Srb5/Med18-mediated Termination of Transcription Is Dependent on Gene Looping

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Background: Mediator subunit Srb5/Med18 is required for termination of transcription by RNAP II.

Results: Srb5/Med18 does not affect CTD-Ser2 phosphorylation but affects recruitment of the CF1 complex on a gene. Srb5/Med18 is required for gene looping.

Conclusion: Srb5/Med18 facilitates termination of transcription through gene looping.

Significance: Gene looping may have a general role in the termination of transcription.

We have earlier demonstrated the involvement of Mediator subunit Srb5/Med18 in the termination of transcription for a subset of genes in yeast. Srb5/Med18 could affect termination either indirectly by modulating CTD-Ser2 phosphorylation near the 3′ end of a gene or directly by physically interacting with the cleavage and polyadenylation factor. That the Srb5/Med18-dependent genes remains unchanged in the absence of Srb5 in cells. Combinatorial analysis revealed the physical interaction of Srb5/Med18 with the cleavage and polyadenylation factor complex, however, could be detected. The Srb5/Med18-CF1 interaction was not observed in the looping defective sua7-1 strain. Srb5/Med18 cross-linking to the 3′ end of genes was also abolished in the sua7-1 strain. Chromosome conformation capture analysis revealed that the looped architecture of Srb5/Med18-dependent genes was abrogated in srb5− cells. Furthermore, Srb5-dependent termination of transcription was compromised in the looping defective sua7-1 cells. The overall conclusion of these results is that gene looping plays a crucial role in Srb5/Med18 facilitated termination of transcription, and the looped gene architecture may have a general role in termination of transcription in budding yeast.

The role of Mediator in the recruitment and assembly of the preinitiation complex is well established (1–4). Although Mediator was discovered as a factor that helps the activator recruit the general transcription machinery onto the promoter, it is required for basal transcription as well (5–10). The recruitment function of Mediator is an evolutionarily conserved feature, being observed in yeast as well as in mammalian systems.

Recent studies, however, have revealed that the repertoire of Mediator functions extends beyond the recruitment of the preinitiation complex into the initiation and early elongation steps of transcription (11–18). These additional post-recruitment functions of Mediator have been observed both in yeast and in higher eukaryotes.

Mediator is composed of ~22–28 subunits organized into four submodules (19–21). These are the head, middle, tail, and kinase submodules. Each of these submodules has an assigned function. The subunits of the tail module are the targets of gene specific transcription activators; the middle module interacts with chromatin remodelers; whereas the head module predominantly interacts with RNAP II and general transcription factors (22–24). Genetic analysis in yeast has revealed that many of these subunits exhibit interactions with the termination factors occupying the 3′ end of a gene. Ssu72, which is a component of the CPF 3′ end processing complex required for both cleavage polyadenylation of mRNA as well as the termination of transcription, has been found to interact with Mediator subunits Med20/Srb2, Med8, Med7, and Med31/Soh1 (25–28). Similarly, Rtt103, which is required for Rat1-dependent termination of transcription by the “Torpedo” mechanism, exhibits a synthetic genetic interaction with the Med8, Med31/Soh1, Med15/ Gal11, Med16/Sfn4, and Med3/Pgd1 subunits (25, 26, 29). Yra1, which is known to couple 3′ end processing with mRNA export to the cytoplasm, interacts with the Med19/Rox3 and Med1 subunits of the Mediator complex (26, 30). Thus, subunits of at least three Mediator submodules, head, middle, and tail, have been found to interact with the terminator-associated factors.

The significance of Mediator termination factor interaction remained a puzzle until recently, when it was reported that Mediator has an influence on the termination of transcription (31). Mediator subunit Srb5/Med18, which interacts with the Rpb4 subunit of RNAP II in the crystal structure, is required for transcriptional activation of a subset of genes in yeast (32–35). Chromatin immunoprecipitation analysis revealed cross-linking of Srb5/Med18 to the 3′ end of genes, where it facilitated the recruitment of the CF1 and CPF 3′ end processing-termination complexes (31). In the absence of Srb5/Med18, RNAP II read through the termination signal leading to a termination defect. The molecular mechanism of Srb5/Med18-facilitated termination of transcription, however, remained unclear.

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** This article contains supplemental Tables S1–S3, Figs. S1 and S2, and an additional reference.

