Elongating RNA Polymerase II Is Disassembled through Specific Degradation of Its Largest but Not Other Subunits in Response to DNA Damage in Vivo*

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Although previous biochemical studies have demonstrated global degradation of the largest subunit, Rpb1p, of RNA polymerase II in response to DNA damage, it is still not clear whether the initiating or elongating form of Rpb1p is targeted for degradation in vivo. Further, whether other components of RNA polymerase II are degraded in response to DNA damage remains unknown. Here, we show that the Rpb1p subunit of the elongating, but not initiating, form of RNA polymerase II is degraded at the active genes in response to 4-nitroquinoline-1-oxide-induced DNA damage in Saccharomyces cerevisiae. However, other subunits of RNA polymerase II are not degraded in response to DNA damage. Further, we show that Rpb1p is essential for RNA polymerase II assembly at the active gene, and thus, the degradation of Rpb1p following DNA damage disassembles elongating RNA polymerase II. Taken together, our data demonstrate that Rpb1p but not other subunits of elongating RNA polymerase II is specifically degraded in response to DNA damage, and such a degradation of Rpb1p is critical for the disassembly of elongating RNA polymerase II at the DNA lesion in vivo.

The genomic DNA is continuously attacked by numerous damaging agents (1). The cellular lesions interfere with various types of DNA transacting processes, leading to extensive degenerative diseases (2–5). However, the integrity of the genomic DNA is protected from genotoxic factors by an intricate network of mechanisms in both prokaryotic and eukaryotic organisms (6–13). The most versatile cellular mechanism to deal with a large variety of DNA lesions is nucleotide excision repair (8), whereas the double-stranded DNA breaks are processed by homology-dependent recombination or DNA end-joining (9, 10).

A severe ramification of DNA damage is the formation of lesions in the transcribing strands of the active genes. These lesions cause the distortion of local helical DNA structure, pausing the elongating RNA polymerase II (14–18), thereby leading to a transcriptional arrest. Such a transcriptional blockage interferes with cell function or triggers apoptosis (19, 20). Fortunately, the cell employs a specific repair mechanism to efficiently remove lesions from the transcribing strands of the active genes for normal cellular activities. Such a repair mode is referred to as transcription-coupled DNA repair (6, 21–25). Transcription-coupled DNA repair is present in both prokaryotic and eukaryotic organisms (26). In prokaryotes, transcription repair coupling factor displaces the stalled RNA polymerase, facilitating recruitment of the DNA repair machinery to the lesion. However, the mechanisms, regulation, and intricate connections of the events involved in eukaryotic transcription-coupled DNA repair are considerably more complex and poorly understood.

Several studies (16, 27–29) in eukaryotes have indicated that the stalled RNA polymerase II covers the DNA lesion, making it less accessible to the repair machinery. Two models have been postulated for the accessibility step of the repair machinery in eukaryotic transcription-coupled DNA repair. The first one is backward displacement of RNA polymerase II from the damaged site to facilitate the access of DNA repair machinery to the DNA lesion. Following DNA repair, RNA polymerase II continues elongation to finish the incomplete mRNA (29–31). The second model involves the ubiquitination of Rpb1p, the largest subunit of RNA polymerase II, upon transcriptional arrest and subsequent proteolysis by the 26 S proteasome complex (17, 29, 32, 33). In this model, the incomplete mRNA is discarded, and transcription is reinitiated to generate a new transcript. The occurrence of both models in vitro has been reported in the literature (30), suggesting that both options are perhaps present in living eukaryotic cells. However, what exactly occurs at the DNA lesions in the transcriptionally active genes in vivo remains mostly unknown. Here, we have analyzed the fate of RNA polymerase II at the transcriptionally active genes in vivo in response to 4NQO (a UV-mimetic agent)-induced DNA damage in vivo. Our data demonstrate that only Rpb1p, and not other subunits, of elongating RNA polymerase II is degraded to disassemble RNA polymerase II at the coding sequences of the active genes in response to 4NQO-

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induced DNA damage, thus supporting the second model in vivo.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—The plasmid pFA6a-13Myc-KanMX6 (34) was used for genomic Myc epitope tagging of the proteins of interest.

