UV damage regulates alternative polyadenylation of the RPB2 gene in yeast

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UV damage regulates alternative polyadenylation of the RPB2 gene in yeast

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ABSTRACT

Alternative polyadenylation (APA) is conserved in all eukaryotic cells. Selective use of polyadenylation sites appears to be a highly regulated process and contributes to human pathogenesis. In this article we report that the yeast RPB2 gene is alternatively polyadenylated, producing two mRNAs with different lengths of 3′ UTR. In normally growing wild-type cells, polyadenylation preferentially uses the promoter-proximal poly(A) site. After UV damage transcription of RPB2 is initially inhibited. As transcription recovers, the promoter-distal poly(A) site is preferentially used instead, producing more of a longer form of RPB2 mRNA. We show that the relative increase in the long RPB2 mRNA is not caused by increased mRNA stability, supporting the preferential usage of the distal poly(A) site during transcription recovery. We demonstrate that the 3′ UTR of RPB2 is sufficient for this UV-induced regulation of APA. We present evidence that while transcription initiation rates do not seem to influence selection of the poly(A) sites of RPB2, the rate of transcription elongation is an important determinant.

INTRODUCTION

Transcription of the protein-coding genes in eukaryotic cells is performed by RNA polymerase II and can be functionally divided into multiple stages, including transcription initiation, elongation and termination (1,2). Transcription termination is initiated by the cleavage/polyadenylation complexes at polyadenylation sites, followed by a series of coupled events including cleavage of the nascent RNA, release of the RNA polymerase, and addition of the poly(A) tail (3–5). Over 85 proteins have been found to be involved in the transcription termination process and most of them are conserved from yeast to humans (6). Although divergent cis-elements are required for 3′ RNA processing in different organisms, more than half of human genes and 72.1% of yeast genes are alternatively polyadenylated, suggesting that alternative polyadenylation (APA) plays an important role in increasing transcript diversity in eukaryotic cells (7–9). The selective use of different polyadenylation signals changes the length of the 3′ untranslated regions (3′ UTRs) of affected mRNAs, and thus could influence translation efficiency, mRNA stability and nuclear export of the mature mRNA (10–12).

The molecular mechanism that determines the selection of one polyadenylation site over another is not completely understood. Studies have suggested that 3′ processing factors may be regulated to modulate APA in the cell. For example, regulation of the cleavage stimulation factor CstF-64 (Rna15 in yeast) promotes the use of the promoter-proximal polyadenylation site of the immunoglobulin M heavy-chain gene (13), reduced level of the cleavage factor Im (CFIm) leads to increased use of the promoter-proximal polyadenylation site of several genes in human cells (14), and increased levels of polyadenylation factors are associated with shortening of 3′ UTRs of many mRNAs in cancer cells, etc. In plants the RNA 3′ processing factors can be specifically targeted to the promoter-proximal site of the FLC antisense gene to control the flowering time (15,16). In yeast RNA processing factors have also been reported to modulate APA (17–19). For example, Nab4, the yeast ortholog of CFIm, promotes cleavage site selection near its binding site on the 3′ UTR (18,20). Recently transcription activity and transcription elongation rate have been shown to play an important role in poly(A) site selection (21–23).

APA has been reported to be a highly regulated process in embryonic development (24), cancerous transformation (25), neuronal synapse development (26) and different tissues (27). This suggests that cells possess the ability to sense various environmental stimuli and initiate a change in polyadenylation site selection. In this regard, yeast cells have been demonstrated to modulate APA in response to various stimuli. For example, the yeast CBP1 gene is transcribed into two forms of transcripts by APA and the short transcript is specifically enriched when switched to respiratory growth (28). Similarly, different growth
conditions modulate APA of several yeast genes including SUA7, AEP2/ATP13, RNA14 and SIR1 (28–30). UV irradiation is better known for its damaging effects on DNA and triggering cellular responses in DNA repair and transcription (31–33). UV damage has been shown to inhibit mRNA 3’-end cleavage in vitro (34–36), suggesting that mRNA 3’ processing may be generally affected by UV damage in the cell. It has been reported that UV damage affects the polyadenylation site selection for the tropoelastin gene in mammalian cells. However, whether any other genes show differential 3’-end processing after UV damage has not been examined thus far. It remains to be determined what is the molecular mechanism of the UV-induced change in APA.

