3104–3114 *Nucleic Acids Research, 2013, Vol. 41, No. 5* *Published online 25 January 2013*
doi:10.1093/nar/gkt020

UV damage regulates alternative polyadenylation of the *RPB2* gene in yeast

Lijian Yu and Michael R. Volkert*

Microbiology and Physiological Systems, University of Massachusetts Medical School, 55 Lake Ave. North, Worcester, MA 01655, USA

Received October 18, 2012; Revised December 12, 2012; Accepted December 26, 2012

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

*To whom correspondence should be addressed. Tel: +508 856 2314; Fax: +508 856 5920; Email: michael.volkert@umassmed.edu

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 SacUra (5′-GCGCCCGCGGTGCACCATACCACAGCTTTT) and BamUra (5′-CGGCGGATCCTTAGTTTTGCTGGCCGCA), then inserted the *URA3* DNA into plasmid pMV1351 between the SacII and BamHI restriction sites. Plasmid pMV1351 was derived from pRS315 (42) by inserting the *RPB2* 3′UTR DNA which was amplified by PCR from the yeast genome using primers BamRPB2-4653(5′-GCGCGGATCCGATCGTTCGAGAGATTTT) and SalRPB2-5148 (5′-CGGCGTCGACCTTTTTGCAGTCTTCAATCC), then inserting the PCR fragment into the BamHI and SalI sites of the vector. Plasmid pMV1352 was used to transform yeast strain MVY101 to obtain strain MVY897.

To construct plasmid pMV1365, we first amplified the *URA3* gene from plasmid pRS416 (42) using primers NotIUra (5′-GACTGCGGCCGCATGTCGAAAGCTACATATAAGGAACG) and BamUra (5′-CGGCGGATCCTTAGTTTTGCTGGCCGCA), 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′-CTGAGCGGCCGCCCTTATAATTATTTTTCTATACTTGTTTGTGTTCTTTTTC) and SacIIRPB2-1KB (5′-GCGCCCGCGGAGGCGACAAAAATCGCTATC) 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′-GCGC-CCGCGG-GTCGGCGAAGAACAAAAATG) and CUP1r (5′-CTGA-GCGGCCGC-AC-TGGCACTCATGACCTTCATT), and inserted the *CUP1* promoter into plasmid pMV1364 between the SacII site and the 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 SacII 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 KpnI-up500-f (5′-CGGCGGTACCGACACATGGTGGATGACAAGA) and up500-Xma-r (5′-GCGCCCCGGGTTGGTAAAATGCGAAACAAGG), and the second, using primers

Table 1. Yeast strains and plasmids used in this study

Strain	Original name, genotype (annotation)	Reference
MVY101	FY833, *MATa ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2Δ202*	(40)
MVY150	W303-1B, *MATα ade2-1 trp1-1 can1-100 leu2-3, 112 his3-11,15 ura3-1*	(37)
MVY818	MVY150 with *KanMX* inserted in *RPB2* 3′UTR, #1	this study
MVY819	MVY150 with *KanMX* inserted in *RPB2* 3′UTR, #2	this study
MVY836	MVY150 with *RPB2* 3′UTR replaced by *CYC1* 3′UTR	this study
MVY896	MVY101 with pRS416	this study
MVY897	MVY101 with pMV1352	this study
MVY898	MVY101 with pMV1365	this study
MVY1001	MVY101 with pMV1390	this study
pMV1352	plasmid with the *URA3-RPB2* construct	this study
pMV1365	plasmid with the *RPB2-URA3-RPB2* construct	this study
pMV1390	plasmid with the *CUP1-URA3-RPB2* construct	this study

SacII-down500-f (5′-GCCACCGCGGCGGTGTTCATTT TGGAACAA) and down500-SacI-r (5′-GACGGAGCTC CATTGGGTAGATTGGCTTCAG). These three fragments 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′-CGGCGGTAC CCCTCTCCTTTCACGGACATT) and Xma-Xho-RPB2 (5′-GCGCCCCGGGCTCGAGTTAAAAATCTCTCGA ACGATCGGTATATAAACG) 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′-CGGCCTCGAGACAGGCCCC TTTTCCTTTG) and Bgl-CYC1-1586 (5′-GCGCAGATC TCGTCCCAAAACCTTCTCAAG) and inserted this PCR product into pMV1346 to insert the *CYC1* terminator to produce pMV1347. Digestion with SnaB1 and KpnI releases a 2739 bp fragment containing the C-terminal region of *RPB2*, followed by the *CYC1* terminator and the *KanMX6* gene to allow selection of recombinants and the downstream 500 bp *RBP2* targeting sequence containing the *RPB2* promoter-distal polyadenylation site. This fragment was then used to transform yeast strain MVY150 to replace the chromosomal *RPB2* termination regions to obtain strain MVY836.

UV irradiation and northern analysis

Yeast cells in mid log phase are suspended in PBS at an OD_{600} reading of 0.8, irradiated with UV or mock treated, resuspended in YPD medium (44) and cultured for indicated times, and collected and frozen on dry ice. Total yeast RNA is extracted using the hot phenol method and analysed using the northern analysis as described elsewhere (45). ^{32}P-labeled probes are synthesized using the Random Primed DNA labeling kit as instructed by the manufacturer (Roche Applied Science, Indianapolis, IN). Northern blot images are acquired on a BAS-2500 Image Scanner (Fujifilm) and processed using Multi Gauge 3.0 (Fujifilm) or Adobe Photoshop CS4. Gel densitometry is analysed using ImageJ 1.44p (NIH). Statistical analysis is performed using Prism 5 (GraphPad Software).

