

Supplemental Information

Evidence that Transcript Cleavage Is Essential for RNA Polymerase II Transcription and Cell Viability

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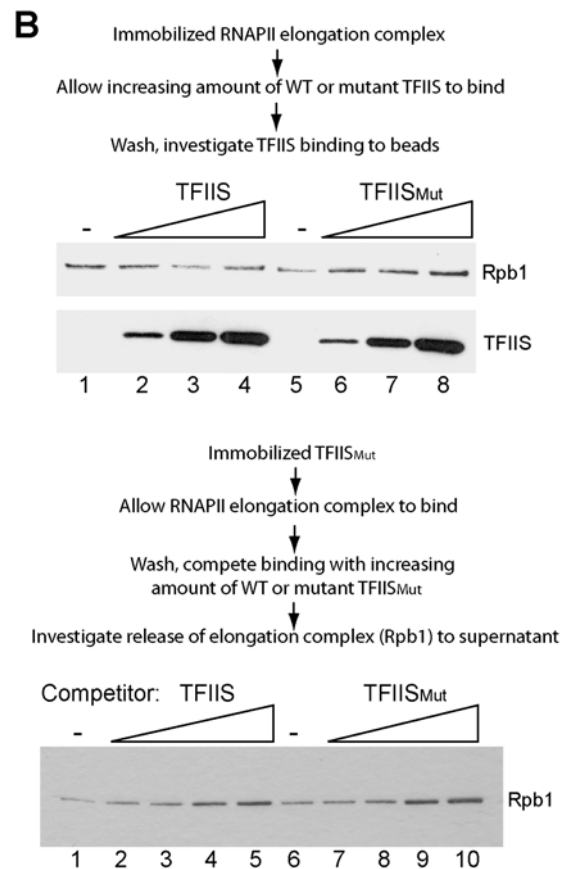
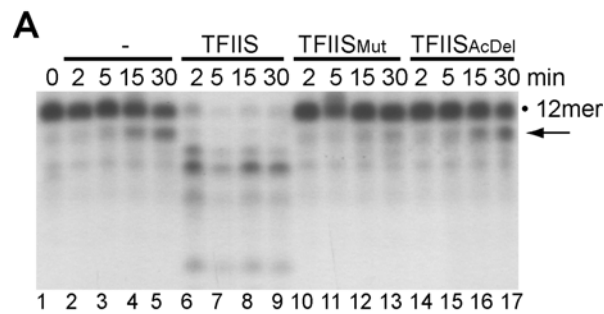


Figure S1, related to Figure 1. (A) RNAPII elongation complexes were assembled with a labelled 12-mer RNA oligonucleotide, and incubated with different TFIIS forms for the times indicated. Migration of full-length (12mer) RNA and the major intrinsic cleavage product (arrow) are indicated. See main text and main Figure 1A for details. Please note that RNAPII from *dst1Δ* cells had no tag, precluding purification of elongation complexes via RNAPII. This means that the quality of the starting material is lower. **(B)(upper)** RNAPII elongation complex, immobilized on Streptavidin-beads, was incubated with TFIIS or TFIIS_{Mut}. After washing, bound proteins were eluted in SDS-PAGE running buffer, and Western blot analysis was performed, using anti-his (TFIIS) and 4H8 (Rpb1) antibodies. As expected from previous studies on TFIIS domain structure (Awrey et al., 1998; Kettenberger et al., 2003; Wang et al., 2009), Asp₂₉₀Ala, Glu₂₉₁Ala mutation (TFIIS_{mut}) (as well as TFIIS_{AcDel} mutation (not shown)) does not affect binding to RNAPII elongation complexes. **(Lower)** TFIIS_{Mut}, biotinylated and immobilized on streptavidin beads, was incubated with RNAPII, and binding to beads competed with increasing amounts of either wild type TFIIS (lanes 2-5) or TFIISMut (7-10). Eluted RNAPII was visualized by Western blot, using 4H8 (Rpb1) antibodies. TFIIS_{mut} and wild type TFIIS compete binding to the TFIIS_{mut} beads equally well, again indicating that Asp₂₉₀Ala, Glu₂₉₁Ala mutation does not affect binding to RNAPII elongation complexes. The same experiment was also done with immobilized wild type TFIIS, with similar outcome (data not shown).

