Functional Analysis of Rad14p, a DNA Damage Recognition Factor in Nucleotide Excision Repair, in Regulation of Transcription *in Vivo**

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Background: Although Rad14p has well known functions in NER, its role in transcription is not clearly known.
Results: We demonstrate that Rad14p associates with active gene in absence of lesion and promotes transcription.
Conclusion: Our results support a new role of Rad14p in transcriptional regulation in addition to its well known function in NER.
Significance: This study implicates Rad14p as a new regulator of gene expression.

Rad14p is a DNA damage recognition factor in nucleotide excision repair. Intriguingly, we show here that Rad14p associates with the promoter of a galactose-inducible *GAL1* gene after transcriptional induction in the absence of DNA lesion. Such an association of Rad14p facilitates the recruitment of TBP, TFIIH, and RNA polymerase II to the *GAL1* promoter. Furthermore, the association of RNA polymerase II with the *GAL1* promoter is significantly decreased in the absence of Rad14p, when the coding sequence was deleted. These results support the role of Rad14p in transcriptional initiation. Consistently, the level of *GAL1* mRNA is significantly decreased in the absence of Rad14p. Similar results are also obtained at other galactose-inducible *GAL* genes such as *GAL7* and *GAL10*. Likewise, Rad14p promotes transcription of other non-*GAL* genes such as *CUP1*, *CTT1*, and *STL1* after transcriptional induction. However, the effect of Rad14p on the steady-state levels of transcription of *GAL* genes or constitutively active genes such as *ADH1*, *PGK1*, *PYK1*, and *RPS5* is not observed. Thus, Rad14p promotes initial transcription but does not appear to regulate the steady-state level. Collectively, our results unveil a new role of Rad14p in stimulating transcription in addition to its well-known function in nucleotide excision repair.

Genomic DNA is a target of incessant attack from an immense number of sources comprising radiation, chemicals, and mutagens as external agents along with various internal biological byproducts. Thus, damaged DNA has to be repaired swiftly and efficiently for the cell to survive and function effectively. Therefore, various repair mechanisms are employed by the cell to maintain its genomic stability. Nucleotide excision

repair (NER)[2] is one such repair mechanism where many proteins work synchronously in a multistep process to coordinate the overall repair pathway (1–8). The importance of this repair mechanism is evidenced by the severe human diseases that result from in-born genetic mutations of NER proteins including xeroderma pigmentosum (XP) and Cockayne syndrome (5, 6, 9–14).

NER is conserved among eukaryotes. In yeast (*Saccharomyces cerevisiae*), the DNA damage recognition step is mediated by a combination of factors, namely Rad14p, Rad4p, Rad23p, and RPA (replication protein A) (1, 15). Then Rad3p and Rad25p DNA helicases (components of TFIIH) unwind the DNA duplex around the lesion (1). Incision of the damaged strand on the 5′ site of the lesion is brought about by the Rad1p-Rad10p complex, whereas Rad2p incises the 3′ end of the lesion, thus releasing ~30-nucleotide fragment of damaged DNA strand (1). Neither Rad1p-Rad10p nor Rad2p nuclease shows a specificity for binding to the UV-damaged DNA (16, 17). Rad14p plays a crucial role in targeting Rad1p-Rad10p nuclease to the DNA (2, 18), whereas TFIIH promotes the targeting of Rad2p nuclease (19). The targeting of Rad1p-Rad10p to the lesion by Rad14p is mediated via physical interaction of Rad14p with Rad1p but not Rad10p (2, 20). Rad14p has a zinc finger domain and recognizes DNA lesion (21, 22). Thus, Rad14p plays a crucial role in NER, and its absence dramatically impairs NER (1, 18, 20–23). Rad14p is highly conserved from yeast to humans. Its human homolog is XPA (XP group A) (24–26). Like in yeast, human XPA recognizes DNA lesion and plays a central role in NER (21, 24–28). Mutations in XPA manifest neurological disorders and XP (a high sun-sensitivity predisposing to skin cancer at an early age). In addition, a polymorphic site in the 5′-untranslated region of XPA has been identified. This polymorphism consists of an A to G substitution in the fourth nucleotide before the ATG start codon and has been implicated to be associated with different types of

[2] The abbreviations used are: NER, Nucleotide excision repair; XP, xeroderma pigmentosum; XPA, XP group A; RPA, replication protein A; PIC, pre-initiation complex; UAS, upstream activating sequence; TBP, TATA-box binding protein.

cancers including lung, skin, breast, gastric, head, and neck cancers (25, 29–50). Thus, XPA and its yeast homologue play critical roles in maintaining genome integrity and, hence, normal cellular functions.

Although Rad14p (or its human homologue) has well known functions in NER, its role in transcription has not been clearly elucidated. A previous study (51) has demonstrated the interaction of Rad14p with RPA and transcription factors such as RNA polymerase II and TFIIH. Furthermore, a recent study (52) has implicated the role of RPA in transcriptional regulation. These studies suggest that Rad14p might be involved in transcriptional regulation through its interaction with RPA, TFIIH, and RNA polymerase II. However, it is not clearly known whether Rad14p regulates transcription. Here, we have carried out a series of experiments including formaldehyde-based *in vivo* cross-linking and chromatin immunoprecipitation (ChIP), transcriptional, and mutational analyses in *S. cerevisiae* to determine Rad14p's function in transcription. Intriguingly, our results reveal that Rad14p associates with the active gene in the absence of lesion and promotes initial transcription. However, the steady-state or constitutive transcription is not altered in the absence of Rad14p. These results unveil a new role of Rad14p in transcriptional regulation in addition to its well-known function in NER.

EXPERIMENTAL PROCEDURES

Plasmids—The plasmids pRS403 and pRS406 (53) were used in the PCR-based disruption of *RAD14* and coding sequence of *GAL1.* The plasmid pFA6a-13Myc-KanMX6 (54) was used for genomic tagging of the proteins of interest by Myc epitope.

Strains—The yeast (*S. cerevisiae*) strain FM391 (Research Genetics) was obtained from the Shilatifard laboratory (Ali Shilatifard; Stowers Institute for Medical Research). The *RAD14* deletion mutant (YMR201C; Open Biosystems) and wild type (BY4741; Open Biosystems) strains were obtained from the Davie laboratory (Judith K. Davie, Southern Illinois University School of Medicine). The wild type strain W303a was obtained from the Green laboratory (Michael R. Green, University of Massachusetts Medical School). The yeast strain PCY25 (Δ*rad14::HIS3*) was generated by deleting *RAD14* from the ZDY2 (W303a expressing Myc-tagged Rad26p) strain using the pRS403 plasmid following PCR-based gene disruption protocol (54). Multiple Myc epitope tags were added to the C terminus of Rad14p at its chromosomal locus in W303a using the pFA6a-13Myc-KanMX6 plasmid to generate the ZDY3 strain. The coding sequence of *GAL1* was deleted in the ZDY2 and PCY25 strains to generate the PCY31 and PCY32 strains, respectively, using the pRS406 plasmid. Multiple Myc epitope tags were added at the C terminus in the chromosomal locus of *RAD3* in the FM391 strain to generate PCY2 using the pFA6a-13Myc-KanMX6 plasmid. The PCY35 strain was generated by deleting the *RAD14* gene in the PCY2 strain following PCR-based gene disruption protocol (54). All these strains with genotypes are listed below in Table 1.

Growth Media—For transcriptional induction of *GAL1, GAL7,* and *GAL10,* yeast strains were initially grown in YPR (yeast extract, peptone plus 2% raffinose) up to an A_{600} of 0.9 (without dilution) and then switched to YPG (yeast extract,

TABLE 1
List of strains used in this study

Strain name	Genotype	Source
BY4741	*MATa his3Δ1 TRP1 leu2Δ0 ura3Δ0 LYS2 met15Δ0*	Open Biosystems
YMR201C	*MATa his3Δ1 TRP1 leu2Δ0 ura3Δ0 LYS2 met15Δ0 rad14Δ*	Open Biosystems
FM391	*MATa his1Δ leu2Δ0 met15Δ0 ura3Δ0*	Research Genetics
PCY2	*MATa his1Δ leu2Δ0 met15Δ0 ura3Δ0 Rad3-myc(KanMX)*	Malik et al. (59)
PCY35	*MATa his1Δ leu2Δ0 met15Δ0 ura3Δ0 Rad3-myc(KanMX) rad14Δ::URA3(pRS406)*	This study
W303a	*MATa his3-11, 15 trp1-1 leu2-3,112 ura3-1 ade2-1 can1-100*	The Green lab, UMass.
ZDY2	*MATa his3-11, 15 trp1-1 leu2-3,112 ura3-1 ade2-1 can1-100 Rad26-myc(KanMX)*	Malik *et al.* (59)
PCY25	*MATa his3-11, 15 trp1-1 leu2-3,112 ura3-1 ade2-1 can1-100 Rad26-myc(KanMX) rad14Δ::HIS3(pRS403)*	This study
ZDY3	*MATa his3-11, 15 trp1-1 leu2-3,112 ura3-1 ade2-1 can1-100 Rad14-myc(KanMX)*	This study
PCY31	*MATa his3-11, 15 trp1-1 leu2-3,112 ura3-1 ade2-1 can1-100 Rad26-myc(KanMX) GAL1-ORFΔ::URA3(pRS406)*	This study
PCY32	*MATa his3-11, 15 trp1-1 leu2-3,112 ura3-1 ade2-1 can1-100 Rad26-myc(KanMX) rad14Δ::HIS3(pRS403) GAL1-ORFΔ::URA3(pRS406)*	This study

peptone plus 2% galactose) for 90 min before formaldehyde-based *in vivo* cross-linking. For continuous induction of *GAL* genes, yeast cells were inoculated in YPG and grown at 30 °C up to an A_{600} of 1.0 before cross-linking for the ChIP assay or harvesting for mRNA analysis. For studies at *ADH1, PGK1, PYK1,* and *RPS5,* yeast cells were grown in YPD (yeast extract, peptone plus 2% dextrose) up to an A_{600} of 1.0. The *CUP1* gene was induced by 1 mM $CuSO_4$ for 15 min in synthetic complete medium (yeast nitrogen base and complete amino acid mixture plus 2% dextrose) at 30 °C. For studies at the *CTT1* and *STL1* genes, yeast cells were initially grown in synthetic complete medium up to an A_{600} of 0.9 and then were induced by 0.45 M NaCl for 7 min before cross-linking or harvesting for mRNA analysis.

