Genetic Interactions of *DST1* in *Saccharomyces cerevisiae* Suggest a Role of TFIIS in the Initiation-Elongation Transition

Francisco Malagon,* Amy H. Tong,[†,‡] Brenda K. Shafer* and Jeffrey N. Strathern*,[1]

**Gene Regulation and Chromosome Biology Laboratory, National Cancer Institute, Frederick, Maryland 21702, [†]Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario M5G 1L6, Canada and [‡]Department of Medical Genetics and Microbiology, University of Toronto, Toronto, Ontario M5S 1A8, Canada*

Manuscript received August 5, 2003
Accepted for publication December 17, 2003

ABSTRACT

TFIIS promotes the intrinsic ability of RNA polymerase II to cleave the 3′-end of the newly synthesized RNA. This stimulatory activity of TFIIS, which is dependent upon Rpb9, facilitates the resumption of transcription elongation when the polymerase stalls or arrests. While TFIIS has a pronounced effect on transcription elongation *in vitro*, the deletion of *DST1* has no major effect on cell viability. In this work we used a genetic approach to increase our knowledge of the role of TFIIS *in vivo*. We showed that: (1) *dst1* and *rpb9* mutants have a synthetic growth defective phenotype when combined with *fyv4*, *gim5*, *htz1*, *yal011w*, *ybr231c*, *soh1*, *vps71*, and *vps72* mutants that is exacerbated during germination or at high salt concentrations; (2) TFIIS and Rpb9 are essential when the cells are challenged with microtubule-destabilizing drugs; (3) among the *SDO* (*s*ynthetic with *D*st *o*ne), *SOH1* shows the strongest genetic interaction with *DST1*; (4) the presence of multiple copies of *TAF14*, *SUA7*, *GAL11*, *RTS1*, and *TYS1* alleviate the growth phenotype of *dst1 soh1* mutants; and (5) *SRB5* and *SIN4* genetically interact with *DST1*. We propose that TFIIS is required under stress conditions and that TFIIS is important for the transition between initiation and elongation *in vivo*.

PREMESSENGER RNA transcription in eukaryotes is driven by the RNA polymerase II (RNApol II) and can be divided into initiation, elongation, and termination (Bentley 1995; Greenblatt 1997). The association of a number of factors with RNApol II occurs via the carboxy terminal domain (CTD) of the Rpb1/Rpo21 subunit of the polymerase (Allison and Ingles 1989; Scafe *et al.* 1990) and thus the CTD plays a major role in the transition and regulation of all the steps, along with RNA processing (Neugebauer and Roth 1997; Bentley 1999). The RNApol II is preassembled with the mediator and other polypeptides into a large complex called the holoenzyme (Kim *et al.* 1994). The mediator integrates and transmits information to the polymerase via CTD, mediating both activation and repression of transcription initiation (Flanagan *et al.* 1991; Koleske and Young 1995; Myers and Kornberg 2000; Davis *et al.* 2002), and at some promoters can be recruited before the loading of the core polymerase (Bhoite *et al.* 2001; Cosma *et al.* 2001; Park *et al.* 2001). RNApol II usually is paused after polymerization of the first nucleotides. These initial pauses are overcome by the action of transcriptional activators (Yankulov *et al.* 1994; Bentley 1995; Mason and Lis 1997; Tang *et al.* 2000) concomitantly with an increase of the phosphorylation level of the CTD and a dissociation of the mediator (Cadena and Dahmus 1987; Weeks *et al.* 1993; O'Brien *et al.* 1994; Otero *et al.* 1999).

The RNA polymerization rate during transcription elongation is dependent on the DNA context and on the action of transcription elongation factors (Uptain *et al.* 1997; Krogan *et al.* 2002; Svejstrup 2002; Shilatifard *et al.* 2003). While the mediator and other initiation factors are dissociated from the RNApol II during promoter clearance, elongation factors are associated with the promoters and travel along the open reading frames (ORFs) during elongation (Zawel *et al.* 1995; Svejstrup *et al.* 1997; Pokholok *et al.* 2002). The mechanism of action of those factors is heterogeneous (Shilatifard 1998). Some factors help the elongation complex to overcome nucleosome barriers (Orphanides *et al.* 1999), speed the polymerization rate up and/or down (Bengal *et al.* 1991; Chafin *et al.* 1991; Hartzog *et al.* 1998; Wada *et al.* 1998), or reactivate arrested polymerases concomitantly to transcript cleavage (Wind and Reines 2000).

TFIIS is an archetypical transcription elongation factor that is highly conserved among eukaryotes and is a functional homolog of the GreA and GreB factors from eubacteria (Labhart and Morgan 1998; Fish and Kane 2002). TFIIS (also called factor SII and P37) was biochemically isolated as an RNApol II transcription stimulatory factor from mice (Sekimizu *et al.* 1979; Ueno *et al.* 1979), humans (Reinberg and Roeder 1987), cow

[1]*Corresponding author:* National Cancer Institute, Bldg. 539, Rm. 151, P.O. Box B, Frederick, MD 21702-1201. E-mail: strather@ncifcrf.gov

(Rappaport *et al.* 1987), and flies (Price *et al.* 1987). In yeast, it was originally isolated as an RNApol II transcription elongation factor named P37 (Sawadogo *et al.* 1980, 1981). TFIIS promotes the reactivation of the RNApol II at arrest sites and was suggested to not have a major role in initiation (Rappaport *et al.* 1987; Reinberg and Roeder 1987; Reines *et al.* 1989). RNApol II pauses are classified as arrests if TFIIS is essential for reactivation of elongation and as stalls if TFIIS is not required. TFIIS induces endonucleolytic cleavage, usually releasing dinucleotides if the polymerase is stalled and four or more nucleotides if arrested (Gu *et al.* 1993; Izban and Luse 1993a,b). The arrested RNA polymerases are formed after backtracking and extrusion of the 3′-end of the RNA from the catalytic center (Reeder and Hawley 1996; Komissarova and Kashlev 1997; Kireeva *et al.* 2000b) and the reactivation of RNApol II involves the stimulation by TFIIS of the intrinsic RNA cleavage activity of the polymerase (Rudd *et al.* 1994; Kettenberger *et al.* 2003; Weilbaecher *et al.* 2003). Blockages of RNA polymerases by failures in elongation not only alter gene expression but also can result in genome instability (Reines *et al.* 1999; Aguilera 2002).

In *Saccharomyces cerevisiae* TFIIS is encoded by the *DST1* gene, also known as *PPR2* (Hubert *et al.* 1983; Clark *et al.* 1991; Archambault *et al.* 1992; Nakanishi *et al.* 1992). With the cloning of the genes or cDNAs it became evident that the factors SII, P37, IIS, and, surprisingly, a factor called DNA strand transfer α (DSTα; Sugino *et al.* 1988) were the same. The C terminus of the protein contains a zinc ribbon that defines the TFIIS domain (Qian *et al.* 1993a,b), also present in the Rpa12, Rpb9, and Rpc11 subunits of RNApol I, II, and III, respectively. TFIIS physically interacts with Rpb1 (Wu *et al.* 1996; Archambault *et al.* 1998), and mutations in *DST1* show genetic interactions with genes involved in transcription elongation such as *CTK1* (Jona *et al.* 2001), *RTF1* (Costa and Arndt 2000), *SPT5* (Lindstrom and Hartzog 2001), *SPT16* (Orphanides *et al.* 1999), or *RPB1* (Archambault *et al.* 1992).

In spite of all the evidence pointing to the importance of TFIIS in transcription, a *dst1* knockout is viable. Moreover, aside from sensitivity to nucleotide-depleting drugs, *dst1* cells have no apparent growth defect under several different conditions (Exinger and Lacroute 1992; Nakanishi *et al.* 1992). In this study we have undertaken a genetic approach to gain further insight into the role of TFIIS *in vivo* in the yeast *S. cerevisiae*. First, to find factors required for cell growth in the absence of TFIIS, we performed a synthetic genetic array (SGA) analysis of a *dst1* knockout against the nonessential gene yeast knockout collection. This approach is complementary to a synthetic lethal screen carried out previously (Davie and Kane 2000). Second, we focused on the characterization of the strongest synthetic interaction uncovered by the SGA.

MATERIALS AND METHODS

Media and growth conditions: Standard media such as rich medium YEPD or synthetic complete (SC) medium with bases and amino acids omitted as specified and sporulation medium were prepared according to standard procedures (Sherman *et al.* 1986). AA synthetic media are similar to SC but contain all 20 amino acids, myo-inositol, para-aminobenzoic acid, adenine, and uracil at a final concentration of 85 mg/liter (170 mg/liter for leucine; 17 mg/liter for para-aminobenzoic acid). Unless specified, all the chemicals were purchased from Sigma (St. Louis). Nourserothricin (Werner BioAgents), geneticin/G418, and hygromycin B were added to rich medium at concentrations of 100, 200, and 300 μg/ml, respectively (Wach *et al.* 1994; Goldstein and McCusker 1999). Benomyl, nocodazole, and thiabendazole (TBZ) were added at final concentrations of 25, 7, and 75 μg/ml, respectively, from stock solutions at 10 mg/ml in dimethyl sulfoxide (benomyl and nocodazole) and 25 mg/ml in *N,N′*-dimethylformamide (TBZ). Doxycycline was added at a final concentration of 20 μg/ml from stock solutions at 5 mg/ml in 50% ethanol. l-Canavanine sulfate and 5-fluoroorotic acid (5-FOA) were added to synthetic medium at concentrations of 100 and 500 μg/ml, respectively. Plates of SC + FOA medium were prepared by using 1 g/liter proline as the nitrogen source. Plates of 6-azauracil (6-AU) medium were prepared by using freshly made 100 mg/ml stock solutions in H_2O added to AA medium lacking uracil at concentrations of 10 or 3 mg/ml. Mycophenolic acid was added to AA complete medium at 50 μg/ml. Formamide was added to the media to final concentration of 3%. All yeast strains were grown at 30°.

