TFIIB is essential for transcription initiation by RNA polymerase II. TFIIB also cross-links to terminator regions and is required for gene loops that juxtapose promoter-terminator elements in a transcription-dependent manner. The Saccharomyces cerevisiae sua7-1 mutation encodes an altered form of TFIIB (E62K) that is defective for both start site selection and gene looping. Here we report the isolation of an ssl2 mutant, encoding an altered form of TFIIH, as a suppressor of the cold-sensitive growth defect of the sua7-1 mutation. Ssl2 (Rad25) is orthologous to human XBP and is a member of the SF2 family of ATP-dependent DNA helicases. The ssl2 suppressor allele encodes an arginine replacement of the conserved histidine residue (H508R) located within the DEVH-containing helicase domain. In addition to suppressing the TFIIB E62K growth defect, Ssl2 H508R partially restores both normal start site selection and gene looping. Moreover, Ssl2, like TFIIB, associates with promoter and terminator regions, and the diminished association of TFIIB E62K with the PMA1 terminator is restored by the Ssl2 H508R suppressor. These results define a novel, functional interaction between TFIIB and Ssl2 that affects start site selection and gene looping.

Promoter recognition and transcription initiation by RNA polymerase II (Pol II) require five general transcription factors: TFIIB, TFIID, TFIIE, TFIIF, and TFIIH (1–3). Promoters containing a TATA box require the TATA-binding protein (TBP) subunit of TFIID for promoter recognition. TFIIB binds the DNA-TBP complex, contacting promoter DNA both upstream and downstream of TATA. Pol II, in association with TFIIF, binds the DNA-TBP-TFIIE ternary complex, forming a closed promoter complex. TFIIE and TFIIF complete formation of the preinitiation complex (PIC). TFIIE and TFIIH are essential for transcription initiation in vivo but are dispensable for Pol II transcription from negatively supercoiled DNA in vitro, indicating that these factors play an essential role in promoter melting (4). Biochemical probes of DNA-protein interactions mapped the path of promoter DNA within human and yeast PICs (5–13). The topology of the PIC deduced from these in vitro experiments agrees remarkably well with the consensus location of the general transcription factors revealed by a genome-wide analysis of their locations in yeast (14).

This paper focuses on TFIIB and TFIIH. TFIIB is a single subunit protein consisting of an N-terminal zinc ribbon (“B-ribbon”) followed by a ~60-amino acid sequence that links the B-ribbon to two cyclin repeats (“B-core”) that form the C-terminal two-thirds of the protein. The yeast gene encoding TFIIB (SUA7) was initially identified based on mutations that shift start site selection downstream of normal (15). The sua7-1 mutation encodes an E62K replacement between the B-ribbon and B-core; other replacements spanning residues Glu-62 to Val-79 were also found to affect start site selection (16–22). X-ray structures of yeast Pol II-TFIIB complexes revealed two alternative structures for this region, denoted the “B-ribbon” and “B-reader” (23–25). The B-reader has been proposed to “scan” template DNA for acceptable start sites (24). A conformational switch between the B-reader and B-finger subsequent to initiation facilitates the transition from abortive initiation to promoter escape (25, 26).

Genetic suppressors of the sua7-1 mutation identified other components of the PIC that affect start site selection. Two tfg1 alleles (sua71-1 and sua71-2), encoding single amino acid replacements in the Tfg1 subunit of TFIIH, shift initiation upstream of normal and partially restore the normal initiation pattern in the sua7-1 mutant (27, 28). Other amino acid replacements in Tfg1 or Tfg2 also affect start site selection, in each case shifting initiation upstream (29). Mutations in the RPB2 and RPB9 genes, which encode two subunits of Pol II, were also identified as suppressors of either sua7-1 or the related sua7-3 mutation (TFIIH R78C) (30, 31). The architecture of the PIC suggests that Rpb2, Rpb9, and TFIIH affect start site selection allosterically, via a protein interaction network that extends from Rpb9 at the leading edge of the Pol II through TFIIH to TFII (9).