ChIP Assay—The ChIP assay was performed as described previously (55–58). Briefly, yeast cells were treated with 1% formaldehyde, collected, and resuspended in lysis buffer. Subsequently, yeast cells were lysed by vortexing with the volume equivalent of glass beads at a maximum speed in a Tomy (MT-360) Vortex mixer at 4 °C for 30 min. The whole cell extract was collected by punching a hole at the bottom of the Eppendorf tube followed by centrifugation and sonicated by a Misonix sonicator for 10 s. The sonication was repeated 4 times with at least a 2 min pause in ice. After sonication, cell lysate (400 μl of lysate from 50 ml of yeast culture) was precleared by centrifugation, and then 100 μl lysate was used for each immunoprecipitation. Immunoprecipitated protein-DNA complexes were treated with proteinase K, the cross-links were reversed, and DNA was purified. 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 analyzed by PCR. PCR reactions contained [α-^{32}P]dATP (2.5 μCi for 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 dissolved in 100 μl of TE 8.0. To compare PCR signal arising from the immunoprecipitated DNA with the input DNA, 1 μl of input DNA was used in the PCR analysis. The PCR analysis was carried out within linear range using 23 PCR cycles as done previously (55, 56). The associations of Rad3p and Rad14p with gene were analyzed by modified ChIP assay as described in our recent publication (59). Briefly, 800 μl of lysate was prepared from 100 ml of yeast culture. 400 μl of lysate was used for each immunoprecipitation (using 10 μl of anti-HA (2 μg) or anti-myc (2 μg) antibody and 100 μl of protein A/G plus-agarose suspension (25 μl agarose beads) from Santa Cruz Biotechnology, Inc.), and immunoprecipitated DNA sample was dissolved in 10 μl of TE 8.0 for PCR analysis. In parallel, the PCR for input DNA was performed using 1 μl of DNA that was prepared by dissolving purified DNA from 5 μl of lysate in 100 μl of TE 8.0.

The primer pairs used for PCR analysis were as follows: *GAL1*(UAS), 5′-CGCTTAACTGCTCATTGCTATATTG-3′ and 5′-TTGTTCGGAGCAGTGCGGCGC-3′; *GAL1* (Promoter), 5′-ATAGGATGATAATGCGATTAGTTTTTTAGCCTT-3′ and 5′-GAAAATGTTGAAAGTATTAGTTAAAGTGGTTATGCA-3′; *GAL1* (ORF), 5′-CAGTGGATTGTCTTCTTCGGCCGC-3′ and 5′-GGCAGCCTGATCCATACCGCCATT-3′; *GAL7* (Promoter), 5′-CTATGTTCAGTTAGTTTGGCTAGC-3′ and 5′-TTGATGCTCTGCATAATAATGCCC-3′; *GAL7* (ORF), 5′-AAAGTGCAATCTGTGAGAGGCAATT-3′ and 5′-TTTTCTCTTGCTTCTCTGGAGAGAT-3′; *GAL10* (Promoter), 5′-GCTAAGATAATGGGGCTCTTTACAT-3′ and 5′-TTTCACTTTGTAACTGAGCTGTCAT-3′; *GAL10* (ORF), 5′-TTAATGCGAATCATAGTAGTATCGG-3′ and 5′-TTACCAATAGATCACCTGGAAATTC-3′; *ADH1* (Promoter), 5′-GGTATACGGCCTTCCTTCCAGTTAC-3′ and 5′-GAACGAGAACAATGACGAGGAAACAAAAG-3′; *ADH1* (ORF), 5′-CTGGTTACACCCACGACGGTTCTT-3′ and 5′-CAGACTTCAAAGCCTTGTAGACG3′; *RPS5* (Promoter), 5′-GGCCAACTTCTACGCTCACGTTAG-3′ and 5′-CGGTGTCAGACATCTTTGGAATGGTC-3′; *CTT1* (Promoter), 5′-GGCTGCAGGCTAGCCTAGCCGAT-3′ and 5′-GGAATAGAGGTAAAGCAACGACTTC-3′; *CTT1* (ORF), 5′-TGCTGAACTGTCAGGCTCCCACC-3′ and 5′-GGGGAATTCCTTGTGTGGCCATATT-3′; *STL1* (Promoter), 5′-ACCTTTGATAGGGCTTTCATTGGGGC-3′ and 5′-TCTAAGGCCAAGCAGCGTTGAAG-3′; *STL1* (ORF), 5′-ACACTAGACGCGGATCCAAAT-3′ and 5′-AGCGTTACAACCAGTAAATTGCTGG-3′; *CUP1* (Promoter), 5′-TCTTCTAGAAGCAAAAAGAGCGATG-3′ and 5′-CGCTGAACATTTTATGTGATGATTG3′; *CUP1* (ORF), 5′-ACTTCCAAAATGAAGGTCATGAGTG-3′ and 5′-AGCAGCATGACTTCTTGGTTTCTTC-3′; *PGK1* (Promoter), 5′-GAATCGTGTGACAACAACAGCCTG-3′ and 5′-CTTGCATTGACCAATTTATGC-3′; *PGK1* (ORF), 5′-GACGAAGTTGTCAAGAGCTCTGC-3′ and 5′-GAAAGCAACACCTGGCAATTCCT-3′; *PYK1* (Promoter), 5′-CTCGCCATCAAAACGATATTCG-3′ and 5′-TAACTTTGAAAGGGGACCATG-3′; *PYK1* (ORF), 5′-AAGTTTCCGATGTCGGTAACGCTAT-3′ and 5′-TTGGCAAGTA-

AGCGATAGCTTGTTC-3′. UAS is the upstream activating sequence, and "promoter" is the core promoter.

Autoradiograms were scanned and quantitated by the National Institutes of Health Image 1.62 program. The ChIP signal is the ratio of immunoprecipitate over the input in the autoradiogram. The experiments were carried out multiple times. These experiments are biologically independent. The average ChIP signal of the biologically independent experiments is reported with standard deviation.

Both the wild type and Δ*rad14* mutant strains grew similarly in YPR up to an A_{600} of 0.9 before transfer to YPG for 90 min of transcriptional induction of *GAL* genes before cross-linking. Likewise, both the wild type and Δ*rad14* mutant strains grew similarly in YPD before cross-linking. Whole cell extract preparation and sonication for both the wild type and Δ*rad14* strains were carried out in parallel. Furthermore, the wild type and Δ*rad14* mutant strains had equal amounts of DNA after whole cell extract preparation and sonication as evident by equal input signals of the wild type and mutant strains.

Total RNA Preparation—The total RNA was prepared from yeast cell culture as described by Peterson *et al.* (60). Briefly, 10 ml of yeast culture was harvested and then suspended in 100 μl of RNA preparation buffer (500 mM NaCl, 200 mM Tris-HCl, 100 mM Na_2EDTA, and 1% SDS) along with 100 μl of phenol/chloroform/isoamyl alcohol and 100 μl of a volume equivalent of glass beads (acid-washed; Sigma). Subsequently, yeast cell suspension was vortexed with a maximum speed (10 in VWR Mini-vortexer; catalog no. 58816-121) 5 times (30 s each). Cells suspension was put in ice for 30 s between pulses. After vortexing, 150 μl of RNA preparation buffer and 150 μl of phenol/chloroform/isoamyl alcohol were added to the yeast cell suspension followed by vortexing for 15 s with a maximum speed on VWR Mini-vortexer. The aqueous phase was collected after 5 min of centrifugation at a maximum speed in microcentrifuge machine. The total RNA was isolated from the aqueous phase by ethanol precipitation.