Genetic analysis, manipulations, and strains: Genetic analysis and manipulations were performed according to published procedures (Orr-Weaver *et al.* 1981; Ito *et al.* 1983; Sherman *et al.* 1986; Schiestl and Gietz 1989; Rothstein 1991). Tetrads were dissected on YEPD and incubated at 30° following the growth phenotype of the colonies every day for 3–8 days.

All yeast strains used belong to or are derivatives of the *S. cerevisiae* 288C BY series of the yeast knockout collection (Giaever *et al.* 2002) and were purchased from either EURO SCARF (Frankfurt, Germany) or ResGen. All the mutations relevant to this study are replacements of the corresponding ORFs by the kanMX4, hphMX4, or natMX4 cassettes. All the strains and details of any particular strain used in this work will be provided upon request. Relevant strains are shown in Table 1.

To facilitate the analysis of synthetic interactions, we routinely changed the markers of the deletions. The kanMX4, hphMX4, and natMX4 cassettes were replaced *in vivo* in yeast by transforming with PCR fragments obtained using the oligos Cassette-i-A (GTCACCCGGCCAGCGACATGGA) and Cassette-i-B (CGAATCGACAGCAGTATAGCGACCAGCATT) and by selecting for the corresponding drug resistance. We amplify the cassettes with 358 bp 5′ to the ATG and 214 bp 3′ to the end of the ORF. We took advantage of the fact that the cassettes were made using the same promoter and terminator providing homology regions for the PCR and for homologous recombination in yeast. As templates for the PCR we used the plasmids pFA6a-kanMX4 (containing the kanMX4 cassette), pAG32 (containing the hphMX4 cassette), and pAG25 (containing the natMX4 cassette) (Wach *et al.* 1994; Goldstein and McCusker 1999).

We constructed GRY3001 to allow us to follow the 6-AU phenotype of the different mutants. Yeast strains carrying the *trp1Δ::hisG-URA3-hisG* deletion, in which 546 bp of the *TRP1* ORF plus 139 bp of the region upstream of the ATG was removed, were obtained by transforming the yeast strain GRY3000 with a *TRP1* blaster plasmid (D. Gotte and J. N.

TABLE 1

Yeast strains

Strain	Genotype[a]	Reference
BY4741	*MAT***a** *met15Δ0*	Brachmann *et al.* (1998)
BY4742	*MATα lys2Δ0*	Brachmann *et al.* (1998)
Y3656	*MATα lys2Δ0 can1Δ::MFA1pr-HIS3 mfα1Δ::MF1α1 pr-LEU2*	This work
Y3803	*MATα lys2Δ0 can1Δ::MFA1pr-HIS3 mfα1Δ::MF1α1 pr-LEU2 dst1Δ::natMX4*	This work
GRY3000	*MATα lys2Δ0 met15Δ0 dst1Δ::natMX4*	This work
GRY3001	*MATα lys2Δ0 met15Δ0 dst1Δ::natMX4 trp1Δ::hisG-URA3-hisG*	This work
GRY3002	*MATα lys2Δ0 met15Δ0 trp1Δ::hisG-URA3-hisG*	This work
GRY3003	*MATα lys2Δ0 met15Δ0 trp1Δ::hisG-URA3-hisG PtetDST1::kanMX4*	This work
GRY3004	*MATα lys2Δ0 met15Δ0 trp1Δ::hisG-URA3-hisG PtetDST1::hphMX4*	This work

[a] All the strains are *his3Δ1 leu2Δ0 ura3Δ0.*

Strathern, unpublished results) containing the *hisG-URA3-hisG* direct repeats (Alani *et al.* 1987) and selecting for Ura+ Trp− transformants. The proper integration was demonstrated genetically by cosegregation of Ura+ Trp− in tetrad analysis, papillation on FOA, and linkage to the *TRP1* locus. The loss of the *URA3* marker by direct repeat recombination can be selected by growth on FOA. Other strains carrying *trp1Δ::hisG-URA3-hisG* were made by genetic crosses with GRY3001 or derivatives.

We constructed a conditional allele of *DST1* by replacing its natural promoter from position −138 to −1 (both included) by the kanMX4 cassette, the doxycycline-sensitive tTA activator gene plus the bacterial *tetO2* promoter. This allele of *DST1* (*PtetDST1*) is expressed constitutively and can be repressed by the addition of doxycycline to the media. GRY3003 was constructed by transforming GRY3002 with a 3.9-kb PCR fragment obtained using pCM224 (Belli *et al.* 1998) and the oligos dst1tet-A (**TTGATATA TAATATCCAATTTCATTATAGGGAAATTTTCA**CAGCTGAAG CTTCGTACGCT) and dst1tet-B (**CTAGATTCTTAACATGTACC AGTACTTCCTTACTATCCAT**ACTAGTGGATCTGATAAACG) and by selecting for G418R transformants. The sequences in boldface type correspond to the region of homology with the *DST1* locus. The proper integration and the absence of mutations was monitored by PCR using oligos tet-A (AAGC GAATTTCTTATGATTT) and dst1-Bintern (CGGACTCACC TACAGTTGTC) and by subsequent sequencing of the PCR fragment. The kanMX4 cassette was replaced by the hphMX4 as described above, creating the strain GRY3004.

SGA analysis: SGA analysis was carried out as described (Tong *et al.* 2001; Jorgensen *et al.* 2002). The starting strain, Y3803, which contains a marked deletion of *DST1*, was constructed by crossing the Y3656 strain with the *MAT***a** *dst1Δ::kanMX4* strain from the yeast BY4741 knockout collection, replacing the kanMX4 cassette with the natMX4 cassette, transforming the diploid with the p4339 plasmid cut with *Eco*RI and selecting for the desired markers after sporulation. The *MATα dst1Δ::natMX4* starting strain was mated by ~4700 individual *MAT***a** xxxΔ::kanMX4 haploid deletion strains.

Oligonucleotides, plasmids, and genomic libraries: Oligonucleotides were purchased from Invitrogen (San Diego). The oligos, presented always in 5′–3′ orientation with artificial sequences introduced for cloning purposes underlined, are described below.

YEplac181 is a 2μ multicopy plasmid carrying the *LEU2* gene for selection in yeast (Gietz and Sugino 1988).

pRS415 is a centromeric plasmid carrying the *LEU2* gene for selection in yeast (Sikorski and Hieter 1989).

pRS415-SOH1 was constructed by cloning a 1.1-kb *Xba*I-*Xho*I *SOH1*-containing fragment obtained by PCR into pRS415 opened with *Xba*I and *Xho*I. PCR amplification was made using the oligos SOH1-5′XbaI (AAATCCACTATTCCATC TAGACCACACTGTCAGTATGGCAT), SOH1-3′XhoI (GAT TTGGATGGATTTCTCGAGATCTTCTAATGTCTTGC GAG), and S288C genomic DNA as template.

pRS415-soh1-nsiI* was constructed by opening pRS415-SOH1 with *Nsi*I, subsequently treating with Klenow, and religating the plasmid. The original *Nsi*I site lies in the position +213 of *SOH1* ORF.

YEplac181-SOH1 was constructed by cloning a 1.1-kb *XhoI-XbaI SOH1* fragment obtained by PCR using the oligos SOH1-5′XbaI and SOH1-3′XhoI (described above) of plasmid pRS415-SOH1 into YEplac181 opened with *SmaI* and *Xba*I. The *Xho*I site of the insert was made blunt with Klenow prior to ligation.

YEplac181-DST1 was constructed by cloning a 1.8-kb *Bam*HI-*Sal*I *DST1* fragment obtained by PCR using the oligos dst1-5′bam (AATACGACTATTCCAGGATCCCGTATGGTATAGAACCC AGAT) and dst1-3′xho1 (GATTTGGATGGATTTCTCGAG ATCTTAAATTGTATTTCTTTA) into YEplac181 opened with *Bam*HI and *Sal*I.

YEplac181-TAF14 was constructed by cloning a 1.6-kb *Eco*RI-*Hpa*I *TAF14* fragment obtained by cutting a 1.8-kb PCR using the oligos TAF14-A (TTCAGACGTCACAGGAATTCAT ATTGTTAATA) and TAF14-B (AAGAGGATTCACATGGG CAAAAT) into YEplac181 opened with *Eco*RI and *Sma*I.

YEplac181-RTS1 was constructed by cloning a 3.4-kb *RTS1* fragment obtained by PCR using the oligos RTS1-A (TTTCACGACTTGACTGTGAG) and RTS1-B (CGAAGA TATATTTGGAGAAA) into YEplac181 opened with *Sma*I. The insert was made blunt with Klenow prior to ligation.

YEplac181-SUA7 was constructed by cloning a 1.9-kb *SUA7* fragment obtained by cutting with *Nsi*I after treatment with Klenow a 2.7-kb PCR using the oligos SUA7-A (AAT GATCCGTTTTATTGG) and SUA7-B (GTTCTCGCCTAA GTCATT) into YEplac181 opened with *Pst*I and *Sma*I.