TFIIH is an 11-subunit complex that includes two DNA helicases and a kinase-cyclin subcomplex that targets the C-termi-

**Background:** TFIIB and TFIIH are required for transcription initiation by RNA polymerase II.

**Results:** TFIIB and the Ssl2 helicase of TFIIH functionally interact at both promoter and terminator.

**Conclusion:** Ssl2 affects TFIIB-mediated gene looping and start site selection.

**Significance:** These results underscore the intriguing role of promoter-terminator interactions during the transcription cycle.
nal repeat domain of the Rpb1 subunit of Pol II (32). XPB and XPD are the helicase subunits of human TFIIH and are orthologs of yeast Ssl2 (also known as Rad25) and Rad3, respectively. XPB/Ssl2 is the only TFIIH subunit in immediate proximity to promoter DNA and is located at the leading edge of Pol II (5, 7). XPB is required for open complex formation (33), although not as a conventional helicase but apparently by acting as a “molecular wrench” that rotates DNA downstream of the promoter relative to a fixed upstream position (5). The location of Ssl2 within the yeast PIC is consistent with this model for promoter opening (7). The location and function of Ssl2/XPB suggest that it might affect start site selection, perhaps by feeding template DNA into the active center (7), although no direct evidence for TFIIH involvement in start site selection has been reported.

In addition to the role of TFIIH in start site selection, we have discovered a function for TFIIH in the formation of gene loops that juxtapose promoter and terminator regions (34). Furthermore, TFIIH occupies the terminator region of Pol II-transcribed genes and does so in a manner dependent upon transcription. The sua7-1 mutation adversely affects gene looping and TFIIH-terminator association without affecting PIC assembly. The physiological role of gene loops remains to be established, although mutations that block gene looping, including sua7-1, adversely affect “transcriptional memory” of GAL genes (35, 36). In an effort to learn more about the role of TFIIH in transcription and to further investigate the significance of gene loops, we have isolated additional suppressors of sua7-1. Here we report the identification of ssl2-508, encoding an altered form of the Ssl2 subunit of TFIIH, as a suppressor of TFIIB as described. 6-Azauracil was added to −Ura medium at a final concentration of 50 μg/ml. Yeast X-gal indicator medium was prepared as described (41). For growth assays, strains were grown to saturation at 30 °C in liquid YPD medium, harvested, and diluted in sterile water to ~1 × 10⁶ cells/ml, followed by spotting of 10-fold serial dilutions onto the indicated medium. Csm− (cold-sensitive) and Tsm− (heat-sensitive) phenotypes refer to distinctly impaired growth at 12 °C (Csm−) and 39 °C (Tsm−), respectively.

**Isolation and Genetic Analysis of sua7-1 Suppressors**—Strain YMH893 (sua7-1) was streaked on YPD medium and incubated at 30 °C. Single colonies were picked and inoculated into separate 5-ml YPD liquid culture tubes and grown to stationary phase at 30 °C. Cultures were harvested, washed with sterile water, and diluted to 1 × 10⁶ cells/ml. One hundred μl of cells were spread onto YPD plates and incubated at 12 °C for at least 7 days. Single colony Csm+ revertants (a maximum of one colony per plate) were picked, subcloned on YPD medium, and incubated at 12 °C. Dominance/recessiveness of suppressor mutations and linkage of the Csm+ suppressor and Tsm− pleiotropic phenotypes were determined by standard yeast genetic methods involving backcrosses, diploid selection, sporulation, and tetrad dissection, as described (38).

**Isolation of SSL2 and ssl2-508 Allele**—The SSL2 gene was isolated from a YCp50 genomic library (42) by complementation of the Tsm− phenotype conferred by the ssl2-508 mutation using strain YMH1138 as the host. The complementing DNA was delimitled to the SSL2 gene using plasmid pN861 (pEP23), which carries the SSL2 (RAD25) gene (43). The ssl2-508 allele was recovered from genomic DNA by gap repair (44). Briefly, pN861 was digested to completion with restriction enzymes PvuII and Swal, thereby deleting most of the SSL2 open reading frame. Linear DNA was purified by agarose gel electrophoresis and introduced into strain YMH1138 (ssl2-508 ura3). Ura− colonies were selected and screened for retention of the Csm+ and Tsm− suppressor phenotypes. Plasmid DNA was recovered, amplified in *Escherichia coli*, and analyzed by restriction digestion to confirm the presence of the ssl2 ORF. The resulting plasmid (pM1930) failed to complement the Csm− and Tsm− phenotypes when reintroduced into strain YMH1138, thereby confirming recovery of the ssl2-508 allele. The DNA sequence of the entire ssl2-508 ORF was determined using SSL2-specific primers that span the entire ORF at ~500-bp intervals. The suppressor mutation was identified by aligning the ssl2-508 sequence with SSL2 sequence obtained from the Saccharomyces Genome Database Web site.