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Here, we show that Srb5/Med18 has a direct role in the termination of transcription. Srb5/Med18 facilitates recruitment of the CF1 complex at the 3′ end of genes. Srb5/Med18 is also essential for gene looping. In the looping defective strain, there was no recruitment of CF1 complex at the 3′ end of genes even in the presence of Srb5/Med18, leading to a termination defect. We, therefore, propose that Srb5/Med18-dependent gene looping is required for the termination of transcription of a subset of genes. Our results suggest that gene looping may be generally required for proper termination of transcription in budding yeast.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains**—The yeast strains used in this study are listed in supplemental Table S1. Strain pairs FY23 (WT) and BPM2 (srb5Δ), SLW3 and BPM41, and SAM51 and BPM33 are isogenic. BPM2 and BPM41 were constructed by replacing the entire ORF of **SRB5** by KANMX6, as described in Ref. 36. The C-terminal Myc-tagged Rna15 (SAM51, BPM33), HA-tagged Pta1 (BPM9, BPM36), and Myc-tagged Srb4 (BPM34, BPM35) strains were derived from the FY23 and BPM2 strains by transforming with a PCR product amplified from pFA6–13Myc-TRP1, pFA6–3HA-TRP1, pFA6–13Myc-TRP1, or pBS1479 (TRP marker) respectively. The primers used for the C-terminal tagging of proteins are listed in the supplemental Table S3.

**Cell Culture**— Cultures were grown and processed for RT-PCR, capture chromosome conformation assay (CCC), and ChIP as described in Ref. 37. Briefly, cultures were started from freshly streaked plates and grown overnight in 5 ml of YP-dextrose at 30 °C with shaking. The next day, the cells were diluted 100 times in 200 ml of YP-dextrose until the A₆₀₀ reached ~0.3. One-half of the cells were pelleted and resuspended in inositol-depleted medium and the other half in inositol-rich (100 µg/ml) medium for **INO1**. For **CHA1**, the cells were transferred to ammonium sulfate (5 g/liter) containing medium and serine threonine (1 g/liter) containing medium as described in Ref. 38. For **ASCI1**, cells were grown in YP-dextrose medium. All the cultures were now allowed to grow at 30 °C with constant shaking for 120 min (A₆₀₀ ~ 0.8) and then processed for RT-PCR, ChIP, or CCC. The primers/probes used in the following analyses are listed in the supplemental Table S2.

**ChIP**—ChIP experiments were performed as described previously (37). Srb5 ChIP was carried out using anti-Myc antibodies in a strain with a C terminus Myc-tagged Srb5. For Rna15 and Pta1, a HA tag was inserted at the carboxy-terminus of each subunit, and ChIP was performed using anti-HA antibodies. Chromatin immunoprecipitated DNA was PCR-amplified (30 cycles) by appropriate primer pairs and subjected to quantification and statistical analysis as described below. Each experiment was repeated with at least two independently grown cultures. In case of Srb5/Med18 deleted strains, the experiment was performed with at least two biological replicates. Anti-phosphoserine2-CTD (3E10) and anti-Rpb1 monoclonal antibodies (8WG16) were purchased from Millipore and Santa Cruz Biotechnology, respectively.

**CCC—CCC** experiments were performed exactly as described previously (39). Briefly, 50 ml of cells were grown at 30 °C until A₆₀₀ of ~0.8. Cells were formaldehyde cross-linked for 15 min at 25 °C. Formaldehyde cross-linking is done to make sure that observed interaction signals are not due to random interaction of chromosomal regions. The cross-linked crude chromatin was digested with restriction endonuclease(s) (AluI for **INO1**; NlaIV and Alu1 for **CHA1** and **ASCI1**). After restriction digestion, the reaction volume was diluted by 7.5-fold to minimize intermolecular ligation in the next step. Ligation reactions were performed at room temperature for 90 min. The cross-links were reversed by incubating at 65 °C overnight. DNA was extracted with phenol-chloroform followed by ethanol precipitation. 300 ng of DNA was used as template in the PCR reaction using P1-T1 divergent primer pair as indicated in Fig. 4, A and D. Control PCR products were generated using convergent primer pair (F2-R1). PCR reactions and detection of products were performed exactly as described in Ref. 39. Each experiment was performed with at least four independently grown cultures and from two biological replicates in case of Srb5/Med18 deleted strains. P1-T1 PCR signals are normalized with respect to F2-R1 PCR signals.