The initial goal of this work was to characterize the transcription recovery process following a UV-induced transcription arrest in yeast. We chose to study the transcript dynamics of the RPB2 gene following UV damage because the DNA repair process has been well studied using RPB2 as a repair target by us and others (37–39). We found that RPB2 encodes two mRNA species as a result of APA and that UV damage regulates selection of the poly(A) sites. We provide evidence that the rate of transcription elongation but not transcription induction affects poly(A) selection.

### MATERIALS AND METHODS

#### Yeast strains and plasmids

Yeast strains and plasmids used in this study are listed in Table 1 and the construction details of key strains are described below. Yeast transformation methods are as described (41). All plasmids constructed in this study were sequenced to confirm that they contain no mutations.

To construct plasmid pMV1352, which contains the URA3 gene followed by the RPB2 3’UTR, we first amplified the URA3 gene from plasmid pRS416 (42) using primers NotIURA (5’-GACTGGCAGCAATGCTCAATGTAAGCTA CATATAAGGACAAG) and BamURA (5’-CGGCAGTTATCTTGGTCGTGCGCGCA), then inserted the URA3 DNA into plasmid pMV1351 between the NotI and BamHI restriction sites to create plasmid pMV1364. Plasmid pMV1364 was digested with SacII and NotI, purified with the Qiagen PCR purification kit, and ligated with the RPB2 promoter DNA that was amplified from yeast genomic DNA using primers NotI-RPB2-0 (5’-CTGAGCCGCGCCCTTATAATTATTCTCAT ACTTTTTGCTAGCATTGTTTTTCTTCTTTC) and SacIRPB2-1KB (5’-GCCGCCGCGCAGGACAAATGCTATAC) to create the plasmid pMV1365. Plasmid pMV1365 was used to transform yeast strain MVY101 to obtain strain MVY898.

To construct plasmid pMV1390, we amplified the CUP1 promoter from the yeast genomic DNA using primers CUP1f (5’-GCCGCCGCGCAGGACAAATGCTAC-TGGCATCATGACCTTCATT) and CUP1r (5’-CTGAGCCGCGCCCTTATAATTATTCTCAT ACTTTTTGCTAGCATTGTTTTTCTTCTTTC), and inserted the CUP1 promoter DNA that was amplified between the SacII and NotI site into plasmid pMV1364 between the SacII and NotI site. Plasmid pMV1390 was used to transform yeast strain MVY101 to obtain strain MVY1001.

To construct yeast strains MVY818 and MVY819, which have the KanMX gene inserted into the chromosomal RPB2 3’UTR between the two polyadenylation sites (Figure 1D), we assembled three DNA fragments: the KanMX6 gene which was obtained as a XmaI fragment from plasmid pFA6a-KanMX6 (43) and two PCR products produced from the downstream region of the RPB2 gene and its 3’ UTR using primer pairs. One PCR product was produced using primers Kpm1-up500-f (5’-GGGCCAGTACCAGACATGATGAGTCTGACAAAG) and up500-Xma-r (5’-GCCGCCGCGCGTGGTGTTAAATCGGACAAAGGG), and the second, using primers

### Table 1. Yeast strains and plasmids used in this study

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<th>Strain</th>
<th>Original name, genotype (annotation)</th>
<th>Reference</th>
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<tr>
<td>MVY150</td>
<td>W303-1B, MATa ade2-1 trpl1-1 can1-100 leu2-3, 112 his3-11,15 ura3-1</td>
<td>(37)</td>
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<td>this study</td>
</tr>
<tr>
<td>MVY819</td>
<td>MVY150 with KanMX inserted in RPB2 3’UTR, #2</td>
<td>this study</td>
</tr>
<tr>
<td>MVY836</td>
<td>MVY150 with RPB2 3’UTR replaced by CYC1 3’UTR</td>
<td>this study</td>
</tr>
<tr>
<td>MVY896</td>
<td>MVY101 with pRS416</td>
<td>this study</td>
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<tr>
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<td>MVY1001</td>
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<td>pMV1390</td>
<td>plasmid with the CUP1-URA3-RPB2 construct</td>
<td>this study</td>
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</table>
SacII-down500-f (5'¬GCCACCGCGCGGTTTCACTTGAAACCAA) and down500-Sacl-r (5'¬GACGGAGCTC CATTGGTAGTTAGGCTTAC) These three digested plasmid were then assembled together with KpnI and SacI digested pBluescript II SK plasmid to produce plasmid pMV1343, which was sequenced to confirm its structure. It carries the KanMX6 gene flanked by the two 500 bp RPB2 targeting sequences. This fragment can be released as a single linear DNA fragment of 2630 bp using SnaBI and KpnI. After gel purification, yeast cells were transformed with this fragment and KanMX6 carrying clones were selected by G418 resistance. Such clones carry the KanMX6 gene between the two polyadenylation sites shown in Figure 1C.