Reverse Transcriptase (RT)-PCR Analysis—RT-PCR analysis was performed according to the standard protocols (61). Briefly, total RNA was prepared from 10 ml of yeast culture. Ten micrograms of total RNA was used in the reverse transcription assay for both the wild type and mutant strains. RNA was treated with RNase-free DNase (M610A, Promega) and then reverse-transcribed into cDNA using oligo(dT) as described in the protocol supplied by Promega (A3800, Promega). PCR was performed using synthesized first strand as template and the primer pairs targeted to the *GAL1*, *GAL7*, *GAL10*, *ADH1*, *PGK1*, *PYK1*, *RPS5*, *CTT1*, *STL1*, and *CUP1* ORFs. RT-PCR products were separated by 2.2% agarose gel electrophoresis and visualized by ethidium bromide staining. The primer pairs used in the PCR analysis were as follows: *GAL1*, 5′-CAGAGGGCTAAGCATGTGTATTCT-3′ and 5′-GTCAATCTCTGGACAAGAACATTC-3′; *GAL7*, 5′-TGAGACCTTGGTCATTTCAAAGAAG-3′ and 5′-ATGGATACCCATTGAGTATGGGAAA-3′; *GAL10*, 5′-TTAATGCGAATCATAGTAGTATCGG-3′ and 5′-TTACCAATAGATCACCTGGAAATTC-3′; *ADH1*, 5′-CGGTAACAGAGCTGACACCAGAGA-3′ and 5′-ACGTATCTACCAACGATTTGACCC-3′; *RPS5*, 5′-AGGCTCAATGTCCAATCATTGAAAG-3′ and 5′-CAACAACTTGGATTGGG-

TTTTGGTC-3′; *CUP1*, 5′-ACTTCCAAAATGAAGGTCAT-GAGTG-3′ and 5′-AGCAGCATGACTTCTTGGTTTCTTC-3′; *CTT1*, 5′-TGCTGAACTGTCAGGCTCCCACC-3′ and 5′-GGG-GAATTCCTTGTGTGGCCATATT-3′; *STL1*, 5′-ACACTA-GACGCGGATCCAAAT-3′ and 5′-AGCGTTACAACCAGTA-AATTGCTGG-3′; *PGK1*, 5′-GACGAAGTTGTCAAGAGCTC-TGC-3′ and 5′-GAAAGCAACACCTGGCAATTCCT-3′; *PYK1*, 5′-AAGTTTCCGATGTCGGTAACGCTAT-3′ and 5′-TTGG-CAAGTAAGCGATAGCTTGTTC-3′.

Whole Cell Extract Preparation and Western Blot Analysis—For analysis of global levels of TBP, Rpb1p, Rad3p, and actin in the *RAD14* deletion mutant and its isogenic wild type equivalent, the yeast cells were grown in YPR up to an A_{600} of 0.9 and then induced for 90 min in YPG. The harvested cells were lysed and sonicated to prepare the whole cell extract with solubilized chromatin following the protocol as described previously for the ChIP assay (55–58). The whole cell extract was run on SDS-polyacrylamide gel and then analyzed by Western blot. The anti-TBP (obtained from Michael R. Green, University of Massachusetts Medical School), anti-Rpb1p (8WG16; Covance), anti-myc (9E10; Santa Cruz Biotechnology), and anti-actin (A2066; Sigma) antibodies against TBP, Rpb1p, myc-tagged Rpb3p, and actin, respectively, were used for Western blot analysis.

RESULTS

Rad14p Associates with the Promoter and Coding Sequence of the Transcriptionally Active, but Not Inactive, GAL1 Gene—To determine whether Rad14p associates with active gene in the absence of lesion, we employed the ChIP assay to analyze the level of Rad14p at the promoter and coding sequence of a galactose-regulated gene, *GAL1*, under inducible (galactose-containing growth medium) and non-inducible (raffinose-containing growth medium) conditions. For the ChIP experiments, we tagged Rad14p by Myc epitope at the C terminus in its chromosomal locus in the W303a strain. The generated yeast strain expressing Myc epitope-tagged Rad14p (ZDY3) was grown in either raffinose- or galactose-containing growth medium up to an A_{600} of 1.0 before formaldehyde-based *in vivo* cross-linking. Subsequently, immunoprecipitation was performed using an anti-Myc antibody against Myc epitope-tagged Rad14p. Immunoprecipitated DNA was analyzed by PCR using the primer pairs targeted to the promoter and coding sequence (ORF) of *GAL1* (Fig. 1*A*). A primer pair targeted to the transcriptionally inactive region of chromosome V was also used in the PCR analysis as a nonspecific DNA control (62). Our ChIP analysis revealed that Rad14p was associated with the promoter and coding sequence of *GAL1* under transcriptionally inducible conditions in galactose-containing growth medium (Fig. 1, *B* and *C*). However, Rad14p did not associate with the *GAL1* promoter and coding sequence (Fig. 1, *B* and *C*) when transcription of *GAL1* was off in raffinose-containing growth medium (58, 63). Furthermore, Rad14p was not associated with the transcriptionally inactive region of chromosome V in both raffinose and galactose-containing growth media (Fig. 1, *B* and *C*). Together, these results support that Rad14p associates with the promoter and coding sequence of the transcriptionally active, but not inactive, *GAL1* gene in the absence of DNA lesion.

How does Rad14p associate with the transcriptionally active, but not inactive, *GAL1* gene? It is quite likely that Rad14p interacts with RNA polymerase II, and such interaction plays an important role to bring down Rad14p to the active gene. In fact, a previous biochemical study demonstrated the interaction of Rad14p with RNA polymerase II (51). Furthermore, RNA polymerase II (Rpb1p) is associated with the promoter and coding sequence of *GAL1* under inducible conditions (Fig. 1, *D* and *E*), consistent with previous studies (58, 63). However, RNA polymerase II does not associate with the *GAL1* promoter and coding sequence under non-inducible conditions (Fig. 1*E*; Refs. 58 and 63). Thus, the interaction of RNA polymerase II with Rad14p is attributed for the association of Rad14p with the active gene. However, we observed a higher level of Rad14p association with the *GAL1* coding sequence than the promoter. This could be due to a high level of RNA polymerase II at the *GAL1* coding sequence in comparison to the promoter. Indeed, a high level of RNA polymerase II was observed at the *GAL1* coding sequence when compared with the promoter (Fig. 1, *D* and *E*). Furthermore, the association of Rad14p with the active *GAL1* gene can also be attributed to be mediated via RPA, as a previous biochemical study (51) demonstrated the interaction of Rad14p with RPA and a recent study (52) showed the association of RPA with active gene via its interaction with ssDNA at the transcription bubble.

Rad14p Promotes Transcription of GAL1—Because Rad14p is associated with the transcriptionally active *GAL1* gene, it may play an important role to promote *GAL1* transcription. To test this possibility, we analyzed *GAL1* transcription in the Δ*rad14* strain (PCY25) using the RT-PCR assay. In this direction, we isolated total RNA from the wild type and Δ*rad14* strains after a 90-min transcriptional induction in galactose-containing growth medium. Subsequently, we analyzed *GAL1* mRNA levels in these strains. Interestingly, we find that the absence of Rad14p significantly lowered the level of *GAL1* mRNA (Fig. 1*F*). Thus, our results support the role of Rad14p in stimulation of *GAL1* transcription.