5′-RACE assay

The 5′-RACE assay is performed as described (46). Briefly, yeast mRNA is purified from yeast total RNA using the Qiagen Oligtex mRNA miniPrep kit, reverse transcribed using Promega ImProm-II reverse transcriptase (Promega) and primer RPB2-13r (5′-GGTGGAATCC TCGCAAATAA), purified by the QIAQuick PCR purification kit (QIAGEN, Valencia, CA), polyadenylated using terminal transferase (NEB). The resulting polyadenylated cDNA is subjected to two rounds of PCR amplification, first using primers RPB2-14R (5′-AA AGCGGATATAACAGCCCA) and RACE1 (5′-GCTC GATGTGCACTGCTTTTTTTTTTTTTTT) followed by a second round of PCR using primers anchorP (5′-G CTCGATGTGCACTGC) and RPB2-17R (5′-GCACTT TCATCCTCGAATCC). The final PCR product is gel purified and sequenced using primer RPB2-20R (5′-GA GTTTGCAAGGTCTGACATCC) to determine the 5′ transcription start sites.

3′-RACE assay

The 3′-RACE assay is performed as described (47). To determine the 3′-ends of RPB2 mRNAs, cDNA is prepared as in the 5′-RACE assay except that primer RACE1 (5′-GCT CGATGTGCACTGCTTTTTTTTTTTTTTT) is used in the reverse transcription and no polyadenylation step is included. Primers 529 (5′-CGGCGGTACCGACACATG GTGGATGACAAGA) and anchorP (5′-GCTCGATGT GCACTGC) are used to PCR amplify the 3′-UTR of the *RPB2* gene, followed by a second round PCR amplification using primers 466 (5′-GCTGATGACAGTTATCGCG) and the anchorP primer. The PCR products are gel purified and sequenced using primer RPB2-13 (5′-GCCG CGAAGTTATTATTCCAAG) 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 primer set KanMX-717f (5′-GCAAAACAGCATTCCAG GTA) / anchorP and primer set KanMX-845f (5′-GTATT TCGTCTCGCTCAGGC) / anchorP. The PCR products are gel purified and sequenced by primer KanMX-905 (5′-TTTGATGACGAGCGTAATGG). A unique polyadenylation site is found after the sequence TTAGCGTGA TTTA.

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 (TGCCCTC CAATTGTTCCTCG) was added to reverse transcribe the ribosomal *RDY18* RNA.cDNA of the long *RPB2* mRNA was amplified with primer 861 (TCATTTGTGCTGATCT TGCCA) and primer 862 (TGCTTGAAAGTTCTCTCT GCT), cDNA of the total *RPB2* mRNA with primer 876 (GCCTGTAGAGGGTAGATCGAG) and primer 877 (T CAGCCCGCAAATACCACAA), cDNA of the *RDY18* RNA with primer 870 (GTGCTGGCGATGGTTCAT TC) and primer 871 (CCTTGGATGTGGTAGCCGTT), cDNA of the *CBP1* mRNA with primer 878 (GCGCACT GCAGATAGGAAGA) and primer 879 (GTAAATGTG CGTTTGGCCGT), and cDNA of the total *CBP1* mRNA with primer 867 (GGCGGAATAAAGTTAACGAGG) and primer 868 (GCACTTGATCATCCCGAAGC). RT-PCR was performed on a ViiA 7 QPCR machine with the following thermocycler protocol: 95°C for

10 min to nitially denature, followed by 40 cycles of (95°C for 15 s, 60°C for 20 s, 72C 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 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′-GCTCGATGTGCACTGCTTTTTTTTTTTT TTTT), then PCR-amplified the 3′UTR of *RPB2* using *RPB2* specific primers and the anchor primer [5′-GCTC GATGTGCACTGC, 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 *RBP2* 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.

