Evidence that the Tfg1/Tfg2 dimer interface of TFIIF lies near the active center of the RNA polymerase II initiation complex

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ABSTRACT
The ssu71 alleles of the TFG1 gene, which encodes the largest subunit of TFIIF, were isolated as suppressors of a TFIIB defect that affects the accuracy of transcription start site selection in the yeast Saccharomyces cerevisiae. Here we report that ssu71-1 also suppresses the cell growth and start site defects associated with an altered form of the Rpb1 subunit of RNA polymerase II (RNAP II). The ssu71-1 and ssu71-2 alleles were cloned and found to encode single amino acid replacements of glycine-363, either glycine to aspartic acid (G363D) or glycine to arginine (G363R). Two other charged replacements, G363E and G363K, were constructed by site-directed mutagenesis and suppress both TFIIB E62K and Rpb1 N445S, whereas neither G363A nor G363P exhibited any effect. G363 is phylogenetically conserved and its counterpart in human TFIIF (RAP74 G112) is located within the RAP74/RAP30 dimerization domain. We propose that the TFIIF dimerization domain is located in proximity to the B-finger of TFIIB near the active center of RNAP II where the TFIIB–TFIIF–RNAP II interface plays a key role in start site selection.

INTRODUCTION
Promoter-dependent transcription by RNA polymerase II (RNAP II) requires a set of general transcription factors that include the TATA-binding protein (TBP), TFIIB, TFIIE, TFIIF and TFIH (1–3). TBP binds to the TATA element of promoter DNA to nucleate assembly of the preinitiation complex (PIC), followed by binding of TFIIB both upstream and downstream of TATA. The DNA–TBP–TFIIB ternary complex forms the binding site for RNAP II, which enters the complex in association with TFIIF. TFIIE and TFIH complete PIC assembly and are required for ATP-dependent promoter melting by RNAP II (4). These steps define the pathway of PIC assembly in vitro. A similar pathway might be followed in vivo as PIC formation has been shown to proceed via formation of structural intermediates in yeast (5).

RNAP II comprises 12 subunits, encoded by the RPB1–RPB2 genes in Saccharomyces cerevisiae (6,7). The Rpb1, Rpb2, Rpb3 and Rpb11 subunits form the catalytic core of RNAP II and are the counterpart of the bacterial αβ′′′ core enzyme. High-resolution X-ray structures of 10- (lacking Rpb4/Rpb7) and 12-subunit yeast RNAP II complexes have been solved (8–10), as have minimal transcript elongation complexes (11–13). These structures, in combination with high-resolution structures of bacterial RNAPs (14), offer insight into the mechanism of transcription and a framework for the interpretation of a wealth of genetic and biochemical data.

TFIIB plays a key role in transcription initiation. The SUA7 gene, which encodes yeast TFIIB, was initially identified by mutations that alter start site selection (15). The sua7-1 and sua7-3 alleles shift initiation at the CYC1 and ADH1 promoters downstream of normal and encode, respectively, glutamate-62 to lysine (E62K) and arginine-78 to cysteine (R78C) replacements (16). TFIIB E62 replacements do not affect PIC assembly, but abolish transcription in vitro by adversely affecting RNAP II promoter clearance (5,17,18). The N-terminal domain of TFIIB forms a zinc-ribbon that interacts with TFIIF and the ‘dock’ domain of RNAP II (19,20). A phylogenetically conserved domain lies immediately downstream of...
the zinc-ribbon and is critical for accurate start site selection in yeast (2), human (21) and Archaea (22). The recent high-resolution X-ray structure of a yeast RNAP II–TFIIB complex defined the conserved domain as a ‘B-finger’ that encompasses E62 and R78. The B-finger projects into the RNAP II active center via the ‘saddle’ between the ‘clamp’ and ‘wall’ domains (20). This structure suggests that steric clash between the B-finger and nascent RNA on the saddle accounts for abortive initiation (20), a proposal consistent with the effect of the R78C replacement on promoter clearance (18).