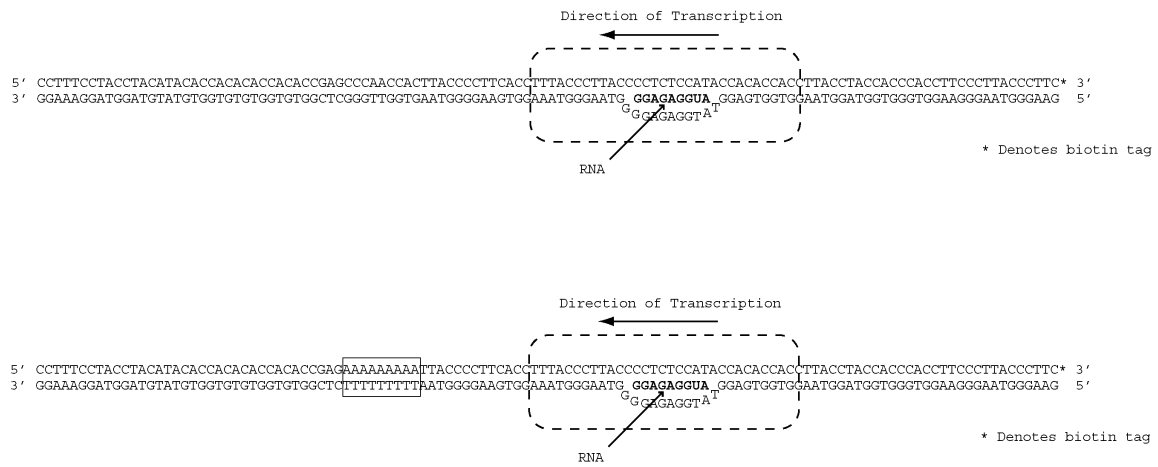


Figure S2, related to Figure 2. Schematic of reconstituted elongation complexes. Stippled line denotes RNAPII.

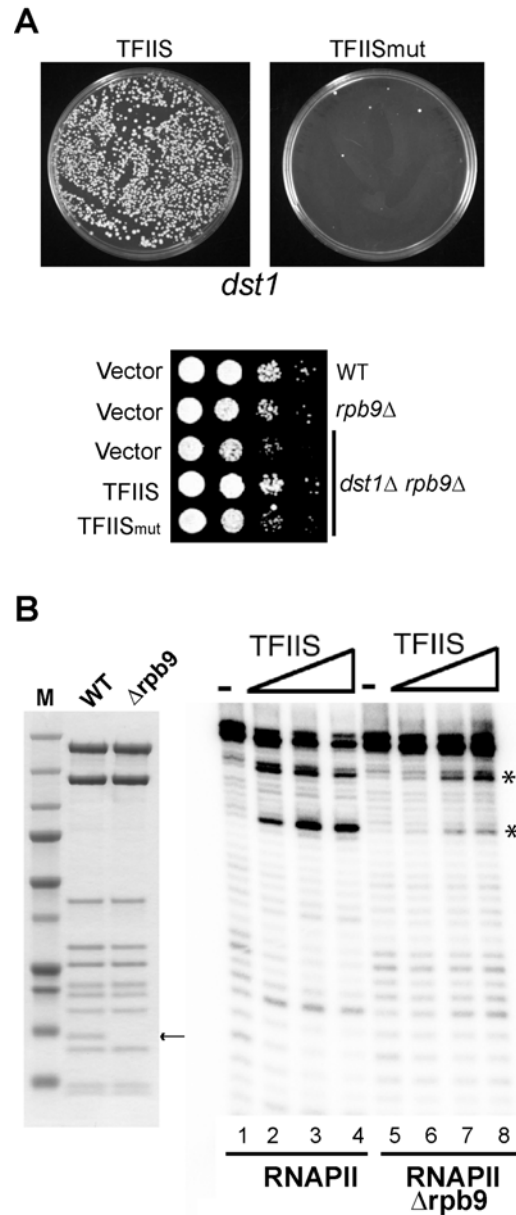


Figure S3, related to Figure 4. (A)(upper) A *dst1* Δ strain was transformed with equal amounts of *CEN* plasmids carrying either wild type TFIIS or TFIIS_{mut} (both expressed from the endogenous *DST1* promoter). A significant number of colonies fails to form with the plasmid expressing TFIIS_{mut}, unless cells also carry WT TFIIS expressed from the *GAL* promoter (and then only if grown on galactose)(See Figure 4B). (Lower) Strains of the genotype indicated on the right were transformed with TFIIS *CEN* plasmids (or a vector control), as indicated on the left, and grown on galactose plates where the difference in growth between the *rpb9* Δ and the *dst1* Δ *rpb9* Δ strain is clearer (in this experiment the difference is less obvious than in Figure 4D). TFIIS_{Mut} is not lethal in *dst1* Δ *rpb9* Δ cells, but, in contrast to WT TFIIS, fails to rescue the slower growth of this strain (compared

with *rpb9Δ*). **(B)** RNAPII, or RNAPII lacking the Rpb9 subunit (shown after SDS-PAGE and Coomassie staining on the left), were allowed to transcribe (in the absence of CTP) to the first guanine in the immobilized template strand (see Figure 1B). Nucleotides were removed, and TFIIS (5, 25, 50 ng) added to induce transcript cleavage. Asterisks indicate the major transcript cleavage products. Note that very little transcript cleavage is seen with RNAPII Δ rpb9 under conditions where substantial TFIIS-promoted transcript cleavage is observed with wild type RNAPII (compare lanes 2-4 and 6-8).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Yeast strains and plasmids

