPD from the gene. In undifferentiated cells, the MYC gene is actively transcribed producing full length mRNA. In differentiated cells, expression of MYC is reduced. The expression of MYC in early differentiation is regulated at the level of transcription elongation; later in differentiation, regulation of the gene also occurs at the level of transcription initiation. We analyzed the repair characteristics of MYC when the cells were either undifferentiated (maximally transcribed) or differentiated for 48 h (maximally down-regulated transcription throughout the gene).

Removal of CPD from the actively transcribed MYC gene in undifferentiated cells was rapid; within 2 h nearly 30% of the CPD were removed. By 16 h the repair of CPD in the MYC gene began to plateau, with almost 60% of the dimers removed. Furthermore, the transcribed strand was repaired more rapidly than the nontranscribed strand and undifferentiated cell types, although little or no mRNA is being produced in the undifferentiated cell type, suggesting that there may be some post- or cotranscriptional regulation of the gene (24, 35). Since the gene is very large (see Fig. 5), we used probes that hybridize to fragments at the 5' end of the gene as well as probes that hybridize to the 3' end of the gene. Our results point out several interesting features: (a) proficient removal of CPD occurs in the FMS gene in cells differentiated for 48 h (Fig. 3b). This suggests that preferential repair is occurring in the differentiated cell type and that the relatively poor removal of CPD seen in the MYC gene is not due to a global repression of the DNA repair mechanisms responsible for preferential repair; (b) differences in the removal of CPD were consistently seen in the 5' end of the gene versus the 3' end (Fig. 6). The 5' end of the gene was repaired either at a faster rate or to a better extent in both the differentiated and undifferentiated cell types; and (c) the repair of the FMS gene was somewhat more proficient in differentiated cells than in the undifferentiated cells.

Fig. 1. RNA analysis of the MYC gene. a, steady state RNA levels of the MYC gene probed for exon 1 (A) and exon 3 (B) transcripts. The graph shows the relative decrease of MYC transcripts as a function of time. b, autoradiograms of nuclear run-ons probed for exon 1 and exon 3 nascent transcripts in undifferentiated and cells differentiated with 32 nm TPA for 16 and 48 h.
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dered dramatically in the maximally down-regulated MYC gene. Few CPD were removed from the MYC gene within 2 h. The removal of CPD continued at later times but still was much reduced from that in the actively transcribed gene in the undifferentiated cell type. We were concerned that the decrease in repair seen in the MYC gene as the cells differentiated might be due to a global reduction of repair capacity. In order to test whether any repair was occurring during differentiation, we measured the removal of CPD from the actively transcribed FMS gene. We showed that in cells differentiated for 48 h, when the MYC gene is transcriptionally repressed and repair is poor, the transcribed FMS gene is still being repaired proficiently. At early repair times the rate of CPD removal was similar to that seen in the actively transcribed MYC gene. These results show that there does not

Fig. 4. Strand-specific repair of the maximally transcribed MYC gene in undifferentiated HL60 cells. The entire c-myc gene fragment was probed with strand-specific 32P-labeled riboprobes using pBSCE (exon 3) to detect the transcribed strand (•) and nontranscribed strand (U).

strand, consistent with previous studies showing strand specific removal of CPD from the DHFR gene in human cells (4). The fact that not all CPD are removed from the gene is consistent with the results of other studies using relatively high UV doses (e.g., 20 J/m²) (36, 37). As higher doses are used the cellular DNA repair capacity evidently becomes saturated, resulting in incomplete repair.