YEplac181-GAL11 was constructed by cloning a 4.4-kb *GAL11* fragment obtained by PCR using the oligos GAL11-A (GCAA AAGAAGCGGCGAGG) and GAL11-B (CGGCCTCATCAAAA CATT) into YEplac181 opened with *Sma*I. The insert was made blunt with Klenow prior to ligation.

YEplac181-TYS1 was constructed by cloning a 2.2-kb *Pst*I-*Sac*I *TYS1* fragment obtained by PCR using the oligos TYS1-A (TTCAGACGTCACAGCTGCAGCATCGGCCTCGACAC) and TYS1-B (CTGTGACGTCTGAAGAGCTCGGTGGTAA AAAAACT) into YEplac181 opened with *Pst*I and *Sac*I.

p4339 was made by cloning into pCR2.1-TOPO (Invitrogen) a PCR fragment obtained using the oligos MX4-Forward (ACATGGAGGCCCAGAATACCC) and MX4-Reverse (CAG

TATGCGACCAGCATTCAC), which provided 55 bp of homology on both sides of the cassette ORFs and using pAG25 as template.

We used two multicopy genomic libraries to isolate multicopy suppressors. The MW90 library is a YEp351-based episomic *LEU2* gene bank (Waldherr *et al.* 1993). The second library is a 2μ *URA3* library based on pHR81 (Nehlin *et al.* 1989). MW90 candidates were sequenced using oligos YEp351-A (GCGGATAACAATTTCACACAGGAAA) and YEp351-B (ATT AAGTTGGGTAACGCCAGGGTTT). pHR81 candidates were sequenced using oligos pHR81-A (AAAGGGGGATGTGCTGC AAG) and pHR81-B (CTGCCACTCCTCAATTGGATTAGTC).

RESULTS

SGA analysis reveals synthetic growth phenotype of *dst1* with deletions of *YAL011w*, *YBR231c*, *VPS72*, *SOH1*, *FYV4*, *VPS71*, *GIM5*, and *HTZ1*: To identify genes with functions related to TFIIS, we crossed a *dst1* mutant with the arrayed collection of nonessential gene knockout mutants. The resulting diploids were scored for the ability to give rise by meiosis to the corresponding double mutants by the protocol described previously (Tong *et al.* 2001). The SGA showed a reproducible synthetic growth phenotype (SGP) of a *dst1*Δ*::natMX4* allele with deletions of the following 30 ORF/genes: *YAL011w*, *YBR231c*, *YDL066w/IDP1*, *YDL068w*, *YDR485c/VPS72*, *YGL066w/SGF73*, *YGL124c/MON1*, *YGL127c/SOH1*, *YGL215w/CLG1*, *YHR013c/ARD1*, *YHR059w/FYV4*, *YHR108w/GGA2*, *YJL115w/ASF1*, *YJR145c/RPS4A*, *YJL211c*, *YLR085c/ARP6*, *YLR087c/CSF1*, *YLR114c*, *YLR174w/IDP2*, *YLR268w/SEC22*, *YLR384c/IKI3*, *YLR410w/VIP1*, *YML032c/RAD52*, *YML041c/VPS71*, *YML094w/GIM5*, *YMR035w/IMP2*, *YMR038c/LYS7*, *YNL297c/MON2*, *YOL012c/HTZ1*, and *YPR024w/YME1*.

All candidates were retested after dissection of at least 15 tetrads of each cross onto YEPD medium by visualizing the SGP. Even though some synthetic interactions with *dst1* were evident on minimal medium, such as *rad52*, or under different stress conditions, only those that showed a clear SGP in rich medium were selected for further characterization. This reduced the number of positive candidates for genetic interaction with *DST1* to eight *SDO* (*s*ynthetic growth with *D*st *o*ne) genes: *YAL011w*, *YBR231c*, *VPS72*, *SOH1*, *FYV4*, *VPS71*, *GIM5*, and *HTZ1* (see Figure 1A). Among the *SDO* genes the synthetic growth phenotype is significantly stronger in the case of *soh1* where we were hardly able to see the formation of microcolonies 5 days after the dissection of the tetrads (Figure 1A).

Deletion of both *YML094w/GIM5* and the complementary ORF *YML094c-A* shows a SGP with *dst1*. Gim5 is a subunit of the prefolding chaperone or GIM complex (Geissler *et al.* 1998; Vainberg *et al.* 1998). Some of the other subunits of this complex are Yke2/Gim1 and Gim3. To ensure that the SGP with *yml094w* mutant was due to the absence of Gim5 and not to the putative Yml094c protein, we analyzed the crosses *dst1* by *gim3* and *dst1* by *yke2*. Both *yke2* and *gim3* showed a synthetic phenotype with *dst1* (not shown).

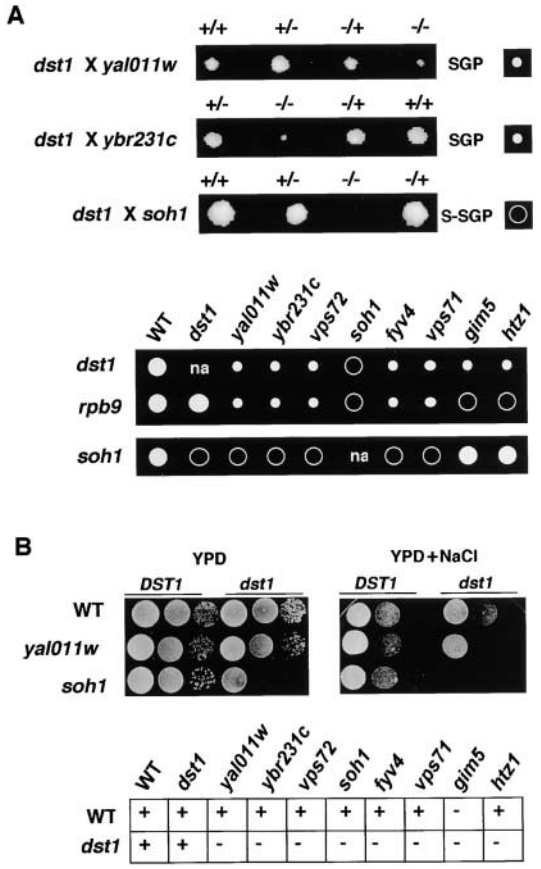

Figure 1.— SGP and salt sensitivities. (A) SGP of *dst1*, *rpb9*, and *soh1* with *sdo* mutants. (Top) Tetrads of the crosses of *dst1* by *sdo1*, *sdo2*, and *soh1*, followed by (bottom) a representation of multiple synthetic phenotypes. The absence of SGP is represented by an open circle, the presence of SGP as a small open circle, and severe SGP (S-SGP) as an outlined circumference of a black circle. na, not applicable. (B) Sensitivities to salt of single *sdo* and double *dst1 sdo* mutants. Fiftyfold dilutions for each strain were placed on YPED and YPED + 1 M NaCl and pictures were taken after 2 and 3 days, respectively.

Some mutants previously described to have a synthetic lethal phenotype with *dst1* were not uncovered by the SGA approach. Among them are *kex2*, *ctk1*, *snf2*, and *snf5* (Davie and Kane 2000; Jona *et al.* 2001). To evaluate the level of false negatives obtained by this method, we crossed *dst1* by *kex2*, *ctk1*, *snf2*, and *snf5* deletions. All the crosses showed a very clear and strong SGP but not lethality (F. Malagon and J. N. Strathern, unpublished results). Since we followed the tetrads' behavior for 5 days or more, this discordance may be due to differences in criteria. In any case, the fact that *kex2*, *ctk1*, *snf2*, and *snf5*, as well as *srb5* (see *Synthetic growth phenotype of dst1 with deletions of SRB5 and SIN4*) did not show up as positive candidates suggests that the scrutiny for nonessential synthetic lethal mutants with *dst1* is still incomplete.

We tested the significance of the SGP found with *dst1* by crossing the *sdo* mutants by an *rpb9*Δ*::hph*MX4

deletion strain. We chose this gene because Rpb9 is an RNApol II subunit and acts coordinately with TFIIS. Rpb9 is essential for TFIIS to stimulate the readthrough and transcript cleavage activity of the RNApol II (Awrey *et al.* 1997) and *rpb9* mutants are epistatic to *dst1* (Van Mullem *et al.* 2002). The double-mutant *dst1 rpb9* did not show an SGP by our criteria. The results of the crosses by *rpb9*, summarized in Figure 1A, are very similar to those obtained with *dst1* except that *rpb9* has a stronger synthetic phenotype with *gim5* and *htz1*. The synthetic interactions of *RPB9* with *YAL011w*, *YBR231c*, *VPS72*, *SOH1*, *FYV4*, *VPS71*, *GIM5*, and *HTZ1* reinforce the SGA results and also the importance of Rpb9 for TFIIS activity.

Finally, we tested the SGP during vegetative growth. We did that by plating for single colonies on rich medium of the single *dst1*, *sdoX* (any of the single *sdo* mutants), and double *dst1 sdoX* mutants and comparing the growth. All the double *dst1 sdoX* mutants showed a slight but consistent retardation of colony growth with respect to the parental strains (not shown). A remarkable exception was the *dst1 soh1* mutant, which clearly grew more slowly than the single *dst1* or *soh1* strains (see Figure 1B). It has been reported that TFIIS is recruited to the transcribed genes under stress but not under optimal growth conditions (Pokholok *et al.* 2002). This led us to test the SGP phenotype at 37° in minimal media and in high salt concentration. We found no differences for growth at 37° or in minimal media but a clear and generalized accentuation of the SGP on 1 M NaCl (Figure 1B). Neither *dst1* nor *sdoX*, with the exception of *gim5*, is sensitive to 1 M NaCl. This may indicate that TFIIS is required during germination and at high salt concentrations in the absence of the *SDO* genes and in the absence of *SOH1* during optimal growth conditions.