**Determination of Transcription Start Sites**—Reporter plasmids pM50 and pM107 were used to assess transcription start site changes at the CYC1 promoter, as described previously (15,
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FIGURE 1. Phenotypes associated with sua7-1 mutant and ssl2-508 suppressor. 10-Fold serial dilutions of SUA7 (YMH14), sua7-1 (YMH893), and sua7-1 ssl2-508 (YMH138) haploid strains and diploid strain sua7-1/ssl2-508 SSL2-508 (YMH1138 × YMH124) were spotted onto YPD medium and photographed after incubation at the indicated temperature for 2 days (30 °C), 5 days (12 °C), or 3 days (39 °C).

TABLE 1

| Strain* | Genotype | Source |
|---------|----------|--------|
| W303-1B | MATa leu2-3,112 his3-11,15 trp1-1 ade2-1 can1-100 ura3-1 | Ref. 44 |
| YMH114 | MATa cyd1-5000 cyc7-67 ura3-52 leu2-3,112 | Ref. 16 |
| YMH124 | MATa cyc1-5000 cyc7-67 ura3-52 leu2-3,112 sua7-1 | Ref. 16 |
| YMH893 | MATa cyc1-5000 cyc7-67 ura3-52 trp5-48 his5-2 sua7-1 ssl2-508 | Ref. 16 |
| YMH1138 | MATa cyc1-5000 cyc7-67 trp5-48 his5-2 ura3-52 sua7-1 ssl2-508 | This study |
| YMH1141 | MATa leu2-3,112 his3-11 trp1-1 ade2-1 can1-100, ura3-1 ssl2-KanMX [pN861: SSL2-IRA3] | This study |
| YMH1142 | MATa leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 ura3-1 ssl2-KanMX [pN863: SSL2-TRP1] | This study |
| YMH1143 | MATa leu2-3,112 his3-11, trp1-1 ade2-1 can1-100, ura3-1 ssl2-KanMX [pM1930: ssl2-508-IRA3] | This study |
| YMH1165 | MATa cyc1-5000 cyc7-67 ura3-52 leu2-3,112 SSL2-TAP | This study |
| YMH1166 | MATa cyc1-5000 cyc7-67 ura3-52 trp5-48 his5-2 sua7-1 SSL2-TAP | This study |
| YMH1167 | MATa cyc1-5000 cyc7-67 trp5-48 his5-2 ura3-52 sua7-1 ssl2-508-TAP | This study |
| DG1657 | MATa ura3-167 his3-200 trp1-1 histg leu2-1 hisg Tyl1-270 his3-1 AI Tyl1-146 [tyb1: lacZ] | Ref. 56 |
| DG1772 | DG1657 ssl2-TRP1 [pRS416: URA3-CEN SSL2] | Ref. 56 |
| DG1773 | DG1657 ssl2-TRP1 [BDG853: LEU2-CEN-SSL2] [pCS9: URA3-CEN-SSL2] | Ref. 56 |
| DG1774 | DG1657 ssl2-TRP1 [BDG853: LEU2-CEN-SSL2] | Ref. 56 |
| DG1775 | DG1657 ssl2-TRP1 [pRS416: URA3-CEN-sal2-rtt] | Ref. 56 |
| DG1776 | DG1657 ssl2-TRP1 [pRS416: URA3-CEN-sal2-DEAD] | Ref. 56 |
| DG1777 | DG1657 ssl2-TRP1 [URA3-CEN-sal2-XP] | Ref. 56 |
| DG1778 | DG1657 ssl2-TRP1 [LEU2-CEN-sal2-1] | Ref. 56 |

* Strains YMH14 (Y16), YMH124 (YDV546), and YMH893 (YMH71-9C) were described previously using the names indicated in parentheses (16).