Rad14p Enhances the Association of RNA Polymerase II with the Promoter and Coding Sequence of GAL1—Because transcription of *GAL1* was significantly reduced in the absence of Rad14p, it is likely that the association of RNA polymerase II with active *GAL1* would be decreased in the Δ*rad14* strain in comparison to the wild type equivalent. To test this, we analyzed the association of RNA polymerase II with the *GAL1* promoter and coding sequence in the wild type and Δ*rad14* (PCY25) strains using the ChIP assay. We find that the association of RNA polymerase II (Rpb1p) with the *GAL1* promoter and coding sequence was significantly decreased in the Δ*rad14* strain (Fig. 2*A*). Such a reduction in the association of RNA polymerase II with *GAL1* in the Δ*rad14* strain could be due to the decreased stability of Rpb1p. However, our Western blot analysis revealed that the global level of Rpb1p was not altered in the Δ*rad14* strain (Fig. 2*B*). The level of actin was monitored as a loading control (Fig. 2*B*). Thus, our results demonstrate that Rad14p promotes the association of RNA polymerase II with *GAL1* (Fig. 2, *A* and *B*), and hence, the transcription of *GAL1* was significantly decreased in the Δ*rad14* strain as compared with the wild type equivalent (Fig. 1*F*). However, there is a possibility that a hidden mutation, but not null, mutation of
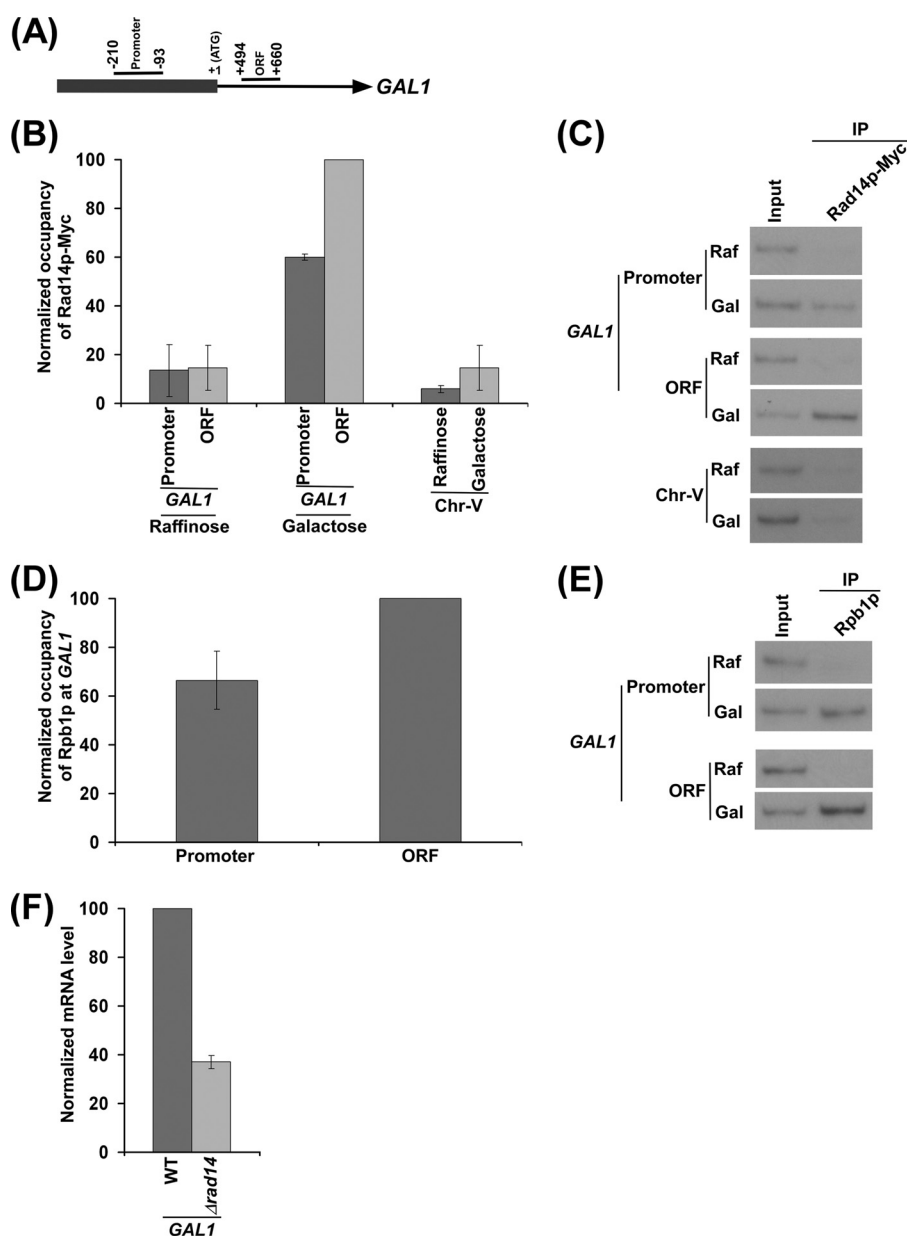
FIGURE 1. **Rad14p is associated with the promoter and coding sequence of the *GAL1* gene in a transcription-dependent manner.** *A*, a schematic diagram shows the PCR primer pairs located at the promoter and coding sequence (or ORF) of the *GAL1* gene in the ChIP assay. The *numbers* are presented with respect to the position of the first nucleotide of the initiation codon ($+1$). *B*, Rad14p is associated with the promoter and coding sequence of the active *GAL1* gene. The yeast strain expressing Myc epitope-tagged Rad14p (ZDY3) was grown in raffinose (YPR)- or galactose (YPG)-containing growth medium up to an A_{600} of 1.0 before cross-linking. Immunoprecipitation was performed using a mouse monoclonal antibody against the c-myc epitope-tag (9E10; Santa Cruz Biotechnology). Primer pairs targeted to the promoter and coding sequence of the *GAL1* gene and an inactive region of the chromosome-V (*Chr-V*) were used for PCR analysis of the immunoprecipitated DNA samples. The maximum ChIP signal was set to 100, and other ChIP signals were normalized with respect to 100. The normalized ChIP signals (represented as normalized occupancy) were plotted in the form of a histogram. *C*, the autoradiograms for the data presented in the *panel B* are shown. *IP*, immunoprecipitate. *D*, RNA polymerase II is associated with the promoter and coding sequence of the active *GAL1* gene. The yeast strain was grown in YPR up to an A_{600} of 0.9 and then switched to YPG for 90 min before cross-linking. Immunoprecipitation was performed using a mouse monoclonal antibody 8WG16 (Covance) against the C-terminal domain of the largest subunit (Rpb1p) of RNA polymerase II. The maximum ChIP signal was set to 100, and another ChIP signal was normalized with respect to 100. The normalized ChIP signal was plotted in the form of a histogram. *E*, the autoradiograms for the data presented in the *panel D*. *F*, RT-PCR analysis. Both the wild type and Δ*rad14* strains were grown as in *panel D*. The Δ*rad14* strain (PCY25) was generated in the W303a wild type background. *GAL1* mRNA level in the wild type strain was set to 100, and the mRNA level in the Δ*rad14* strain was normalized with respect to 100. The normalized mRNA level was plotted in the form of a histogram.

RAD14 in our strain reduced *GAL1* transcription. To address this issue we have performed similar experiments using commercial Δ*rad14* strain (YMR201C; Open Biosystems) and its isogenic wild type equivalent (BY4741; Open Biosystems). We find that the association of RNA polymerase II with *GAL1* was significantly impaired in this commercial Δ*rad14* strain as compared with the wild type equivalent (Fig. 2, *C* and *D*). Consistently, transcription of *GAL1* was significantly decreased in this Δ*rad14* strain (Fig. 2*E*). Furthermore, we have generated a *RAD14* deletion mutant strain (PCY35) in the FM391 wild type background and performed similar experiments. Again, using this new Δ*rad14* strain in the FM391 wild type background, we
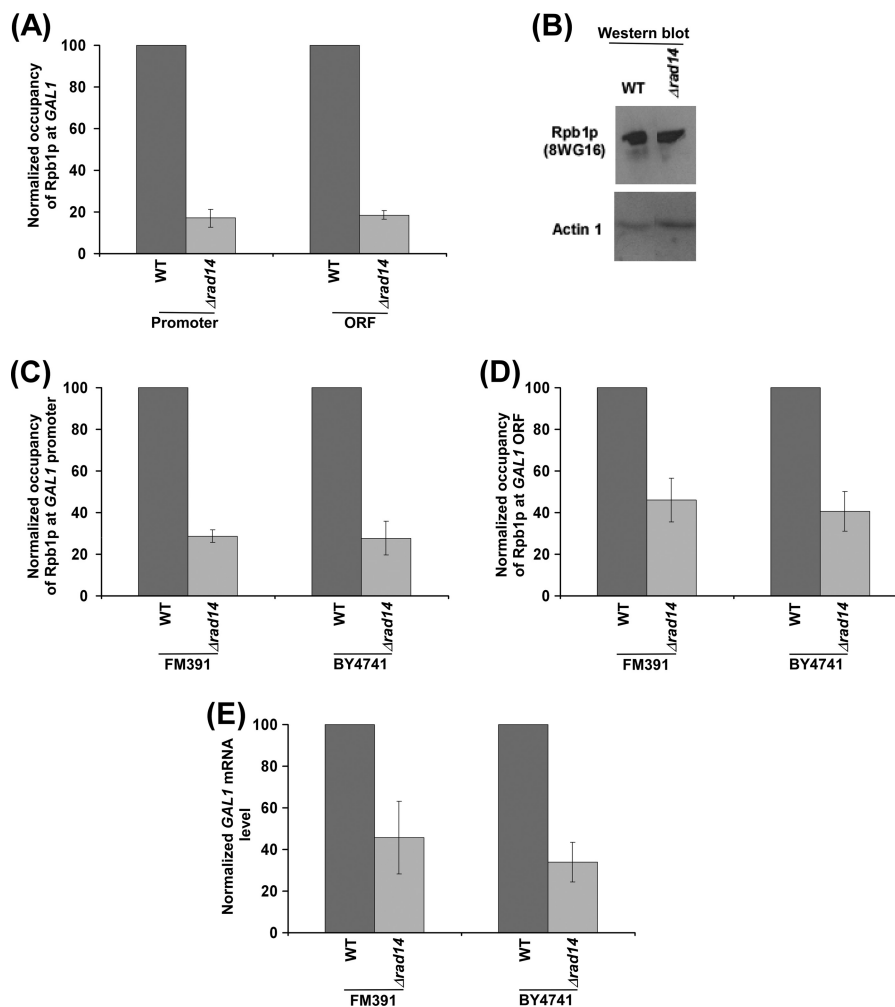
FIGURE 2. **Rad14p promotes the association of RNA polymerase II with *GAL1*.** *A*, analysis of Rpb1p association with the *GAL1* promoter and coding sequence in the Δ*rad14* strain (PCY25) and its isogenic wild type equivalent is shown. The wild type and Δ*rad14* strains were grown, cross-linked, and immunoprecipitated as in Fig. 1*D*. The maximum ChIP signal for the wild type strain was set to 100, and the ChIP signal of the Δ*rad14* strain was normalized with respect to 100. The normalized ChIP signal was plotted in the form of a histogram. *B*, Western blot analysis. *C* and *D*, analysis of Rpb1p association with *GAL1* in the Δ*rad14* strains (PCY35 and YMR201C) derived from the FM391 and BY4741 wild type backgrounds is shown. Yeast strains were grown, cross-linked, and immunoprecipitated as in *panel A*. *E*, analysis of *GAL1* transcription in the Δ*rad14* strains (PCY35 and YMR201C) is shown. Yeast strains were grown as in *panel A*.

found that Rad14p promotes the association of RNA polymerase II with *GAL1* (Fig. 2, *C* and *D*) and, hence, transcription (Fig. 2*E*). Collectively, we find a significant impairment of RNA polymerase II association with *GAL1* and hence transcription in the Δ*rad14* strains generated from three different parental backgrounds (W303a, BY4741, and FM391), thus supporting the role of Rad14p in promoting transcription.