**Coominunoprecipitation**—For affinity purification of Myc-tagged Srb5, HA-tagged Rna15, HA-tagged Rna14, HA-tagged Ssu72, and Myc-tagged Srb4, cells were grown in 1 liter of YP-dextrose to an A₆₀₀ of 1.5. The cell pellet obtained from the 1-liter culture was washed with 50 ml of ice-cold 1× TBS followed by a wash with 50 ml of ice-cold double distilled water. The pellet was resuspended in 10 ml of lysis buffer (20 mM Tris-HCl, pH 8.0, 150 mM KCl, 0.5 mM EDTA, 1 mM MgCl₂, 0.1% Triton X-100, 1 mM PMSF, and 10% glycerol (v/v)). The cell suspension was flash frozen in liquid nitrogen as described in Ansari and Schwer (40). The frozen cells were homogenized into a fine powder in a chilled mortar. The powder was then transferred to an ice-cold beaker and allowed to thaw slowly. The resulting cell lysate was then centrifuged at 16,400 rpm for 20 min in a Sorvall SS-34 rotor. The supernatant (~10 ml) was allowed to bind to 40 µl of either anti-HA-agarose or anti-Myc-agarose beads (Sigma) in a 15-ml tube for 4 h at 4 °C with gentle shaking. The beads were washed three times with 1 ml each of ice-cold lysis buffer. Elution was performed with 100 µg of either HA oligopeptides or Myc oligopeptides resuspended in 200 µl of lysis buffer at 25 °C. The resulting eluate was then used for SDS-PAGE followed by Western blotting.

**Western Blot Analysis**—Anti-HA antibodies were purchased from Neomarkers. Anti-Myc antibodies were purchased from Upstate. Western blotting protocol was performed as described previously (37).

**Transcription Analysis**—Transcription analysis of **CHA1** and **ASCI1** in wild type and srb5Δ strains was performed by the RT-PCR approach as described previously (37).

**TRO Assay**—Transcription run-on (TRO) assay was performed following the protocols described in Refs. 41 and 42.

**Strand-specific RT-PCR**—Strand-specific RT-PCR was performed to identify read-through transcripts that indicate the transcription termination defect. Total RNA for this procedure

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3 The abbreviations used are: CCC, chromosome conformation capture; CF1, cleavage factor 1; CFP, cleavage and polyadenylation factor; TRO, transcription run-on; RNAP II, RNA polymerase II; CTD, carboxy-terminal domain.
was extracted using TRIzol reagent. Cell pellet was resuspended in 500 μl of TRIzol. Acid-washed glass beads (~250 μl) were added to the cell suspension. Cells were lysed by vigorous shaking for 20 min on an agitator at 4 °C. 500 μl more of TRIzol reagent and 200 μl of chloroform were added on top of it and centrifuged at high speed for 10 min. The supernatant was extracted two times with an equal volume of phenol/chloroform (pH 4.3), followed by an extraction with chloroform only. RNA was precipitated using 0.1 volumes 10 M LiCl and 3 volumes of cold ethanol in the presence of glycogen as a carrier. The precipitated RNA was collected by centrifugation at 13,000 rpm for 15 min. Air-dried RNA pellet was resuspended in 50 μl of diethylpyrocarbonate (DEPC)-treated water, and the concentration was estimated using a spectrophotometer.

Strand-specific RT-PCR was now performed as described in El Kaderi et al. (37). 1 μg of RNA was used to make cDNA using strand-specific primers for CHA1 as shown in Fig. 7. Primers cDNA primer1 and cDNA primer2 were used to reverse-transcribe sense mRNA. cDNA prepared using cDNA primer1 was used in PCR amplification of regions W and X. cDNA prepared from cDNA primer2 was used in PCR amplification of regions Y and Z. A minus-RT control (without reverse transcriptase) was always performed to ensure that the strand-specific RT-PCR signal was not due to contaminating DNA in the RNA preparation.

Quantification—The quantification was performed as described in Ref. 39. In ChIP, CCC, and RT-PCR experiments described above, PCR products were fractionated on a 1.5% agarose gel and visualized by ethidium bromide using Gel Logic 200 system. The net intensity of the bands was calculated using the Kodak one-dimensional software. Using the scaled net intensities, a minimum of eight trials were analyzed under the Univariate analysis of variance model in the SPSS statistical software to verify that there was no significant gel interaction ($p < 0.005$). Each trial was also duplicated to ensure that there was no significant trial interaction ($p < 0.005$). Scaled net intensities were then used to generate ratio data comparing the experimental test with that of the control PCR, which was then used to generate the mean and S.D. as shown in the graphs. For all of the quantification graphs, the error bars represent one unit of S.D. based on at least eight independent PCRs from four separate immunoprecipitations or CCC reactions or reverse-transcribed RNA samples from two independently grown cultures. In the case of Srb5/Med18-deleted strains, the experiment was repeated with at least two biological replicates.