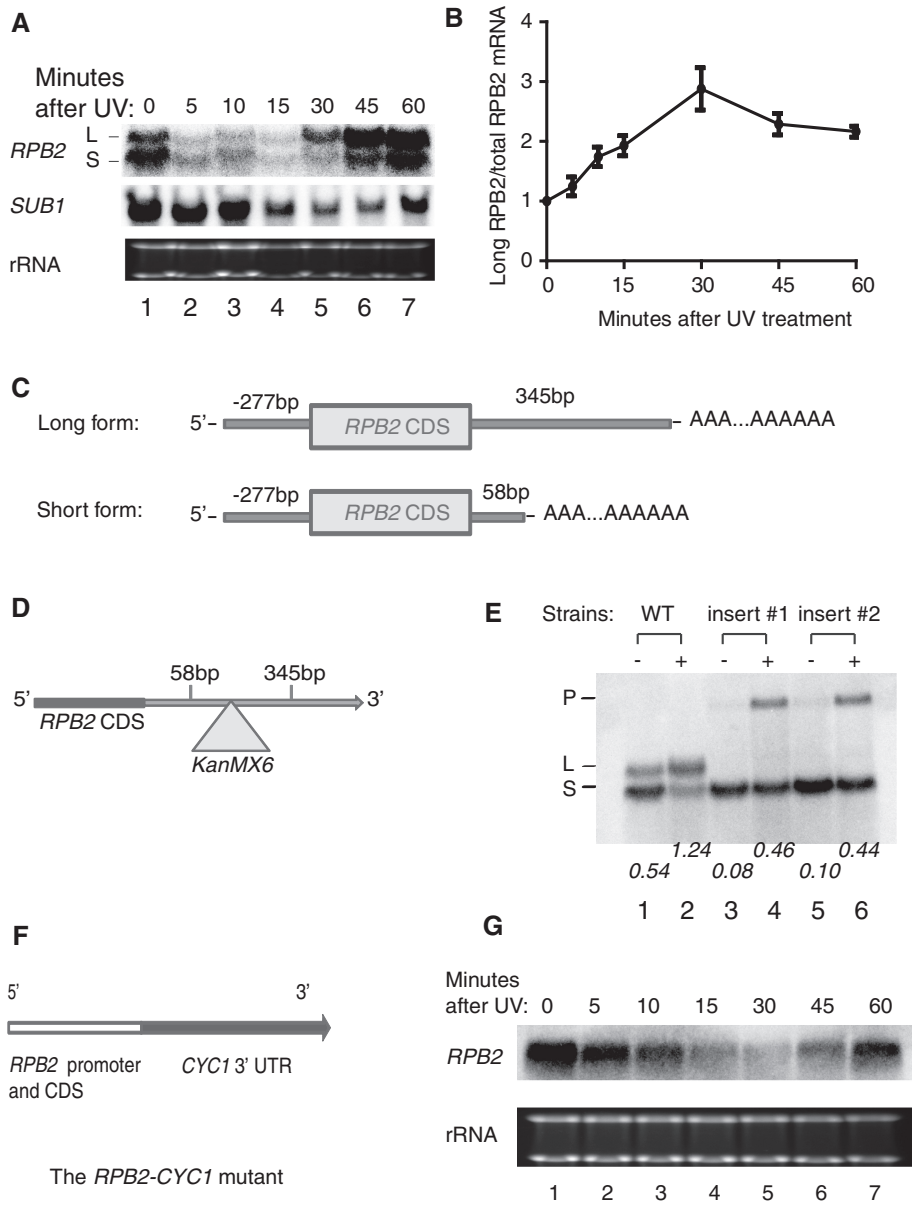

Figure 1. Transcription of *RPB2* after UV damage preferentially produces the long mRNA. (**A**) Northern blot image showing *RPB2* mRNA levels after UV irradiation. Cells are irradiated with UV (70 J/m^2) and samples are taken after the indicated incubation time for recovery. The symbol 'L' indicates the long *RPB2* mRNA and 'S' indicates the short mRNA. Ribosomal RNA (rRNA) is shown as a loading control. (**B**) The ratios of the long *RPB2* mRNA in total *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 *RPB2* mRNAs determined by RACE assays. Both mRNAs share a unique transcription start site 277 bp upstream of the translational start codon. The long *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 *RPB2* gene by inserting the *KanMX6* gene between the two polyadenylation sites in the chromosome. The size of *KanMX6* gene is 1.5 kb. The *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 *RPB2* mRNAs before UV damage or 30 min after UV damage (70 J/m^2). The first two lanes are the wild-type strain carrying the normal *RPB2* gene. 'Insert #1' and 'Insert #2' are two individual clones with the insertion of *KanMX6* in the chromosome as depicted in Figure 1D. 30 μg of total RNA is loaded in each lane. L: position of the long *RPB2* mRNA, S: position of the short *RPB2* mRNA, P: position of the polycistronic *RPB2-KanMX6* RNA. Numbers below the gel are the ratios of the long *RBP2* mRNA to the short *RPB2* mRNA determined by densitometry. (**F**) Schematic representation of the *RPB2-CYC1* mutant in which the *CYC1* 3′UTR has been used to replace the endogenous *RPB2* 3′UTR. (**G**) Northern blot image showing *RPB2* mRNA levels after UV irradiation. Cells are irradiated with UV (70 J/m^2) and samples are taken after the indicated incubation times for recovery. Note only one form of *RPB2* mRNA is seen in this construct. rRNA is shown as a loading control.