The same genetic selection that yielded the sua7 mutants yielded the sua8 alleles of RPB1 (23). The sua7 and sua8 mutants confer similar growth defects, show identical effects on start site selection and a double sua7-1 sua8-1 mutant is synthetically lethal. These results define a functional interaction between Rpb1 and TFIIF, and imply that this interaction is critical for accurate initiation. The sua8-1 allele encodes an asparagine-445 to serine (N445S) replacement in the active center of Rpb1, which is near the B-finger in the RNAP II active center via the ‘saddle’ between the ‘clamp’ and ‘wall’ domains (20). This structure suggests that steric clash between the B-finger and nascent RNA on the saddle accounts for abortive initiation (20), a proposal consistent with the effect of the R78C replacement on promoter clearance (18).

In an effort to elucidate the mechanism of start site selection by yeast RNAPII and to determine how mutations in components of the transcription initiation machinery alter start sites, we have defined the sua7-1-encoded TFIIF mutations and analyzed these replacements in the context of crystal structures of yeast RNAP II–TFIIB and human TFIIF complexes. Our results suggest that the Tfg1–Tfg2 dimer interface of TFIIF is located within the active center of RNAP II initiation complex.

**MATERIALS AND METHODS**

**Yeast strains and nomenclature**

The yeast strains used in this study are listed in Table 1. Plasmid-shuffle strains YM904 (SUA7 tfg1::kanMX4 [TFG1-URA3]), YM910 (sua7-1 tfg1::kanMX4 [TFG1-URA3]) and YM916 (sua8-1 tfg1::LEU2 [TFG1-URA3]) were derived from isogenic strains T16 (SUA7), YDW546 (sua7-1) and YDW383 (sua8-1), respectively. The TRP1 gene was first disrupted by one-step disruption with either trpl::hphMX4 (T16, YDW546) or trpl::kanMX6 (YDW383), which were generated by PCR amplification of the drug-resistant cassettes pAG34-hphMX4 (27) or pFA6a-kanMX6 (28) with primer pairs carrying TRP1 5'- and 3'-tails. Strains were then transformed with plasmid pM435 (TFG1-URA3), followed by one-step disruption of the chromosomal TFG1 gene either with tfg1::LEU2 (sua8-1) using a tfg1::LEU2 DNA fragment that was generated by

| Table 1. List of yeast strains |
|-----------------------------|
| Strain | Genotype* | Reference |
| T16  | MATα cycl-1:5000 cycl7-67 leu3-112 ura3-52 cys2-1 | (15) |
| YDW546 | MATα cycl-1:5000 cycl7-67 leu3-112 ura3-52 cys2-1 sua7-1 | (15) |
| YDW383 | MATα cycl-1:5000 cycl7-67 leu3-112 ura3-52 cys2-1 sua8-1 | (23) |
| YMH917 | MATα cycl-5000 cycl7-67 trp5-48 his5-2 ura3-52 sua7-1 | (16) |
| YZS14 | MATα cycl-1:5000 cycl7-67 trp5-48 his5-2 ura3-52 sua7-1 sua7-1 | (25) |
| YZS45 | MATα cycl-1:5000 cycl7-67 trp5-48 his5-2 ura3-52 sua7-1 sua7-1 | (25) |
| YZS96 | MATα cycl-1:5000 cycl7-67 leu3-112 ura3-52 cys2-1 sua8-1 tfg1::LEU2 [pM482: sua7-1-URA3] | this study |
| YMH904 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::kanMX4 [pM435: TFG1-URA3-CEN] | this study |
| YMH905 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::kanMX4 [pM441: TFG1-TRP1-CEN] | this study |
| YMH906 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::kanMX4 [pM1874: trpl::G363D-TRP1-CEN] | this study |
| YMH907 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::kanMX4 [pM1876: tfg1::G363E-TRP1-CEN] | this study |
| YMH908 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::kanMX4 [pM1877: tfg1::G363K-TRP1-CEN] | this study |
| YMH909 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::kanMX4 [pM1879: tfg1::G363R-TRP1-CEN] | this study |
| YMH910 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::kanMX4 [pM1887: tfg1::G363E-TRP1-CEN] | this study |
| YMH911 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::kanMX4 [pM1890: tfg1::G363K-TRP1-CEN] | this study |
| YMH912 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::kanMX4 [pM1897: trpl::G363D-TRP1-CEN] | this study |
| YMH913 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::kanMX4 [pM1876: trpl::G363D-TRP1-CEN] | this study |
| YMH914 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::kanMX4 [pM1877: trpl::G363K-TRP1-CEN] | this study |
| YMH915 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::kanMX4 [pM1879: trpl::G363R-TRP1-CEN] | this study |
| YMH916 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::kanMX4 [pM435: TFG1-URA3-CEN] | this study |
| YMH917 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::LEU2 [pM441: TFG1-TRP1-CEN] | this study |
| YMH918 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::LEU2 [pM1874: trpl::G363D-TRP1-CEN] | this study |
| YMH919 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::LEU2 [pM1876: tfg1::G363E-TRP1-CEN] | this study |
| YMH920 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::LEU2 [pM1877: tfg1::G363K-TRP1-CEN] | this study |
| YMH921 | MATα cycl-1:5000 cycl7-67 ura3-52 leu2-3,112 cys2-2 trpl::hphMX4 tfg1::LEU2 [pM1879: tfg1::G363R-TRP1-CEN] | this study |