When the cells were allowed to differentiate for 48 h, repair
polymerases are occasionally proceeding past the block of elongation (20). The small amount of transcription occurring downstream of the block might be sufficient to facilitate the repair of the transcribed strand in this region, but that would not explain why repair occurs in the nontranscribed strand as well. Alternatively, since most of the elongation block may be due to promoter proximal pausing of RNA polymerase molecules within the first 30 nucleotides of the major (P2) promoter (21–23), not enough transcription may be taking place over a large enough area to have an effect on repair of the restriction fragment. This would explain the little difference seen between the upstream and downstream MYC strands; however, it would not explain the similar repair characteristics seen in the strand encoding the divergent upstream transcript, unless that too showed extremely localized transcription. It would be interesting to dissect the MYC gene

![Graph](image1)

**Fig. 7.** Repair of the MYC domain upstream and downstream of the block to transcription elongation in HL60 cells differentiated for 16 h. The HindI/HpaI fragment upstream of the transcription elongation block ( ● ) and the XbaI/EcoRI fragments downstream of the block ( □ ) were probed.

![Graph](image2)

**Fig. 8.** Strand-specific repair of the MYC domain upstream and downstream of the elongation block. Removal of CPD was measured in each strand from two Xbal DNA fragments, one fragment mostly upstream of the elongation block and the other fragment completely downstream of the elongation block. Both strands upstream of the elongation block, the strand encoding MYC exon 1 ( ● ) and the strand encoding the divergent upstream transcript ( □ ), showed higher levels and rates of repair than the strands downstream of the elongation block. Both the transcribed ( ● ) and nontranscribed ( □ ) strands downstream of the elongation block show nearly identical removal of CPD.

appear to be a general reduction of the ability of the cells to preferentially remove damage from expressed genes. These results further confirm that removal of CPD is dependent on the transcriptional state of the gene.

We were interested in the effect of the block of transcription elongation on the removal of CPD downstream as well as upstream of the block. Our initial hypothesis was that if the repair machinery must be coupled physically to the transcription elongation complex to recognize and repair lesions in expressed genes, then as the polymerases stalled at the elongation block, little or no repair would occur beyond that point. Our findings did not appear to support this model. Repair downstream of the block was as efficient as that upstream of the block. In addition to the transcription of the first exon in MYC, the fragment studied also contains a divergent upstream transcript which is not affected by the block of transcription elongation in the MYC gene (34). Therefore, transcription is occurring on both strands of the upstream fragment. In contrast, transcription is drastically reduced downstream of the elongation block, although it is possible that a few
even further, by determining the repair of CPD within that region of the MYC exon 1 that is highly transcribed; however, there are several factors that would make that study difficult, including the extremely high UV dose one would need to detect CPD in such a small fragment. Alternatively, the transcription 5′ of the elongation block may be sufficient to direct processive repair machinery to the vicinity of the damage, even if the damage is 3′ of the elongation block. It may be that because of transcription occurring in the 5′ end of the gene, the entire MYC domain is poised so that the nearby repair machinery has greater access to any damage in that region. The chromatin structure may be altered in a such a way that lesions on both strands of the DNA downstream of the block of elongation could be more easily recognized by the repair machinery, allowing repair to occur on both strands. This would explain why both strands downstream of the elongation block show the same level of repair. It should be noted, however, that several other studies in our laboratory, using differentiated cell systems, also have reported no strand-specific repair. Using an L8 rat myoblast cell line, Ho and Hanawalt (38) saw no strand-specific repair in a number of expressed genes investigated, both prior to and during differentiation. A similar absence of strand-specific repair was seen in differentiating PC12 rat pheochromocytoma cells (39). Although both studies showed some enhanced repair of transcribed sequences relative to silent sequences, the repair was seen in both the transcribed and nontranscribed strand. Although these studies were done in rat tissue culture cells and very little is known about preferential repair in rat cells, these studies may point to a unique feature in the repair of terminally differentiated cells. The genomic organization of terminally differentiated cells may be such that the domains containing actively transcribed genes are sufficiently accessible, because of a more open chromatin structure, that efficient repair may be possible on both strands in the sequences flanking expressed genes. In postmitotic cells it may be unnecessary to remove damage from the overall genome, concentrating instead on those regions in which the DNA is in more open chromatin configurations such as domains of active transcription.

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REFERENCES


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