**Yeast Strains and Media**—Yeast strain harboring a temperature-sensitive (ts) mutation in RPB1 was obtained from the Young laboratory (Richard A. Young, MIT). The ts mutant and its isogenic wild-type equivalent were gifts from Ali Shilatifard (Stowers Institute for Medical Research, Kansas City, MO). Multiple Myc epitope tags were added at the original chromosomal loci of RPB1, RPB2, RPB3, RPB4, RPB5, RPB7, RPB8, RPB9, RPB10, and RPB11 in W303a to generate ZDY4 (Rpb1p-myc, KAN), ZDY5 (Rpb2p-myc, KAN), NSY17 (Rpb3p-myc, KAN), ZDY6 (Rpb4p-myc, KAN), ZDY8 (Rpb6p-myc, KAN), ZDY9 (Rpb7p-myc, KAN), ZDY10 (Rpb8p-myc, KAN), ZDY11 (Rpb9p-myc, KAN), ZDY12 (Rpb10p-myc, KAN), and ZDY13 (Rpb11p-myc, KAN), respectively. The strains ZDY15 (Rpb3p-myc, KAN; rpb1 -ts) and ZDY16 (Rpb4p-myc, KAN; rpb1 -ts) were generated by adding multiple Myc epitope at the C termini of Rpb3p and Rpb4b, respectively, in the rpb1-ts strain.

The DEFI deletion mutant and its isogenic wild-type equivalent were grown in YPR (yeast extract-peptone plus 2% raffinose) up to an A600 of 0.8 at 30 °C prior to formaldehyde cross-linking. For ts mutants and yeast cells were grown in YPR (yeast extract-peptone plus 2% galactose) for 90 min to induce GAL1 prior to 4NQO treatment. Similarly, the mutant and its isogenic wild-type equivalent were grown in W303a to generate ZDY4 (Rpb1p-myc, KAN), ZDY5 (Rpb2p-myc, KAN), NSY17 (Rpb3p-myc, KAN), ZDY6 (Rpb4p-myc, KAN), ZDY8 (Rpb6p-myc, KAN), ZDY9 (Rpb7p-myc, KAN), ZDY10 (Rpb8p-myc, KAN), ZDY11 (Rpb9p-myc, KAN), ZDY12 (Rpb10p-myc, KAN), and ZDY13 (Rpb11p-myc, KAN), respectively. The strains ZDY15 (Rpb3p-myc, KAN; rpb1-ts) and ZDY16 (Rpb4p-myc, KAN; rpb1-ts) were generated by adding multiple Myc epitope at the C termini of Rpb3p and Rpb4b, respectively, in the rpb1-ts strain.

**ChIP Assay**—The ChIP assay was performed as described previously (35, 36). Briefly, yeast cells were cross-linked by 1% formaldehyde and harvested. Following sonication, 100 μl of whole cell lysates (400 μl of lysate from 50 ml of yeast culture) was used for each immunoprecipitation. Immunoprecipitated DNA was dissolved in 20 μl of TE 8.0 (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA), and 1 μl of immunoprecipitated DNA was used for PCR analysis. PCRs contained [α-32P]dATP (2.5 μCi for each 25-μl reaction), and the PCR products were detected by autoradiography after separation on a 6% polyacrylamide gel. As a control, “input” DNA was isolated from 5 μl of lysate without going through the immunoprecipitation step and suspended in 100 μl of TE 8.0. To compare PCR signal arising from the immunoprecipitated DNA with the input DNA, 1 μl of the input DNA was used in PCR analysis. Serial dilutions of the input and immunoprecipitated DNAs were used to assess the linear range of PCR amplification as described previously (36). All of the PCR data presented in this paper are within the linear range of PCR analysis.