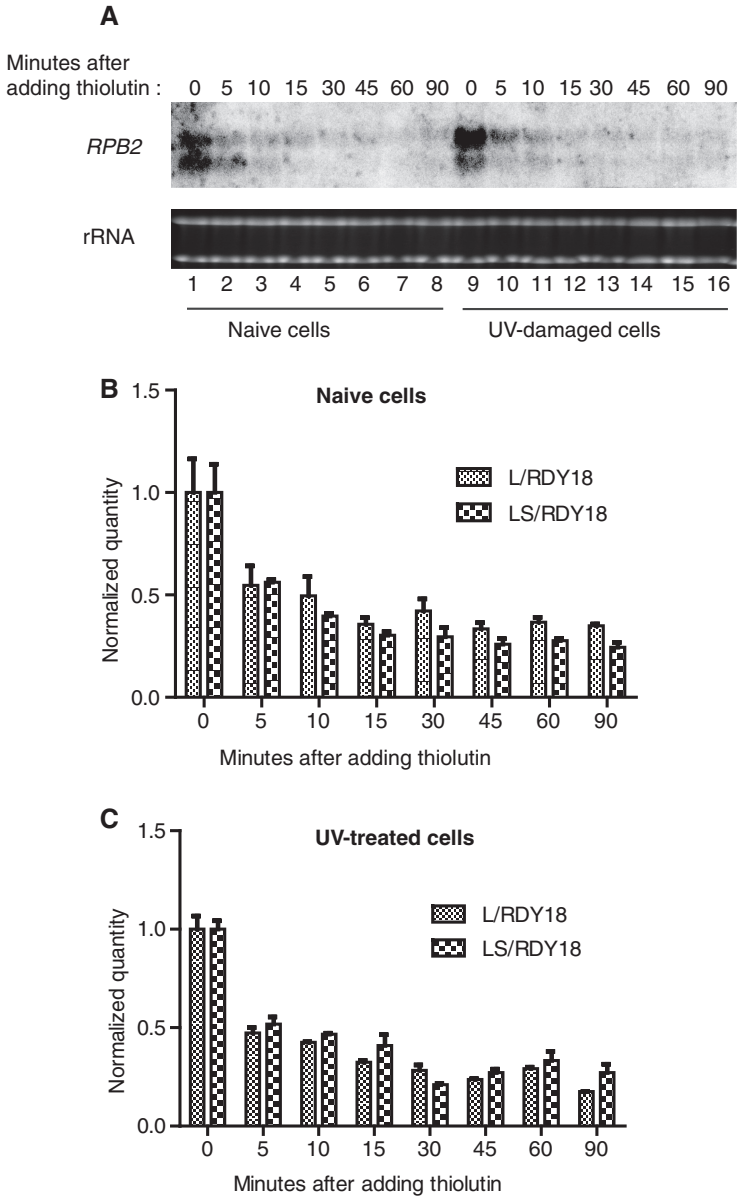

Figure 2. Turnover of *RPB2* is rapid and does not change after UV irradiation. (**A**) *RPB2* mRNA is analysed by northern blot after RNA transcription is inhibited by adding thiolutin into the cell culture. Naïve cells (lanes 1–8) are wild-type yeast cells without UV treatment and UV-damaged cells (lanes 9–16) have been irradiated with UV (70 J/m^2) 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.

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.

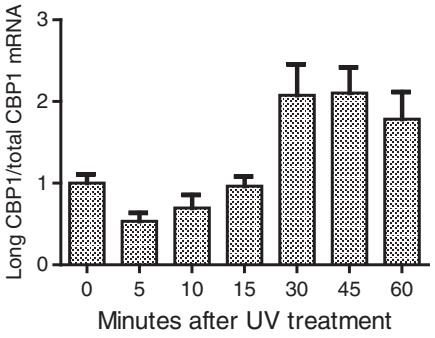

Figure 3. UV damage regulates APA of *CBP1*. Quantitative RT-PCR analysis of the *CBP1* mRNA after UV treatment (70 J/m^2). The ratios of the long *CBP1* mRNA in total *CBP1* mRNA are normalized to time point 0. Shown are the means of three independent experiments and error bars represent standard errors.

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) 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