Rad14p Promotes the Recruitment of TBP, TFIIH, and RNA Polymerase II to the GAL1 Promoter and Hence Transcriptional Initiation—So far we find that the absence of Rad14p decreases the association of RNA polymerase II with the *GAL1* promoter and coding sequence and hence transcription. Next, we asked whether the decrease in the association of RNA polymerase II with the *GAL1* coding sequence in the Δ*rad14* strain is a result of the defect of RNA polymerase II recruitment to the promoter. To address this question, we analyzed the role of Rad14p in recruitment of initiating RNA polymerase II to the *GAL1* promoter. In this direction, we deleted the coding sequence of *GAL1* from its chromosomal locus and then analyzed the

recruitment of RNA polymerase II to the *GAL1* promoter in the presence and absence of Rad14p. We found that the recruitment of RNA polymerase II (Rpb1p) to the *GAL1* promoter was significantly decreased in the absence of Rad14p, when the coding sequence was deleted (Fig. 3, *A* and *B*). This observation supports that Rad14p promotes the recruitment of RNA polymerase II to the *GAL1* promoter (and hence transcriptional initiation). Otherwise, the recruitment of RNA polymerase II to the *GAL1* promoter would not have been altered in the Δ*rad14* strain, when the coding sequence was deleted, consistent with our recent studies (64) demonstrating the role of Rad26p in promoting the association of elongating but not initiating RNA polymerase II with *GAL1*.

How does Rad14p promote the association of RNA polymerase II with the *GAL1* promoter? A previous biochemical study (51) demonstrated the interaction of Rad14p with RNA polymerase II and TFIIH. TFIIH is known to be recruited to the promoter after RNA polymerase II during the formation of the pre-initiation complex (PIC) (65–67). Because Rad14p inter-
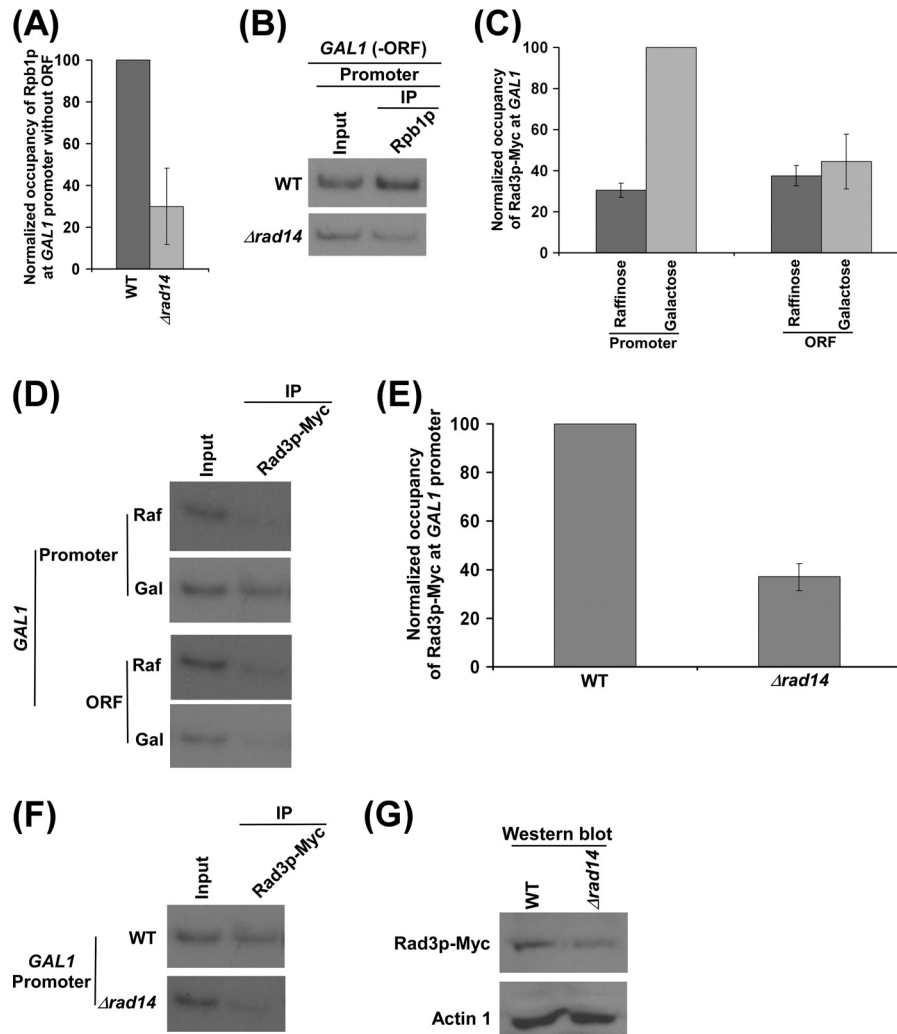
FIGURE 3. **Rad14p promotes the formation of the PIC assembly.** *A*, regulation of Rpb1p occupancy at the *GAL1* promoter by Rad14p in the absence of the coding sequence. Both the wild type and Δ*rad14* strains without *GAL1* ORF (PCY31 and PCY32) were grown, cross-linked, and immunoprecipitated as in Fig. 1*D*. *B*, the autoradiograms for the data presented in *panel A*. *C*, Rad3p is predominantly associated with the *GAL1* promoter. The yeast strain expressing Myc epitope-tagged Rad3p (PCY2) was grown, cross-linked, and immunoprecipitated as in Fig. 1*B*. The maximum ChIP signal was set to 100, and other ChIP signals were normalized with respect to 100. The normalized ChIP signals were plotted in the form of a histogram. *D*, the autoradiograms of the data presented in the *panel C*. *E*, Rad14p promotes the recruitment of Rad3p. Both the wild type and Δ*rad14* strains expressing myc-tagged Rad3p (PCY2 and PCY35) were grown and cross-linked as in Fig. 1*D*. *F*, the autoradiograms for the data presented in the *panel E*. *G*, Western blot analysis is shown.

acts with both TFIIH and RNA polymerase II (51), it is likely to enhance their recruitment and/or stabilize the PIC. Furthermore, the decreased association of TFIIH with the promoter would weaken the transition of the initiating RNA polymerase II to the elongating form, leading to the low level of RNA polymerase II at the coding sequence, as previous studies (67–71) implicated TFIIH-mediated phosphorylation of RNA polymerase II for entering into the elongation phase. Thus, Rad14p is likely to promote/stabilize the PIC formation for transcriptional initiation. Therefore, we observed a significant decrease in the recruitment of RNA polymerase II to the *GAL1* promoter in the Δ*rad14* strain in the absence of the coding sequence (Fig. 3, *A* and *B*). Because TFIIH joins the PIC assembly after RNA polymerase II, we would also expect to observe a decrease in the recruitment of TFIIH in the absence of Rad14p. To test this, we tagged the Rad3p component of TFIIH by Myc epitope in its chromosomal locus in the wild type and Δ*rad14* strains and subsequently performed the ChIP analysis at the

GAL1 promoter. Our ChIP analysis revealed that the absence of Rad14p significantly lowered the recruitment of Rad3p to the *GAL1* promoter (Fig. 3, *C–F*). However, such a decreased level of Rad3p to the *GAL1* promoter was not due to an altered stability of Rad3p in the Δ*rad14* strain as evident by the Western blot analysis (Fig. 3*G*). Thus, our data support that the recruitment of TFIIH to the *GAL1* promoter is significantly impaired in the absence of Rad14p.

TBP nucleates the PIC formation, and RNA polymerase II and TFIIH join toward the end of the PIC assembly (65–67). Thus, the defect in the recruitment of RNA polymerase II and TFIIH during PIC formation may also have an effect on the recruitment of TBP due to the loss of reciprocal cooperativity. Indeed, we observe a decrease in the recruitment of TBP to the *GAL1* promoter in the absence of Rad14p (Fig. 4, *A* and *B*). However, such a decreased level of TBP recruitment in the Δ*rad14* strain is not mediated via an altered stability of TBP as evident by the Western blot analysis (Fig. 4*C*). Taken together,
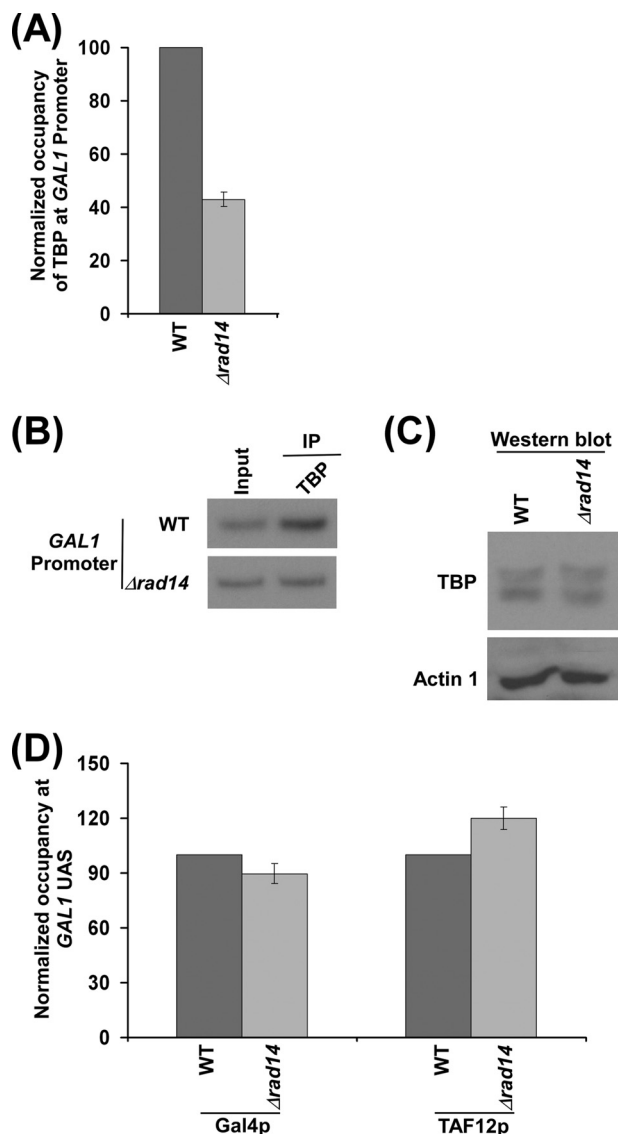
FIGURE 4. **Rad14p regulates the recruitment of TBP, but not Gal4p and SAGA, to GAL1.** *A*, Rad14p facilitates the recruitment of TBP. Both the wild type and Δ*rad14* strains were grown and cross-linked as in Fig. 3*E*. Immunoprecipitation was performed using an anti-TBP antibody against TBP. *B*, the autoradiograms for the data presented in the *panel A*. *C*, Western blot analysis. *D*, Rad14p does not regulate the recruitment of Gal4p and SAGA (TAF12p) to the *GAL1* UAS. Both the wild type and Δ*rad14* strains were grown and cross-linked as in Fig. 3*E*. Immunoprecipitations were performed using anti-Gal4p (Santa Cruz Biotechnology) and anti-TAF12p (Michael R. Green, University of Massachusetts Medical School) antibodies against Gal4p and TAF12p, respectively. Immunoprecipitated DNAs were analyzed by PCR using the primer pair targeted to the *GAL1* UAS.