***dst1* mutants are sensitive to microtubule-destabilizing drugs:** To understand why the *SDO* genes show the SGP with *dst1*, we tried to find common features among them. According to the *Saccharomyces* Genome Database (http://www.yeastgenome.org/), only *GIM5* and *HTZ1* have a molecular function assigned (summarized in Figure 2A). Gim5 has tubulin-binding activity and is involved in the folding of actin and tubulin (Geissler *et al.* 1998; Vainberg *et al.* 1998) and Htz1 is a histone variant involved in silencing and regulation of transcription from RNApol II promoters (Jackson and Gorovsky 2000; Santisteban *et al.* 2000; Adam *et al.* 2001; Meneghini *et al.* 2003). *SOH1* is involved in genome stability and genetically interacts with components of the RNApol II holoenzyme (Fan and Klein 1994; Fan *et al.* 1996). *VPS71* and *VPS72* were isolated in a screening for protein-vacuolar targeting mutants (Bonangelino *et al.* 2002) and *FYV4* was isolated as sensitive to K1 killer toxin (Page *et al.* 2003).

The fact that the absence of Gim5 and other components of the GIM complex increases the requirement

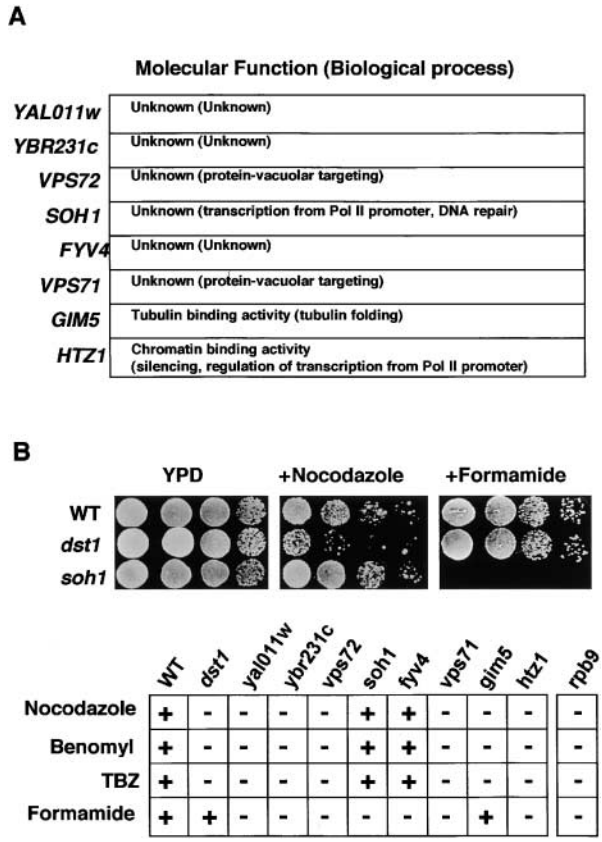

Figure 2.—Sensitivities to microtubule-destabilizing drugs and formamide of *dst1*, *rpb9*, and *sdo* mutants. (A) Molecular functions and biological processes assigned to the *SDO* genes (http://www.yeastgenome.org). (B) Sensitivity of *dst1*, *rpb9*, and *sdo* mutants to nocodazole, benomyl, TBZ, and formamide. Tenfold dilutions for each strain were placed on YPED or YEPD plus the correspondent chemical, and pictures were taken after 3 days for YEPD and YEPD + formamide and after 4 days for YPD + nocodazole, benomyl, and TBZ.

of TFIIS for cell growth may indicate that improper folding of actin and tubulin interferes with transcription. The correct microtubule and microfilament network architecture in the cell are important for a large number of different biological processes (Yarm *et al.* 2001; Barr 2002; Schott *et al.* 2002). A coordinated input-output of tubulin subunits is essential for the biological function of the microtubules for such processes as chromosome disjunction in mitosis. Actin cables have a major role in organelle transport, and actin patches function in endo- and exocytosis. Additionally, a direct interaction has been shown between the microtubules/microfilaments and mRNA distribution (Nasmyth and Jansen 1997; Takizawa *et al.* 1997; Oleynikov and Singer 1998; Beach *et al.* 1999; Jansen 1999) and also a direct role of nuclear actin in transcription elongation (Percipalle *et al.* 2003). We reasoned that the phenotypes of some of the *sdo* mutants might also be explained by a deficiency in actin/tubulin organization. This is particularly likely in the cases of *GIM5*, *VPS71*, and *VPS72*. To test this hypothesis, we checked the sensitivity

of all of them to the microtubule-destabilizing drug nocodazole. Six of the *sdo* mutants were highly sensitive to nocodazole, while the *soh1* and *fyv4* mutants were not (Figure 2B). Furthermore, *dst1* and *rpb9* mutants showed a strong hypersensitivity to nocodazole. The fact that *rpb9* is also sensitive to nocodazole, along with the described biochemical activities of TFIIS, suggests that this new phenotype for *dst1* is directly related to its role in transcription rather than to a direct structural function in microtubule organization. Similar results were obtained with other microtubule-destabilizing drugs such as benomyl and TBZ (see Figure 2B), with the only exception that *soh1* and *fyv4* mutants are slightly more resistant than the wild type to benomyl.

It has been reported that *htz1* mutants show a strong sensitivity to formamide (Jackson and Gorovsky 2000). The molecular mechanism that renders cells sensitive to formamide is unknown. We wondered whether this phenotype could be generalized to the rest of the *sdo* mutants and to *dst1*. As indicated in Figure 2B, all the *sdo* mutants except *gim5* show a strong sensitivity to formamide. Interestingly, we found discordance in the behavior of the *dst1* (resistant) and *rpb9* (sensitive) mutants for this phenotype. We used the formamide sensitivity as an experimental tool for dissecting genetic interactions with *dst1* and *soh1* (see below).

Characterization of the genetic interaction of *soh1* and *dst1*: The *SOH1* gene is highly conserved in evolution from yeast to humans. It was originally isolated as a suppressor of the thermosensitivity of *hpr1* mutants in yeast (Fan and Klein 1994) and has a role in genome integrity. In mammals, the role of Soh1 in transcription is well defined as an integral component of the mediator complexes (Gu *et al.* 1999). In Saccharomyces, *SOH1* is synthetically lethal with mutations in genes encoding two subunits of the RNApol II, Rpo21/Rpb1 and Rpb2, the transcription initiation factor II B (Sua7/TFIIB; Fan *et al.* 1996), and with the histone H3 methyl transferase factor Set2 (Krogan *et al.* 2003). We focused specifically on the interaction *DST1-SOH1* for three reasons: (1) it showed the strongest SGP; (2) *soh1* mutants are not hypersensitive to nocodazole, decreasing the probability of an indirect SGP mediated by a microtubule effect; and (3) the published data on *SOH1* suggest a direct role in RNApol II transcription.

First, we asked if *soh1* mutants show synthetic interaction with the other *sdo* genes. *soh1* has a severe SGP with *yal011w*, *ybr231c*, *vps71*, *vps72*, and *fyv4* and no synthetic phenotype with *gim5* and *htz1* (Figure 1A). Moreover, among the other mutants originally pulled out in the SGA with *dst1*, *soh1* has a SGP with *ard1*, *arp6*, and *yme1* (not shown). The common synthetic behavior of *soh1* and *dst1* with other mutants reinforces the genetic interaction between *SOH1* and *DST1*.

Second, we tested the sensitivity of *soh1* and the other *sdo* mutants to the nucleotide-depleting drug 6-azauracil and/or to mycophenolic acid. Transcription elongation

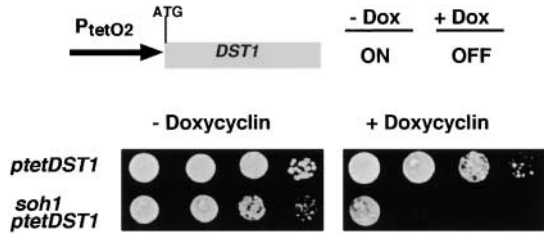

Figure 3.—Effect of the repression of *DST1* in a *soh1* mutant. Diagram of the *PtetDST1* conditional allele and effect of repression of the tet promoter with doxicyclin in a *PtetDST1* and a *PtetDST1 soh1* strain. The behavior of wild type, *dst1*, and *soh1* strains is similar to that of the single *PtetDST1* (not shown). Tenfold dilutions for each strain were placed on YPED or YEPD + doxycyclin and pictures were taken after 3 days.

mutants usually show sensitivity to one or both of those drugs due to a deficient induction of *IMD2/PUR5* (Exinger and Lacroute 1992; Shaw and Reines 2000). Neither *soh1* nor the rest of the *sdo* mutants are sensitive to 6-azauracil by our criteria and only *htz1* is sensitive to mycophenolic acid (not shown). These results argue against a direct role of *soh1* in transcription elongation.