All *Saccharomyces cerevisiae* strains used in this study were grown and manipulated using standard techniques (Sherman, 1991), and were congenic with W303-1A (Thomas and Rothstein, 1989). The *dst1Δ* strain (Otero et al., 1999) and the yeast strain carrying a *GAL-VPS13* fusion gene (Kristjuhan and Svejstrup, 2004) have been described previously. The *rpb9Δ*, *dst1Δ rpb9Δ*, *rpo21-18* and *rpo21-24* strains were kindly provided by David Jansma and Jim Friesen and have also been described previously (Archambault et al., 1992; Hemming et al., 2000).

For expression of endogenous levels of TFIIS, a PCR product containing the gene along with its promoter was cloned into the *SpeI* and *HindIII* restriction sites of centromeric vector pRS415 (New England Biolabs; (Sikorski and Hieter, 1989)), generating pRS415-TFIIS. Based on this plasmid, pRS415-TFIIS_{Mut} (D₂₉₀A, E₂₉₁A) and pRS415-TFIIS_{AcDel} (D₂₉₀-E₂₉₁ deletion) was generated by site-directed mutagenesis using the QuickChange II XL mutagenesis kit (Statagene). Correct mutagenesis was confirmed by DNA sequencing.

The open reading frames of TFIIS, TFIIS_{Mut} and TFIIS_{AcDel} (from the *E. coli* plasmids below) were also cloned into the vector pYC2/CT (galactose-inducible expression)(Invitrogen). Sequences of oligonucleotides used for cloning and mutagenesis are available upon request.

Proteins

The His-TFIIS expression plasmid (Awrey et al., 1998) was a kind gift from Caroline Kane, University of California, Berkeley. TFIIS_{Mut} and TFIIS_{AcDel} were generated by site-directed mutagenesis using the same oligonucleotides described above. Correct mutagenesis was confirmed by DNA sequencing. The proteins were expressed in *E. coli* and purified via Ni-NTA Superflow (Qiagen) chromatography, followed by fractionation on MonoS HR 5/5. GST-Dsk2, immobilized on glutathione sepharose 4B, was produced as described (Anindya et al., 2007). RNAPII, RNAPII carrying a FLAG-tag on the Rpb3 subunit (RNAPII Rpb3-FLAG), and RNAPII lacking the Rpb9 subunit (RNAPII Δ rpb9) were purified from yeast as described (Cramer et al., 2000).

Oligonucleotides used for elongation complex formation

Sequences of the DNA oligonucleotides were: (template/transcribed strand) 5'CCTTTCCTACCTACATACACCACACACCGAGCCCAACCACTTACC CCTTCACCTTTACCCTTACCCCTCTCCATACCACACCACCCTACCTACCACC CACCTTCCCTTACCCTTC* 3' (where asterisk denotes biotin tag), and (non-transcribed strand) 5'GAAGGGTAAGGGAAGGTGGGTGGTAGGTAGGGTGGTGTGGTATGGAGA GGGGTAAGGGTAAAGGTGAAGGGGTAAGTGGTTGGGCTCGGTGTGGTGTG

TGGTGTATGTAGGTAGGAAAGG3'. The sequence of the RNA oligonucleotides were (9mer) 5' AUGGAGAGG 3', and (12mer) 5' GGAGAGGGGUAA 3'. The sequences of the A/T-tract DNA oligonucleotides were: 5'CCTTTCCTACCTACATACACCACACACCGAGAAAAAAAAATTACCCCTTCACCTTTACCCTTACCCCTCTCCATACACACACCTTACCTACCACCCACCTTCCCTTACCCTTC3', and 5'GAAGGGTAAGGGAAGGTGGGTGGTAGGTAGGGTGGTGTGGTATGGAGAGGGGTAAGGGTAAAGGTGAAGGGGTAAATTTTTTTTCTCGGTGTGGTGTGTGGTGTATGTAGGTAGGAAAGG 3', using the same 9mer RNA oligonucleotide.

SUPPLEMENTAL REFERENCES

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