Multiple Mechanisms Contribute to Inhibit Transcription in Response to DNA Damage*

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Cellular DNA damage elicits the phosphorylation and ubiquitination of RNA polymerase II (RNAPII), leading to the global repression of transcription. In this report we show that there are at least two different pathways to transcriptional repression, depending on the type of DNA damage. After H2O2 treatment, transcription was rapidly inhibited and rapidly restored. On the other hand, UV irradiation caused a much slower transcriptional inhibition, with a corresponding depletion of unphosphorylated RNAPII. We found that after UV treatment, but not treatment with H2O2, the inhibition of transcription was dependent on both the proteasome and new protein synthesis. In addition, RNAPII activity and ubiquitination were regulated through the phosphorylation of RNAPII by the P-TEFb kinase. These results highlight that multiple cellular pathways exist to globally repress transcriptional processes that might interfere with the repair of DNA damage.

Transcription and the response to DNA damage are interconnected processes, but the mechanisms linking these are unknown. Following DNA damage, transcription by RNA polymerase II (RNAPII) is globally repressed (1–3). Cockayne syndrome (CS) proteins are required to ubiquitinate RNAPII at the site of a DNA lesion and signaling transcription-coupled repair (4). Interestingly, CS proteins are not required for the global repression of transcription after DNA damage, but in CS mutant cells transcription remains repressed for prolonged periods (1). There is limited information about what proteins participate in mammalian cells in establishing the global repression of transcription after DNA damage.

It is known that the RNAPII becomes phosphorylated on the carboxyl-terminal domain (CTD) of its largest subunit, Rpb1. The CTD contains a repeating unit of seven amino acids with serines at positions 2 and 5. The phosphorylation of these two serines is tightly regulated and has important functional consequences. Only unphosphorylated RNAPII assembles into the preinitiation complex that forms at core promoters (5). During the initiation of transcription, RNAPII is phosphorylated on multiple repeats at serine 5 (Ser5p). During the transition to the elongating polymerase, the phosphorylation switches to serine 2 (6).

DNA damage causes the specific phosphorylation of the CTD and consequent ubiquitination of Rpb1. The specificity of the phosphorylation, interestingly, diverges between Saccharomyces cerevisiae and higher eukaryotes. In yeast, DNA damage causes serine 2 phosphorylation and Rpb1 ubiquitination. The proteins that execute these functions in the yeast cell have been identified (7–12). In mammalian cells, unlike yeast, serine 5 is the primary phosphoaceptor after DNA damage and the ubiquitin ligases are unknown. However, it has been shown that the BRCA1-ubiquitin ligase can execute the Rpb1 Ser5p-specific ubiquitination function (13, 14). Some CTD kinases involved in the damage response have been identified (15–17). Although the P-TEFb (Cdk9) kinase regulates the phosphorylation of serine 2 for transcription elongation under normal regulatory conditions (18, 19), P-TEFb along with JNK kinases phosphorylate serine 5 after UV-induced DNA damage. The MEK1/2 pathway phosphorylates serine 5 in response to H2O2 treatment. Additionally, cells that are deficient for CS proteins or the VHL protein do not ubiquitinate Rpb1 after UV damage. In response to H2O2 exposure, ubiquitination of Ser5p-Rpb1 was observed in the CS- and VHL-deficient cell lines, indicating a second pathway toward ubiquitinating RNAPII (15).

Previous work has shown that RNAPII becomes extensively modified in response to DNA damage (4, 15, 20). In this study, we set out to determine the causal relationship between RNAPII modifications and transcriptional inhibition. We find that there are two pathways to repress the global transcription initiation potential of the cell, just as there are at least two pathways to induce CTD phosphorylation in response to different types of DNA damage (15). Which pathway operates depends on the type of DNA damage inflicted. We focused on the UV-induced pathway and found that this pathway caused a depletion of unphosphorylated RNAPII, blocking transcription initiation. In addition, this depletion of unphosphorylated
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RNAPII in response to UV is complex and depends on the activity of P-TEFb, the proteasome, and new protein synthesis.