Finally, we constructed a conditional *soh1 dst1* double mutant for subsequent genetic approaches (see next section). For this purpose, we replaced the endogenous *DST1* promoter with an artificial promoter that can be partially repressed by the drug doxycycline (see materials and methods). As expected, the addition of doxycycline to a *Ptet-DST1* strain has no noticeable growth phenotype, while repression of *Ptet-DST1* in a *soh1* background causes a severe growth phenotype (Figure 3). This phenotype can be rescued by the introduction of the *SOH1*-carrying plasmid pRS415-SOH1, but not with pRS415-soh1-nsiI* carrying a 4-bp deletion of *SOH1* (not shown).

Isolation and identification of multicopy suppressors of the synthetic growth defect of *dst1 soh1*: To understand the function of *DST1* and *SOH1* in transcription and the mechanism by which those genes are required for cell viability, we searched for genes that suppressed the synthetic growth phenotype of a *dst1 soh1* double mutant by overexpression. Such genes might have functions partially related to *DST1, SOH1*, or both. To do so, we isolated genes that suppressed the inability of a *PtetDST1 soh1* strain to grow under repression of the tet promoter. We used two different multicopy libraries (see materials and methods). After isolation and retransformation of the candidates, we selected those with a clear suppression phenotype. We also obtained several other candidates with a reproducible but very weak phenotype that we did not characterize. We sequenced the selected candidates and subcloned the genes into YEplac181 to have a single gene insert and to homogenize the multicopy vector harboring them. The genes that are able to suppress the SGP in the *PtetDST1 soh1* strain are *DST1*, *SOH1*, *TAF14*, *RTS1*, *SUA7*, *GAL11*, and

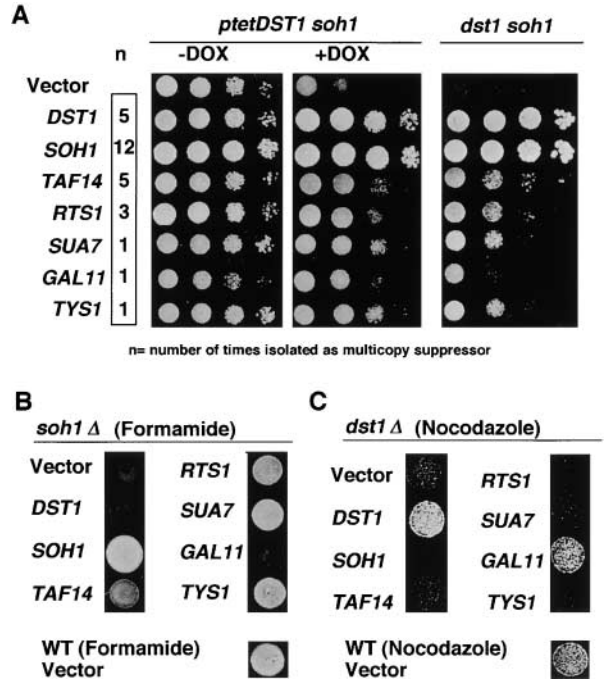

FIGURE 4.—Suppression of growth phenotypes of *dst1*, *soh1*, and *dst1 soh1* by the presence of multiple copies of *TAF14*, *RTS1*, *SUA7*, *GAL11*, or *TYS1*. (A) Growth phenotype of *PtetDST1 soh1*, under expression or repression of the tet promoter, and double *dst1 soh1* strains transformed with YEplac181 (*LEU2* containing multicopy vector) or the corresponding gene cloned in the same vector. Tenfold dilutions for each strain were placed on AA-Leu or AA-Leu + doxicyclin and pictures were taken after 3 days. (B) Sensitivity to formamide of a *soh1* strain transformed with the plasmids described in A. The spots contained ~105 cells plated on AA-Leu + formamide and the pictures were taken after 4 days. (C) Sensitivity to nocodazole of a *dst1* strain transformed with the plasmids described in A. The spots contained $\sim 5 \times 10^3$ cells plated on YEPD + formamide and the pictures were taken after 4 days.

TYS1 (see Figure 4A). To eliminate any possible artifact due to an effect on the artificial Ptet promoter, we tested the ability of all of them to suppress the SGP in a *soh1 dst1* strain. As shown in Figure 4B, all can complement the double-deletion strain.

Sua7, Taf14, and Gal11 proteins are integral components of the RNApol II holoenzyme (KOLESKE and YOUNG 1995; MYER and YOUNG 1998). *SUA7* (*s*uppressor of *u*pstream *A*TG; HAMPSEY *et al.* 1991) is an essential gene and functions in site selection for transcriptional initiation, and mutants in *SUA7* shift start-site selection downstream of normal (PINTO *et al.* 1992). *SUA7* was also isolated as a suppressor of *hpr1* (an alias of *SUA7* is *SOH4*) and point mutants of this gene are synthetic lethal with *soh1* (FAN and KLEIN 1994; FAN *et al.* 1996). Taf14/Taf30 is a promiscuous protein that physically interacts with or belongs to several components of the RNApol II or accessories complexes such as TFIID, TFIIF, Swi/Snf, and the mediator (GAVIN *et al.* 2002; SANDERS *et al.* 2002). Gal11, also found associated with several complexes of the RNApol II, has a major role in the recruitment of the holoenzyme to the promoters and is an integral component of the mediator (MYER and YOUNG 1998; LEE *et al.* 1999; SANDERS *et al.* 2002).

Rts1 is a protein serine/threonine phosphatase 2A regulatory subunit (SHU *et al.* 1997; EVANS and HEMMINGS 2000) involved in stress-related responses (EVANGELISTA *et al.* 1996; ZABROCKI *et al.* 2002). While Rts1 is not directly associated with the holoenzyme, it should be pointed out that *RTS1* (*R*OX *t*hree *s*uppressor) is a multicopy suppressor of another component of the mediator (EVANGELISTA *et al.* 1996; GUSTAFSSON *et al.* 1997). There is no obvious explanation for the isolation of *TYS1*, an essential gene that encodes a tyrosyl-tRNA synthetase with a role in tRNA export and cell wall formation (SARKAR *et al.* 1999; AZAD *et al.* 2001; DAGKESSAMANSKAIA *et al.* 2001).

We used the differential sensitivity of *dst1* and *soh1* mutants to nocodazole and formamide to define the multicopy suppressors as specific to any of the two genes or to the double-mutant interaction. Among them *SOH1*, *TAF14*, *RTS1*, *SUA7*, and *TYS1* were able to suppress, at least partially, the formamide sensitivity of a *soh1* single mutant (Figure 4B). Only *DST1* suppressed the sensitivities to 6-azauracil or mycophenolic acid of a single *dst1* mutant (not shown). Nevertheless, *GAL11* suppressed the sensitivity to nocodazole of a *dst1* mutant in spite of the toxicity of overexpressing *GAL11* (see Figure 4B).

Synthetic growth phenotype of *dst1* with deletions of *SRB5* and *SIN4*: The deletion of *DST1* can be partially suppressed by overexpression of a well-defined subunit of the mediator, Gal11, and shows synthetic growth phenotype with *soh1* mutants lacking a putative subunit of the mediator. To determine further interactions with mediator subunits, we crossed a *dst1* mutant with strains carrying deletions of *GAL11*, *HRS1/PGD1*, *SIN4*, *MED1*, *SRB8*, *SRB9*, *ROX3*, *SRB2*, and *SRB5*. Among them, *dst1* showed a severe synthetic growth phenotype with *srb5* (see Figure 5A) and a synthetic phenotype with *sin4* at 37° (see Figure 5B). The rest of the mutants did not show a synthetic phenotype with *dst1* in the conditions tested. Srb5 belongs to the Srb4 subcomplex of the mediator involved in basal transcription (THOMPSON *et al.* 1993; KIM *et al.* 1994; KANG *et al.* 2001), and Sin4 belongs to the Gal11 module of the Rgr1 subcomplex of the mediator (LI *et al.* 1995; KANG *et al.* 2001). These results extend the genetic interactions of *DST1* to other genes encoding initiation factors.

DISCUSSION

Function of the *SDO* genes in transcription: We identified new synthetic interactions of *DST1* with genes involved in transcription, vesicular transport, folding of

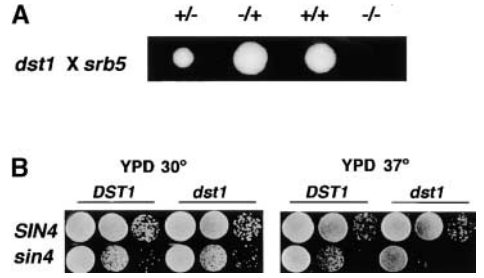

FIGURE 5.—Genetic interaction of *DST1* with *SRB5* and *SIN4*. (A) SGP of *dst1* with *srb5* mutants. We show one tetratype tetrad of the cross of *dst1* by *srb5* as an example. The picture was taken after 3 days. (B) Growth phenotype of single *dst1* and *sin4* and double *dst1 sin4* mutant. Fiftyfold dilutions for each strain were placed on YPD at 30° and 37°. The pictures were taken after 2 days.

actin and tubulin, resistance to killer toxin, and two ORFs of unknown function. Among the genes uncovered using the SGA approach, *HTZ1* and *SOH1* participate in RNApol II transcription, *GIM5* encodes for a chaperone of actin and tubulin, and the rest are very poorly characterized. The fact that all the *SDO* genes also show synthetic interaction with *RPB9*, encoding a subunit of the RNApol II, may suggest that some other genes revealed by the SGA may also have a transcription-related function.