45). Primer extension experiments were performed using the ADH1-specific primer oIP87; primer extension products were resolved in an 8% polyacrylamide gel and visualized by autoradiography (15).

Chromatin Immunoprecipitation—Cross-linking and isolation of chromatin were performed as described previously (34) using antibodies directed against either TFII B or Protein A (Ssl2-TAP) (IgG-agarose; Sigma). Conditions for PCRs to quantify PMA1 DNA were as described previously (46) using [α-32P]dATP in 25-μl reactions containing 1× Standard Taq Buffer (New England Biolabs). PCR products were resolved in 6% polyacrylamide, 1× TBE gels and quantified by a PhosphorImager (Amersham Biosciences). Conditions for PCRs to detect PYK1 DNA were performed as described previously (47), except that PCR products were fractionated in 1.5% agarose gels and visualized by ethidium bromide staining using an Alphalmager 2000 (34). The immunoprecipitate/input ratio of each gene-specific product was normalized to the immunoprecipitate/input ratio of an intergenic region of chromosome V (for PMA1 ChIP) or to the non-transcribed HMR gene (for PYK1 ChIP). Accordingly, the numbers on the y axis depict the -fold enrichment of the ChIP signal over the background signal such that y = 1 represents background. All PCR primer pairs are identical to those described previously (46) except for the HMR primers (48).

Chromosome Conformation Capture—DNA loops were analyzed by a modified version of 3C (49, 50), as described previously (51). Chromatin was extracted and digested overnight at 37 °C with gentle shaking in the presence of the restriction enzyme HindIII (New England Biolabs). Juxtaposition of the altered forms of the largest subunit of TFIIF, as suppressors
of the sua7-1 Csm phenotype; an allele of the RPB2 was also isolated as a suppressor of the related sua7-3 mutation (R78C). In light of new information about TFIIB, including its association with terminator regions and its role in gene looping, we have isolated new suppressors of sua7-1 in an effort to more fully understand the role of TFIIB in the transcription cycle.

Strain YMH1138 (Table 1) was isolated as a spontaneous Csm+/H11001 revertant of YMH893 (MATα sua7-1) at 12°C; this strain also acquired a distinct Tsm− phenotype at 39°C (Fig. 1). When back-crossed to strain YMH124 (MATα sua7-1), the resulting diploid strain reacquired the Csm− and Tsm+ phenotypes of the primary mutant, indicating that both phenotypes are the result of a recessive mutation(s). The YMH1138 × YMH124 diploid strain was sporulated and dissected, and segregants were scored for growth at 12, 30, and 39°C. The Csm− and Tsm− co-segregated, thereby defining Tsm− as a pleiotropic marker of the sua7-1 suppressor.
To identify the suppressor gene in strain YMH1138, a YCp50 genomic library (URA3) was introduced into YMH1138, and Ura⁺ transformants were selected at 39 °C. Two Tsm⁺ transformants were isolated that were also Csm−. When cured of plasmid DNA, the Csm⁺ and Tsm⁺ phenotypes were restored, indicating that complementation was due to plasmid DNA rather than strain reversion. Plasmid DNA from both strains included identical 13-kb fragments encompassing five ORFs from chromosome IX. Retransformation of YMH1138 with plasmid pN861 (SSL2-URA3-CEN) established that complementation was due solely to the SSL2 (RAD25) gene.

Suppressor of sua7-1 Encodes Ssl2 H508R Replacement—Ssl2 is a subunit of TFIH and member of the SF2 family of DNA helicases. SF2 proteins are defined by seven helicase motifs, denoted I, Ia, II, III, IV, V, and VI, that form two distinct subdomains, designated H1 and H2 (Fig. 2A). To identify the Ssl2 defect that suppresses TFIIB E62K, we cloned the Ssl2 suppressor allele from strain YMH1138 by gap repair (see “Experimental Procedures”). DNA sequence analysis of the entire sssl2 open reading frame revealed a single base pair substitution encoding a histidine to arginine replacement at position 508 (H508R) (Fig. 2B). Accordingly, we designated the suppressor allele sssl2-508.