Primer pairs used for PCR analysis were as follows: GAL1 (UAS), 5′-CGCTTAACTGCTCATGTGATTG-3′ and 5′-TTGTTCGGAGACTGTCGGGC-3′; GAL1 (Core), 5′-ATAGATGAATACTGCTGACGTTAATGCGATTAGTTTTTAGGCTT-3′ and 5′-GAAAATGTGAAATGAATGTTTAAGTTG-3′; GAL7 (ORF), 5′-CAGAGGCTAAGCATTGTTATTC-3′ and 5′-GTCAATCTCTGGACAAAGACATT-3′; GAL7 (Core), 5′-CTATGTTCTAATTGTGGCCTAGC-3′ and 5′-TGTAGTCCTCTGATATAATGCCC-3′; GAL7 (ORF), 5′-TGACGTGCTTCACTTCAAGAAG-3′ and 5′-AATGATAACCAATTGAGTTGAAA-3′; GAL7 (ORF), 5′-GCTAAGATATGGGCCTTATACAT-3′ and 5′-TTTCATTTTGTAAGCCTGTCAT-3′; GAL10 (ORF), 5′-TTAATGGCAATCTATAGTATCGCAGG-3′ and 5′-TTAACCAATAGATCCTCCGAAATTTCC-3′.

Autoradiograms were scanned and quantitated by NIH Image version 1.62 (National Institutes of Health). The ratio of immunoprecipitated DNA over the input was presented as %IP. For mutant strains, DNA immunoprecipitated relative to wild type is presented as %WT.

**DNA Damage Protocol**—Yeast cells were grown to a desired A600 and then a concentrated solution of 4NQO in ethanol (0.4 mg/ml) was added to the growing yeast culture to a final concentration of 4 μg/ml. The 4NQO-treated cells in liquid YPG medium were allowed to grow at 30 °C for different times and then these cells were processed for either ChIP or Western blot analysis.

**Genomic DNA Preparation**—Five ml of 4NQO-treated yeast cells was harvested at different time points. The genomic DNAs were extracted from the 4NQO-treated yeast cells. Briefly, the harvested cells were suspended in 200 μl of lysis buffer (50 mM HEPE, pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, and 0.1% sodium deoxycholate) with a 200-μl volume equivalent of glass beads and then were vortexed for 30 min at 4 °C using a Tommy vortexer. The whole cell extract (WCE) was collected by punching a hole at the bottom of the Eppendorf tube and then was extracted with 200 μl of phenol/chloroform/isooamy alcohol. The aqueous phase following phenol/chloroform extraction was treated with ethanol to precipitate genomic DNA.

The genomic DNAs were analyzed for 4NQO-induced DNA damage at GAL1, GAL7, and GAL10 loci, using the following primer pairs: GAL1 (whole gene), 5′-CGCTTAACTGCTCATGTGATTG-3′ and 5′-TTGTTCGGAGACTGTCGGGC-3′; GAL1 (promoter), 5′-CGCTTAACTGCTCATGTGATTG-3′ and 5′-GAAAATGTGAAATGTTTAAGTTG-3′; GAL1 (ORF), 5′-CAGAGGCTAAGCATTGTTATTC-3′ and 5′-GTCAATCTCTGGACAAAGACATT-3′; GAL7 (whole gene), 5′-CTATGTTCTAATTGTGGCCTAGC-3′ and 5′-TGTAGTCCTCTGATATAATGCCC-3′; GAL7 (ORF), 5′-TGACGTGCTTCACTTCAAGAAG-3′ and 5′-AATGATAACCAATTGAGTTGAAA-3′; GAL7 (ORF), 5′-GCTAAGATATGGGCCTTATACAT-3′ and 5′-TTTCATTTTGTAAGCCTGTCAT-3′; GAL10 (whole gene), 5′-TTAATGGCAATCTATAGTATCGCAGG-3′ and 5′-TTAACCAATAGATCCTCCGAAATTTCC-3′.

**Analysis of Global Levels of RNA Polymerase II Subunits**—The yeast culture (5 ml) was harvested following 4NQO treatment. The harvested cells were lysed to prepare the
results