Even though the precise molecular functions of Htz1 and Soh1 are not clear, both proteins seem to have a major role in transcription initiation. Htz1 performs redundant functions with the Swi/Snf complex (SANTISTEBAN *et al.* 2000), localizes mainly in the promoters, and has been suggested to act as a transcription activator (LAROCHELLE and GAUDREAU 2003). Several lines of evidence suggest that Soh1 belongs to the RNApol II holoenzyme. First, *SOH1* was isolated as a suppressor of *hpr1* (FAN and KLEIN 1994). Other genes isolated as suppressors of mutations of *HPR1* are components of the holoenzyme such as *SOH2/RPB2*, *SOH4/SUA7*, *HRS1/PGD1/MED3*, and *HRS2/SRB2* (SANTOS-ROSA and AGUILERA 1995; FAN *et al.* 1996; PIRUAT and AGUILERA 1996; SANTOS-ROSA *et al.* 1996; PIRUAT *et al.* 1997). Second, *soh1* mutants are synthetically lethal with mutations in *RPB1*, *RPB2*, and *SUA7* (FAN *et al.* 1996). And third, *SOH1* is a highly conserved gene found to be an integral part of the mediator in humans and in Drosophila (BOUBE *et al.* 2000; GU *et al.* 2002). The high conservation of the mediator subunits in evolution suggests that Soh1 is also a component of the mediator in yeast (ASTURIAS *et al.* 1999; DOTSON *et al.* 2000; BOUBE *et al.* 2002). Moreover, the Srb5 and Sin4 subunits of the mediator are required for cell growth in the absence of TFIIS at 30° and 37°, respectively.

Sensitivity to microtubule-destabilizing drugs and salt: The SGA and tetrad analysis of the *sdo* mutants helped us to define a new phenotype for *dst1* mutants. We found a connection with microtubule metabolism for TFIIS and showed that *dst1* and *rpb9* mutants are sensitive to nocodazole, benomyl, and TBZ. The simplest hypothesis to explain the nocodazole hypersensitivity phenotype of *dst1* is that TFIIS is required for expression of tubulin-related genes. We cannot exclude other possibilities such as a direct interaction of TFIIS with proteins controlling the modulation of microtubules/microfilaments. In this sense, a Ca^{2+}-dependent association of TFIIS with Cmd1/calmodulin has been reported (STIRLING *et al.* 1994).

The same type of hypothesis can be formulated to explain the salt sensitivity of the double mutants *dst1 sdoX*. The simplest hypothesis is that TFIIS regulates the expression of some genes involved in salt tolerance. In agreement with this idea, it has been reported that mRNA levels in general and specifically of *ENA1*, a gene required for salt tolerance, are severely affected in a *dst1* background when combined with the *rpb2-10* allele (LENNON *et al.* 1998; WIND-ROTOLO and REINES 2001).

Function of the multicopy suppressors of *soh1 dst1*: To further characterize the strong genetic interaction between *DST1* and *SOH1*, we isolated multicopy suppressors of the SGP of *dst1 soh1* mutants and found five genes that were strong suppressors without fully complementing the wild-type growth level: *GAL11*, *TAF14*, *SUA7*, *RTS1*, and *TYS1*. The isolation of *GAL11*, *TAF14*, and *SUA7*, encoding components of the RNApol II holoenzyme, as multicopy suppressors of the SGP of *dst1 soh1* mutants clearly points out that the growth defect of the double mutant is most likely due to a defect in initiation of transcription. Especially relevant is the isolation of *GAL11*, a subunit of the mediator, as a specific suppressor of *dst1*. Genetic interactions, such as suppression of *gal11* by overexpression of the CTD kinase domain of Sgv1/Bur1, suggest a role for Gal11 specifically in the initiation-elongation transition (BADI and BARBERIS 2001).

Does TFIIS participate in transcription initiation? Our genetic characterization of *dst1* mutants indicates an interaction of TFIIS and functions involved in the initiation of transcription. We and others (DAVIE and KANE 2000) have shown that the absence of Htz1, Soh1, or the Swi/Snf complex increases the requirement of TFIIS for cell viability. Surprisingly, neither DAVIE and KANE (2000) nor we were able to pull out transcription elongation genes as synthetic with *dst1*. On the other hand, overexpression of Gal11 can partially rescue phenotypes associated with the absence of TFIIS, and the growth defect of the *dst1 soh1* double mutant is suppressed by high copy number of *SUA7* or *TAF14*. Could this indicate that TFIIS has a role in initiation? Some reports show that the functional homologs of TFIIS in eubacteria, GreA and GreB, have a role in initiation of transcription (HSU *et al.* 1995; SEN *et al.* 2001). There is, to our knowledge, no molecular evidence for a role of TFIIS in initiation. Nevertheless, some data, like the

synthetic interaction of *dst1* with *kin28* point mutants (Lindstrom and Hartzog 2001), suggest that TFIIS may contact the initiation complex.

What features of initiation might require the action of TFIIS? After the abortive initiation mode is finished, there are promoter-proximal blocks in the first 50 nucleotides in the absence of gene-specific activators that may facilitate the mediator-elongator exchange (Otero *et al.* 1999). This kind of arrest or halt of the RNApol II after the synthesis of 20–40 nucleotides is a well-established mechanism of control of transcription at certain genes like *c-myc* (Strobl and Eick 1992; Krumm *et al.* 1995; Plet *et al.* 1995; Schneider *et al.* 1999), *c-fos* (Fivaz *et al.* 2000), and *DHRF* and *ACTG1* (Cheng and Sharp 2003) in humans or *hsp70* in Drosophila (Lee *et al.* 1992; Rasmussen and Lis 1995; Li *et al.* 1996; Tang *et al.* 2000). Certainly the pausing of the RNApol II during initiation is an important regulatory point in transcription of specific genes and, consequently, antiarrest activities may play a role in initiation.

A model for a role of TFIIS in the initiation-elongation transition: We imagine two mechanisms for the transition from initiation to elongation, one dependent on initiation factors like the mediator complex and another dependent upon TFIIS. In addition to a possible role in transcription elongation in stressed cells (Pokholok *et al.* 2002), we propose that TFIIS provides a mechanism to restart arrested initiation complexes in *soh1* or *srb5* mutants. We envision the arrest of the RNApol II shortly after polymerization of the first ribonucleotides as the cause of the SGP of *soh1 dst1* mutants. The overexpression of initiation factors such as TFIIB/Sua7 or Gal11 may alter the interactions in the initiation complex promoting the advance of the RNApol II toward the elongation phase of transcription. Soh1 and Srb5 may act to prevent the arrest of the polymerase by either enhancing promoter escape or inhibiting the backtracking of the polymerase.

The TFIIS activity facilitating the cleavage and readthrough of the nascent RNA may be directly required to overcome RNApol II initiation-elongation arrests. The hypothesis for this role of TFIIS *in vivo* is strongly supported by *in vitro* studies with RNApol II carried out by D. S. Luse's lab. Researchers in this lab have shown that the RNApol II is arrest prone during promoter clearance (Pal *et al.* 2001) and that this phenomenon can be recreated in promoter-distal locations by shortening the nascent RNA or by hybridizing oligonucleotides to the transcript between 30 and 45 bp upstream of the 3′-end (Ujvari *et al.* 2002). Those arrests are accompanied by upstream translocation of the RNA polymerase as shown by the increase in the proportion of long TFIIS cleavage products (Ujvari *et al.* 2002). These *in vitro* data clearly show that TFIIS promotes the resumption of arrested polymerases in the interphase between initiation and elongation of transcription. One appealing possibility is that TFIIS acts not just to reactivate arrested complexes, but also to prevent the pausing of the polymerase during initiation by avoiding the hybridization of the 5′-end of the nascent RNA with the transcribing DNA strand. The inhibition of transcription by an overextended RNA-DNA hybrid *in vitro* has been previously reported (Kireeva *et al.* 2000a). In fact, TFIIS/DSTα has a DNA strand annealing activity that theoretically can avoid the formation of the overextended RNA-DNA hybrid (Sugino *et al.* 1988; Clark *et al.* 1991; Kipling and Kearsey 1991).

We thank S. Chavez, M. Kashlev, K. Christie, M. Kireeva, and A. Rattray for the critical reading of the manuscript. We also thank A. Aguilera and D. Garfinkel for providing the genomic libraries, D. Gotte for sequencing assistance, and M. Grau and M. Mills for editorial and administrative help. This work was sponsored by the National Cancer Institute, Department of Health and Human Services. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organization imply endorsement by the U.S. government.

Note added in proof: While this article was in press, two articles showed that the proteins encoded by *VPS72*, *YAL011w*, *YBR231c*, and *VPS71* (renamed as Swc2, Swc3, Swc5, and Swc6, respectively) belong to a protein complex involved in the deposition of Htz1 at specific chromosome locations *in vivo* (N. J. Krogan, M. C. Keogh, N. Datta, C. Sawa, O. W. Ryan *et al.*, 2003, A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htzl. Mol. Cell **12:** 1565–1576; G. Mizuguchi, X. Shen, J. Landry, W. H. Wu, S. Sen *et al.*, 2004, ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science **303:** 343–348).

LITERATURE CITED

Adam, M., F. Robert, M. Larochelle and L. Gaudreau, 2001 H2A.Z is required for global chromatin integrity and for recruitment of RNA polymerase II under specific conditions. Mol. Cell. Biol. **21:** 6270–6279.

Aguilera, A., 2002 The connection between transcription and genomic instability. EMBO J. **21:** 195–201.

Alani, E., L. Cao and N. Kleckner, 1987 A method for gene disruption that allows repeated use of *URA3* selection in the construction of multiply disrupted yeast strains. Genetics **116:** 541–545.