our results reveal that Rad14p promotes the formation of the PIC, and hence a significantly decreased level of RNA polymerase II was observed at the *GAL1* coding sequence in the absence of Rad14p (Fig. 2*A*). However, the decreased association of RNA polymerase II with the *GAL1* coding sequence may also be due to the defect in transcriptional elongation in addition to an altered transcriptional initiation in the Δ*rad14* strain. This possibility remains to be further elucidated.

Next, we asked whether an impairment of the PIC formation at the *GAL1* promoter in the absence of Rad14p is mediated via activator Gal4p or co-activator SAGA. To address this question, we analyzed the recruitment of Gal4p and SAGA

(TAF12p) to the *GAL1* UAS in the Δ*rad14* strain and its isogenic wild type equivalent. Our ChIP analysis revealed that the absence of Rad14p did not alter the recruitment of Gal4p and SAGA to the *GAL1* UAS (Fig. 4*D*). Thus, Rad14p appears to promote the PIC formation directly (and hence transcription initiation) without altering the recruitment of activator or co-activator.

Rad14p Associates with the Active GAL7 and GAL10 Genes to Promote Transcription—We find above that Rad14p associates with the promoter and coding sequence of *GAL1* and stimulates its transcription. To determine whether Rad14p also associates with other active genes and subsequently promotes their transcription, we analyzed the association of Rad14p with the promoters and coding sequences of other *GAL* genes such as *GAL7* and *GAL10* (Fig. 5*A*) under transcriptionally inducible and non-inducible conditions. Similar to the results at *GAL1*, we found that Rad14p associated with the promoters and coding sequences of *GAL7* and *GAL10* under transcriptionally inducible conditions (Fig. 5, *B* and *C*). However, Rad14p did not associate with these genes under non-inducible conditions (Fig. 5, *B* and *C*). Thus, Rad14p associates with active *GAL7* and *GAL10* genes in the absence of lesion. Next, we analyzed the role of Rad14p in regulation of transcription of *GAL7* and *GAL10* genes. In this direction, we isolated total RNA from the wild type and Δ*rad14* strains and then analyzed the levels of *GAL7* and *GAL10* mRNAs. We found that the levels of *GAL7* and *GAL10* mRNAs were significantly decreased in the absence of Rad14p when compared with the wild type equivalent (Fig. 5*D*). Thus, similar to the results at *GAL1*, we find that Rad14p promotes the transcription of *GAL7* and *GAL10*. Consistently, the association of RNA polymerase II with the active *GAL7* and *GAL10* genes was significantly decreased in the absence of Rad14p (Fig. 5, *E* and *F*). Taken together, our results demonstrate that Rad14p associates with active *GAL7* and *GAL10* genes and promotes their transcription, similar to the results obtained at *GAL1*.

Effect of Rad14p on the Steady-state Level of Transcription—We next asked whether the occupancy of RNA polymerase II at the *GAL* gene in the Δ*rad14* strain could reach to the wild type level if given enough time for attaining the steady state. To address this question, we analyzed the association of RNA polymerase II with *GAL1* at different times (1, 3, 7, and 12 h) after transcriptional induction. Fig. 6, *A* and *B*, show that the association of RNA polymerase II with *GAL1* in the Δ*rad14* strain reached to the wild type level after long transcriptional induction (*e.g.* 7 and 12 h). Consistently, transcription of *GAL1* in the Δ*rad14* strain became the same as the wild type level after longer transcriptional induction (Fig. 6*C*). Likewise, we found that the effect of Rad14p on the association of RNA polymerase II with the *GAL7* and *GAL10* promoters and coding sequences (and hence transcription) was minimal (or absent) after a long induction in galactose-containing growth medium (Fig. 6, *D–F*). Therefore, it is expected that there would not be a dramatic effect of Rad14p on the association of RNA polymerase II (and hence transcription) with constitutively active genes. Indeed, we found that Rad14p did not alter the association of RNA polymerase II with a constitutively active gene, *ADH1*, in dextrose-containing growth medium (Fig. 7, *A* and *B*). There-
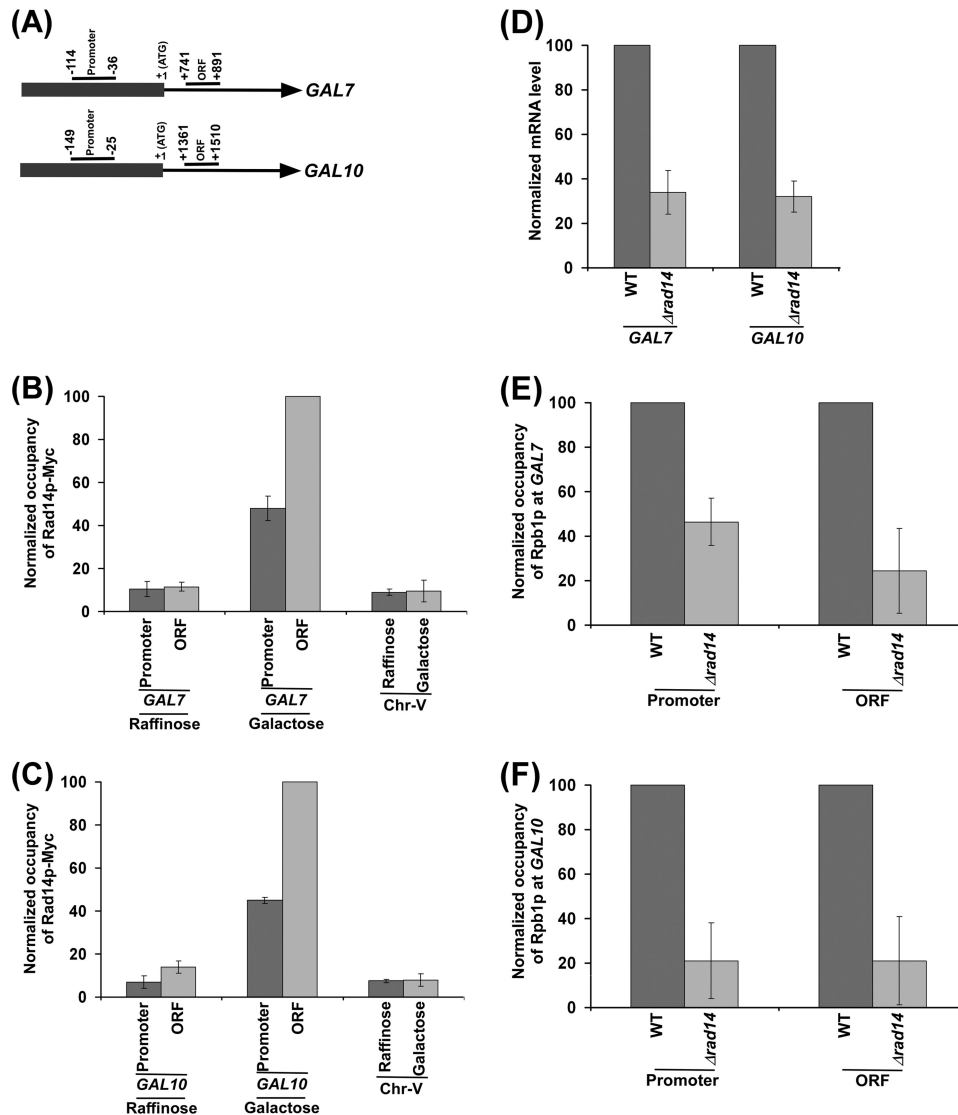
FIGURE 5. **Rad14p associates with the promoters and coding sequences of the *GAL7* and *GAL10* genes in a transcription-dependent manner.** *A*, the schematic diagrams show the PCR primer pairs located at the promoters and ORFs of the *GAL7* and *GAL10* genes in the ChIP assay. The numbers are presented with respect to the position of the first nucleotide of the initiation codon (+1). *B* and *C*, Rad14p associates with the promoters and coding sequences of the active *GAL7* and *GAL10* genes. The yeast strain expressing Myc epitope-tagged Rad14p (ZDY3) was grown, cross-linked, and immunoprecipitated as in Fig. 1*B*. *D*, RT-PCR analysis is shown. Both the wild type and Δ*rad14* strains (ZDY2 and PCY25) were grown for analysis of *GAL7* and *GAL10* transcriptions as in Fig. 1*F*. *E* and *F*, analysis of Rpb1p association with the promoters and coding sequences of the *GAL7* and *GAL10* genes in the Δ*rad14* strain (PCY25) and its isogenic wild type equivalent. The wild type and Δ*rad14* strains were grown, cross-linked, and immunoprecipitated as in Fig. 2*A*.

fore, transcription of *ADH1* was not changed in the absence of Rad14p (Fig. 7*C*). Likewise, we found that the transcription of other constitutively active genes such as *PGK1* and *PYK1* was not changed in the absence of Rad14p (Fig. 7*D*). Therefore, the association of RNA polymerase II with these genes was not changed in the Δ*rad14* strain (Fig. 7, *E* and *F*). Similar results were also obtained at another constitutively active gene, *RPS5* (Fig. 7, *G* and *H*). Thus, Rad14p appears to promote the initial rate of transcription. However, its effect is minimal or absent on the steady-state level or constitutive transcription.