To replace the RPB2 3'UTR with the CYC1 3'UTR sequences, we first amplified by PCR 500 bp of RPB2 DNA using primers Kpn-RPB2-4131 (5'¬CGGCCGTAC CCGCGCCCCGGGCTCGAGTTAAAAATCTCTCAG ACGATCGTATATATACG) and Xma-Xho-RPB2 (5'¬GGGCCGGGCGGCTCGAGTTAAAAATCTCTCAG ACGATCGTATATATACG) and used this fragment to replace the KpnI-XmaI fragment of pMV1343 to produce plasmid pMV1346. We then amplified the CYC1 terminator sequence from the yeast genome using primers Xho-CYC1 (5'¬CCCTCTTCCTTTACGGGACATT) and the anchorP primer. The PCR products are gel purified and sequenced using primer RPB2-20R (5'¬GGTGGAATCC) to determine the 5'UTR of the RPB2 gene, followed by a second round PCR amplification using primers 466 (5'¬GCTGATGAGCTTACGACATGCTGATGAAAG) and primerP (5'¬GTCGATGAGCGTCACTG) to use PCR amplify the 3'UTR of the RPB2 gene, followed by a second round PCR amplification using primers 466 (5'¬GCTGATGAGCTTACGACATGCTGATGAAAG) and anchorP primer. The PCR products are gel purified and sequenced using primer RPB2-13 (5'¬GGCGCGAATTTATATCAAG) and the anchorP primer. The PCR products are gel purified and sequenced by primer KanMX-905 (5'¬TTTGATGAGCGGCTATG) to determine the polyadenylation sites.

To determine the 3' ends of the polycistronic mRNAs in MVY818/819, cDNA is generated using the procedure described above, followed by two rounds of PCR using primers KanMX-717f (5'¬GCGGAACACGCTATTCGACATGCTGATGAAAG) and KanMX-845f (5'¬GTCGATGAGCGTCACTG) / anchorP to determine the 5'UTR of the RPB2 gene, followed by a second round PCR amplification using primers 466 (5'¬GCTGATGAGCTTACGACATGCTGATGAAAG) and anchorP primer. The PCR products are gel purified and sequenced by primer KanMX-905 (5'¬TTTGATGAGCGGCTATG) to determine the polyadenylation sites. A unique polyadenylation site is found after the sequence TTAGCGTGA TTAA.

Quantitative real-time PCR

The procedure of real-time PCR (RT-PCR) has been described (48). Briefly, 1 μg of yeast total RNA was digested with DNase I, reverse transcribed with the poly(T) primer to make cDNA. Primer 869 (TGCCCTCT CCTTACTTGACT) was added to reverse transcribe the ribosomal RDIY18 RNA. cDNA of the long RPB2 mRNA was amplified with primer 861 (TCTTTTGTGCTATGCTGCGCA) and primer 862 (TGCTTCAACTTACTTACTGCTGCGCA) / anchorP to determine the 3'UTR of the RPB2 gene, followed by a second round PCR amplification using primers 466 (5'¬GCTGATGAGCTTACGACATGCTGATGAAAG) and anchorP primer. The PCR products are gel purified and sequenced by primer KanMX-905 (5'¬TTTGATGAGCGGCTATG) to determine the polyadenylation sites. A unique polyadenylation site is found after the sequence TTAGCGTGA TTAA.
10 min to initially denature, followed by 40 cycles of (95°C for 15 s, 60°C for 20 s, 72°C for 20 s), and terminated by a standard melting curve to verify quality of the PCR product.