Allison, L. A., and C. J. Ingles, 1989 Mutations in RNA polymerase II enhance or suppress mutations *in GAL4*. Proc. Natl. Acad. Sci. USA **86:** 2794–2798.

Archambault, J., F. Lacroute, A. Ruet and J. D. Friesen, 1992 Genetic interaction between transcription elongation factor TFIIS and RNA polymerase II. Mol. Cell. Biol. **12:** 4142–4152.

Archambault, J., D. B. Jansma, J. H. Kawasoe, K. T. Arndt, J. Greenblatt *et al.*, 1998 Stimulation of transcription by mutations affecting conserved regions of RNA polymerase II. J. Bacteriol. **180:** 2590–2598.

Asturias, F. J., Y. W. Jiang, L. C. Myers, C. M. Gustafsson and R. D. Kornberg, 1999 Conserved structures of mediator and RNA polymerase II holoenzyme. Science **283:** 985–987.

Awrey, D. E., R. G. Weilbaecher, S. A. Hemming, S. M. Orlicky, C. M. Kane *et al.*, 1997 Transcription elongation through DNA arrest sites. A multistep process involving both RNA polymerase II subunit Rpb9 and TFIIS. J. Biol. Chem. **272:** 14747–14754.

Azad, A. K., D. R. Stanford, S. Sarkar and A. K. Hopper, 2001 Role of nuclear pools of aminoacyl-tRNA synthetases in tRNA nuclear export. Mol. Biol. Cell **12:** 1381–1392.

Badi, L., and A. Barberis, 2001 Proteins that genetically interact with the *Saccharomyces cerevisiae* transcription factor Gal11p emphasize its role in the initiation-elongation transition. Mol. Genet. Genomics **265:** 1076–1086.

Barr, F. A., 2002 Inheritance of the endoplasmic reticulum and Golgi apparatus. Curr. Opin. Cell. Biol. **14:** 496–499.

Beach, D. L., E. D. Salmon and K. Bloom, 1999 Localization and anchoring of mRNA in budding yeast. Curr. Biol. **9:** 569–578.

Belli, G., E. Gari, M. Aldea and E. Herrero, 1998 Functional analysis of yeast essential genes using a promoter-substitution cassette and the tetracycline-regulatable dual expression system. Yeast **14:** 1127–1138.

Bengal, E., O. Flores, A. Krauskopf, D. Reinberg and Y. Aloni, 1991 Role of the mammalian transcription factors IIF, IIS, and IIX during elongation by RNA polymerase II. Mol. Cell. Biol. **11:** 1195–1206.

Bentley, D. L., 1995 Regulation of transcriptional elongation by RNA polymerase II. Curr. Opin. Genet. Dev. **5:** 210–216.

Bentley, D., 1999 Coupling RNA polymerase II transcription with pre-mRNA processing. Curr. Opin. Cell Biol. **11:** 347–351.

Bhoite, L. T., Y. Yu and D. J. Stillman, 2001 The Swi5 activator recruits the Mediator complex to the HO promoter without RNA polymerase II. Genes Dev. **15:** 2457–2469.

Bonangelino, C. J., E. M. Chavez and J. S. Bonifacino, 2002 Genomic screen for vacuolar protein sorting genes in *Saccharomyces cerevisiae*. Mol. Biol. Cell **13:** 2486–2501.

Boube, M., C. Faucher, L. Joulia, D. L. Cribbs and H. M. Bourbon, 2000 *Drosophila* homologs of transcriptional mediator complex subunits are required for adult cell and segment identity specification. Genes Dev. **14:** 2906–2917.

Boube, M., L. Joulia, D. L. Cribbs and H. M. Bourbon, 2002 Evidence for a mediator of RNA polymerase II transcriptional regulation conserved from yeast to man. Cell **110:** 143–151.

Brachmann, C. B., A. Davies, G. J. Cost, E. Caputo, J. Li *et al.*, 1998 Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast **14:** 115–132.

Cadena, D. L., and M. E. Dahmus, 1987 Messenger RNA synthesis in mammalian cells is catalyzed by the phosphorylated form of RNA polymerase II. J. Biol. Chem. **262:** 12468–12474.

Chafin, D. R., T. J. Claussen and D. H. Price, 1991 Identification and purification of a yeast protein that affects elongation by RNA polymerase II. J. Biol. Chem. **266:** 9256–9262.

Cheng, C., and P. A. Sharp, 2003 RNA polymerase II accumulation in the promoter-proximal region of the dihydrofolate reductase and gamma-actin genes. Mol. Cell. Biol. **23:** 1961–1967.

Clark, A. B., C. C. Dykstra and A. Sugino, 1991 Isolation, DNA sequence, and regulation of a *Saccharomyces cerevisiae* gene that encodes DNA strand transfer protein alpha. Mol. Cell. Biol. **11:** 2576–2582.

Cosma, M. P., S. Panizza and K. Nasmyth, 2001 Cdk1 triggers association of RNA polymerase to cell cycle promoters only after recruitment of the mediator by SBF. Mol. Cell **7:** 1213–1220.

Costa, P. J., and K. M. Arndt, 2000 Synthetic lethal interactions suggest a role for the *Saccharomyces cerevisiae* Rtf1 protein in transcription elongation. Genetics **156:** 535–547.

Dagkessamanskaia, A., H. Martin-Yken, F. Basmaji, P. Briza and J. Francois, 2001 Interaction of Knr4 protein, a protein involved in cell wall synthesis, with tyrosine tRNA synthetase encoded by *TYS1* in *Saccharomyces cerevisiae*. FEMS Microbiol. Lett. **200:** 53–58.

Davie, J. K., and C. M. Kane, 2000 Genetic interactions between TFIIS and the Swi-Snf chromatin-remodeling complex. Mol. Cell. Biol. **20:** 5960–5973.

Davis, J. A., Y. Takagi, R. D. Kornberg and F. A. Asturias, 2002 Structure of the yeast RNA polymerase II holoenzyme: mediator conformation and polymerase interaction. Mol. Cell **10:** 409–415.

Dotson, M. R., C. X. Yuan, R. G. Roeder, L. C. Myers, C. M. Gustafsson *et al.*, 2000 Structural organization of yeast and mammalian mediator complexes. Proc. Natl. Acad. Sci. USA **97:** 14307–14310.

Evangelista, C. C., Jr, A. M. Rodriguez Torres, M. P. Limbach and R. S. Zitomer, 1996 Rox3 and Rts1 function in the global stress response pathway in baker's yeast. Genetics **142:** 1083–1093.

Evans, D. R., and B. A. Hemmings, 2000 Mutation of the C-terminal leucine residue of PP2Ac inhibits PR55/B subunit binding and confers supersensitivity to microtubule destabilization in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **264:** 425–432.

Exinger, F., and F. Lacroute, 1992 6-Azauracil inhibition of GTP biosynthesis in *Saccharomyces cerevisiae*. Curr. Genet. **22:** 9–11.

Fan, H. Y., and H. L. Klein, 1994 Characterization of mutations that suppress the temperature-sensitive growth of the *hpr1*Δ mutant of *Saccharomyces cerevisiae*. Genetics **137:** 945–956.

Fan, H. Y., K. K. Cheng and H. L. Klein, 1996 Mutations in the RNA polymerase II transcription machinery suppress the hyperrecombination mutant *hpr1*Δ of *Saccharomyces cerevisiae*. Genetics **142:** 749–759.

Fish, R. N., and C. M. Kane, 2002 Promoting elongation with transcript cleavage stimulatory factors. Biochim. Biophys. Acta **1577:** 287–307.

Fivaz, J., M. C. Bassi, S. Pinaud and J. Mirkovitch, 2000 RNA polymerase II promoter-proximal pausing upregulates *c-fos* gene expression. Gene **255:** 185–194.

Flanagan, P. M., R. J. Kelleher, III, M. H. Sayre, H. Tschochner and R. D. Kornberg, 1991 A mediator required for activation of RNA polymerase II transcription *in vitro*. Nature **350:** 436–438.

Gavin, A. C., M. Bosche, R. Krause, P. Grandi, M. Marzioch *et al.*, 2002 Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature **415:** 141–147.

Geissler, S., K. Siegers and E. Schiebel, 1998 A novel protein complex promoting formation of functional alpha- and gamma-tubulin. EMBO J. **17:** 952–966.

Giaever, G., A. M. Chu, L. Ni, C. Connelly, L. Riles *et al.*, 2002 Functional profiling of the Saccharomyces cerevisiae genome. Nature **418:** 387–391.

Gietz, R. D., and A. Sugino, 1988 New yeast-*Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. Gene **74:** 527–534.

Goldstein, A. L., and J. H. McCusker, 1999 Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. Yeast **15:** 1541–1553.

Greenblatt, J., 1997 RNA polymerase II holoenzyme and transcriptional regulation. Curr. Opin. Cell Biol. **9:** 310–319.

Gu, J. Y., J. M. Park, E. J. Song, G. Mizuguchi, J. H. Yoon *et al.*, 2002 Novel Mediator proteins of the small Mediator complex in *Drosophila* SL2 cells. J. Biol. Chem. **277:** 27154–27161.

Gu, W., W. Powell, J. Mote, Jr. and D. Reines, 1993 Nascent RNA cleavage by arrested RNA polymerase II does not require upstream translocation of the elongation complex on DNA. J. Biol. Chem. **268:** 25604–25616.

Gu, W., S. Malik, M. Ito, C. X. Yuan, J. D. Fondell *et al.*, 1999 A novel human SRB/MED-containing cofactor complex, SMCC, involved in transcription regulation. Mol. Cell **3:** 97–108.