Degradation of Rpb1p but Not Other Subunits of RNA Polymerase II in 4NQO-treated Yeast Cells—To analyze the fate of RNA polymerase II at the DNA lesions in the transcriptionally active genes in vivo, we developed a protocol to cause and analyze 4NQO-induced DNA damage in living yeast cells. 4NQO forms bulky adducts primarily with guanine bases and to a lesser extent with adenine bases in DNA. The bulky 4NQO adducts are processed by nucleotide excision repair. To analyze the formation of 4NQO-induced lesions in the yeast genomic DNA, we extracted genomic DNA from yeast cells following 4NQO treatment (+). As a control, the genomic DNA was also isolated from 4NQO-untreated (−) yeast cells. These genomic DNAs were analyzed by PCR at the active genes GAL1 within 10 min following 4NQO treatment, indicating the presence of DNA lesions at the gene locus; 4NQO forms bulky adducts primarily with guanine bases and to a lesser extent with adenine bases in DNA. The bulky 4NQO adducts are processed by nucleotide excision repair. To analyze the formation of 4NQO-induced lesions in the yeast genomic DNA, we extracted genomic DNA from yeast cells following 4NQO treatment (+). As a control, the genomic DNA was also isolated from 4NQO-untreated (−) yeast cells. These genomic DNAs were analyzed by PCR at the GAL1 gene locus. The DNA lesions caused by 4NQO will inhibit PCR at the GAL1 locus; thus, the PCR signal would be significantly reduced or completely lost, depending on the extent of DNA damage at GAL1.

The data presented in Fig. 1A demonstrate that 4NQO induced DNA damage at GAL1 within 10 min following 4NQO treatment. However, severe DNA damage was observed at a later time point.

We next analyzed the global level of Rpb1p in response to 4NQO-induced DNA damage in yeast cells. In this direction, we treated the growing yeast cells in YPG with 4NQO, and then we analyzed the fate of RNA polymerase II at the DNA lesions in the transcriptionally active genes in vivo, we developed a protocol to cause and analyze 4NQO-induced DNA damage in living yeast cells. 4NQO forms bulky adducts primarily with guanine bases and to a lesser extent with adenine bases in DNA. The bulky 4NQO adducts are processed by nucleotide excision repair. To analyze the formation of 4NQO-induced lesions in the yeast genomic DNA, we extracted genomic DNA from yeast cells following 4NQO treatment (+). As a control, the genomic DNA was also isolated from 4NQO-untreated (−) yeast cells. These genomic DNAs were analyzed by PCR at the GAL1 gene locus. The DNA lesions caused by 4NQO will inhibit PCR at the GAL1 locus; thus, the PCR signal would be significantly reduced or completely lost, depending on the extent of DNA damage at GAL1.

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FIGURE 2. Analysis of the level of Rpb1p at the GAL1 gene following 4NQO-induced DNA damage. The yeast strain expressing Myc epitope-tagged Rpb1p was grown in YPR up to an A600 of 0.8 at 30 °C and then transferred to YPG for 90 min to induce GAL1 prior to 4NQO treatment. Following 4NQO treatment, yeast cells were grown in YPG for different times (10 and 40 min). As a control, the yeast cells were also grown under similar growth conditions without 4NQO treatment. These cells were used for the ChIP assay to analyze the level of Rpb1p at the GAL1 core promoter and ORF in 4NQO-treated (+) and untreated (−) cells. The ChIP assay was performed as described previously (35), using a mouse monoclonal antibody against the Myc epitope tag and a polyclonal antibody against TBP. Primer pairs located at the promoter and ORF of GAL1 were used for PCR analysis of the immunoprecipitated DNA samples. The percentage of DNA immunoprecipitated relative to input DNA is represented as %IP. The normalized %IP is plotted in the form of a histogram.

RESULTS

Degradation of Rpb1p but Not Other Subunits of RNA Polymerase II in 4NQO-treated Yeast Cells—To analyze the fate of RNA polymerase II at the DNA lesions in the transcriptionally active genes in vivo, we developed a protocol to cause and analyze 4NQO-induced DNA damage in living yeast cells. 4NQO forms bulky adducts primarily with guanine bases and to a lesser extent with adenine bases in DNA. The bulky 4NQO adducts are processed by nucleotide excision repair. To analyze the formation of 4NQO-induced lesions in the yeast genomic DNA, we extracted genomic DNA from yeast cells following 4NQO treatment (+). As a control, the genomic DNA was also isolated from 4NQO-untreated (−) yeast cells. These genomic DNAs were analyzed by PCR at the GAL1 gene locus. The DNA lesions caused by 4NQO will inhibit PCR at the GAL1 locus; thus, the PCR signal would be significantly reduced or completely lost, depending on the extent of DNA damage at GAL1.