The His-508 residue lies within the DEHV nucleotide-binding motif II and motif III and is phylogenetically invariant. When modeled onto the x-ray structure of the Archaeoglobus fulgidus XPB homolog (AxFPB) (52), His-508 is located near the central groove, between the H1 and H2 helicase subdomains (Fig. 2C). The location of DNA within helicases of known structure, combined with hydroxylradical cleavage of yeast Ssl2, suggests that promoter DNA immediately downstream of the transcription bubble lies in the central groove between the two helicase subdomains (10). From this data, it appears likely that the functional interaction between TFIIB and TFIH involves the helicase activity of Ssl2, affecting the structure of the PIC downstream of the Pol II active center.

Phenotypes Associated with sssl2 Mutants—We next asked whether the sssl2-508 mutation confers cell growth phenotypes independent of the sua7-1 primary mutation. We constructed an isogenic SSL2 (YMH1141) and sssl2-508 (YMH1143) strain pair in a SLA7 wild type background by plasmid shuffle (see “Experimental Procedures”). The sssl2-508 mutant is viable and, in contrast to the sua7-1 sssl2-508 suppressor strain, does not exhibit a Tsm− phenotype (Fig. 3A). Thus, the Tsm− phenotype of the sua7-1 sssl2-508 strain is synthetic, dependent upon both mutations, a result that underscores the functional interaction between TFIIB and Ssl2. This interaction does not necessarily reflect a direct TFIIB-Ssl2 physical interaction because these two components of the PIC interact with distinct regions of promoter DNA and Pol II (10).

We also assayed growth of the sssl2-508 mutant on medium containing galactose as the sole carbon source and on medium containing 6-azauracil. A Gal− phenotype is often associated with defects in Pol II transcription initiation or termination (53), whereas sensitivity to 6-azauracil is associated with defects in elongation (54, 55). The sssl2-508 mutant exhibited no discernable sensitivity to 6-azauracil but displayed a distinct Gal− phenotype (Fig. 3A). The previously isolated sssl2-rrt (E556K), sssl2-DEAD (V490A/H491D), and sssl2-2 (W427L) mutants, encoding the indicated (in parentheses) amino acid replacements within the Ssl2 helicase domain (56, 57), also display Gal− phenotypes. We also note, however, that the sssl2-XP mutant, which encodes a C-terminal truncation of Ssl2 that was designed to mimic the truncated form of XPB from patients suffering from xeroderma pigmentosum (58), displays a mild Gal− phenotype (Fig. 3B).

The sssl2-508 Suppressor Affects Transcription Start Site Selection—TFIIB Glu-62 and Arg-78 lie on opposite sides of the B-finger, forming a salt bridge at the base of the finger (16, 23). In addition to conferring Csm− phenotypes, the sua7-1 (E62K) and sua7–3 (R78C) mutations shift start site selection downstream of normal at the ADH1 and CYC1 genes (16). The previously defined sua71 (Tfg1) and sua73 (Rpb9) suppressors of sua7-1 and sua7-3 shift initiation upstream of normal, partially restoring...
the normal initiation patterns at these genes (28, 30, 31). To determine whether the ssl2-508 suppressor exerts a similar effect on initiation, we assessed start site selection using a CYC1-lacZ reporter plasmid (Fig. 4A) (15, 45). Plasmid pM50 contains the normal CYC1 promoter and leader region fused in frame to lacZ. The E. coli LacZ gene is fused in-frame with the normal CYC1 ATG start codon. Plasmid pM107 is identical to pM50, except for a single base pair substitution (position −20 relative to the ATG) that creates a short ORF, upstream and out-of-frame with the normal CYC1 gene. The normal CYC1 transcription start site at −46 is indicated by the arrow; the downstream start site associated with the sua7-1 mutation is indicated at position −14 of pM107 (15). B, primer extension analysis of ADH1 transcription start sites. Strains (Table 1) are identical to those described in Fig. 3 plus YMH1141 (WT, lane 4) and YMH1143 (ssl2-508, lane 5). In the wild type strains (lanes 1, 4, and 6), transcription initiates equally at two sites, −37 and −27 (A of ATG is denoted +1). Transcripts initiating at −37 and −27 were quantified by determining the ratio of −37 to −27 and normalized to a ratio of 1.0 in the wild type strains.