Rad14p Promotes Transcriptional Induction of Non-GAL Genes—So far, we have demonstrated that Rad14p promotes transcriptional induction of *GAL* genes but not the steady-state level or constitutive transcription. To determine whether Rad14p can also promote transcriptional induction of other non-*GAL* gene, we analyzed the association of RNA polymerase

II with an inducible *CUP1* gene in the presence of CuSO4 in the wild type and Δ*rad14* strains. Fig. 8*A* shows that the occupancy of RNA polymerase II at *CUP1* was significantly impaired in the Δ*rad14* strain. Consistently, transcription of *CUP1* was also dramatically reduced after transcriptional induction in the absence of Rad14p (Fig. 8*B*). *ADH1* transcript levels in the wild type and Δ*rad14* strains were monitored under the similar inducible growth conditions as controls. As expected, transcription of the constitutively active *ADH1* gene was not altered in the absence of Rad14p (Fig. 8*B*). Thus, Rad14p promotes transcription of *CUP1* after transcriptional induction. Likewise, Rad14p promotes transcription of *STL1* and *CTT1* after transcriptional induction by NaCl (Fig. 8*C*). Consistently, the association of RNA polymerase II with these genes was also significantly impaired in the absence of Rad14p (Fig. 8*D*). Together, our data support that Rad14p
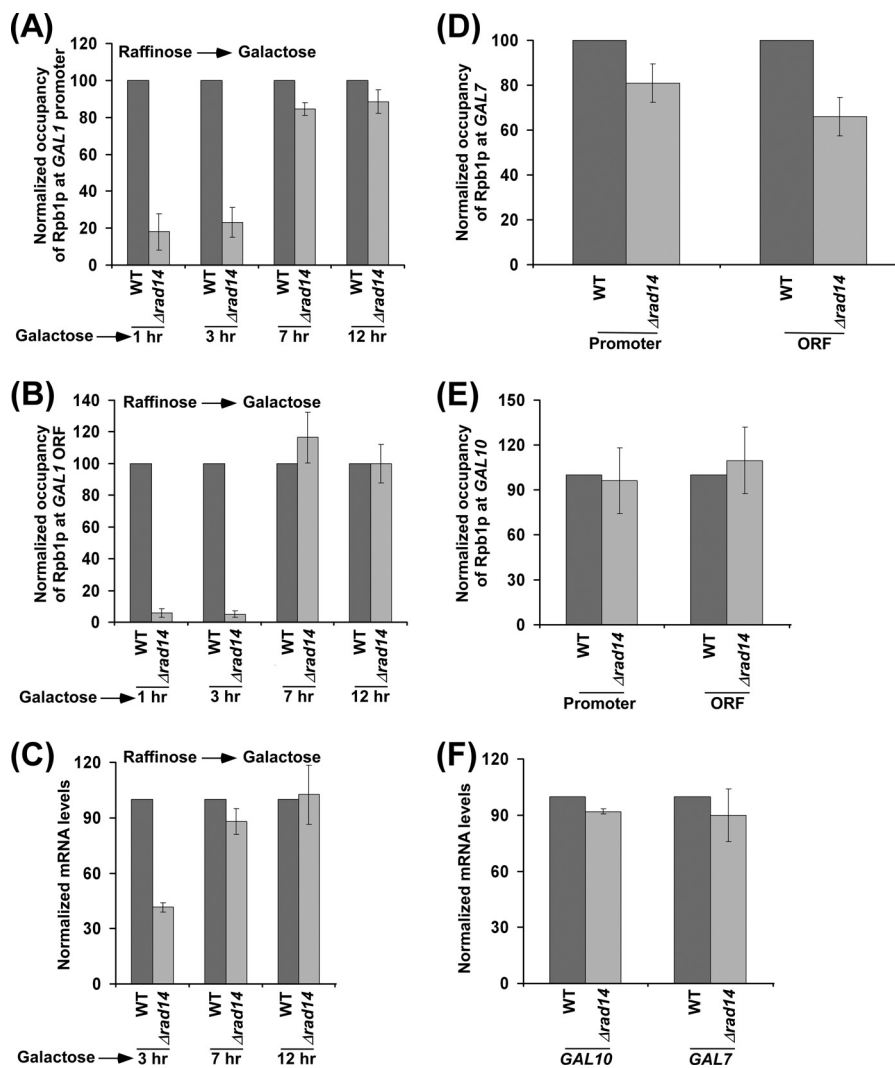
FIGURE 6. **Effect of Rad14p on the association of RNA polymerase II with *GAL* genes under steady-state conditions.** *A* and *B*, kinetic analysis for the association of Rpb1p with the *GAL1* promoter and coding sequence following transcriptional induction. Yeast cells were initially grown in YPR at 30 °C and then switched to YPG for different induction time periods before cross-linking. *C*, RT-PCR analysis is shown. *D* and *E*, analysis of Rpb1p association with the *GAL7* and *GAL10* promoters and coding sequences in the Δ*rad14* strain (PCY25) and its isogenic wild type equivalent after continuous growth in YPG up to an A_{600} of 1.0 before cross-linking. *F*, RT-PCR analysis is shown.

promotes transcriptional induction of *GAL* as well as non-*GAL* genes.

DISCUSSION

Previous studies have implicated Rad14p in recognizing DNA lesions (21, 22), and subsequently Rad14p interacts with DNA repair factors for NER (1, 18, 20–23). Like Rad14p, Rad26p recognizes the DNA lesion at the active gene (59) and promotes repair (72). Intriguingly, Rad26p also associates with active gene in a transcription or elongating RNA polymerase II-dependent manner in the absence of lesion and promotes transcriptional elongation (59, 64). However, it is not clearly known whether, like Rad26p, the DNA lesion recognition factor Rad14p can also associate with active gene in the absence of lesion and promote transcription. Here, we demonstrate that like Rad26p, Rad14p associates with active gene independently of lesion. Such an association of Rad14p with active genes promotes transcription. Thus, this study demonstrates a new role

of Rad14p in promoting transcription in addition to its well known function in NER.

Although Rad14p has been implicated to recognize DNA lesion (21, 22), it has not been investigated whether Rad14p can also interact with active genomic region in the absence of lesion. Here, we show that Rad14p is not recruited to the promoters and coding sequences of several *GAL* genes when these genes are transcriptionally silent or not induced in raffinose-containing growth medium (Figs. 1, *B* and *C*, and 5, *B* and *C*). Intriguingly, we find that Rad14p associates with the promoters and coding sequences of several *GAL* genes when these genes are transcriptionally active in galactose-containing growth medium (Figs. 1, *B* and *C*, and 5, *B* and *C*). These observations support that Rad14p associates with active *GAL* genes even in the absence of lesion.

How does Rad14p associate with the promoter and coding sequence of the active gene? Because Rad14p, like RNA polymerase II, associates with the promoter and coding sequence of
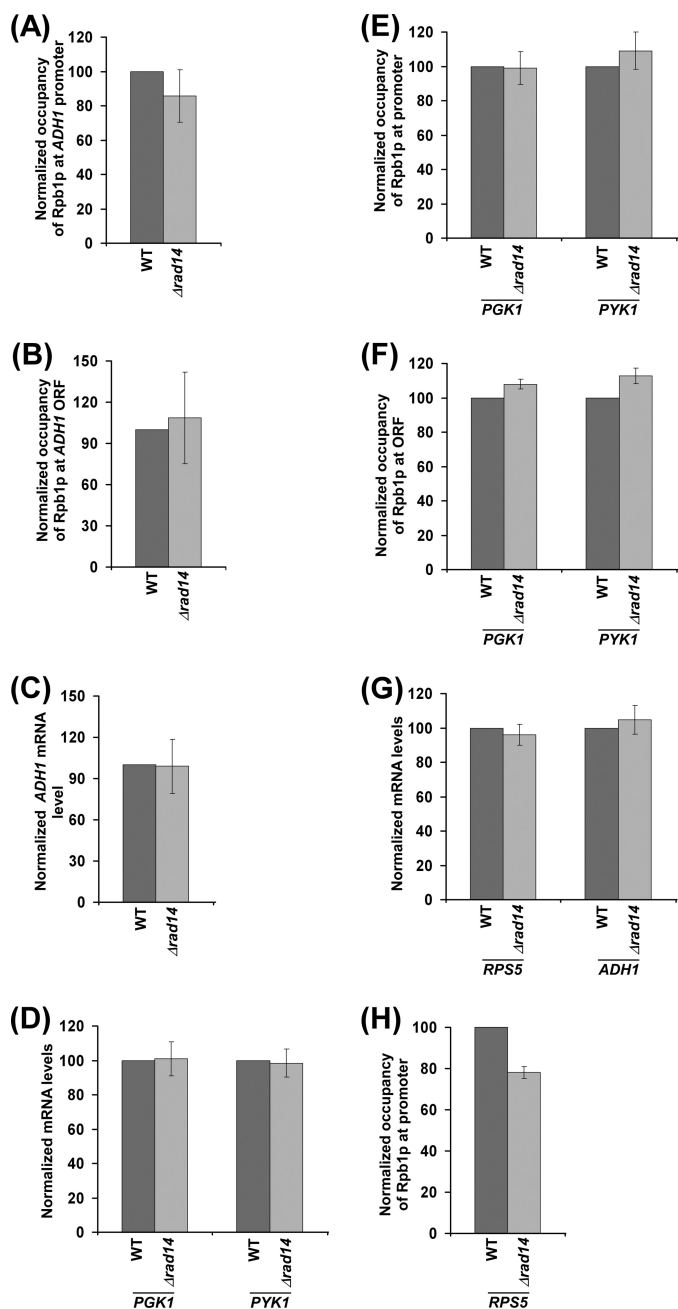
FIGURE 7. **Effect of Rad14p on transcription of constitutively active genes.** *A* and *B*, analysis of Rpb1p association with the *ADH1* promoter and coding sequence in the Δ*rad14* strain (PCY25) and its isogenic wild type equivalent following continuous growth in YPD up to an A_{600} of 1.0 before cross-linking. *C*, RT-PCR analysis of *ADH1* is shown. *D*, RT-PCR analysis of *PGK1* and *PYK1*. *E* and *F*, analysis of Rpb1p association with the promoters and coding sequences of *PGK1* and *PYK1* in the wild type and Δ*rad14* strains. *G*, RT-PCR analysis of *RPS5*. *H*, analysis of Rpb1p association with *RPS5* in the wild type and Δ*rad14* strains.