**RESULTS**

*Srb5/Med18 Does Not Affect CTD-Ser2 Phosphorylation at the 3′ End of a Gene—*We have earlier demonstrated a role for Srb5/Med18 in the termination of transcription (31). Here, we examine the mechanism of Srb5/Med18-mediated termination of transcription. The termination of transcription by RNAP II is facilitated by CTD-Ser$^2$ phosphorylation and requires CF1 and CPF complexes in yeast (41, 43–48). The phosphorylation of CTD-Ser$^2$ starts during early elongation and continues toward the 3′ end of a transcriptionally active gene (49). The Ser$^2$-phosphorylated CTD serves as a loading dock for the recruitment of CF1 and CPF complexes near the 3′ end of a gene, which then bring about termination of transcription (48, 50). Srb5/Med18 could affect termination of transcription either indirectly by affecting phosphorylation of CTD-Ser$^2$ near the 3′ end of genes or directly by facilitating the recruitment of CF1 or CPF complex to the terminator site. To test the first scenario, we checked CTD-Ser$^2$ phosphorylation status in different regions of *INO1* and *CHA1* in the wild type and *srb5/med18*− cells by the ChIP approach. There was no change in the CTD-Ser$^2$ phosphorylation pattern near the 3′ end of *INO1* in the absence of Srb5/Med18 under induced conditions (Fig. 1B, regions D and E). A marginal decrease in Ser$^2$ phosphorylation, however, was observed toward the 3′ end of *CHA1* in the absence of Srb5/Med18 (Fig. 1E, regions D and E), which could be attributed to a decrease in RNAP II density in the region. The normalization of CTD-Ser$^2$ phosphorylation signal with respect to RNAP II density in the region clearly shows that there is no net decrease in the CTD-Ser$^2$ phosphorylation near the 3′ end of either *INO1* or *CHA1* in *srb5/med18*− cells (Fig. 1, C and F). These results suggest that Srb5/Med18 may be playing a more direct role in the termination of transcription.

*Srb5/Med18 Interacts with the CF1 Complex—*Srb5/Med18 can affect termination directly by interacting with either CF1 complex or CPF complex or both and facilitating their recruitment near the terminator region of a gene. We therefore examined whether Srb5/Med18 exhibits a physical interaction with the CF1 or CPF complex in yeast cells. Our experimental approach involved coimmunoprecipitation of Srb5/Med18 followed by detection of CF1 and CPF subunits in the immunoprecipitate by Western blot. To facilitate coimmunoprecipitation, a Myc tag was inserted at the carboxyl terminus of Srb5/Med18. Insertion of the Myc tag did not interfere with the biological activity of Srb5/Med18 as the transcription of both *INO1* and *CHA1* remained unaffected in the tagged strain (data not shown). Additionally, the HA tag was integrated at the carboxyl terminus of either the CF1 subunits Rna15 and Rna14 or the CPF subunit Ssu72 for their detection by Western blot. Our results show that the Myc-tagged Srb5/Med18 was able to coimmunoprecipitate the CF1 subunit Rna15 (Fig. 2A, lane 2). No signal for Rna15 was observed when coimmunoprecipitation was performed using anti-Myc antibodies in a strain carrying HA-tagged Rna15 but Srb5/Med18 without Myc tag (supplemental Fig. S1). To further substantiate the Srb5/Med18-CF1 interaction, we performed the reciprocal experiment by coimmunoprecipitating HA-tagged Rna15 and looking for the presence of Srb5/Med18 in the pulldown. The HA-tagged Rna15 was able to coimmunoprecipitate Srb5/Med18 although with slightly reduced efficiency (Fig. 2A, lane 3). To corroborate Srb5/Med18-CF1 interaction, we checked the interaction of another CF1 subunit Rna14 with Srb5/Med18. Coimmunoprecipitation was performed for HA-tagged Rna14, exactly as described for Rna15 above. Our results clearly demonstrate that Srb5/Med18 exhibits a physical interaction with Rna14 as well (Fig. 2A, lanes 5 and 6). In contrast, no interaction of Srb5/Med18 with the CPF subunit Ssu72 could be detected (Fig. 2B, lanes 8 and 9). Thus, Srb5/Med18 is able to physically interact with the CF1 complex but not with the CPF complex.

It is possible that Srb5/Med18 is interacting with the CF1 complex independently, outside the context of Mediator com-
plex. We reasoned that if Srb5/Med18 is interacting with the CF1 complex as a component of Mediator, then other Mediator subunits will also exhibit a physical interaction with the CF1 subunits. We therefore examined the interaction of another Mediator subunit Srb4/Med17 with the CF1 complex. The coimmunoprecipitation was performed using Myc-tagged Srb4/Med17 as described above. The results show that Srb4/Med17 was able to coimmunoprecipitate CF1 subunit Rna15 (Fig. 2C, lane 12). In the absence of Srb5/Med18, however, Srb4/Med17 failed to interact with Rna15 (Fig. 2C, lane 13). These results suggest that the Mediator complex does not interact with the CF1 subunit in the absence of Srb5/Med18. Further experimentation, however, is needed to demonstrate that Srb5/Med18 is the link that bridges Mediator-CF1 interaction.