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these cells were allowed to grow for different times as mentioned in the legend to Fig. 1B. These time points were chosen based on the previous studies that demonstrated global degradation of yeast Rpb1p at around 1 h in dextrose-containing growth medium in response to DNA damage (17, 37). The WCEs were prepared from the yeast cultures of different post-treatment time points and were analyzed by a Western blot assay using an anti-Myc antibody (9E10; Santa Cruz Biotechnology) against Myc epitope-tagged Rpb1p. Fig. 1, B and C, shows that Rpb1p began to degrade 10 min after 4NQO treat-
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An Elongating Form of Rpb1p Is Degraded at the Active Genes in 4NQO-treated Yeast Cells—Rpb1p is globally degraded in response to 4NQO-induced DNA damage. Rpb1p is present within the initiating and elongating RNA polymerase II. However, it is not clear in vivo which form of Rpb1p gets degraded in response to DNA damage. To address this question, we performed a ChIP assay to analyze the level of Rpb1p within the initiating and elongating RNA polymerase II at a transcriptionally active gene, GAL1, at early time points (10 and 40 min) following 4NQO treatment of yeast cells, since the GAL1 gene at the later time point is severely damaged, which might inhibit preinitiation complex formation and hence transcriptional initiation. Fig. 2 shows a nearly 2-fold reduction in TBP recruitment at the GAL1 core promoter at 10 min post-4NQO treatment, possibly due to partial damage at the core promoter by 4NQO. Consistently, recruitment of the initiating Rpb1p (and hence RNA polymerase II) at the GAL1 core promoter was reduced by around 2-fold (Fig. 2). Strikingly, a nearly 7-fold reduction in the association of elongating Rpb1p with the GAL1 ORF was observed at 10 min post-4NQO treatment (Fig. 2). Such a dramatic decrease of Rpb1p association with the GAL1 ORF indicates the degradation of the elongating form of Rpb1p at GAL1 in vivo.

Next, we analyzed the association of initiating and elongating Rpb1p as well as TBP with GAL1 at 40 min post-4NQO treatment. Recruitment of TBP at the GAL1 core promoter was almost lost (Fig. 2). This might be due to severe damage of the GAL1 core promoter 40 min after 4NQO treatment, leading to the inhibition of the preinitiation complex (TBP and RNA polymerase II are the two important components of the preinitiation complex) formation and hence transcriptional initiation. Consistently, association of initiating and elongating Rpb1p with GAL1 was dramatically lost (Fig. 2).

Collectively, our data indicate that elongating Rpb1p is degraded at the GAL1 gene at the 10 min time point following 4NQO treatment. However, at the later post-4NQO treatment time point (40 min), preinitiation complex was not formed due to severe damage of the core promoter, and hence RNA polymerase II was unable to enter the elongation cycle. Thus, we next performed a time course for the association of Rpb1p with GAL1 within the first 20 min (0, 5, 10, and 20 min) following 4NQO treatment. The whole GAL1 gene was significantly damaged in yeast cells within 20 min of 4NQO treatment (Fig. 3A). Similarly, whole promoter and ORF of GAL1 were also damaged within 20 min (Fig. 3A). However, the smaller regions (termed as ChIP fragments) at GAL1 that were used for PCR analysis in the ChIP experiments (see below) were not damaged within 20 min of 4NQO treatment (Fig. 3A), thus allowing the ChIP analysis following 4NQO treatment.

Fig. 3, B and C, shows that the association of Rpb1p with the GAL1 coding sequence was dramatically reduced as compared with that with the core promoter. Likewise, the reduction of TBP association with the GAL1 core promoter is much less than that of the elongating Rpb1p following 4NQO treatment (Fig. 3D), consistent with the recruitment pattern of initiating Rpb1p in response to DNA damage (Fig. 3, B and C). However, recruitment of factors upstream of TBP binding, namely activator (Gal4p) and co-activator (SAGA-TAF12p), was not sig-