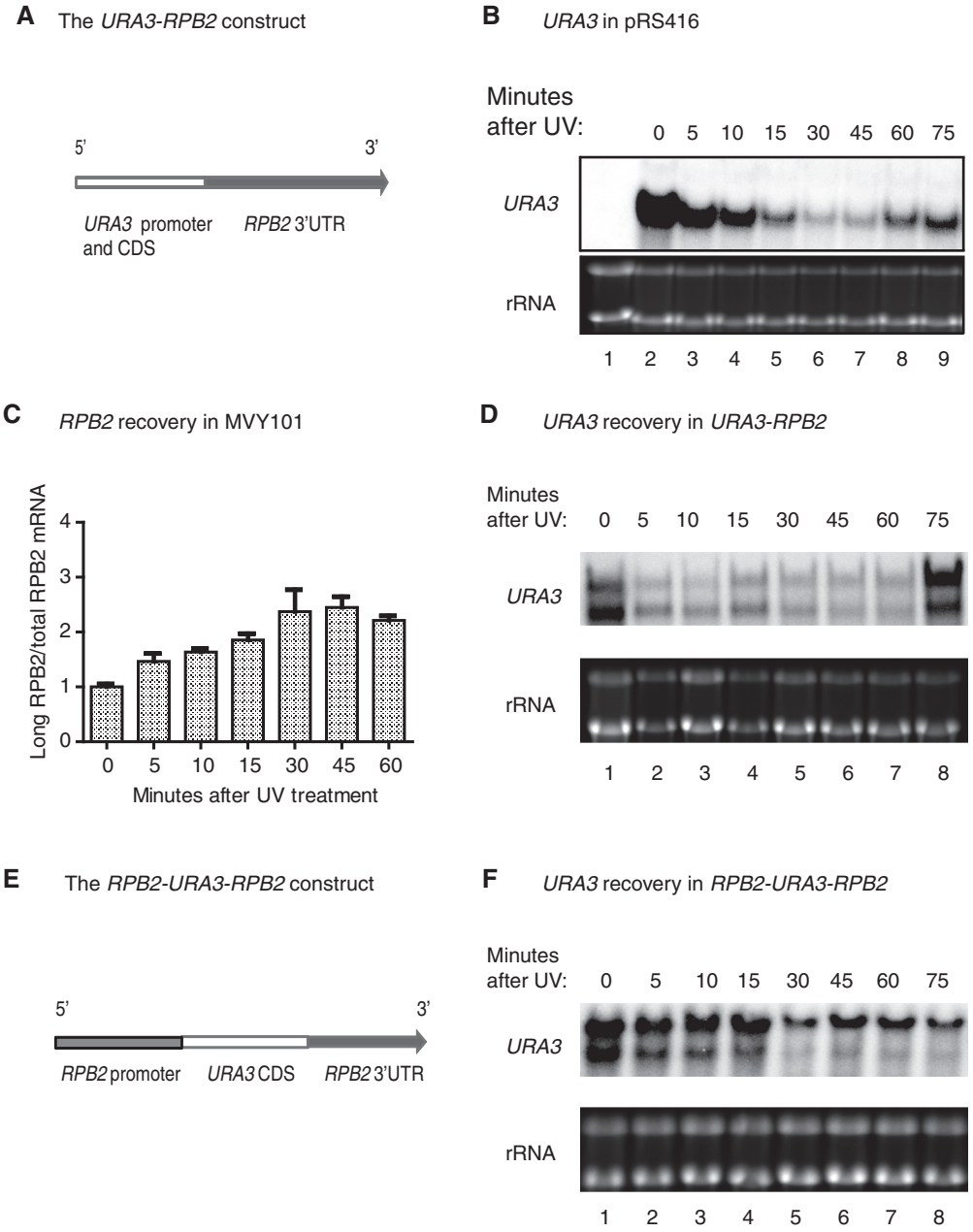

Figure 4. The 3′UTR of *RPB2* is sufficient for UV-induced regulation of APA. (**A**) Schematic representation of the *URA3-RPB2* construct in which the *RPB2* 3′UTR is appended after the *URA3* CDS in plasmid pRS416 to replace the original *URA3* 3′UTR. (**B**) Northern blot image showing that the wild-type 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/m^2). 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.

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 polyadenlyation 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, Nagaike *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

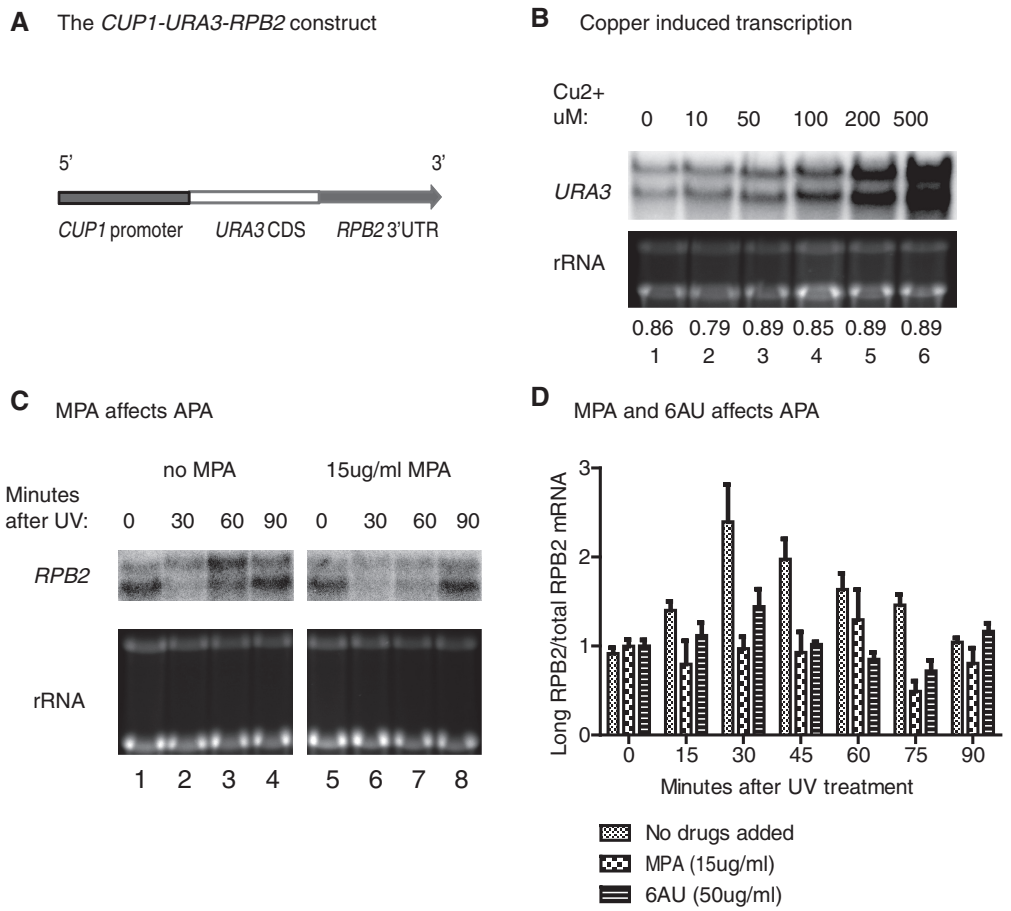

Figure 5. Reduction of transcription elongation by MPA and 6AU abolishes the UV-induced transcription of the long *RPB2* mRNA. (**A**) Schematic representation of the *CUP1-URA3-RPB2* construct in which *URA3-RPB2* is driven by the copper-ion sensitive *CUP1* promoter. (**B**) Northern blot images showing *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/m^2). 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.