Srb5/Med18 Interaction with the 3' End of a Gene Occurs in a Looping-dependent Manner—To recruit CF1 complex to a transcribing gene, Srb5/Med18 must physically interact with the 3' end of the gene. We have previously demonstrated that Srb5/Med18 does bind to the terminator end of a gene (31). There are two possible approaches Srb5/Med18 can use to interact with the 3' end of a gene. One possibility is that the promoter-bound Mediator makes contact with the 3' end due to gene looping, which is the physical interaction of the promoter and terminator regions of a gene in a transcription-dependent manner (68). When a gene is in a looped conformation, the promoter and terminator regions of a gene are juxtaposed, which makes it feasible for the promoter-associated Mediator to interact with the terminator end of a gene. In such a scenario, the same Mediator complex is contacting both ends of a gene. An alternative possibility is that two separate molecules of Mediator are recruited onto the gene, one at the promoter and the other at the terminator. This could be accomplished while the gene remains in a linear conformation. The cross-linking of Srb5/Med18 to the terminator in this case should not be dependent on gene looping. We reasoned that if Srb5/Med18 is making contact with the 3' end of genes due to gene looping, cross-linking of the Mediator subunit to the 3' end will be compromised in a looping deficient strain. We therefore performed chromatin immunoprecipitation of Srb5/Med18 for INO1 and

![Figure 1](image-url)
Srb5/Med18-mediated Termination through Gene Looping

FIGURE 2. Srb5/Med18 interacts with CF1 subunits Rna15 and Rna14. A, immunoprecipitation was performed using antibodies against Myc-tagged Srb5 in strains carrying HA-tagged Rna15 or HA-tagged Rna14. Proteins coimmunoprecipitating with Srb5/Med18 were detected by Western blot using anti-HA and anti-Myc antibodies (lanes 2 and 5). In the reciprocal experiment, immunoprecipitation was performed using antibodies against either HA-tagged Rna15 or HA-tagged Rna14, and coimmunoprecipitated proteins were detected by Western blot (lanes 3 and 6). B, immunoprecipitation was performed using antibodies against Myc-tagged Srb5 in a strain carrying HA-tagged Ssu72. Proteins coimmunoprecipitating with Srb5/Med18 were detected by Western blot as described above (lane 8). In the reciprocal experiment, immunoprecipitation was performed using antibodies against HA-tagged Ssu72, and coimmunoprecipitated proteins were detected as described previously (lane 9). C, proteins coimmunoprecipitating with Myc-tagged Srb4 in srb5/med18− and isogenic wild type strains were detected by anti-HA and anti-Myc antibodies (lanes 12 and 13). Arrows indicate the position of molecular weight marker proteins of 100, 90, 75, 60, 50, or 35 kDa. IP, immunoprecipitation.

CHA1 in the looping defective sua7-1 and isogenic wild type strains. The sua7-1 is a mutant of the general transcription factor TFIIB with glutamic acid at position 62 being replaced by lysine (TFIIB-E62K) (51). The binding affinity of TFIIB-E62K for the promoter region as well as its interaction with TBP and RNAP II are comparable to that of wild type TFIIB (52). The recruitment of general transcription factors onto the promoter during initiation of transcription is also normal in the presence of TFIIB-E62K (51, 52). The cross-linking of mutated TFIIB to the terminator region of actively transcribed genes, however, is severely compromised in sua7-1 cells (53). The mutant TFIIB is unable to interact with the terminator-bound factors, and consequently, there is no gene looping in sua7-1 cells (53, 54). The sua7-1 strain has been used previously to demonstrate the role of gene looping in transcription memory, intron-mediated transcriptional regulation and transcription directionality (55–58). To determine whether the presence of Srb5/Med18 at the 3′ end of genes was due to gene looping, we performed Srb5/Med18 ChIP in the looping defective sua7-1 strain. Our results show that the Srb5/Med18 signal at the 3′ end of both INO1 and CHA1, during induced transcription decreased by ~2.5–3-fold in the sua7-1 strain compared with an isogenic wild type strain (Figs. 3B and 3D, region D). The signal at the 5′ end of both genes, however, remained unaffected in the absence of gene looping (Figs. 3B and 3D, region A). These results suggest that gene looping is required for the interaction of Srb5/Med18 with the 3′ end of genes. Furthermore, coimmunoprecipitation revealed that the interaction of Srb5/Med18 with the CF1 subunit Rna15 was also compromised in the looping defective strain (Fig. 3E lane 4). The overall conclusion of these results is that the Srb5/Med18 interaction with the 3′ end of a gene as well as with the CF1 complex is dependent on gene looping.