FIGURE 3. The time course analysis of Rpb1p association with the GAL1 gene within the first 20 min following 4NQO treatment. A, analysis of DNA damage at the GAL1 locus within the first 20 min of 4NQO treatment. The yeast cells were grown and treated with 4NQO as in Fig. 2. The whole GAL1 locus, promoter, ORF, and Chip specific smaller regions (ChIP fragments) of the genomic DNAs were amplified by PCR, using the specific primer pairs as mentioned under "Experimental Procedures." B, analysis of Rpb1p association with GAL1 at early time points following 4NQO treatment. The yeast strain expressing Myc epitope-tagged Rpb1p was grown and treated with 4NQO as in Fig. 2. Following 4NQO treatment, yeast cells were grown in YPG for different time points (0, 5, 10, and 20 min) prior to cross-linking. Immunoprecipitations were performed as in Fig. 2. Primer pairs located at the core promoter and ORF (toward 3′ end) of GAL1 were used for PCR analysis of the immunoprecipitated DNA samples. The percentage of DNA immunoprecipitated relative to input DNA is represented as %IP. The normalized %IP is plotted in the form of a histogram. C, the normalized %IP of Rpb1p at the GAL1 core promoter and ORF in the 4NQO-treated cells in B was plotted against 4NQO treatment times. D, analysis of TBP recruitment at the GAL1 core promoter at early time points following 4NQO treatment. The yeast strain was grown, cross-linked and immunoprecipitated as in B and Fig. 2. Immunoprecipitation was performed using a polyclonal antibody against TAF12p (obtained from Michael R. Green, University of Massachusetts Medical School). A mouse monoclonal antibody against the DNA binding domain of Gal4p (RK51C) and Santa Cruz Biotechnology, Inc.) was used. The normalized %IP is plotted in the form of a histogram. E, analysis of Rpb1p level at the GAL1 ORF in the DEF1 deletion mutant and its isogenic wild-type equivalent. The normalized %IP is plotted in the form of a histogram.
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FIGURE 4. The analysis of the levels of Rpb1p and TBP at the GAL7 and GAL10 genes following 4NQO treatment. A, analysis of DNA damage at the GAL7 and GAL10 loci at 20 min following 4NQO treatment. The yeast cells were grown and treated with 4NQO as in Fig. 2. The GAL7 and GAL10 loci of the genomic DNAs were amplified by PCR, using the specific primer pairs as mentioned under “Experimental Procedures.” B, analysis of Rpb1p association with GAL10 following 4NQO treatment. Yeast strain expressing Myc epitope-tagged Rpb1p was grown, cross-linked, and immunoprecipitated as in Fig. 3B. Primer pairs located at the core promoter and ORF (toward the 3′-end) of GAL10 (see “Experimental Procedures”) were used for PCR analysis of the immunoprecipitated DNA samples. The normalized %IP is plotted in the form of a histogram. C, analysis of Rpb1p association with GAL7 following 4NQO treatment. Yeast strain expressing Myc epitope-tagged Rpb1p was grown, cross-linked and immunoprecipitated as in B. Primer pairs located at the core promoter and ORF (toward the 3′-end) of GAL7 (see “Experimental Procedures”) were used for the PCR analysis of the immunoprecipitated DNA samples. D, analysis of TBP recruitment at the GAL7 and GAL10 core promoters following 4NQO treatment. The yeast strain was grown, cross-linked, and immunoprecipitated as in B. Primer pairs located at the core promoters of GAL7 and GAL10 were used for PCR analysis of the immunoprecipitated DNA samples.

Together, these results suggest that Rpb1p in the GAL1 coding sequence is preferentially degraded in response to DNA damage. However, the decrease in the level of Rpb1p at the GAL1 coding sequence could be due to the defect in the promoter clearance or indirect effect of the changes in general transcription patterns. To rule out this possibility, we analyzed recruitment of Rpb1p to the GAL1 coding sequence at 20 min post-4NQO treatment in the presence and absence of Def1p that is essential for Rpb1p degradation (17). If the degradation of elongating Rpb1 occurs in response to DNA damage, the level of Rpb1p at the GAL1 coding sequence would increase in Δdef1 in comparison with that in the isogenic wild-type equivalent. Indeed, Fig. 3F shows a significant increase in the level of Rpb1p at the GAL1 coding sequence in the absence of Def1p. Thus, our data clearly demonstrate that the elongating Rpb1p is degraded at GAL1 in response to DNA damage in vivo.