*The symbol sua7 or tfg1 denotes alleles of TFG1.*
KpnI–SphI digestion of plasmid pM521 or with tfg1::kanMX6 (WT and ssu71-1), which was generated from pFA6a-kanMX6 using TFG1-tailed primer pairs. Derivatives of YMH904, YMH910 and YMH916 that carry plasmid-borne TFG1+ or tfg1 alleles encoding G363 replacements were generated by transformation of the respective strains with either pM441 (TFG1+-TRP1) or site-directed derivatives of pM441 encoding TFG1 G363 replacements, followed by counter-selection of pM435 (TFG1-URA3) on 5-FOA medium. Strain YZS96 (ssu71-1 tfg1::LEU2 [tfg1-G363D]) was constructed by transformation of YDW383 with plasmid pM482 [tfg1-G363D-URA3], followed by one-step disruption of TFG1, using plasmid pM521, as described above.

Standard yeast nomenclature is used throughout to designate genotypes and phenotypes. The symbol ssu71 denotes the original alleles of the TFG1 gene (25). The symbols Csm+/− (cold sensitive) and Tsm+/− (heat sensitive) refer to impaired growth on yeast extract/peptone/dextrose (YPD) medium at 16 and 37°C, respectively, relative to growth on YPD at 30°C. YPD, synthetic complete (SC) and SC lacking inositol (SC−Ino) media were prepared using standard recipes (29).

Site-directed mutagenesis of TFG1

Site-directed mutations in the TFG1 gene were made by the QuickChange mutagenesis kit (Stratagene) using plasmid pM441 (TFG1-TRP1) as template DNA and complementary primer pairs that encode amino acid replacements at Tfg1 position G363. All sequence changes were confirmed by DNA sequence analysis using TFG1-specific primers.

Determination of transcript start site sites

Primer extension was performed as described previously using total RNA and the ADH1-specific primer oIP87 (15). Primer extension products were resolved in a 6% polyacrylamide DNA sequencing gel and visualized by autoradiography. Radioactive signals were quantified using ImageQuant software (Molecular Dynamics).