To confirm the effect of the ssl2-508 mutation on start site selection, we mapped initiation at the ADH1 gene by primer extension. Results are shown in Fig. 4B. The wild type strain (YMH14) displayed the normal pattern of ADH1 initiation, defined by a 1:1 ratio of start sites at positions −37 and −27 (lane 1). As observed previously, the sua7-1 primary mutant showed diminished initiation at −37 and enhanced initiation downstream of −27 (lane 2). Consistent with the lacZ reporter assay (Fig. 4A), the ssl2-508 allele resulted in a modest upstream shift in the sua7-1 background (lane 3). Furthermore, the ssl2-508 allele alone, as well as the ssl2-rtt, ssl2-DEAD, and ssl2-XP mutants, exhibited upstream shifts in the SLU7 wild type background, in each case increasing the −37/−27 start site ratio from 1.0 to 1.3–2.0 (lanes 4–9). The ssl2-1 mutant, on the other hand, exhibited little or no effect on initiation (lane 10). Taken together, these results demonstrate that the Ssl2 subunit of TFIIH is an effector of start site selection, presumably in a manner dependent upon its helicase activity and via functional interaction with TFIIB.

**Ssl2 Cross-links to Promoter and Terminator Regions**—In addition to occupying the promoter, TFIIB also localizes to the 3′-ends of genes near the poly(A) site in yeast (34–36, 59) and in mammalian cells (60). To determine whether Ssl2 also occupies terminator regions, we performed ChIP of Ssl2 using a TAP-tagged Ssl2 strain (YMH1165). As expected, Ssl2 cross-links to the promoter of the PMA1 gene but also cross-links to regions 7 and 8, just downstream of the two poly(A) sites (Fig. 5). These results do not reflect the presence of a cryptic promoter because TBP does not cross-link to these regions (34). Ssl2-terminator association is not unique to PMA1 because Ssl2 also cross-links to the terminator region of the PYK1 gene (supplemental Fig. S1). To our knowledge, this is the first demonstration of Ssl2 or XBP occupancy of a terminator region, although earlier ChIP data revealed terminator occupancy of GAL-FMP27 by the Kin28 subunit of TFIIH (61).

We also asked whether Ssl2-terminator occupancy is affected by the sua7-1 mutation and by the ssl2-508 suppressor. Remarkably, sua7-1 significantly enhanced Ssl2 cross-linking to region 7 yet had no apparent effect on cross-linking to either region 1 or region 8 (Fig. 5, middle). Furthermore, the ssl2-508 suppressor restored Ssl2 cross-linking to its normal level at region 7 in the sua7-1 background (Fig. 5, right). The ssl2-508 suppressor appeared to adversely affect Ssl2 cross-linking to the promoter (region 1), although we do not know whether this effect requires the sua7-1 allele.

**Ssl2 H508R Affects TFIIB-Terminator Occupancy**—We next asked whether the ssl2-508 suppressor affects TFIIB occupancy of the PMA1 gene. Results are shown in Fig. 6. As noted above and consistent with previous results (34), TFIIB cross-links to the promoter and terminator regions in the wild type strain (Fig. 6, left); furthermore, occupancy of the terminator, but not the promoter, is diminished in the sua7-1 mutant (middle). The ssl2-508 mutation suppressed this effect, restoring TFIIB-terminator occupancy (Fig. 6, right). Thus, the sua7-1 primary mutation enhances Ssl2-terminator occupancy (Fig. 5) while diminishing TFIIB-terminator occupancy (Fig. 6), whereas the
ssl2-508 suppressor reverses both effects, diminishing Ssl2 occupancy while enhancing TFIIB occupancy. These results demonstrate that Ssl2, presumably as a component of TFIIH, occupies not only the promoter but also the terminator of a Pol II-transcribed gene and does so in a manner that involves functional interaction with TFIIB.