EXPERIMENTAL PROCEDURES

Cell Culture and Treatments—HeLa-S3 human cervical carcinoma cells were maintained using standard methods. For UV treatment, each 10-cm plate was covered in 1 ml of phosphate-buffered saline, irradiated with 254-nm UV light at 25 J/m² (Hoefer UVC-500), and fresh media was added for various recovery times. For H₂O₂ treatment, H₂O₂ (Sigma) was added directly to culture medium (10 mM final concentration) and incubated at 37 °C for 15 min. H₂O₂-containing medium was replaced with fresh culture media and incubated at 37 °C for various recovery times. Reagents for treatment of cells were prepared in ethanol and used at the following concentrations: 20 μM MG132, 25 μg/ml cycloheximide, 50 μM 5,6-dichlorobenzimidazole 1-β-D-ribofuranoside (DRB), 30 μM U0126, or 30 μM SP600125. Cells were treated with reagents 30 min before DNA damage and maintained in media during recovery periods. Stock solutions of MG132 were prepared using Me₂SO for UV treatment or ethanol for H₂O₂ treatment to avoid any nonspecific oxidizing affects in the cells due to the presence of Me₂SO.

Transcription Reactions and Western Blots—For nuclear extract preparations, each 10-cm dish was washed twice with ice-cold phosphate-buffered saline and pelleted at 14,000 × g in 1 ml of phosphate-buffered saline. The mini-nuclear extracts were prepared as previously described (21). Transcription reactions using 10 μg of nuclear extract were performed essentially as previously described (22, 23). Reactions were performed in 20 mM Hepes pH 7.9, 1 mM EDTA, 5% glycerol, 60 mM NaCl, 300 μM ATP, 100 μM UTP, 2.5 μM CTP, 50 μM 3′-O-methyl-GTP, 1 mM dithiothreitol, 400 ng of poly(dG-dC) (Amersham Biosciences), 4 mM MgCl₂, 400 ng of plasmid (pAML-200) (24), 0.15 mg/ml bovine serum albumin, 8 units of RNasin (Promega), 500 μM creatine phosphate, 1 unit of creatine kinase (Sigma), 10 μCi of [α-32P]CTP (800 Ci/mmol; PerkinElmer Life Sciences) in a total of 25 μl. The following transcription factors were purified as previously described (25–27) and added to the above reaction mixtures for the add back experiments: 60 ng of TFIIB, 4 ng of TFIIE, 100 ng of TFIIF, 0.5 μl of TFIIH fraction, 16 ng of TBP, and 1.0 μl of calf thymus RNAPII. Transcription reactions were incubated at 30 °C for 75 min with RNase T1 (0.4 units) added to the reactions after 30 min. The reactions were terminated by adding 200 μl of transcription stop mixture (7 mM urea, 0.5% SDS, 2 mM EDTA, 0.1 mM LiCl, 0.35 M NH₄OAc), followed by phenol/chloroform extraction and ethanol precipitation. Transcription products were resolved on 6% polyacrylamide gels in 8.3 M urea. Gels were dried and exposed to films with intensifying screens.

For Western blots, 15 μg of nuclear extracts were resolved using standard SDS-PAGE and Western techniques. The following antibodies were used: Rpb1 (8WG16; Promega), the Ser5p Rpb1 (H14; Covance), Ser2p-Rpb1 (H5; Covance), and various rabbit polyclonal antibodies (Cocalico) specific to TFIIB, TFIIE, TFIIF, TFIIH, and TBP.

Isolation of Ubiquitinated Proteins—To isolate ubiquitinated proteins, HeLa cells expressing a doubly tagged His-biotin-ubiquitin (32) was used. HeLa cells were treated as described above and allowed to recover for 60 min. A total of 500 μg of nuclear extract was added to avidin-agarose beads (Pierce) and incubated for 2 h at room temperature, with rotation. Beads were extensively washed with a buffer containing 1 M urea, 0.3 M NaCl, 0.5% Nonidet P-40, and 0.1 μM sodium phosphate, pH 7.5. Proteins were eluted from the avidin beads by boiling in 1% SDS and subjected to SDS-PAGE and immunoblotting as described above.