Next, we asked whether elongating Rpb1p is also degraded at other active genes in response to 4NQO-induced DNA damage. To address this question, we analyzed association of Rpb1p with the core promoters and coding sequences of two other active GAL genes, such as GAL7 and GAL10, at 20 min following 4NQO treatment. These genes were significantly damaged when yeast cells were treated with 4NQO for 20 min (Fig. 4A). Fig. 4, B–D, shows that the recruitment of Rpb1p as well as TBP at the core promoters of the GAL7 and GAL10 genes was modestly reduced following 4NQO treatment. Strikingly, the association of Rpb1p with the coding sequences of these genes was dramatically reduced following 4NQO treatment (Fig. 4, B and C), consistent with the results obtained at the GAL1 coding sequence (Fig. 3, B and C). Collectively, our data demonstrate that the elongating Rpb1p is degraded at the active genes in response to 4NQO-induced DNA damage.

Rpb1p Is Essential for the Assembly of RNA Polymerase II at the Active Gene—We next inquired into the fate of RNA polymerase II following degradation of its Rpb1p subunit at the active gene in response to DNA damage. To address this question, we analyzed association of the Rpb3p and Rpb4p subunits of RNA polymerase II with the GAL1 ORF in the wild-type and ts mutant strains at the nonpermissive temperature. Both of the subunits of RNA polymerase II were associated with the GAL1 ORF in the wild-type strain (Fig. 5, A and B). However, association of these subunits with the GAL1 ORF was almost lost in the rpb1-ts mutant strain (Fig. 5, A and B), indicating that Rpb1p is essential for the assembly of RNA polymerase II subunits at the active gene in vivo.

Elongating RNA Polymerase II Is Disassembled at the Active Genes in 4NQO-treated Yeast Cells—RNA polymerase II is disassembled at the active gene when rpb1-ts strain was grown at nonpermissive temperature for 1 h (Fig. 5, A and B). This observation indicates that the degradation of Rpb1p at the DNA lesion would disassemble RNA polymerase II.
We analyzed association of the Rpb4p subunit of RNA polymerase II with the core promoter and coding sequence of the \textit{GAL1} gene following 4NQO treatment. Fig. 6A shows that the association of Rpb4p with the \textit{GAL1} ORF was dramatically more reduced as compared with the core promoter following 4NQO treatment, consistent with the association pattern of Rpb1p with \textit{GAL1} in response to DNA damage (Fig. 3, B and C). However, unlike Rpb1p, Rpb4p was not globally degraded following 4NQO treatment.

**FIGURE 5.** Rpb1p is essential for recruitment of the Rpb3p and Rpb4p subunits of RNA polymerase II at the active gene. 

**A.** Association of Rpb3p with the \textit{GAL1} ORF is dependent on Rpb1p. The yeast strains expressing Myc epitope-tagged Rpb3p in \textit{rpb1}-ts and its wild-type equivalent were grown in YPG at 23 °C up to an \textit{A}$_{600}$ of 0.85 and then switched to 37 °C for 1 h prior to cross-linking. Immunoprecipitation (IP) was performed using a mouse monoclonal antibody against the Myc epitope tag. The anti-HA was used as a non-specific antibody control. The primer pair located at the \textit{GAL1} ORF was used for PCR analysis of the immunoprecipitated DNA samples. The percentage of DNA immunoprecipitated relative to wild type (%WT) is indicated below the band of the mutant strain. 

**B.** Association of Rpb4p with the \textit{GAL1} ORF is dependent on Rpb1p. The yeast strains expressing Myc epitope-tagged Rpb4p in \textit{rpb1}-ts and its wild-type equivalent were grown, cross-linked, and immunoprecipitated as in **A**.

**FIGURE 6.** The analysis of Rpb4p association with \textit{GAL1}, \textit{GAL7}, and \textit{GAL10} following 4NQO treatment.

**A.** Analysis of Rpb4p association with \textit{GAL1} at early time points following 4NQO treatment. The yeast strain expressing Myc epitope-tagged Rpb4p was grown and treated with 4NQO as in Fig. 2. Following 4NQO treatment, yeast cells were grown in YPG for different times (0, 5, 10, and 20 min) prior to cross-linking. Immunoprecipitations were performed as in Fig. 2. Primer pairs located at the core promoter and ORF of \textit{GAL1} were used for PCR analysis of the immunoprecipitated DNA samples. The normalized %IP is plotted in the form of a histogram.