RESULTS

UV damage regulates APA of the RPB2 gene

UV irradiation is known to induce bulky DNA lesions that block elongating RNA polymerases (49) and consequently induce a specialized DNA repair activity, named transcription-coupled DNA repair (TCR) (50). It has been demonstrated that UV damage on the transcribed strand of the RPB2 gene is removed more rapidly than damage on the non-transcribed strand (37–39). However, transcription recovery of the RPB2 gene following repair of the UV damage has not been investigated. To test this, we irradiated yeast cells with UV and monitored levels of the RPB2 mRNA by northern blot. As expected, we found that levels of the RPB2 mRNA show an immediate decline after UV irradiation followed by a recovery of transcription that can be clearly seen 30 min after UV treatment (Figure 1A). Another control RNA SUB1 exhibits similar but delayed pattern of inhibition and recovery, presumably due to different mRNA stability after UV inhibition. Interestingly, we noted that there are two species of the RPB2 mRNAs. Prior to UV treatment, the short, fast migrating form is predominant in the cell. After UV irradiation, however, the newly synthesized long form becomes the predominant RPB2 mRNA. The dynamics of the two RPB2 mRNAs can be better observed in Figure 1B as the ratio of the long RPB2 mRNA to total RPB2 mRNA increases after UV damage. We ask two questions pertinent to this result: what are the two RPB2 mRNAs and how does post-damage transcription preferentially produce the long form?

It has been reported that the RPB2 gene is not subject to alternative splicing and the two RPB2 mRNAs differ only in the 3′ UTR (51). To precisely determine the 3′ UTR of the RPB2 mRNAs, we reverse transcribed the polyadenylated mRNAs using the anchored-polyT primer (5′-GCTCGATGTCAGTACCCCTTTTTTTTTTTTTTTTTT), then PCR-amplified the 3′ UTR of RPB2 using RPB2 specific primers and the anchor primer [5′-GCTC GATGTCAGTACCCCTTTTTTTTTTTTTTTTTTT], for a detailed protocol of the Rapid Amplification of cDNA Ends (RACE) assay, see reference (47)]. DNA sequencing of the PCR products revealed two distinct transcription cleavage sites that are followed by a multitude of adenosines (see Figure 1C and Supplementary Figure S1). Similar determination of the 5′UTR using 5′RACE (46) identified only a single transcription start site that is 277 bp upstream of the RPB2 translational start codon. Therefore RPB2 is alternatively polyadenylated to generate two mRNAs of different length.

To confirm that the two RPB2 mRNA species that we found to be differentially synthesized after UV damage are indeed the two alternatively polyadenylated mRNAs that we determined in the RACE assays, we modified the RPB2 3′UTR by inserting a 1.5 kb KanMX6 gene between the two polyadenylation sites of the RPB2 gene (see Figure 1D for illustration). If post-UV transcription in the insertional mutant favorably bypasses the first poly(A) site and polyadenylates at a distal site, a polycistronic mRNA is expected to be produced due to transcriptional read-through of the inserted KanMX6 gene. Figure 1E shows that UV damage induces a long RPB2 transcript in both clones of the mutants that contain the KanMX6 gene inserted into the 3′ UTR of RPB2, demonstrating a UV-induced increase in the use of the second poly(A) site. Prior to UV treatment little of the long transcript is detected indicating that the KanMX6 insertion reduces the frequency with which the Poly(A) site 2 is used in the absence of UV (Figure 1E). RACE analysis was performed to determine which poly(A) site is used when KanMX6 is present and it was found that the long mRNA is polyadenylated within the 3′ UTR of the inserted KanMX6 gene (data not shown). This observation raises several points. It clearly indicates that site selection of APA of RPB2 is regulated as transcription of RPB2 recovers after UV damage. It suggests that UV inhibits cleavage and polyadenylation at site 1 of RPB2 rather than actively selecting poly(A) site 2, since the KanMX6 poly(A) site, but not the RPB2 poly(A) site 2 is increasingly used after UV treatment.

Substitution of the 3′UTR of RPB2 with the 3′ UTR of the CYC1 gene abolishes the production of two forms of RPB2 mRNAs, confirming that the alternative form of RPB2 is not caused by alternative transcription start or alternative mRNA splicing (Figure 1F and G).