Srb5/Med18 Is Required for Gene Looping—Since Srb5/Med18, like TFIIB, contacts both the ends of a transcriptionally active gene (31), this prompted us to investigate if Srb5/Med18 is required for looping of these genes. CCC analysis was therefore carried out for two Srb5/Med18-regulated genes, INO1 and CHA1, in srb5/med18− and isogenic wild type strains. We have previously used this approach to demonstrate gene looping in budding yeast (37, 39, 54). The P1T1 PCR product is taken as a measure of gene looping in these experiments. We found that both INO1 and CHA1 assume a looped conformation upon induced transcription in wild type cells as indicated by P1T1 PCR products (Figs. 4B, lane 2 and 4E, lane 6; Figs. 4C and 4F). The P1T1 looping signal decreased by about 8 fold for both genes in the absence of Srb5/Med18 in cells (Figs. 4B, lane 4 and 4E, lane 8; Figs. 4C and 4F). RT-PCR analysis was performed in the same batch of cells (supplemental Fig. S2) in which CCC analysis was done to check that loss of looping signal was accompanied by loss of transcription as reported earlier (31). On the basis of these results, we conclude that Srb5/Med18 is required for transcription-dependent looping of a subset of genes.

Srb5/Med18 Is Not Required for Looping or Termination of Transcription of All Yeast Genes—Transcription of about 16% of genes in budding yeast is adversely affected in the absence of Srb5/Med18 (33). We showed that INO1 and CHA1, two genes that are dependent on Srb5/Med18 for their transcriptional
Srb5/Med18-mediated Termination through Gene Looping

activation, exhibited a termination defect in the srb5/med18− cells (31). It was, however, not clear from that study if Srb5/Med18 is generally required for termination of RNAP II-mediated transcription in yeast. To address the issue, we carried out transcription analysis of ASC1 gene, which does not require Srb5/Med18 for its transcription. RT-PCR analysis of ASC1 in wild type and srb5/med18− cells did not reveal any decrease in the transcript level of ASC1 in the absence of Srb5/Med18 as expected (Fig. 5B). To investigate the potential role of Srb5/Med18 in the termination of transcription of ASC1, we examined the recruitment of CPF and CF1 3′ end processing complexes at the 3′ end of the gene by the ChIP approach in the wild type and Srb5/Med18 deleted cells. There was no decrease in the crosslinking of CF1 subunit Rna15 and CPF subunit Pta1 to the 3′ end of ASC1 in srb5/med18− cells compared with isogenic wild type strain (Fig. 5C, region D). TRO analysis revealed that RNAP II did not read through the termination signal of ASC1 in the absence of Srb5/Med18 in the cells (Fig. 5D, lanes 1, 2, 3, and 4). These results affirmed that Srb5/Med18 is not required for termination of transcription of ASC1. Accordingly, Srb5 failed to crosslink to the 3′ end of transcriptionally active ASC1 in wild type cells (Fig. 5E, lane 4). Gene looping of ASC1 also remained unaffected in srb5/med18− cells (Fig. 5F). The overall conclusion of these results is that Srb5/Med18 is required neither for gene looping nor the termination of transcription of Srb5/Med18-independent genes. Since different Mediator subunits respond to different transcription activators, it is possible that the role of Mediator subunits in the termination of transcription is gene specific.

Srb5/Med18-facilitated Termination of Transcription Requires Gene Looping—The results presented so far show that; (i) Srb5/Med18 interacts with the 3′ end of a subset of genes in a looping-dependent manner, (ii) Srb5/Med18 is required for looping of these genes, and (iii) Srb5/Med18 interaction with the CF1 complex is dependent on gene looping. On the basis of these results, we propose that the promoter-bound Srb5/Med18 interacts with the 3′ end of a gene resulting in a looped conformation. The terminator-bound Srb5/Med18 simultaneously interacts with the CF1 complex. The CF1 complex then brings about termination of transcription. A corollary of these results is that Srb5/Med18-dependent gene looping is essential for termination of transcription. If Srb5/Med18 is using gene looping to bring about termination of transcription, then loss of gene looping by some other means will cause a termination defect even in the presence of Srb5/Med18. We therefore monitored the termination defect during transcription of INO1 and CHA1 in the looping defective sua7−1 strain. We first measured recruitment of the CF1 subunit Rna15 at the 3′ end of two genes. In the wild type cells, Rna15 is recruited at the 3′ end of transcriptionally active ASC1 in wild type and looping defective sua7−1 cells compared with iso- genetic wild type strain (Fig. 5C, region D). Accordingly, Srb5/Med18-facilitated Termination of Transcription Re-quires Gene Looping—The results presented so far show that; (i) Srb5/Med18 interacts with the 3′ end of a subset of genes in a looping-dependent manner, (ii) Srb5/Med18 is required for looping of these genes, and (iii) Srb5/Med18 interaction with the CF1 complex is dependent on gene looping. On the basis of these results, we propose that the promoter-bound Srb5/Med18 interacts with the 3′ end of a gene resulting in a looped conformation. The terminator-bound Srb5/Med18 simultaneously interacts with the CF1 complex. The CF1 complex then brings about termination of transcription. A corollary of these results is that Srb5/Med18-dependent gene looping is essential for termination of transcription. If Srb5/Med18 is using gene looping to bring about termination of transcription, then loss of gene looping by some other means will cause a termination defect even in the presence of Srb5/Med18. We therefore monitored the termination defect during transcription of INO1 and CHA1 in the looping defective sua7−1 strain. We first measured recruitment of the CF1 subunit Rna15 at the 3′ end of two genes. In the wild type cells, Rna15 is recruited at the 3′ end of INO1 and CHA1 upon induction of transcription as expected (Figs. 6B and 6D, region D). In the looping defective sua7−1 cells, however, the recruitment of Rna15 onto these genes registered a 70−75% decline (Fig. 6B and 6D, region D). The decrease in CF1 signal at the 3′ end of two genes in sua7−1 cells