**Ssl2 Affects Gene Loops**—In addition to the sua7-1 effects on start site selection and TFIIB-terminator occupancy, TFIIB E62K adversely affects gene loops that juxtapose promoter and terminator regions (34). To determine whether Ssl2 also affects looping, we assayed gene loops by 3C at the BLM10, SEN1, and HEM3 genes, each of which was shown previously to form transcription-dependent promoter-terminator loops (34, 62). Following cell growth in YPD medium to midlog phase, the wild type (YM114), sua7-1 (YM893), and sua7-1 ssl2-508 (YM1138) strains were assayed for gene looping by 3C using primer pairs that are specific to promoter and terminator regions of these genes (Fig. 7A). P1-T1 PCR products were quantified by dividing P1-T1 PCR signals by control PCR signals representing an intergenic region of chromosome V (51). Consistent with earlier results, we observed a significant reduction in the P1-T1 PCR signals for all three genes in the sua7-1 mutant (Fig. 7B). At each gene, however, the looping defect is suppressed by the ssl2-508 mutation,
restoring looping signals to near wild type levels (Fig. 7, B and C). Thus, Ssl2 H508R suppresses all defects associated with the TFIIB E62K replacement, including cell growth rates, changes in transcription start site selection, diminished TFIIB-terminator association, and impaired gene looping. These results define a novel role for the Ssl2 helicase subunit of TFIIH in start site selection and underscore the intriguing, albeit ill defined, role of promoter-terminator interaction during the transcription cycle.

DISCUSSION

The results presented in this paper define a novel, functional interaction between TFIIB and the Ssl2 subunit of TFIIH. First, the ssl2-508 allele suppresses the cold-sensitive growth phenotype of the sua7-1 mutant at 12 °C. Second, the sua7-1 ssl2-508 double mutant displays a synthetic heat-sensitive growth defect at 37 °C, a phenotype dependent upon both mutations. Third, the ssl2-508 mutation affects start site selection by compensating for the downstream shift conferred by sua7-1. Fourth, TFIIB and Ssl2 occupy both the promoter and terminator regions of the PMA1 and PYK1 genes. Fifth, the sua7-1 mutation exerts reciprocal effects on TFIIB- and Ssl2-terminator occupancy, and these effects are reversed by the ssl2-508 suppressor. Finally, ssl2-508 restores gene looping in the sua7-1 mutant, an effect that could position the terminator-TFIIB-Ssl2 complex proximal to the promoter to facilitate transcription reinitiation.
Ssl2 at Promoter—Helicases perform critical roles in essentially all aspects of DNA (replication, repair, and recombination) and RNA (transcription, splicing, and translation) metabolism (63). More than 80 genes in the *S. cerevisiae* genome include conserved helicase motifs and affect a range of RNA and DNA metabolic activities. The activities of these proteins are not limited to classical helicase activity, defined as NTP-driven duplex unwinding. Instead, many SF2 helicases are processive translocases that catalyze directional movement along either single- or double-stranded nucleic acids. Although yeast Ssl2 and human XPB exhibit classical 3′→5′ DNA helicase activities, their principal roles in transcription initiation are likely to include translocase activities (64).

How does Ssl2 affect start site selection? Based on protein-DNA cross-linking data, human XPB was proposed to act as a “ratchet wrench” that rotates DNA at the leading edge of the PIC relative to a fixed upstream site (5). The ensuing torsional stress melts the promoter, enabling single-stranded template DNA to descend into the Pol II active site cleft (65, 66). Hydroxyradical cleavage data supports this model by showing that DNA at the leading edge of the promoter appears to be positioned within the groove between the two helicase subdomains of Ssl2 (10). Regions of TFIIE and TFIIF are also positioned in proximity to DNA, where they are likely to facilitate and stabilize DNA strand separation (9). X-ray structures of Pol II-TFIIB suggest that the B-reader of TFIIB, located within the active site cleft, then scans single-stranded template DNA for acceptable start sites, a conclusion consistent with the positions of TFIIB amino acid replacements that alter start site selection.