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 H_2O_2, that induce fewer transcription-blocking lesions, have no

detectable effect on APA (Supplementary Figure S2). Therefore, an increased transcription elongation rate may be uniquely induced 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 promoter-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.

ACKNOWLEDGEMENTS

We thank Jaap Brouwer and Michael Hampsey for yeast strains, Craig Peterson and Feng He for discussions and advice, XiuFeng Wu for help with RT-PCR, and Lingtao Peng and Hong Jia for technical help with the RACE assays.

FUNDING

National Institutes of Health (NIH; in part) [CA100122]. Funding for open access charge: Waived by Oxford University Press.

Conflict of interest statement. None declared.

REFERENCES

1. Woychik,N.A. and Hampsey,M. (2002) The RNA polymerase II machinery: structure illuminates function. *Cell*, **108**, 453–463.
2. Cramer,P. (2004) RNA polymerase II structure: from core to functional complexes. *Curr. Opin. Genet. Dev.*, **14**, 218–226.
3. Rondón,A.G., Mischo,H.E., Kawauchi,J. and Proudfoot,N.J. (2009) Fail-safe transcriptional termination for protein-coding genes in S. cerevisiae. *Mol. Cell*, **36**, 88–98.
4. Kuehner,J.N., Pearson,E.L. and Moore,C. (2011) Unravelling the means to an end: RNA polymerase II transcription termination. *Nat. Rev. Mol. Cell Biol.*, **12**, 283–294.
5. Kim,M., Krogan,N.J., Vasiljeva,L., Rando,O.J., Nedea,E., Greenblatt,J.F. and Buratowski,S. (2004) The yeast Rat1 exonuclease promotes transcription termination by RNA polymerase II. *Nature*, **432**, 517–522.
6. Shi,Y., Di Giammartino,D.C., Taylor,D., Sarkeshik,A., Rice,W.J., Yates,J.R. III, Frank,J. and Manley,J.L. (2009) Molecular architecture of the human pre-mRNA 3′ processing complex. *Mol. Cell*, **33**, 365–376.
7. Tian,B., Hu,J., Zhang,H. and Lutz,C.S. (2005) A large-scale analysis of mRNA polyadenylation of human and mouse genes. *Nucleic Acids Res.*, **33**, 201–212.
8. Ozsolak,F., Kapranov,P., Foissac,S., Kim,S.W., Fishilevich,E., Monaghan,A.P., John,B. and Milos,P.M. (2010) Comprehensive polyadenylation site maps in yeast and human reveal pervasive alternative polyadenylation. *Cell*, **143**, 1018–1029.
9. Ulitsky,I., Shkumatava,A., Jan,C.H., Subtelny,A.O., Koppstein,D., Bell,G.W., Sive,H. and Bartel,D.P. (2012) Extensive alternative polyadenylation during zebrafish development. *Genome Res.*, **22**, 2054–2066.
10. Millevoi,S. and Vagner,S. (2010) Molecular mechanisms of eukaryotic pre-mRNA 3′-end processing regulation. *Nucleic Acids Res.*, **38**, 2757–2774.
11. Proudfoot,N. and O'Sullivan,J. (2002) Polyadenylation: a tail of two complexes. *Curr. Biol.*, **12**, R855–R857.
12. Moore,M.J. (2005) From birth to death: the complex lives of eukaryotic mRNAs. *Science*, **309**, 1514–1518.
13. Peterson,M.L. (2007) Mechanisms controlling production of membrane and secreted immunoglobulin during B cell development. *Immunol. Res.*, **37**, 33–46.
14. Kubo,T., Wada,T., Yamaguchi,Y., Shimizu,A. and Handa,H. (2006) Knock-down of 25 kDa subunit of cleavage factor Im in Hela cells alters alternative polyadenylation within 3′-UTRs. *Nucleic Acids Res.*, **34**, 6264–6271.
15. Liu,F., Marquardt,S., Lister,C., Swiezewski,S. and Dean,C. (2010) Targeted 3′ processing of antisense transcripts triggers Arabidopsis FLC chromatin silencing. *Science*, **327**, 94–97.
16. Brown,K.M. and Gilmartin,G.M. (2003) A mechanism for the regulation of pre-mRNA 3′ processing by human cleavage factor Im. *Mol. Cell*, **12**, 1467–1476.
17. Seoane,S., Lamas-Maceiras,M., Rodríguez-Torres,A.M. and Freire-Picos,M.A. (2009) Involvement of Pta1, Pcf11 and a KlCYC1 AU-rich element in alternative RNA 3′-end processing selection in yeast. *FEBS Lett.*, **583**, 2843–2848.
18. Kim Guisbert,K.S., Li,H. and Guthrie,C. (2007) Alternative 3′ pre-mRNA processing in Saccharomyces cerevisiae is modulated by Nab4/Hrp1 in vivo. *PLoS Biol.*, **5**, e6.
19. Kyburz,A., Sadowski,M., Dichtl,B. and Keller,W. (2003) The role of the yeast cleavage and polyadenylation factor subunit Ydh1p/Cft2p in pre-mRNA 3′-end formation. *Nucleic Acids Res.*, **31**, 3936–3945.
20. Minvielle-Sebastia,L., Beyer,K., Krecic,A.M., Hector,R.E., Swanson,M.S. and Keller,W. (1998) Control of cleavage site selection during mRNA 3′-end formation by a yeast hnRNP. *EMBO J.*, **17**, 7454–7468.
21. Ji,Z., Luo,W., Li,W., Hoque,M., Pan,Z., Zhao,Y. and Tian,B. (2011) Transcriptional activity regulates alternative cleavage and polyadenylation. *Mol. Syst. Biol.*, **7**, 534.
22. Pinto,P.A.B., Henriques,T., Freitas,M.O., Martins,T., Domingues,R.G., Wyrzykowska,P.S., Coelho,P.A., Carmo,A.M., Sunkel,C.E., Proudfoot,N.J. *et al.* (2011) RNA polymerase II kinetics in polo polyadenylation signal selection. *EMBO J.*, **30**, 2431–2444.
23. Moreira,A. (2011) Integrating transcription kinetics with alternative polyadenylation and cell cycle control. *Nucleus*, **2**, 556–561.
24. Ji,Z., Lee,J.Y., Pan,Z., Jiang,B. and Tian,B. (2009) Progressive lengthening of 3′ untranslated regions of mRNAs by alternative polyadenylation during mouse embryonic development. *Proc. Natl Acad. Sci. USA*, **106**, 7028–7033.
25. Mayr,C. and Bartel,D.P. (2009) Widespread shortening of 3′UTRs by alternative cleavage and polyadenylation activates oncogenes in cancer cells. *Cell*, **138**, 673–684.
26. Flavell,S.W., Kim,T.-K., Gray,J.M., Harmin,D.A., Hemberg,M., Hong,E.J., Markenscoff-Papadimitriou,E., Bear,D.M. and Greenberg,M.E. (2008) Genome-wide analysis of MEF2 transcriptional program reveals synaptic target genes and neuronal activity-dependent polyadenylation site selection. *Neuron*, **60**, 1022–1038.
27. Wang,E.T., Sandberg,R., Luo,S., Khrebtukova,I., Zhang,L., Mayr,C., Kingsmore,S.F., Schroth,G.P. and Burge,C.B. (2008) Alternative isoform regulation in human tissue transcriptomes. *Nature*, **456**, 470–476.
28. Mayer,S.A. and Dieckmann,C.L. (1991) Yeast CBP1 mRNA 3′-end formation is regulated during the induction of mitochondrial function. *Mol. Cell. Biol.*, **11**, 813–821.