RESULTS

The Transcriptional Initiation Potential of the Cell Changes in Response to DNA Damage—Previous reports have shown that RNAPII is specifically phosphorylated, ubiquitinated, and degraded in response to DNA damaging agents such as H₂O₂, cisplatin, and UV light (2, 4, 15, 28, 29). We tested whether these DNA damaging events affect the transcription activity of the cell. Following treatment of cells with H₂O₂, UV, or other agents, mini-nuclear extracts were prepared, and the transcriptional initiation potential of the cell was assayed in vitro. The mini-nuclear extract protocol was important for this assay system because protein samples highly active for transcription assays could be rapidly purified from a small number of cells. For this assay, 10-cm plates of HeLa cells were treated with a damaging agent, H₂O₂ (10 mM), or irradiated with UV-C light (254 nm; 25 J/m²), and allowed to recover for various times. Reduction of the H₂O₂ concentration to 0.3 mM or UV dose to 10 J/m² resulted in similar effects (data not shown). Next, nuclear extracts were prepared from each dish, normalized for total protein content, and used for in vitro transcriptional assays in the presence of an undamaged plasmid DNA template. The nuclear extracts were also analyzed by Western blot to determine the relative abundance, phosphorylation state, and ubiquitination status of RNAPII and other proteins.

Treatment of cells with H₂O₂ elicits a very rapid repression of transcription at 0 min post-treatment with recovery of RNA synthesis within 30 min and followed by hyperactive transcription at 120 min (Fig. 1, top panel, lanes 1–6). To rule out the possibility that the H₂O₂ oxidized proteins essential for transcription, H₂O₂ was added directly to nuclear extracts followed by the transcription assay. Transcription was inhibited by the direct addition of H₂O₂ to the extract, however, this inhibition was fully recovered by the addition of the reducing agent dithiothreitol at similar concentrations used in preparing the nuclear extracts (data not shown). Because the nuclear extracts are prepared in the presence of reducing agent, we conclude that the inhibition of transcription in response to H₂O₂ is due to a cellular response and not due to direct oxidation of proteins by H₂O₂.

Next, we investigated the effects of UV-induced DNA damage on cellular transcription. HeLa cells were irradiated with UV and allowed to recover for the indicated times. In contrast to cells treated with H₂O₂, UV treatment of cells repressed transcription by a significantly slower time course, with full repression at 120 min post-treatment (Fig. 1, top panel, lanes 7–12). The inhibition of transcription following UV treatment continued past 3 h and was fully recovered by 16 h after UV treatment (data not shown). Treatment of the cells with 0.025...
mM cisplatin or 50 milliunits of bleomycin for 60 min resulted in transcription after H2O2 or UV treatments, we were due to the differential inhibition of transcription along a time course similar to that of mammalian cells (4, 14, 15, 28). When low DNA damage, but not after oxidative damage. The faster migrating band detected with this 8WG16 monoclonal antibody, at a position consistent with 210 kDa, was the unphosphorylated form. The slower migrating band detected with this 8WG16 antibody co-migrated with the phosphorylated Rpb1 at a position consistent with 240 kDa. Unlike what was observed for the UV-irradiated samples, the level of unphosphorylated Rpb1 in the H2O2-treated samples did not drop toward undetectable levels (see below), and transcription was active at time points with the highest level of Ser5p-Rpb1 ubiquitination (Fig. 1, lane 4). In contrast to H2O2 treatment, Western blot analysis of cells irradiated with UV show that at 15 min post-treatment, the Ser5p-Rpb1 begins to accumulate and does not decrease over the time points tested here (Fig. 1). In addition, the ubiquitinated Ser5p-Rpb1 peaked at 60 min and was still detectable at 120 min post-treatment when transcription was fully suppressed. In contrast to H2O2-treated cells, Western blots of UV-treated cells showed a gradual decrease in the unphosphorylated Rpb1, which was undetectable at 120 min post-treatment (Fig. 1). The depletion of the unphosphorylated RNAII in UV-treated cells correlates well with the suppression of transcription. Together, these data suggest that H2O2 and UV treatment result in different DNA damage responses leading to the inhibition of transcription. We also tested for Ser2p-Rpb1, and it was found to be at very low levels in the nuclear extracts, and changes in or ubiquitination of this specific phosphorylation state of RNAII were not observed (data not shown).

Because RNAII works in concert with several other basal transcription factors, we tested whether other factors were also affected by treatment of cells with H2O2 or UV treatment. The minimal set of purified factors required for transcription includes TFIIH, RNAPII, and TBP (33). As can be seen in the series of Western blots shown in Fig. 1, none of these basal transcription factors are affected by treatment with either of the DNA damaging agents used here. These data indicate that the modification of RNAII could limit the transcriptional activation in response to DNA damage.