The quantitative increase in the RPB2 mRNA after UV damage can be influenced by a change in the stability of the RNA. Figure 1A shows that both forms of RPB2 mRNA are degraded quickly after UV damage (compare lane 2 to lane 1), suggesting RPB2 mRNA has a very short half life. To accurately determine the stabilities of the RPB2 mRNA, we used the transcription inhibitor thiolutin to shutdown mRNA synthesis and measured the decay rate of the RPB2 mRNA (52,53). As shown in Figure 2A, both forms of RPB2 mRNA are quickly degraded after transcription inhibition in the absence of UV (lanes 1–8), corroborating the rapid degradation seen after UV damage. To test if UV damage changes the decay rate of the RPB2 mRNA, we irradiated the cells with UV, allowed mRNA synthesis to recover for 60 min, then inhibited transcription immediately by adding thiolutin. Figure 2 shows that the long form of RPB2 mRNA is preferentially recovered (lane 9) as previously shown, but both forms are rapidly degraded (lanes 10–16), suggesting that UV damage does not increase the RPB2 mRNA half lives. Quantitative RT-PCR experiments show that the half lives of the long RPB2 mRNA and total RPB2 mRNAs are about 5 min and do not increase after UV treatment (Figure 2B and C). The rapid turnover of the RPB2 mRNA both before and after UV damage suggests that the UV-induced increase in the long RPB2 mRNA results from enhanced synthesis during transcription recovery. Therefore APA is modulated to preferentially use the distal poly(A) site after UV damage.
Figure 1. Transcription of \textit{RPB2} after UV damage preferentially produces the long mRNA. (A) Northern blot image showing \textit{RPB2} mRNA levels after UV irradiation. Cells are irradiated with UV (70 J/m²) and samples are taken after the indicated incubation time for recovery. The symbol ‘L’ indicates the long \textit{RPB2} mRNA and ‘S’ indicates the short mRNA. Ribosomal RNA (rRNA) is shown as a loading control. (B) The ratios of the long \textit{RPB2} mRNA in total \textit{RPB2} mRNA determined by quantitative RT-PCR. The ratios are normalized to time point 0. Shown are the means of three independent experiments, error bars represent standard errors. (C) A graphic representation of the two \textit{RPB2} mRNAs determined by RACE assays. Both mRNAs share a unique transcription start site 277 bp upstream of the translational start codon. The long \textit{RPB2} mRNA is polyadenylated 345 bp downstream of the translational stop codon and the short mRNA is polyadenylated 58 bp downstream of the translational stop codon. (D) Schematic representation of the strategy to disrupt the 3' UTR of the \textit{RPB2} gene by inserting the \textit{KanMX6} gene between the two polyadenylation sites in the chromosome. The size of \textit{KanMX6} gene is 1.5 kb. The \textit{KanMX6} gene is inserted in the 3'UTR and replaces the DNA sequence from 226 bp to 332 bp after the translational stop codon. (E) Northern blot image showing the \textit{RPB2} mRNAs before UV damage or 30 min after UV damage (70 J/m²). The first two lanes are the wild-type strain carrying the normal \textit{RPB2} gene. ‘Insert #1’ and ‘Insert #2’ are two individual clones with the insertion of \textit{KanMX6} in the chromosome as depicted in Figure 1D. 30 µg of total RNA is loaded in each lane. L: position of the long \textit{RPB2} mRNA, S: position of the short \textit{RPB2} mRNA, P: position of the polycistronic \textit{RPB2-KanMX6} RNA. Numbers below the gel are the ratios of the long \textit{RPB2} mRNA to the short \textit{RPB2} mRNA determined by densitometry. (F) Schematic representation of the \textit{RPB2-CYC1} mutant in which the \textit{CYC1} 3'UTR has been used to replace the endogenous \textit{RPB2} 3'UTR. (G) Northern blot image showing \textit{RPB2} mRNA levels after UV irradiation. Cells are irradiated with UV (70 J/m²) and samples are taken after the indicated incubation times for recovery. Note only one form of \textit{RPB2} mRNA is seen in this construct. rRNA is shown as a loading control.
To test if UV damage regulates APA of other yeast genes, we tested the CBP1 mRNA after UV damage. APA of CBP1 produces two messages, a long 2.2 kb mRNA terminating at the end of the gene and a 1.2 kb mRNA terminating near the middle of the gene (28). Figure 3 shows that the long CPB1 mRNA is preferentially induced after UV damage. This result suggests that APA of CBP1 is a regulated process during recovery from UV damage.