FIGURE 3. Srb5/Med18 interaction with the 3′ end of genes as well as with CF1 subunit Rna15 is compromised in looping defective sua7−1 strain. A and C, schematic depiction of INO1 and CHA1 indicating the positions of ChIP primer pairs. B and D, ChIP analysis showing cross-linking of Myc-tagged Srb5 to different regions of INO1 and CHA1 in isogenic wild type and looping defective sua7−1 strains. The input signal represents DNA prior to immunoprecipitation. The error bars represent one unit of S.D. The results shown are an average of at least eight independent PCRs from four separate immunoprecipitates from two independently grown cultures. E, coimmunoprecipitation of Rna15 with Srb5/Med18 in wild type and sua7−1 cells. HA-tagged Rna15 coimmunoprecipitating with Myc-tagged Srb5 in sua7−1 and isogenic wild type strains was visualized using anti-HA antibodies (lanes 2 and 4). Arrows indicate the position of molecular weight marker proteins of 75, 60, and 50 kDa. IP, immunoprecipitation.

APRIL 19, 2013 • VOLUME 288 • NUMBER 16

JOURNAL OF BIOLOGICAL CHEMISTRY 11389
**FIGURE 4.** Gene looping of *INO1* and *CHA1* requires Srb5/Med18. 

A and D, schematic depiction of *INO1* and *CHA1* indicating the positions of restriction sites (vertical lines) and PCR primers (arrows) used in CCC analysis. 

B and E, CCC analysis of *INO1* and *CHA1* to detect gene looping in wild type and *srb5−/−* cells under induced (−Ino for *INO1*; ST for *CHA1*) and uninduced conditions (+Ino for *INO1*; AS for *CHA1*). Control PCR represents amplification of an internal region of the gene by F2-R1 primer pairs indicating that an equal amount of DNA template was used in all CCC experiments. 

C and F, quantification of the CCC results shown in B and E representing P1-T1 PCR signal normalized with respect to F2-R1 control PCR. The P1-T1 PCR signals under uninduced conditions are background signals due to intermolecular ligation. Any P1-T1 PCR signal that is detected above the background signal is a measure of looping interaction. The error bars represent one unit of S.D. The results shown are an average of at least eight independent PCRs from four separate CCC replicates from two independently grown cultures. AS, ammonium sulfate; ST, serine and threonine.

**FIGURE 5.** Srb5/Med18 not required for termination and gene looping of *ASC1*. 

A, schematic depiction of *ASC1* indicating the positions of primer pairs/probes used in RT-PCR, CCC, ChIP, and TRO analysis. Vertical lines indicate positions of AluI and NlaIV restriction sites used in CCC analysis. 

B, quantification of the transcript level of *ASC1* as detected by RT-PCR analysis. 

C, ChIP analysis showing cross-linking of HA-tagged Rna15 and HA-tagged Pta1 to the D and E regions of *ASC1* during transcription. 

D, TRO analysis of *ASC1* in wild type and Srb5/Med18-deleted cells. 

E, ChIP analysis showing cross-linking of Myc-tagged Srb5/Med18 to different regions of *ASC1* during transcription. 