Retrieval of the ssu71 alleles by gap repair

The ssu71-1 and ssu71-2 alleles were cloned by gap repair from strains YZS14 and YZS45, respectively (30). Plasmid pM436 (TFG1-LEU2-2μ) was digested with BamHI and BglII, thereby creating a sequence gap in the TFG1 open reading frame (ORF), and introduced into YZS14 and YZS45. Leu+ transformants were isolated and shown to retain the Csm+ and Tsm− suppressor phenotypes. Plasmid DNA was isolated from each strain and confirmed to include the tfg1 BamHI–BglII DNA fragment; these plasmids were designated pM484 (ssu71-1) and pM538 (ssu71-2). pM484 and pM538 were reintroduced into YZS14 and YZS45 and the resulting transformants retained the suppressor phenotypes, consistent with having isolated the ssu71 suppressor mutations. The entire BamHI–BglII DNA fragments of pM484 and pM538 were sequenced and a single base pair substitution encoding different amino acid replacements of the same residue (G363D and G363R) were identified for the two clones. The TFG1 ORFs flanking the BamHI–BglII DNA fragment of each allele were subsequently amplified by PCR from YZS14 and YZS45 genomic DNA and sequenced in their entirety. No other sequence changes were found.

**Protein sequence and structural analyses**

Protein sequence comparisons were performed using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST). TFIIF crystallographic structural information was acquired through the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/). Amino acid replacements at position G112 of RAP74 were visualized using PyMol.

**RESULTS**

The ssu71 suppressors encode Tfg1 G363 amino acid replacements

Two independent ssu71 mutants were isolated as suppressors of the Csm phenotype associated with ssu71-1 (25). The ssu71-1 and ssu71-2 alleles were retrieved from strains YZS14 and YZS45 by gap repair. DNA sequence analysis of the entire ssu71 ORF revealed that both alleles are the result of single base pair substitutions encoding different amino acid replacements at the same position in the Tfg1 subunit of TFIIF: ssu71-1 encodes glycine-363 to aspartic acid (G363D), whereas ssu71-2 encodes glycine-363 to arginine (G363R) (Figure 1A). These two different replacements of the same residue underscore the importance of position G363 and point to this region of Tfg1 as playing an important role in the functional interaction between TFIIB, TFIIF and RNAPII during transcription start site selection.

Tfg1 G363 lies at the Tfg1/Tfg2 interface

The Tfg1 subunit of yeast TFIIF shows limited amino acid sequence similarity to the RAP74 subunit of human TFIIF (24,25). However, alignments based on hydrophobic cluster analysis suggested that Tfg1 residues 312–441 are structurally related to region I near the N-terminus of RAP74 (residues 66–187) (31). This alignment is also supported by domain searches using the Pfam database (32). Pfam analysis identified a single domain common to Schizosaccharomyces pombe Tfg1 and its counterpart in human TFIIF, which was generated from pFA6a-kanMX6 as described previously (25). The symbols Csm+/− (cold sensitive) and Tsm+/− (heat sensitive) refer to impaired growth on yeast extract/peptone/dextrose (YPD) medium at 16 and 37°C, respectively, relative to growth on YPD at 30°C. YPD, synthetic complete (SC) and SC lacking inositol (SC−Ino) media were prepared using standard recipes (29).

An X-ray structure for the RAP74(2-172)/RAP30(2-119) dimerization domain has been solved to 1.7 Å resolution for human TFIIF (34). This complex forms a novel ‘triple barrel’ that has been proposed to form loops and extensions important for interaction with other components of the PIC. The RAP74/RAP30 dimer interface comprises β-sheets β1, β2, β3, β6, β7 and β8 of RAP74 and the corresponding β-sheets of RAP30 (Figure 1C) (34). G112 of human RAP74 lies within the β7-sheet at the RAP74/RAP30 interface (Figure 1B and C). Thus, sequence alignments and comparison with the human RAP74/RAP30 crystal structure place G363 at the interface between Tfg1 and Tfg2. These results suggest that the Tfg1/Tfg2 dimerization region functionally interacts with TFIIB to affect transcription start site selection.
Tfg1 G363D suppresses the Rpb1 N445S replacement in the RNAP II active center