**B.** Analysis of Rpb4p association with \textit{GAL10} following 4NQO treatment. The yeast strain expressing Myc epitope-tagged Rpb4p was grown, cross-linked, and immunoprecipitated as in **A**.

**C.** Analysis of Rpb4p association with \textit{GAL7} following 4NQO treatment. The yeast strain expressing Myc epitope-tagged Rpb4p was grown, cross-linked, and immunoprecipitated as in **A**.
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Following 4NQO treatment (Fig. 1D). Together, these observations support the fact that the association of Rpb4p with the GAL10 ORF was lost following degradation of the elongating Rpb1p in response to DNA damage, consistent with the results obtained with the rpb1-ts mutant strain (Fig. 5, A and B). Thus, elongating RNA polymerase II is disassembled at GAL1 following degradation of Rpb1p in response to DNA damage. Similar results were also obtained at other genes, such as GAL7 and GAL10 (Fig. 6, B and C).

**DISCUSSION**

Several studies have demonstrated that the Rpb1p subunit of RNA polymerase II is globally degraded in response to DNA damage (16, 17, 32, 33, 37). However, it is not known whether the initiating or elongating form of Rpb1p is degraded in response to DNA damage. Recently, Somesh *et al.* (37) have biochemically demonstrated that the Rpb1p subunit of the elongating, but not initiating, form of RNA polymerase II is ubiquitinated in response to DNA damage, which subsequently becomes the target for proteasomal degradation. Further, they have demonstrated that Rpb1p is globally ubiquitinated when yeast cells are treated with 6-azauracil, suggesting the ubiquitination of elongating Rpb1p in vivo. However, there is no direct demonstration of the degradation of elongating Rpb1p at the DNA lesion in the active gene in vivo. Here, we provide direct in vivo evidence for the degradation of Rpb1p within the elongating, but not initiating, form of RNA polymerase II in response to DNA damage. The degradation of elongating Rpb1p eventually leads to global decrease of the Rpb1p level following DNA damage.

Elongating RNA polymerase II along with several associated transcription factors stalls at the DNA lesion, thus making it inaccessible to the DNA repair machinery. However, it remains unclear how DNA repair machinery can access the DNA lesion. One possibility would be to disassemble RNA polymerase II and associated factors from the damage site, making it accessible to the DNA repair machinery. Indeed, our data show that RNA polymerase II is disassembled in the absence of Rpb1p. Further, RNA polymerase II has been shown to be the loading dock for several transcription factors at the coding sequences of the active genes (38–41). Thus, the absence of RNA polymerase II at the coding sequence following Rpb1p degradation in response to DNA damage would disassemble transcription elongation machinery, allowing the DNA repair factors to access the damage site, as suggested previously (16, 27, 28).

Although the Rpb1p subunit of RNA polymerase II is degraded in response to DNA damage, it remains unknown whether only Rpb1p or the other subunits of RNA polymerase II is targeted for degradation. To address this question, we have analyzed in this study the global levels of the Rpb2p, Rpb3p, Rpb4p, Rpb6p, Rpb7p, Rpb8p, Rpb9p, Rpb10p, and Rpb11p subunits of RNA polymerase II following 4NQO-induced DNA damage. Interestingly, the levels of these RNA polymerase II subunits remain unaltered in response to DNA damage. Thus, our data demonstrate that only Rpb1p, and not the whole RNA polymerase II complex, is degraded following DNA damage. Such a highly selective degradation of Rpb1p provides the cell an economical way to efficiently recycle other RNA polymerase II subunits. Consistent with our results, Chen *et al.* (42) have recently demonstrated that Rpb2p and Rpb9p subunits of RNA polymerase II are not degraded in response to UV-induced DNA damage.

In summary, we have demonstrated in vivo that only Rpb1p and not the other subunits of the elongating RNA polymerase II is degraded at the transcriptionally active genes in response to 4NQO-induced DNA damage. Following Rpb1p degradation, elongating RNA polymerase II is disassembled at the DNA lesion (Fig. 7). However, whether such a disassembly of RNA polymerase II promotes DNA repair machinery to access the DNA lesion in vivo remains to be elucidated. Nonetheless, our study provides the direct in vivo evidence for degradation of the elongating, but not initiating, form of Rpb1p to disintegrate RNA polymerase II in response to DNA damage.

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