To confirm the presence of the ubiquitinated phospho-Rpb1 following DNA damage, we isolated ubiquitinated proteins in HeLa cells expressing a biotin-tagged ubiquitin (31, 32). Ubiquitinated proteins were purified using avidin beads and subjected to immunoblot analysis using the anti-Ser5p-Rpb1 antibody H14 (Fig. 2). Cells were pretreated with the proteasome inhibitor MG132, as indicated, for 30 min prior to exposure to DNA damaging agents. After H2O2 or UV treatment, cells were allowed to recover for 60 min, nuclear extracts were generated, and ubiquitinated proteins were isolated. Following H2O2 treatment, the Ser5p-specific Rpb1 had slowed migration, with a smear, consistent with polyubiquitination (Fig. 2, top panel, lanes 2 and 3). In contrast to treatment with H2O2, UV treatment of cells resulted in a discrete Ser5p-Rpb1-specific band and with a limited amount of the slower migrating species (Fig. 2, bottom panel, lanes 5 and 6).
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FIGURE 2. Ubiquitination of Ser5p-Rpb1 in response to DNA damage. Western blots of HeLa cells expressing biotin-tagged ubiquitin in the presence or absence of the proteasome inhibitor MG132. Cells treated for 30 min with MG132 (where indicated) before treatment with 10 mM H$_2$O$_2$ (lanes 2 and 3), 25 J/m$^2$ UV light (lanes 4 and 5), or no treatment (Mock; lane 1). Ubiquitinated proteins were isolated from total cell extract via binding of the biotinylated ubiquitin to avidin beads. The immunoblot of avidin-purified proteins was probed for the Ser5p RNAPII using the H14 antibody (top panel). Input samples for the avidin purification were analyzed by immunoblot and probed with an antibody specific for the type of damage. Interestingly, inhibited for transcription in H$_2$O$_2$-treated cells (0 min of recovery) was not reversed by addition of any of the basal factors (Fig. 3). We interpret this result to indicate that the transcription repression following H$_2$O$_2$ damage is due to an unidentified trans-repressor rather than due to the loss of a basal factor.

The Proteasome and New Protein Synthesis Are Both Required for Inhibition of Transcription in Response to UV, but Not H$_2$O$_2$.—According to previous reports, DNA damage leads to the ubiquitination and subsequent degradation of RNAPII (15, 28, 29, 34). To examine if the inhibition of transcription in response to H$_2$O$_2$ treatment is due to protein degradation via the proteasome, we pretreated cells for 30 min with either the proteasome inhibitor MG132 or vehicle. After H$_2$O$_2$ treatment, cells were allowed to recover for the indicated times and nuclear extracts were generated. Cells treated with H$_2$O$_2$ were inhibited for transcription at 0 min post-treatment regardless of the presence of MG132 (Fig. 4). No changes were detected in the accumulation of Ser5p-Rpb1 nor were any changes detected in its ubiquitination status in cells treated with or without MG132. Additionally, no changes were detected in the accumulation of the unphosphorylated Rpb1 subunit in the MG132 sample when compared with the non-treated sample. The Western blots were also probed with an antibody specific for TFIIH subunit p89 as a gel loading control (Fig. 4). These data suggest that the proteasome is not involved in the inhibition of transcription in response to H$_2$O$_2$ treatment.
Our data indicate that this effect limits transcription initiation potential. In addition, because the effect was partial, these results also show that an additional mechanism other than via the proteasome is required for the inhibition of RNAPII. Collectively, these data highlight the involvement of the proteasome in response to UV-induced DNA damage, but not H$_2$O$_2$ treatment.

Because the treatment of cells with UV in the presence of MG132 did not completely reverse the repression of transcription at 120 min, we reasoned that another factor, not being degraded by the proteasome, could also be involved in the inhibition of transcription. To determine whether this factor is newly translated, cells were treated with the proteasome inhibitor MG132 and/or the ribosome inhibitor cycloheximide for 30 min prior to treatment with UV and allowed to recover for 120 min in the presence of the drug. Interestingly, pretreatment of cells with cycloheximide also partially reversed UV-induced transcriptional inhibition. Moreover, the addition of both MG132 and cycloheximide resulted in a complete restoration of uninhibited transcription levels. Additionally, treatment of cells with cycloheximide partially stabilized the unphosphorylated RNAPII (Fig. 5). Treatment of cells with H$_2$O$_2$ in the presence of cycloheximide had no influence on the inhibition of transcription or accumulation of RNAPII (data not shown). This result is consistent with the concept of unphosphorylated RNAPII being the limiting factor in transcription following UV damage, but an additional factor, likely to be a trans-acting repressor, is contributing to the response.