Based on the similar growth phenotypes and start site defects associated with \( \text{sua7} \) and \( \text{sua8} \) mutants, we asked whether \( \text{ssu71-1} \) would also suppress \( \text{sua8-1} \). The \( \text{sua8-1} \) allele encodes an Rpb1 N445S replacement within the active center of RNAP II (23). An isogenic \( \text{sua8-1 TFG1/SSU71} \) and \( \text{sua8-1 ssu71-1} \) strain pair was constructed as described in the Materials and Methods. The \( \text{ssu71-1} \) allele clearly suppressed \( \text{sua8-1} \), resulting in comparable growth of the \( \text{sua7-1 ssu71-1} \) and \( \text{sua8-1 ssu71-1} \) strains at 16°C (Figure 2, 16°C panel). In contrast to \( \text{sua7} \) mutants, \( \text{sua8} \) mutants are Ino− and this phenotype was also partially suppressed by \( \text{ssu71-1} \) (rows 3–5, +Ino and −Ino panels). Although \( \text{ssu71-1} \) acts as a suppressor of the Csm phenotype of \( \text{sua7-1} \), it confers a synthetic Tsm− phenotype in combination with \( \text{sua7-1} \) at 37°C (rows 1–3, 37°C panel). The \( \text{sua8-1 ssu71-1} \) double mutant also exhibited a synthetic Tsm− phenotype, although this effect is more difficult to discern owing to the inherent Tsm− phenotype of the \( \text{sua8-1} \) primary mutant (rows 4 and 5, 37°C panel). Thus, Tfg1 G363D suppresses the growth phenotypes associated with defects in both TFIIB and Rpb1. Moreover, the location of TFIIB E62 and Rpb1 N445 within the crystal structures of RNAP II and the RNAP II–TFIIB complex imply that the Tfg1/Tfg2 interface of TFIIF is positioned within the active center of the RNAP II PIC.

Suppression is specific for Tfg1 G363 charged residue replacements

In an effort to define the Tfg1 structural requirements at position 363 for suppression of TFIIB and RNAP II defects, we constructed a \( \text{TFG1} \) plasmid-shuffle strain in isogenic \( \text{SUA7} \), \( \text{sua7-1} \) (E62K) and \( \text{sua8-1} \) (N445S) genetic backgrounds and scored site-directed G363 amino acid replacements for suppression of the \( \text{sua7-1} \) and \( \text{sua8-1} \) growth phenotypes. Results are presented in Figure 3. Consistent with the phenotypes of the original \( \text{sua71} \) suppressor strains, G363D and G363R suppressed the \( \text{sua7-1} \) Csm− phenotype (cf. row 6 with rows 7 and 10 at 16°C), confirming that suppression is due solely to these replacements. Furthermore, two other charged replacements,
G363E and G363K, also suppressed the \textit{sua7-1} Csm$^-$ phenotype (rows 8 and 9). The interactions with TFIIB were not identical for all G363 replacements, however, as G363D, G363K and G363R caused synthetic Tsm$^-$ growth defects in combination with \textit{sua7-1} (rows 7, 9 and 10 at 37$^\circ$C) whereas G363E exhibited no synthetic growth defect (row 8). These suppressor (Csm$^-$) and synthetic (Tsm$^-$) growth phenotypes are consistent with those of the original YZS14 and YZS45 strains, which were initially selected based on screening Csm$^-$ revertants for a Tsm$^-$ phenotype (25). In contrast to the four charged residue replacements of G363, neither G363A nor G363P suppressed the \textit{sua7-1} Csm$^-$ phenotype (data not shown).

Although the \textit{sua8-1} mutant exhibits a less severe Csm$^-$ phenotype than the \textit{sua7-1} mutant (Figure 3, cf. rows 1, 6 and 11 at 16$^\circ$C), this phenotype is nonetheless discernable and is clearly suppressed by all four G363 replacements (cf. row 11 with rows 12–15). Comparable with their effects on \textit{sua7-1},
the G363D, G363K and G363R replacements also enhanced the sua8-1 Tsm- defect, resulting in no growth at 37°C. In contrast, but identical to its effect on sua7-1, G363E has no effect on sua8-1 at 37°C (cf. rows 11 and 13). These results underscore the functional importance of Tfg1 residue G363 and suggest that suppression by charged residue replacements might be a consequence of forming alternative salt bridges within the RNAP II active center (Discussion).