29. Hoopes,B.C., Bowers,G.D. and DiVisconte,M.J. (2000) The two Saccharomyces cerevisiae SUA7 (TFIIB) transcripts differ at the 3′-end and respond differently to stress. *Nucleic Acids Res.*, **28**, 4435–4443.
30. Sparks,K.A. and Dieckmann,C.L. (1998) Regulation of poly(A) site choice of several yeast mRNAs. *Nucleic Acids Res.*, **26**, 4676–4687.
31. Ciccia,A. and Elledge,S.J. (2010) The DNA damage response: making it safe to play with knives. *Mol. Cell*, **40**, 179–204.
32. Fu,Y., Pastushok,L. and Xiao,W. (2008) DNA damage-induced gene expression in Saccharomyces cerevisiae. *FEMS Microbiol. Rev.*, **32**, 908–926.
33. Reagan,M.S. and Friedberg,E.C. (1997) Recovery of RNA polymerase II synthesis following DNA damage in mutants of Saccharomyces cerevisiae defective in nucleotide excision repair. *Nucleic Acids Res.*, **25**, 4257–4263.
34. Cevher,M.A., Zhang,X., Fernandez,S., Kim,S., Baquero,J., Nilsson,P., Lee,S., Virtanen,A. and Kleiman,F.E. (2010) Nuclear deadenylation/polyadenylation factors regulate 3′ processing in response to DNA damage. *EMBO J.*, **29**, 1674–1687.
35. Mirkin,N., Fonseca,D., Mohammed,S., Cevher,M.A., Manley,J.L. and Kleiman,F.E. (2008) The 3′ processing factor CstF functions in the DNA repair response. *Nucleic Acids Res.*, **36**, 1792–1804.
36. Kleiman,F.E. and Manley,J.L. (2001) The BARD1-CstF-50 interaction links mRNA 3′-end formation to DNA damage and tumor suppression. *Cell*, **104**, 743–753.
37. Van Gool,A.J., Verhage,R., Swagemakers,S.M., Van de Putte,P., Brouwer,J., Troelstra,C., Bootsma,D. and Hoeijmakers,J.H. (1994) RAD26, the functional S. cerevisiae homolog of the Cockayne syndrome B gene ERCC6. *EMBO J.*, **13**, 5361–5369.
38. Sweder,K.S. and Hanawalt,P.C. (1994) The COOH terminus of suppressor of stem loop (SSL2/RAD25) in yeast is essential for overall genomic excision repair and transcription-coupled repair. *J. Biol. Chem.*, **269**, 1852–1857.
39. Verhage,R.A., Van Gool,A.J., De Groot,N., Hoeijmakers,J.H., Van de Putte,P. and Brouwer,J. (1996) Double mutants of Saccharomyces cerevisiae with alterations in global genome and transcription-coupled repair. *Mol. Cell. Biol.*, **16**, 496–502.
40. Wu,W.H., Pinto,I., Chen,B.S. and Hampsey,M. (1999) Mutational analysis of yeast TFIIB. A functional relationship between Ssu72 and Sub1/Tsp1 defined by allele-specific interactions with TFIIB. *Genetics*, **153**, 643–652.
41. Knop,M., Siegers,K., Pereira,G., Zachariae,W., Winsor,B., Nasmyth,K. and Schiebel,E. (1999) Epitope tagging of yeast genes using a PCR-based strategy: more tags and improved practical routines. *Yeast*, **15**, 963–972.
42. Sikorski,R.S. and Hieter,P. (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. *Genetics*, **122**, 19–27.
43. Wach,A., Brachat,A., Alberti-Segui,C., Rebischung,C. and Philippsen,P. (1997) Heterologous HIS3 marker and GFP reporter modules for PCR-targeting in Saccharomyces cerevisiae. *Yeast*, **13**, 1065–1075.
44. Adams,A., Gottschling,D., Kaiser,C. and Stearns,T. (1997) *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory.