If the position of Ssl2 is “fixed” at the leading edge of the promoter, then its 3′→5′ translocase activity could generate the rotational torque required to melt the promoter and continue to feed template DNA into the active center of Pol II as TFIIB scans for start sites (Fig. 8). As suggested previously (10), DNA might be fed into the active center by a “scrunching” mechanism (67) that could generate long, single-stranded DNA loops emerging from the Pol II central cleft prior to its closure upon initiation. Whereas the TFIIB E62K replacement shifts initiation downstream of normal by scanning past the normal initiator sequence (20, 68), the Ssl2 H508R replacement could suppress this defect by impeding the translocase activity of Ssl2, compensating for the downstream shift by slowing the rate at which template DNA is fed into the active site cleft, past the TFIIB “scanner.”

Ssl2 at Terminator—TFIIB cross-links to the terminator as well as the promoter of Pol II-transcribed genes (34–36, 59, 60). The TFIIB E62K replacement, although without affect on PIC assembly (8), impairs TFIIB-terminator cross-linking as well as promoter-terminator looping (34). Remarkably, Ssl2 exhibits a pattern of cross-linking similar to that of TFIIB, occupying DNA downstream of the two poly(A) sites of *PMA1* and the terminator region of *PYK1* (Fig. 5 and supplemental Fig. S1). Moreover, Ssl2 H508R restores TFIIB E62K occupancy over the
**TFIIB-Ssl2 Functional Interaction**

![Model for Ssl2-mediated promoter melting and start site selection](image)

**FIGURE 8.** Model for Ssl2-mediated promoter melting and start site selection. The model proposes that the Ssl2 helicase subunit of TFIH, located at the leading edge of Pol II, rotates promoter DNA relative to a fixed upstream site, thereby inducing torsional stress that melts the promoter (5). Single-stranded template DNA then descends into the active site cleft, where TFIIB “scans” for acceptable start sites. The translocase activity of Ssl2 might feed template DNA through the active center by a “scrunching” mechanism (67). Amino acid replacements in the Rpb2 lobe domain or deletion of the Rpb9 subunit of Pol II, located near the leading edge of Pol II and proximal to Ssl2, might affect start site selection either by affecting Ssl2 translocase activity or by transducing their effects via TFIIF to the TFIIB-Pol II catalytic center.

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PMA1 terminator (Fig. 6) and also restores looping at the SEN1, BLM10, and HEM3 genes (Fig. 7). Thus, Ssl2 functionally interacts with TFIIB at the promoter, affecting transcription start site selection, and with TFIIB at the terminator, affecting juxtaposition of the terminator to the promoter to form gene loops.

Although we do not yet understand the molecular nature of the promoter-terminator interaction or how the 3′-end of a gene can affect start site selection, these effects are not entirely surprising or without precedent. Of particular interest, Jensen and colleagues (60) recently showed that 3′-end processing and termination of the β-globin gene stimulates transcription initiation in a manner involving recycling of Pol II and TFIIB. Although they did not assay looping at the β-globin locus, they speculate that physical interaction between the promoter and terminator regions could allow recycling by direct hand-off of Pol II and TFIIB from the terminator to the promoter (69).

There is also precedent for helicase function at Pol II terminators. Yeast Sen1 is a member of the SFI family of RNA/DNA helicases and, like Ssl2, exhibits 3′→5′ unwinding activity. Sen1 is required for termination of small nucleolar RNAs and other short transcripts as a member of the Sen1-Nrd1-Nab3 complex and also functionally interacts with the Rat1 exonuclease to promote termination of polyadenylated Pol II transcripts (70–73). Sen1 resolves R-loops (DNA-RNA hybrids) that form in the wake of Pol II, thereby enabling the Rat1 exonuclease to degrade RNA and promote termination (74). A similar function has been attributed to senataxin, the mammalian Sen1 ortholog (75). Furthermore, senataxin depletion reduces Pol II occupancy over the β-actin promoter (75), a result that resonates with cross-talk between terminator and promoter of the β-globin gene (60). Whether the Ssl2 helicase might also be involved in resolution of R-loops and how this might be related to formation or maintenance of gene loops are subjects for future investigations.

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