Effects of Tfg1 G363 amino acid replacements on transcription start site selection

The sua7-1 and sua8-1 alleles cause the selection of transcription start sites to shift downstream of normal in a promoter-specific manner (16,23). We therefore asked whether the Tfg1 G363 amino acid replacements also compensate for this defect. Transcription start sites at the ADH1 gene were mapped by primer extension. Results are shown in Figure 4. The sua7-1 and sua8-1 alleles shifted initiation downstream, as seen by diminished initiation at -37 and enhanced initiation at sites downstream of -27 (cf. lane 1 with lanes 6 and 11). The acidic G363D and G363E replacements suppressed the sua7-1 defect, partially restoring the normal ADH1 start site pattern (lanes 7 and 8). The most notable effects of the basic G363K and G363R replacements were to diminish the overall levels of ADH1 transcripts, although these replacements also weakly compensated for the downstream start site shift associated with sua7-1 (cf. lanes 9 and 10 with lane 6).

Somewhat different effects were observed in the sua8-1 background. In this case, the G363D and G363K replacements weakly compensated for the downstream shift and the principal effect of the G363K replacement was again to diminish overall ADH1 transcript levels (cf. lanes 12 and 14 with lane 11). Conversely, the effects of G363E and G363R on compensation of the downstream start site shift associated with sua8-1 were more pronounced (cf. lanes 13 and 15 with lane 11). Furthermore, G363E consistently enhanced overall ADH1 transcript levels.

In the otherwise wild-type background, the two negatively charged replacements, G363D and G363E, enhanced initiation upstream of normal (cf. lane 1 and lanes 2 and 3), whereas neither of the positively charged replacements had any effect (lanes 4 and 5). Thus, acidic and basic residue replacements of Tfg1 G363 exert differential effects on both the accuracy and level of ADH1 transcription in a manner dependent upon TFIIB and Rpb1. These results support our conclusion that the ‘triple barrel’ structure at the Tfg1/Tfg2 interface lies within the active center of RNAP II where it plays a critical role in the mechanism of transcription initiation.

DISCUSSION

In this study we have defined charged residue replacements of G363 in the largest subunit of TFIIF that are responsible for suppression of the Csm- growth defects and altered initiation pattern associated with TFIIB and Rpb1 defects. These results define a functional interaction among the TFIIB–Tfg1–Rpb1 components of the PIC and implicate the altered regions of these three polypeptides in the mechanism of start site selection.

The topological organization of the yeast RNAP II–TFIIF complex has been defined by cryo-electron microscopy (35). The TFIIF electron density was distributed across the surface of RNAP II and other regions of the complex. Tfg1 was deduced to interact with the Rpb4–Rpb7 subcomplex and with the mobile ‘clamp’ that swings over the active center. These images, however, are not of sufficient resolution to define specific contact points of Tfg1 or Tfg2 with the RNAP II–TFIIF structure. Our identification of Tfg1 G363 replacements as suppressors of start site defects associated with amino acid replacements of TFIIB and Rpb1 residues, both of which lie within the active center of the TFIIB–RNAP II complex, combined with comparative sequence information that places G363 at the Tfg1–Tfg2 dimer interface, make a strong case for the TFIIF dimerization region being located.
within the active center of RNAP II during initiation (Figure 5). Furthermore, these results, and those from the Ponticelli laboratory (26), implicate the triple barrel region of TFIIF as a determinant of start site selection, presumably by interacting with the B-finger–Rpb1 interface.

High-resolution protein–DNA contacts have been determined for the yeast RNAP II transcription initiation machinery (36). The results from that study revealed that Tfg1 and Tfg2 crosslinked to promoter DNA at the transcription start site. Moreover, the TFII B R78C replacement, which shifts start site selection in a manner comparable with the Tfg1 G363 replacements defined here, has no effect on TFII B–DNA interactions, but instead enhances the TFIIB–DNA crosslinking data showing Tfg1 and Tfg2 contact points at the transcription start site (36).