APA of RPB2 is determined by the 3'UTR

Cis-elements that determine APA of a gene in yeast have not been well defined but is known to reside in the 3'UTR of a gene (54,55). To test if the 3'UTR of RPB2 contains sequence elements required for the UV-induced APA change, we annealed the RPB2 3'UTR to the yeast URA3 gene, replacing the original URA3 3'UTR (Figure 4A). The URA3-RPB2 construct is cloned in a yeast centromeric plasmid and expressed in yeast cells. During testing of the URA3-RPB2 construct, we found that the wild-type strain MVY150 used in previous experiments expresses mRNA of the ura3-1 mutant allele, complicating the analysis of the expression of wild-type URA3 (Data not shown). Therefore we chose to use strain MVY101 which contains the ura3-52 allele.

![Figure 2](image_url)

**Figure 2.** Turnover of RPB2 is rapid and does not change after UV irradiation. (A) RPB2 mRNA is analysed by northern blot after RNA transcription is inhibited by adding thiolutin into the cell culture. Naïve cells (lanes 1–8) are wild-type yeast cells without UV treatment and UV-damaged cells (lanes 9–16) have been irradiated with UV (70 J/m²) and incubated in YPD for 60 min to allow transcription to recover. In each case the RPB2 mRNA is quickly degraded within 5 min after addition of thiolutin. rRNA is shown as a loading control. (B) and (C) Levels of the RPB2 mRNAs are normalized to the levels of the ribosomal RNA RDY18 and determined by quantitative RT-PCR. L/RDY18: ratios of long RPB2 mRNA to RDY18 RNA; LS/RDY18: ratios of long and short RPB2 mRNA (total RPB2 mRNA) to RDY18 RNA. Shown are the means of three independent experiments and error bars represent standard errors. (B) Cells without UV treatment; (C) cells treated with UV and incubated in YPD at 30°C for 60 min.
that is driven by the CUP1 promoter. Expression of the CUP1 gene in yeast is regulated by the concentration of copper ions in the culture and the CUP1 promoter has been used in copper-ion concentration-dependent induction experiments (57,58). As shown in Figure 5B, increasing the amount of copper ions induces increasing levels of the URA3 mRNA. However, the relative abundance of the two mRNAs does not appear to change with different degrees of transcription induction (Figure 5B). This suggests that transcription induction does not cause differential APA of RPB2 and post-UV-damage transcription recovery is different from transcription induction.

Transcription termination is a concerted action of many proteins and polyadenylation site selection has been demonstrated to be affected by the rate of transcription elongation (22). The model is that slower elongation may give the transcription termination complex more time to terminate on the weaker promoter-proximal poly(A) site and faster elongation tends to penetrate the first weak proximal site and stop on the distal site (59). Therefore we tested if transcription elongation influences APA during transcription recovery. Mycophenolic acid (MPA) is a drug that inhibits the synthesis of GTP and UTP and has been demonstrated to reduce the rate of transcription elongation in yeast (60). We added MPA to cells undergoing transcription recovery after UV irradiation. As shown in Figure 5C, in the absence of MPA, the long RPB2 mRNA is preferentially recovered after UV (compare lane 3 and lane 1). However, in the presence of 15 μg/ml MPA, the short RPB2 mRNA becomes predominant when the messages are recovered from UV-treated cells. Quantitative RT-PCR experiments show that MPA-treated cells do not show the preferential increase of the long RPB2 mRNA that is seen in untreated cells. We additionally tested 6-azauracil (6AU), which has also been demonstrated to slow down transcription elongation in yeast (60), and it too suppresses the UV-induced induction of the long RPB2 mRNA (Figure 5D). Together these results suggest that the rate of transcription elongation is pivotal in determining which polyadenylation site is used during transcription recovery.