FIGURE 8. **Rad14p promotes transcriptional induction of *CUP1*, *STL1*, and *CTT1*.** *A*, Rad14p promotes the association of RNA polymerase II with *CUP1*. *B*, RT-PCR analysis of *CUP1*. *C*, RT-PCR analysis of *STL1* and *CTT1* in the wild type and Δ*rad14* strains following transcriptional inductions by NaCl is shown. *D*, analysis of Rpb1p association with *STL1* and *CTT1* in the wild type and Δ*rad14* strains.

the active gene, it is likely that Rad14p interacts with RNA polymerase II and gets recruited to the active gene. In support of this possibility, a previous biochemical study (51) revealed the interaction of Rad14p with the largest subunit (Rpb1p) of RNA polymerase II. Thus, the interaction of Rad14p with RNA polymerase II (51) is attributed for the association of Rad14p with the active gene in the absence of lesion. Likewise, RNA polymerase II has been recently shown to play an important
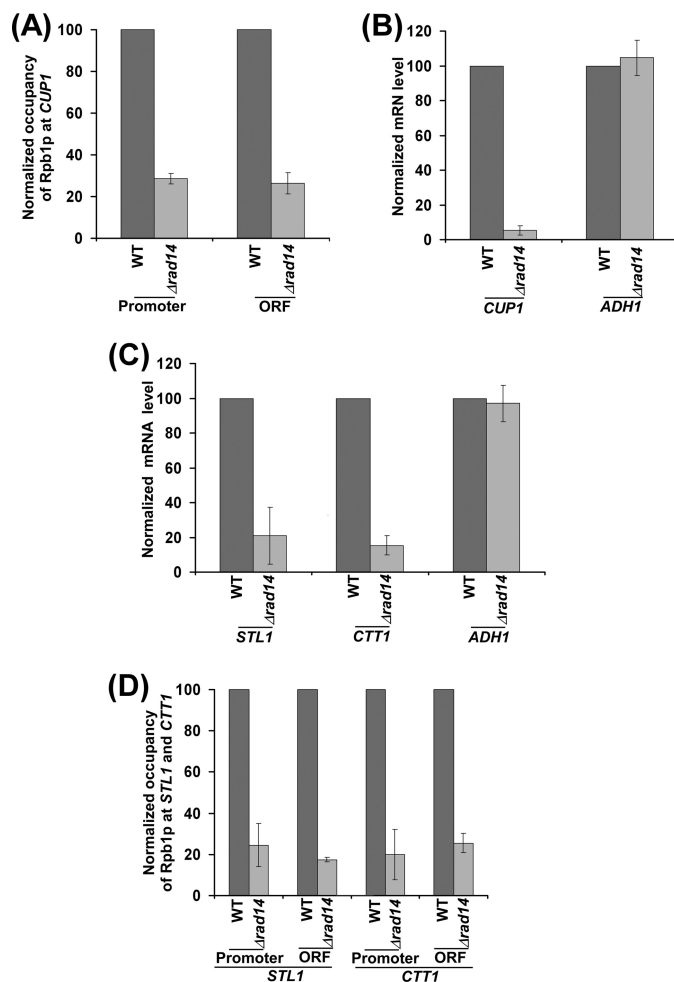
role to bring down Rad26p to the coding sequence of active gene (59, 64). However, unlike Rad14p, Rad26p does not associate with the promoter (59). Thus, Rad26p does not interact with initiating RNA polymerase II but rather with elongating RNA polymerase II (59). Therefore, Rad26p associates with the active gene in an elongating RNA polymerase II-dependent manner (59). On the other hand, Rad14p appears to associate with the active gene in initiating and elongating RNA polymerase II-dependent manner. Furthermore, Rad14p has been previously shown to interact with RPA (51), which associates with active gene (52). Thus, the interaction of RPA with Rad14p is also likely to play an important role to bring down Rad14p to the active gene in the absence of lesion.

Because Rad14p associates with the active gene, it is likely to enhance transcription. Indeed, our data demonstrate that the transcription of several *GAL* genes is significantly decreased in the absence of Rad14p after 90 min of transcriptional induction in galactose-containing growth medium (Figs. 1*F* and 5*D*). Similarly, Rad14p promotes transcriptional induction of non-*GAL* genes such as *CUP1*, *CTT1*, and *STL1* (Fig. 8, *B* and *C*). These results support the role of Rad14p in facilitation of transcrip-

tion. Likewise, human homologue of Rad14p has recently been shown to associate with the *RARβ*2 promoter to facilitate transcription after all-*trans* retinoic acid induction in HeLa cell lines (73). However, in a striking contrast to our results and recent study (73) in HeLa cell lines, Prakash and co-workers (74, 75) did not find a change in *GAL* transcription in the absence of Rad14p in their Northern blot analysis. Such contradictory results raise the possibility that either our strain or the strain used in the Prakash laboratory contained a hidden mutation that impaired transcription in our study or suppressed Δ*rad14* phenotype in the previous studies of Prakash and co-workers (74, 75). To address this issue, we have carried out experiments using three Δ*rad14* mutants generated from three different parental backgrounds (W303a, BY4741, and FM391). We find that Rad14p promotes *GAL1* transcription (Figs. 1*F* and 2, *A*, *C*, *D*, and *E*). Thus, the role of Rad14p in promoting transcription does not appear to be due to a hidden mutation in our strain. Furthermore, the growth conditions in the previous studies of Prakash and co-workers (74, 75) were different from our study. Moreover, the strong signals (or exposures) in the reported northern blots of Prakash and co-workers (74, 75) may not adequately show up a change of transcriptional defect. These factors might have led to the discrepancy in our results and previous studies of Prakash and co-workers (74, 75). Nonetheless, our ChIP and RT-PCR data support the function of Rad14p in promoting transcription, consistent with the recently reported role of its homologue in HeLa cell lines (73).

How does Rad14p regulate transcription? A previous biochemical study (51) demonstrated the interaction of Rad14p with RNA polymerase II and TFIIH. Both RNA polymerase II and TFIIH are involved in transcriptional initiation (65–67). TFIIH has a kinase activity that phosphorylates serine 5 at the C-terminal domain of the Rpb1p subunit of RNA polymerase II. Such phosphorylation is essential for RNA polymerase II to enter into the elongation phase of transcription (68–71). Transcription is initiated via the formation of PIC by RNA polymerase II and a set of general transcription factors (GTFs) such as TBP, TFIIB, TFIIE, TFIIF, and TFIIH. TBP nucleates the assembly of GTFs to form the PIC. RNA polymerase II and TFIIH join the PIC toward the end to initiate transcription. The fact that Rad14p interacts with TFIIH and RNA polymerase II suggests that the absence of Rad14p would lower the formation of the PIC and, hence, transcriptional initiation. Indeed, we find that the absence of Rad14p significantly lowers the association of RNA polymerase II, TFIIH, and TBP with the *GAL1* promoter (Figs. 2*A*, 3, *E* and *F*, and 4, *A* and *B*). Furthermore, we show that the recruitment of RNA polymerase II to the *GAL1* promoter is significantly decreased in the Δ*rad14* strain, when the coding sequence is deleted (Fig. 3, *A* and *B*). These results support the role of Rad14p in promoting the PIC formation (and hence transcriptional initiation). Furthermore, we find that Rad14p associates with the active coding sequence (Figs. 1, *B* and *C*, and 5, *B* and *C*). Hence, Rad14p may have a hidden role in transcriptional elongation, and such function may be mediated via RPA as RPA interacts with Rad14p (51) and regulates transcriptional elongation (52). This possibility remains to be further elucidated. Nonetheless, our results demonstrate the role of a DNA repair factor, Rad14p, in stimulation of transcription in addi-

tion to its well known function in NER, thus implicating Rad14p as an important regulator of gene expression.

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