45. He,F. and Jacobson,A. (1995) Identification of a novel component of the nonsense-mediated mRNA decay pathway by use of an interacting protein screen. *Genes Dev.*, **9**, 437–454.
46. Scotto-Lavino,E., Du,G. and Frohman,M.A. (2006) 5′-end cDNA amplification using classic RACE. *Nat. Protoc.*, **1**, 2555–2562.
47. Scotto-Lavino,E., Du,G. and Frohman,M.A. (2006) 3′-end cDNA amplification using classic RACE. *Nat. Protoc.*, **1**, 2742–2745.
48. Wu,X., Arumugam,R., Baker,S.P. and Lee,M.M. (2005) Pubertal and adult Leydig cell function in Mullerian inhibiting substance-deficient mice. *Endocrinology*, **146**, 589–595.
49. Damsma,G.E., Alt,A., Brueckner,F., Carell,T. and Cramer,P. (2007) Mechanism of transcriptional stalling at cisplatin-damaged DNA. *Nat. Struct. Mol. Biol.*, **14**, 1127–1133.
50. Hanawalt,P.C. and Spivak,G. (2008) Transcription-coupled DNA repair: two decades of progress and surprises. *Nat. Rev. Mol. Cell Biol.*, **9**, 958–970.
51. Sweetser,D., Nonet,M. and Young,R.A. (1987) Prokaryotic and eukaryotic RNA polymerases have homologous core subunits. *Proc. Natl Acad. Sci. USA*, **84**, 1192–1196.
52. Parker,R., Herrick,D., Peltz,S.W. and Jacobson,A. (1991) Measurement of mRNA decay rates in Saccharomyces cerevisiae. *Meth. Enzymol.*, **194**, 415–423.
53. Coller,J. (2008) Methods to determine mRNA half-life in Saccharomyces cerevisiae. *Meth. Enzymol.*, **448**, 267–284.
54. Irniger,S., Egli,C.M. and Braus,G.H. (1991) Different classes of polyadenylation sites in the yeast Saccharomyces cerevisiae. *Mol. Cell. Biol.*, **11**, 3060–3069.
55. Zhao,J., Hyman,L. and Moore,C. (1999) Formation of mRNA 3′-ends in eukaryotes: mechanism, regulation, and interrelationships with other steps in mRNA synthesis. *Microbiol. Mol. Biol. Rev.*, **63**, 405–445.
56. Andersen,P.K., Lykke-Andersen,S. and Jensen,T.H. (2012) Promoter-proximal polyadenylation sites reduce transcription activity. *Genes Dev.*, **26**, 2169–2179.
57. Maya,D., Quintero,M.J., De la Cruz Muñoz-Centeno,M. and Chávez,S. (2008) Systems for applied gene control in Saccharomyces cerevisiae. *Biotechnol. Lett.*, **30**, 979–987.
58. Karin,M., Najarian,R., Haslinger,A., Valenzuela,P., Welch,J. and Fogel,S. (1984) Primary structure and transcription of an amplified genetic locus: the CUP1 locus of yeast. *Proc. Natl Acad. Sci. USA*, **81**, 337–341.
59. Di Giammartino,D.C., Nishida,K. and Manley,J.L. (2011) Mechanisms and consequences of alternative polyadenylation. *Mol. Cell*, **43**, 853–866.
60. Mason,P.B. and Struhl,K. (2005) Distinction and relationship between elongation rate and processivity of RNA polymerase II in vivo. *Mol. Cell*, **17**, 831–840.
61. Nagaike,T., Logan,C., Hotta,I., Rozenblatt-Rosen,O., Meyerson,M. and Manley,J.L. (2011) Transcriptional activators enhance polyadenylation of mRNA precursors. *Mol. Cell*, **41**, 409–418.
62. Muñoz,M.J., Pérez Santangelo,M.S., Paronetto,M.P., De la Mata,M., Pelisch,F., Boireau,S., Glover-Cutter,K., Ben-Dov,C., Blaustein,M., Lozano,J.J. *et al.* (2009) DNA damage regulates alternative splicing through inhibition of RNA polymerase II elongation. *Cell*, **137**, 708–720.