**DISCUSSION**

We describe regulation of APA of RPB2 after UV damage. APA has been found as a ubiquitous process in eukaryotic cells (7–9). Regulation of APA is implicated in embryonic development, tissue formation, and oncogenesis (24,25,27). Our results suggest that APA is a dynamic process that is regulated in response to many cellular stresses including UV damage. Further studies are required to determine whether this UV-induced regulation affects other yeast genes that undergo APA. Previous genomic analysis revealed that APA is systematically modulated throughout the transcriptomes (9,21,59), thus it is likely that UV damage may broadly affect many genes in the genome. The fact that the long mRNA forms of RPB2 and CBP1 are similarly preferentially induced after UV damage indicates that there may be a general change in gene transcription that affects poly(A) site selection, rather than gene

**Figure 3.** UV damage regulates APA of CBP1. Quantitative RT-PCR analysis of the CBP1 mRNA after UV treatment (70 J/m²). The ratios of the long CBP1 mRNA in total CBP1 mRNA are normalized to time point 0. Shown are the means of three independent experiments and error bars represent standard errors.

Lane 1 in Figure 4B shows that there is no URA3 expression in MVY101. We transformed this strain with the yeast plasmid pRS416 that carries the wild-type URA3 gene to confirm that URA3 is not alternatively polyadenylated before and after UV irradiation (Figure 4B, lanes 2–9). We also confirmed that, despite the different genetic background, strain MVY101 like MVY150 preferentially recovers the long form of RPB2 mRNA after UV irradiation (Figure 4C). Expression of the URA3-RPB2 construct in MVY101 results in two species of URA3 mRNA, indicating that the grafted RPB2 3'UTR causes APA of the URA3 (Figure 4D). The URA3-RPB2 expression is inhibited for up to 60 min after UV damage. However, when it finally recovers after 75 min, the long form is predominant, suggesting that the RPB2 3'UTR is sufficient for UV-induced regulation of APA. We then tested the role of the RPB2 promoter in transcription recovery after UV damage by using the RPB2-URA3-RPB2 construct which is driven by the RPB2 promoter (Figure 4E). Figure 4F shows that the mRNA dynamics of the RPB2-URA3-RPB2 construct is quite different from that of the URA3-RPB2 construct in Figure 4D. Levels of the long URA3 mRNA are higher before treatment and stay high after UV treatment, whereas the short form is suppressed by UV damage. Although this result suggests a role of the RPB2 promoter in mRNA recovery and APA, it is unclear why the RPB2-URA3-RPB2 construct behaves differently from the endogenous RPB2 gene shown in Figure 1A. It may be caused by differences in transcription of the chromosomal vs. plasmid genes. Alternatively, the distance between the promoter and the 3'UTR might influence the transcription activity as reported by Andersen et al. (56).

**Roles of transcription induction and transcription elongation in APA**

We tested two hypotheses for the regulated APA after UV damage. First we tested if transcription induction will influence APA. We reasoned that transcription recovery that occurs after UV-induced transcription inhibition may have similarities to transcription induction. We therefore made the CUP1-URA3-RPB2 construct (Figure 5A)
specific regulation mechanisms. In this regard, although we do not observe a great UV sensitivity in the rpb2 mutants that have its RPB2 3'UTR mutated or replaced (data not shown), it is possible that regulation of alternative polyadenylation actually plays a more important role after UV damage via effects on other genes.

Transcriptional activity has been linked to polyadenylation site selection in transcription termination (21). Ji et al. found that highly transcribed genes tend to use the proximal poly(A) sites, producing shorter mRNAs. Furthermore, Nagaie et al. (61) demonstrated that transcriptional activators can enhance the efficiency of polyadenylation of reporter genes in vivo, supporting the notion that transcription initiation may influence transcription termination. Our test of the copper inducible CUP1-URA3-RPB2 construct shows that the short URA3 in pRS416