Inhibition of Transcription in Response to UV Irradiation-Induced DNA Damage Requires the Phosphorylation of Ser-5 of Rpb1—Previous work has shown that the phosphorylation of Ser5p-Rpb1 is required for both damage-independent ubiquitination (28) and UV-induced ubiquitination (14, 15) of RNAPII. In response to UV, phosphorylation can be abrogated by the addition of two kinase inhibitors, DRB, and SP600125, which inhibit P-TEFb, and JNK, respectively (15, 16, 35). We tested whether the activity of any of these kinases is required for the DNA damage-induced inhibition of transcription. HeLa cells were pretreated with three protein kinase inhibitors including U0126 (MEK1/2 inhibitor), DRB (P-TEFb inhibitor), or SP600125 (JNK inhibitor) for 30 min prior to treatment with UV and allowed to recover for 120 min in the presence of the inhibitor. Transcriptional assays of nuclear extracts from these treated cells revealed that cells treated with DRB blocked the UV-induced inhibition of transcription (Fig. 6). Western blots of nuclear extracts probed for the phosphorylation status of Rpb1 revealed a decrease in the accumulation of the Ser5p-Rpb1 and a stabilization of the unphosphorylated RNAPII in the UV-treated DRB sample. Treatment of cells with DRB did not completely abolish the phosphorylation of Ser5p-Rpb1, but this was not entirely unexpected, as TFIIH can also phosphorylate Ser-5. Although the SP600125 was shown previously to inhibit Ser-5 phosphorylation in response to UV treatment after 60 min of recovery (15), we did not detect any effect of this kinase inhibitor at the time point tested here. Similarily, the extracellular signal-regulated kinase (ERK) 1/2 kinase inhibitor, U0126, had no effect on the accumulation of Ser5p-Rpb1 after UV treatment (Fig. 6). In addition, these kinase inhibitors had
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FIGURE 6. UV-induced inhibition of transcription depends on P-TEFb kinase activity. Nuclear extracts were generated from HeLa cells treated either with (+) or without (−) UV light. Where indicated, kinase inhibitors U0126 (lanes 3 and 4), DRB (lanes 5 and 6), and SP600125 (lanes 7 and 8) were added to cells 30 min prior to UV treatment and remained in the media during the recovery period. Nuclear extracts generated after the 120-min recovery period were used for transcription assays (Trxen) and Western blot analysis with the indicated antibodies.

no effect on transcription initiation after H2O2 treatment in our experiments (data not shown). These findings indicate that P-TEFb is the major Ser-5 kinase in response to UV-induced DNA damage and Ser-5 hyperphosphorylation is required for the inhibition of transcription in response to UV irradiation.

DISCUSSION

RNAPII is a target for modification through phosphorylation and ubiquitination in response to DNA damage (2, 4, 15, 28, 29). In this study, we extend this knowledge in several important ways: 1) we find multiple pathways exist to shut down transcription initiation following DNA damage; 2) following UV damage cells lack unphosphorylated RNAPII required for transcription initiation; 3) both protein degradation by the proteasome as well as new protein synthesis control transcription inhibition after UV damage; and 4) the post-UV damage RNAPII levels and ubiquitination are controlled by phosphorylation on Ser5p-Rpb1 by P-TEFb.

UV damage of DNA elicits a number of DNA repair mechanisms. One key mechanism is transcription-coupled repair, which depends on Cockayne syndrome proteins, CSA and CSB, to control the ubiquitination of the RNAPII molecule at a DNA lesion (4, 36). Because we used an undamaged DNA template and assayed for the global effects of DNA damage on transcription factors, our study did not address transcription-coupled repair. There is a global effect on transcription following UV irradiation in which bulk RNAPII initiation of mRNA synthesis is blocked, and, interestingly, the cells mutant for CS proteins are not defective for the global shut down of RNA synthesis but rather fail to re-start transcription following DNA insult (1, 2, 36).

