The positions of TFIIF and TFIIE in the RNA Polymerase II transcription Preinitiation Complex

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SUPPLEMENTARY MATERIAL

Supplementary Methods

Yeast plasmids. The original tRNA/Bpa-tRNA synthetase plasmid pESC-BPZ was a gift from P. Schultz. For more efficient suppression, plasmid pESC-BPZ was modified to replace the promoter of the $tRNA_{CUA}$ gene by the 66 base *S. cerevisiae* tRNA promoter for the N(GTT)PR tRNA gene and this new plasmid is named pLH157. Plasmid pSH375 (*SUA7*, Cen, *LEU2*) was constructed by inserting the gene encoding *SUA7* (TFIIB) into pRS315[1] vector at PstI/SacI sites. This plasmid was used as the wild-type TFIIB plasmid **(Figure 1a)**. The TFIIB-Ser53 amber plasmid, pLH163, was generated through Quickchange mutagenesis (Stratagene) creating a TAG mutation at Ser53. Plasmid pHC2201 (*RPB2*, Cen, *LEU2*) was constructed by subcloning a 5.5-kbp XbaI/SalI fragment containing *RPB2* from pRP212[2] into pRS315. To create plasmid pHC2501 (*RPB2*-Myc, 2μ, *LEU2*), a 5.5-kbp NotI/SalI fragment from pHC2201 was first subcloned into pRS425[3]. Subsequently, the 414-bp of *S. cerevisiae* Adh1 promoter sequence was inserted in front of *RPB2* through PCR sewing and the 13-Myc epitope sequence was inserted at the C-terminus of *RPB2* using Quickchange mutagenesis (Stratagene) with a DNA fragment that was generated by PCR amplification of the 13-Myc epitope sequence in plasmid pFA6a-13Myc-kanMX6[4] with primer pairs carrying 5'- and 3'-sequences flanking *RPB2* C-terminus. Rpb2 amber plasmids containing single TAG codon at the mutation sites were generated by using oligonucleotide-directed phagemid mutagenesis of pHC2501. Rpb1 amber plasmids were generated through phagemid mutagenesis of pHC1601 (*RPB1*-Myc, 2μ, *LEU2*) that contains Rpb1 gene sequence originally from pRP112[5]. Similar to the Rpb2 amber plasmid, Rpb1 is also driven by the Adh1 promoter and contains the 13-Myc epitope sequence at the C-terminus. Plasmid pHC2202 (*RPB2*-Flag, Cen, *LEU2*) was generated by inserting a triple Flag tag at the C-terminus of *RPB2* in pHC2201 (Rpb2, Cen, *LEU2*) by using oligonucleotide-directed phagemid mutagenesis. Radical mutations of the Rpb2 lobe and protrusion domains were subsequently introduced to pHC2202 through phagemid mutagenesis.

Yeast Strains. The following strains were used for TFIIB study: LHy165 (*MATα ade1Δ leu2Δ his4Δ ura3Δ trp1::KanMX4 sua7::his4* [pSH375 and pLH157]); LHy167, isogenic to LHy165 except that plasmid pSH375 is replaced by pLH163 (TFIIB Ser53-amber). Each Rpb2 amber strain was generated from the shuffle strain HCy2501 through plasmid shuffling to contain both the Rpb2 amber plasmid and pLH157 plasmid. HCy2501 (*MATα ade2::hisG his3Δ200 leu2Δ lys2Δ met15Δ trp1Δ63 ura3Δ Δrpb2::kanMX4* [p*RPB2-URA3*] was generated through transformation of a Rpb2-*URA3* plasmid to the strain BY4705 [6], followed by one-step gene disruption of the chromosomal *RPB2* gene with a rpb2::KanMX4 DNA fragment that was generated by PCR amplification of the drug resistant cassette pFA6-kanMX4[7] with primer pairs carrying *RPB2* 5'- and 3'-tails. Similarly, the Rpb1 amber strain containing both Rpb1 amber plasmid and pLH157 was generated from strain HCy1601 (*MATα ade2::hisG his3Δ200 leu2Δ lys2Δ met15Δ trp1Δ63 ura3Δ Δrpb1::KanMX4* [*pRPB1-URA3*] through plasmid shuffling. The following are the strains containing triple Flag-tagged proteins: HCy1201 and HCy2201 contain a triple Flag epitope fused to the C-terminus of Rpb1 and Rpb2, respectively; HCy2202 containing Rpb2 triple Flag-tagged at the N-terminus; SHy384 and SHy564 containing a triple Flag epitope at the C-terminus of Tfg1 and Tfg2, respectively; SHy361 containing a triple Flag epitope at the C-terminus of Tfa1. The strain used for plasmid shuffling of Rpb2 lobe/protrusion mutant plasmids is HCy2501.

Antisera. Anti-Flag M2 monoclonal antibody was from Sigma, anti-Myc monoclonal antibody (9E10) and anti-Rpb1CTD monoclonal antibody (8WG16) were from Covance. Other antisera have been described previously[8,9].

TFIIF purification. The 6xHis-SUMO-TFIIF expression plasmid from above was transformed into *E. coli* strain BL21 (DE3) RIL (Stratagene). The transformed cells were grown in 2 L YT medium containing ampicillin and chloramphenicol. Expression of SUMO-TFIIF fusion protein was induced by addition of IPTG to 0.1 mM when the O.D. of cell density was around 0.8. The cells were harvested by centrifugation 4 hours after induction and the cell paste was subsequently frozen in liquid nitrogen and stored at -70 ˚C. Cells were lysed by sonication in 25 ml lysis buffer of 250 mM KCl, 10% (v/v) glycerol, 20 mM imidazole, 5 mM β-mercaptoethanol (BME), 1 mM PMSF, 0.05% (v/v) NP-40, and 20 mM Tris (pH 7.8) and the lysate was clarified by

centrifugation. After centrifugation, the supernatant was incubated with 3 ml pre-equilibrated Nickel-Sepharose (GE Healthcare) at 4 ˚C. After 1.5-hour incubation, the supernatant was removed and the Nickel-Sepharose beads were washed twice with 25 ml wash buffer containing 1 M KCl, 10% (v/v) glycerol, 5 mM BME, 1 mM PMSF, 0.05% (v/v) NP-40 and 20 mM Tris (pH 7.8). The SUMO fusion protein was eluted with 24 ml elution buffer containing 250 mM imidazole, 250 mM KCl, 10% (v/v) glycerol, 5 mM BME, 1 mM PMSF, and 20 mM Tris (pH 7.8). The eluted protein sample was dialyzed against SUMO digestion buffer 150 mM KCl, 10% (v/v) glycerol, 1 mM PMSF, 1 mM DTT, 20 mM Tris pH 7.8. The resulting protein sample was subjected to SUMO protease digestion with 8 μg protease and 1 hr incubation at RT. The digested protein sample was loaded onto a 6 ml BioRex 70 (BioRad) column pre-equilibrated with the SUMO digestion buffer. The *Sm*Tfg1/*Sc*Tfg2 heterodimer was eluted from the column with a salt gradient of 150 to 600 mM KCl. The fractions containing TFIIF were pooled and concentrated with Centriprep YM-10 concentrator (Amicon) to ~0.8 mg ml^{-1}. The typical yield of a TFIIF variant is approximately from 0.5-1mg from 2-L induction. The protein solution was frozen with liquid nitrogen and stored at -70 ˚C.

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