Figure 5. Model depicting interactions among TF IIB, TF IIF and Rpb1 at the active center of RNAP II. The sus7-1 (TF IIB E62K) and sus8-1 (Rpb1 N445S) alleles confer similar growth defects and altered patterns of transcription start site selection at ADH1. The sus7-1 and sus7-1 alleles of TF G1 encode charged residue replacements of Tfg1 G363, which is predicted to lie at the Tfg1–Tfg2 dimer interface of TF IIF (Figure 1). Based on suppression by Tfg1 G363 replacements of the cell growth and start site defects associated with the TF IIB E62K and Rpb1 N445S replacements (Figures 3 and 4), we place the TF IIF dimer interface at the active center (purple dot) of RNAP II. The dotted black triangle denotes genetic, and presumably physical (Discussion), interactions among Tfg1, TF IIB and Rpb1. This model is consistent with earlier protein–DNA crosslinking data showing Tfg1 and Tfg2 contact points at the transcription start site (36).

How do Tfg1 G363 replacements by positively or negatively charged residues suppress TF IIB E62K? The E62 residue lies within the B-finger opposite to R78 and appears to form an E62–R78 salt bridge important for maintaining the structure of the B-finger (16,20). E62K and R78C replacements confer similar growth phenotypes and start site defects, implying that the structural integrity of the B-finger is critical for accurate initiation. We suggest that Tfg1 remains positioned within the active center of the PIC where charged replacements compensate for disruption of the B-finger E62–R78 salt bridge, perhaps forming a compensatory salt bridge. For example, Tfg1 acidic replacements G363D or G363E might replace TF IIB E62, forming a salt bridge with TF IIB R78. Conversely, basic residue replacements G363K and G363R might form a salt bridge with a different, negatively charged TF IIB residue such as conserved residue D58. In either case, we propose that alternative salt bridges involving Tfg1 G363 replacements partially restore the structural integrity of the B-finger that is critical for accurate start site selection.

How then do Tfg1 G363 replacements compensate for Rpb1 N445S? The X-ray structure of an RNAP II elongation complex revealed that Rpb1 N445 directly contacts the DNA template strand at positions −1/−2, immediately upstream of the active center (11). It is interesting that this is the precise location where Tfg1 and Tfg2 crosslink to promoter DNA in a DNA–TBP–TF IIB–TF IIF–RNAP II complex (36). Moreover, these crosslink sites are altered by the TF IIB R78C replacement. Although this information does not define how Tfg1 G363 charges residue replacements suppress Rpb1 N445S it supports our conclusion that the TF IIF dimerization domain lies within the active center of the RNAP II PIC.

We wish to note that defects distal to the RNAP II active center can also affect start site selection. An altered form of the Rpb9 subunit of RNAP II was identified in the same genetic screen that identified the Tfg1 G363 suppressors (37). Furthermore, deletion of the RPB9 gene shifted transcription start site selection upstream of normal at the ADH1 gene in a manner comparable with the Tfg1 G363 replacements defined here, or with the Tfg1 E364A and W350A replacements defined previously (26,37–39). An Rpb2 replacement (G369S) located near the Rpb9 subunit conferred a similar upstream start site shift and suppressed the TF IIB R78C defect (18). These results define a functional relationship between Rpb9 and its neighboring Rpb2 region with the active center Rpb1– TF IIB–TF IIF interface. Yet in the crystal structure of yeast RNAP II the Rpb2–Rpb9 interface forms part of the upper ‘jaw-lobe’ region of RNAP II, which is distal to the active center (10). This apparent discrepancy can be explained, however, by impaired TF IIF–RNAP II association conferred by deletion of Rpb9 (26,39). Accordingly, Rpb9 is likely to play an indirect role in start site selection by altering interaction of the triple barrel within the active center of RNAP II, effectively mimicking the structural changes associated with the Tfg1 W350, G363 and E364 amino acid replacements.

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