We found evidence of two different pathways in HeLa cells that globally repress transcription after DNA damage. Prior work had hinted at this observation because phosphorylation and ubiquitination of RNAPII after H2O2 depended on different kinases when compared with those required for UV irradiation, but transcription effects were not tested (15). We found peroxide treatment of cells resulted in the rapid inhibition of transcription initiation irrespective of proteasome function, new protein synthesis, or the inclusion of excess basal transcription factors into reactions. These results suggest that H2O2 treatment of cells caused an already present trans-acting inhibitor to become active, transiently shutting down transcription. Subsequently, relief from the inhibition caused hyperactive transcription initiation.

In contrast to H2O2 exposure, treatment of cells with UV, cisplatin, or bleomycin resulted in a slow suppression of transcription initiation that rebounded more than 3 h later. We investigated the UV-induced pathway and found that transcriptional inhibition correlated with the appearance of ubiquitin-modified Ser5p-Rpb1 and consequent loss of unphosphorylated Rpb1, which is known to be required for loading RNAPII into the preinitiation complex (5). Loss of unphosphorylated RNAPII was clearly causative because inclusion of purified unphosphorylated RNAPII in the UV-damaged nuclear extract reconstituted full transcriptional activity. The post-UV damage suppression of transcription initiation required new protein synthesis, proteasome function, and phosphorylation of RNAPII, presumably by P-TEFb. DNA damage induced by cisplatin treatment of HeLa cells revealed that RNAPII contains polyubiquitin chains with Lys-6, Lys-48, and Lys-63 linkages (37), suggesting that multiple E3-ubiquitin ligases act directly on RNAPII in response to DNA damage. This ubiquitination then results in the destruction of RNAPII, resulting in diminished initiation-competent unphosphorylated RNAPII. The requirement for new protein synthesis for complete inhibition of transcription suggests the possibility that a second, newly translated ubiquitin ligase acts on Ser5p-RNAPII. However, because the effects of MG132 and cycloheximide on transcription inhibition were additive, we suggest that the UV response itself results from at least two pathways, only one of which is proteasome-dependent.

The ubiquitination of RNAPII in response to DNA damage depends on the hyper-phosphorylation of Ser5p-Rpb1 (29). Although the kinase activity associated with TFIIH phosphorylates the CTD at Ser-5 for transcriptional initiation (38), the P-TEFb kinase phosphorylates Ser-5 in response to UV irradiation, but not transcriptional initiation (15). The phosphorylation of Ser-5 via P-TEFb signals for the ubiquitination of RNAPII and transcriptional inhibition. Thus, the phosphorylation of Ser-5 is a key regulatory step in the transcriptional inhibition in response to UV.

In response to DNA damage, RNAPII can be ubiquitinated by a number of ubiquitin ligases, which include BRCA1, CSA, and VHL protein complexes (4, 14, 15, 20, 22). During the preparation of this article, a new report linked an E3 ubiquitin ligase, Nedd4, to the ubiquitination of RNAPII after UV damage (39). In that study, the role of RNAPII phosphorylation on the Nedd4 ubiquitination activity was not addressed.

Previous results show that overexpressed BRCA1 can ubiquitinate phospho-RNAPII in response to UV damage (14, 22). BRCA1-dependent ubiquitination of RNAPII could inhibit transcription independent of the proteasome by preventing the assembly of the pre-initiation complex (22). In our unpublished...
observations, we found that silencing BRCA1 by RNA interference resulted in decreased ubiquitination of the Ser5p-Rpb1 subunit, an increase in the content of unphosphorylated RNAPII, and a modest blunting of the effect of UV on transcription inhibition. We infer from these observations that BRCA1 is one of two or more ubiquitin ligases that execute the UV-induced ubiquitination of Ser5p-Rpb1. Because this current study assays the transcription initiation potential from crude extracts, it remains unclear which ubiquitin ligase is required for transcriptional repression via the proteasome. Previous studies indicate that multiple ubiquitin ligases are probably modifying RNAPII in response to UV irradiation (4, 15, 20, 39). The importance of BRCA1 and the ubiquitination of RNAPII to the DNA damage response is indicated by the finding that survival following UV damage depends on BRCA1-mediated ubiquitination of a small subunit of RNAPII, Rpb8 (40).

Why does the cell inhibit transcription globally following DNA damage, and why are there different damage type-specific pathways that repress transcription according to different time courses? It is likely that once significant DNA damage is detected, the transcription process is halted to prevent disruption of repair processes. The existence of multiple, possibly redundant, pathways to repress transcription initiation highlights that this process is vital and suggests the possibility that unless it is turned off, transcription could interfere with the proper repair of DNA.

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