Figure 4. The 3'UTR of RPB2 is sufficient for UV-induced regulation of APA. (A) Schematic representation of the URA3-RPB2 construct in which the RPB2 3'UTR is appended after the URA3 CDS in plasmid pRS416 to replace the original URA3 3'UTR. (B) Northern blot image showing that the wild-type strain MVY101 (lane 1) is completely abolished of the URA3 RNA and pRS416-transformed MVY101 expresses the URA3 gene as a single transcript (lanes 2-9) whether before (lane 2) or after UV irradiation (3-9). (C) Ratios of the long RPB2 mRNA to total RPB2 mRNA determined by quantitative RT-PCR during transcription recovery after yeast strain MVY101 is irradiated with UV (70 J/m²). Ratios have been normalized to time point 0. Shown are the means of three independent experiments and error bars represent standard errors. (D) Transcription recovery of the URA3-RPB2 gene after UV damage. The URA3 probe is used to detect the chimeric URA3 transcripts. rRNA is shown as a loading control. (E) Schematic representation of the RPB2-URA3-RPB2 construct. (F) Northern blot image showing the levels of the RPB2-URA3-RPB2 transcripts after UV damage. The URA3 probe is used to detect the chimeric URA3 transcripts. rRNA is shown as a loading control.
URA3 mRNA is not preferentially produced when the transcription level is upregulated, suggesting that the poly(A) site choice within the RPB2 3'UTR is not determined by transcription activity. Notably, Ji et al.'s measurements of transcript levels were performed on cells in steady states and this suggests that the regulation mechanism of APA may be different between steady state mRNA synthesis and transcription activation. In any case, transcription activation and transcription recovery appear to be different processes and exert different influences on poly(A) site choices.

Transcription elongation rates have also been demonstrated to affect poly(A) site selection (22,23). The proposed model is that slower transcription elongation allows more time for the termination complex to cleave at the ‘weaker’ promoter-proximal poly(A) site (22,59). Our results show that slowing down transcription elongation abolishes the preferential production of the long RPB2 mRNA during UV recovery, supporting the model that transcription kinetics is a determinant for poly(A) site usage. The fact that transcription terminates within the KanMX 3' UTR when it is inserted between the two RPB2 poly(A) sites eliminates the possibility that there is a positive selection for the distal poly(A) site and suggests that the distal RPB2 poly(A) site serves as a fail-safe stop in the event that the first poly(A) site is penetrated. Further work is required to directly test if UV damage alters the rate of transcription elongation and if so what molecular mechanisms cause this change in rate. It seems plausible that accelerated transcription elongation is used to produce more RNA messages to compensate for the vast loss of mRNA once RNA-polymerase blocking DNA damages are repaired. Surprisingly, UV damage has been shown to reduce the rate of transcription elongation and regulate alternative splicing in mammalian cells through phosphorylation of the carboxyl-terminal domain of RNA polymerase II (62). We noted that lower doses of UV irradiation were used in those experiments and UV-induced transcription-blocking DNA lesions are not required for the effects on alternative splicing. Moreover, other DNA damaging agents, such as MMS and H2O2, that induce fewer transcription-blocking lesions, have no

Figure 5. Reduction of transcription elongation by MPA and 6AU abolishes the UV-induced transcription of the long RPB2 mRNA. (A) Schematic representation of the CUP1-URA3-RPB2 construct in which URA3-RPB2 is driven by the copper-ion sensitive CUP1 promoter. (B) Northern blot images showing URA3 mRNA levels in the CUP1-URA3-RPB2 strain after various amounts of cupric sulfate are added to the culture for 1 h. Numbers below the gel are the ratios of the long URA3 mRNA to the short URA3 mRNA determined by densitometry. (C) Northern blot images showing recovery of the RPB2 mRNA after UV damage. MPA is added to slow down the transcription elongation (lanes 5–8); no MPA (lanes 1–4). rRNA is shown as a loading control. (D) Quantitative RT-PCR results showing ratios of the long RPB2 mRNA to total RPB2 mRNA during transcription recovery after UV damage (70 J/m²). The ratios are normalized to time point 0. In addition to MPA, 6AU is used to slow down transcription elongation. Shown are means of three independent experiments and error bars represent standard errors.
detectable effect on APA (Supplementary Figure S2). Therefore, an increased transcription elongation rate may uniquely induce after transcription inhibition and subsequently result in altered poly(A) site usage.

UV damage has been shown to suppress 3'-cleavage, possibly to suppress the production of premature cleavage and polyadenylation of incomplete mRNAs produced when transcription is interrupted by template DNA damage (34–36). It appears that 3' processing is transiently inhibited when DNA is damaged, raising the possibility that transcription termination or cleavage on the promotor-proximal poly(A) site may be inhibited by UV, resulting in greater usage of the distal poly(A) site. Although we show that transcription elongation rate is vitally important for poly(A) site selection, we do not rule out the possibility that the 3' processing machinery is altered in a way that favors the use of the distal poly(A) sites after UV damage, resulting in the production of longer mRNA messages.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Figures 1 and 2.

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