F, CCC analysis of *ASC1* to detect gene looping in wild type and *srb5/med18−/−* cells.
suggest a termination defect. However, it does not prove that the termination is defective in looping deficient cells. A termination defect will cause RNAP II to readthrough into the downstream region. To confirm that loss of looping resulted in defective termination in sua7–1 cells, we performed strand-specific RT-PCR for CHA1 in wild type and sua7–1 strain as described in (59). Induced transcription of CHA1 was about 1.5 fold lower in the sua7–1 cells compared with the wild type cells (Fig. 7B, regions W and X). A 6–8 fold increase in the amount of readthrough transcripts was observed in the sua7–1 cells compared with wild type cells (Fig. 7B, regions Y and Z). These results indicate that RNAP II read-through the terminator region of CHA1 in the absence of gene looping. Although Srb5/Med18 was present in these cells, in the absence of gene looping, it failed to facilitate termination of transcription. A broader conclusion of these results is that gene looping plays a role in termination of transcription in budding yeast.

**DISCUSSION**

The successful accomplishment of transcription involves the cooperative interaction among different steps of the transcription cycle. The integration of initiation and elongation steps and of the elongation and termination steps is well established (45, 60, 61). Until recently, it was not realized that the initiation and termination steps also cross-talk during the transcription cycle (62, 63). A large body of genetic and biochemical evidence suggests that a network of intricate interactions exist between the factors involved in the initiation and termination steps of transcription (25, 26). The Mediator complex, for example, exhibits multiple interactions with the terminator-bound factors and the subunits of RNAP II involved in the termination of transcription (25–28, 32, 34, 64). Accordingly, we recently demonstrated that Mediator subunit Srb5/Med18 physically interacts with the 3′ end of a subset of genes and facilitates termination of transcription of these genes (31). In the absence of Srb5/Med18, there was no recruitment of CFI and CFP complexes at the 3′ end of a subset of genes leading to a transcription read-through phenotype. Mediator is not an aberrant case of an initiation factor engaged in termination. Analogous studies with the general transcription factor TFIIB have revealed its role at the 3′ end of genes as well (37, 51, 54, 65, 66). TFIIB has been shown to facilitate termination of transcription in flies and mammalian systems (37, 53, 66, 67). A complex of TFIIB with a number of 3′ end processing/termination factors has been purified from yeast, thereby suggesting that the termination function of TFIIB is an evolutionarily conserved feature (54). The possibility of other promoter-bound factors playing a similar role in the termination of transcription cannot be ruled out.

Here, we show that Srb5/Med18-mediated termination is dependent on gene looping. Our hypothesis is that the promoter-bound Mediator makes contact with the 3′ end of a gene through Srb5/Med18. This interaction stabilizes a looped gene
conformation. The terminator-linked Srb5/Med18 now interacts with the CF1 complex and facilitates its recruitment to the 3′ end of a gene. CF1 and CPF complexes then bring about termination of transcription. In the absence of gene looping, Srb5/Med18 is unable to contact the 3′ end of a gene leading to its inability to recruit CF1 complex, resulting in defective termination. It is evident from this study that the interaction of the promoter-associated Srb5/Med18 with the 3′ end of genes is also crucial for gene looping of Srb5/Med18-dependent genes. Although TFIIB is the prime promoter-bound factor that interacts with the factors bound to the terminator end of a gene to facilitate gene loop formation, the possibility of other promoter-bound factors like Mediator stabilizing the promoter-terminator interaction cannot be discounted. Thus, gene looping is stabilized by multiple protein-protein interactions between the promoter-bound factors and the terminator-occupying factors. The TFIIB-CF1 interaction is one such interaction, which is required for looping of transcriptionally active genes (54). In addition, there are other interactions, similar to the one involving Srb5/Med18, which may be stabilizing the looped conformation for specific genes.

Gene looping, which was first demonstrated during transcription in budding yeast (68, 69), has been implicated in activator-dependent enhancement of transcription, transcription memory and intron-mediated enhancement of transcription (37, 55–57, 70). A recent study discovered a novel role of gene looping in determining the directionality of bidirectional promoters (58). Here, we report yet another physiologically significant role of gene looping in the termination of transcription. Although gene looping is defective in almost all termination factor mutants that have been investigated so far (37, 54, 68), this is the first instance where a promoter-bound factor has been implicated in facilitating termination through gene loop formation. Similar to Srb5/Med18, TFIIB may also be facilitating termination of transcription through gene looping in yeast. This view received strong support from a recent study that demonstrated a role for TFIIA-mediated gene looping in termination of transcription in flies (67). Although a role for TFIIA-dependent gene looping in termination is yet to be conclusively demonstrated in yeast, the looping defective suat-1 mutant that we used in this study to demonstrate the role of gene looping in Srb5/Med18-dependent termination is a TFIIA mutant. Because different Mediator subunits respond to different transcription activators, the possibility of other Mediator subunits playing a similar role in the termination of transcription of other genes cannot be ruled out. More research is needed to comprehend the molecular mechanism-underlying gene looping facilitated termination of transcription.

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Srb5/Med18-mediated Termination through Gene Looping

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