Gustafsson, C. M., L. C. Myers, Y. Li, M. J. Redd, M. Lui *et al.*, 1997 Identification of Rox3 as a component of mediator and RNA polymerase II holoenzyme. J. Biol. Chem. **272:** 48–50.

Hampsey, M., J. G. Na, I. Pinto, D. E. Ware and R. W. Berroteran, 1991 Extragenic suppressors of a translation initiation defect in the *cyc1* gene of *Saccharomyces cerevisiae*. Biochimie **73:** 1445–1455.

Hartzog, G. A., T. Wada, H. Handa and F. Winston, 1998 Evidence that Spt4, Spt5, and Spt6 control transcription elongation by RNA polymerase II in *Saccharomyces cerevisiae*. Genes Dev. **12:** 357–369.

Hsu, L. M., N. V. Vo and M. J. Chamberlin, 1995 *Escherichia coli* transcript cleavage factors GreA and GreB stimulate promoter escape and gene expression in vivo and in vitro. Proc. Natl. Acad. Sci. USA **92:** 11588–11592.

Hubert, J. C., A. Guyonvarch, B. Kammerer, F. Exinger, P. Liljelund *et al.*, 1983 Complete sequence of a eukaryotic regulatory gene. EMBO J. **2:** 2071–2073.

Ito, H., Y. Fukuda, K. Murata and A. Kimura, 1983 Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:** 163–168.

Izban, M. G., and D. S. Luse, 1993a The increment of SII-facilitated transcript cleavage varies dramatically between elongation competent and incompetent RNA polymerase II ternary complexes. J. Biol. Chem. **268:** 12874–12885.

Izban, M. G., and D. S. Luse, 1993b SII-facilitated transcript cleavage in RNA polymerase II complexes stalled early after initiation occurs in primarily dinucleotide increments. J. Biol. Chem. **268:** 12864–12873.

Jackson, J. D., and M. A. Gorovsky, 2000 Histone H2A.Z has a conserved function that is distinct from that of the major H2A sequence variants. Nucleic Acids Res. **28:** 3811–3816.

Jansen, R. P., 1999 RNA-cytoskeletal associations. FASEB J. **13:** 455–466.

Jona, G., B. O. Wittschieben, J. Q. Svejstrup and O. Gileadi, 2001 Involvement of yeast carboxy-terminal domain kinase I (CTDK-I) in transcription elongation *in vivo*. Gene **267:** 31–36.

Jorgensen, P., B. Nelson, M. D. Robinson, Y. Chen, B. Andrews *et al.*, 2002 High-resolution genetic mapping with ordered arrays of *Saccharomyces cerevisiae* deletion mutants. Genetics **162:** 1091–1099.

Kang, J. S., S. H. Kim, M. S. Hwang, S. J. Han, Y. C. Lee *et al.*, 2001 The structural and functional organization of the yeast mediator complex. J. Biol. Chem. **276:** 42003–42010.

Kettenberger, H., K. J. Armache and P. Cramer, 2003 Architecture of the RNA polymerase II-TFIIS complex and implications for mRNA cleavage. Cell **114:** 347–357.

Kim, Y. J., S. Bjorklund, Y. Li, M. H. Sayre and R. D. Kornberg, 1994 A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell **77:** 599–608.

Kipling, D., and S. E. Kearsey, 1991 TFIIS and strand-transfer proteins. Nature **353:** 509.

Kireeva, M. L., N. Komissarova and M. Kashlev, 2000a Overextended RNA:DNA hybrid as a negative regulator of RNA polymerase II processivity. J. Mol. Biol. **299:** 325–335.

Kireeva, M. L., N. Komissarova, D. S. Waugh and M. Kashlev, 2000b The 8-nucleotide-long RNA:DNA hybrid is a primary stability determinant of the RNA polymerase II elongation complex. J. Biol. Chem. **275:** 6530–6536.

Koleske, A. J., and R. A. Young, 1995 The RNA polymerase II holoenzyme and its implications for gene regulation. Trends Biochem. Sci. **20:** 113–116.

Komissarova, N., and M. Kashlev, 1997 Transcriptional arrest: *Escherichia coli* RNA polymerase translocates backward, leaving the 3′ end of the RNA intact and extruded. Proc. Natl. Acad. Sci. USA **94:** 1755–1760.

Krogan, N. J., M. Kim, S. H. Ahn, G. Zhong, M. S. Kobor *et al.*, 2002 RNA polymerase II elongation factors of *Saccharomyces cerevisiae*: a targeted proteomics approach. Mol. Cell. Biol. **22:** 6979–6992.

Krogan, N. J., M. Kim, A. Tong, A. Golshani, G. Cagney *et al.*, 2003 Methylation of histone H3 by Set2 in *Saccharomyces cerevisiae* is linked to transcriptional elongation by RNA polymerase II. Mol. Cell. Biol. **23:** 4207–4218.

Krumm, A., L. B. Hickey and M. Groudine, 1995 Promoter-proximal pausing of RNA polymerase II defines a general rate-limiting step after transcription initiation. Genes Dev. **9:** 559–572.

Labhart, P., and G. T. Morgan, 1998 Identification of novel genes encoding transcription elongation factor TFIIS (TCEA) in vertebrates: conservation of three distinct TFIIS isoforms in frog, mouse, and human. Genomics **52:** 278–288.

Larochelle, M., and L. Gaudreau, 2003 H2A.Z has a function reminiscent of an activator required for preferential binding to intergenic DNA. EMBO J. **22:** 4512–4522.

Lee, H., K. W. Kraus, M. F. Wolfner and J. T. Lis, 1992 DNA sequence requirements for generating paused polymerase at the start of *hsp70*. Genes Dev. **6:** 284–295.

Lee, Y. C., J. M. Park, S. Min, S. J. Han and Y. J. Kim, 1999 An activator binding module of yeast RNA polymerase II holoenzyme. Mol. Cell. Biol. **19:** 2967–2976.

Lennon, J. C., III, M. Wind, L. Saunders, M. B. Hock and D. Reines, 1998 Mutations in RNA polymerase II and elongation factor SII severely reduce mRNA levels in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **18:** 5771–5779.

Li, B., J. A. Weber, Y. Chen, A. L. Greenleaf and D. S. Gilmour, 1996 Analyses of promoter-proximal pausing by RNA polymerase II on the *hsp70* heat shock gene promoter in a *Drosophila* nuclear extract. Mol. Cell. Biol. **16:** 5433–5443.

Li, Y., S. Bjorklund, Y. W. Jiang, Y. J. Kim, W. S. Lane *et al.*, 1995 Yeast global transcriptional regulators Sin4 and Rgr1 are components of mediator complex/RNA polymerase II holoenzyme. Proc. Natl. Acad. Sci. USA **92:** 10864–10868.

Lindstrom, D. L., and G. A. Hartzog, 2001 Genetic interactions of Spt4-Spt5 and TFIIS with the RNA polymerase II CTD and CTD modifying enzymes in *Saccharomyces cerevisiae*. Genetics **159:** 487–497.

Mason, P. B., Jr., and J. T. Lis, 1997 Cooperative and competitive protein interactions at the *hsp70* promoter. J. Biol. Chem. **272:** 33227–33233.

Meneghini, M. D., M. Wu and H. D. Madhani, 2003 Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. Cell **112:** 725–736.

Myer, V. E., and R. A. Young, 1998 RNA polymerase II holoenzymes and subcomplexes. J. Biol. Chem. **273:** 27757–27760.

Myers, L. C., and R. D. Kornberg, 2000 Mediator of transcriptional regulation. Annu. Rev. Biochem. **69:** 729–749.

Nakanishi, T., A. Nakano, K. Nomura, K. Sekimizu and S. Natori, 1992 Purification, gene cloning, and gene disruption of the transcription elongation factor S-II in *Saccharomyces cerevisiae*. J. Biol. Chem. **267:** 13200–13204.

Nasmyth, K., and R. P. Jansen, 1997 The cytoskeleton in mRNA localization and cell differentiation. Curr. Opin. Cell Biol. **9:** 396–400.

Nehlin, J. O., M. Carlberg and H. Ronne, 1989 Yeast galactose permease is related to yeast and mammalian glucose transporters. Gene **85:** 313–319.

Neugebauer, K. M., and M. B. Roth, 1997 Transcription units as RNA processing units. Genes Dev. **11:** 3279–3285.

O'Brien, T., S. Hardin, A. Greenleaf and J. T. Lis, 1994 Phosphorylation of RNA polymerase II C-terminal domain and transcriptional elongation. Nature **370:** 75–77.

Oleynikov, Y., and R. H. Singer, 1998 RNA localization: Different zipcodes, same postman? Trends Cell Biol. **8:** 381–383.

Orphanides, G., W. H. Wu, W. S. Lane, M. Hampsey and D. Reinberg, 1999 The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. Nature **400:** 284–288.

Orr-Weaver, T. L., J. W. Szostak and R. J. Rothstein, 1981 Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA **78:** 6354–6358.

Otero, G., J. Fellows, Y. Li, T. De Bizemont, A. M. Dirac *et al.*, 1999 Elongator, a multisubunit component of a novel RNA polymerase II holoenzyme for transcriptional elongation. Mol. Cell **3:** 109–118.

Page, N., M. Gerard-Vincent, P. Menard, M. Beaulieu, M. Azuma *et al.*, 2003 A *Saccharomyces cerevisiae* genome-wide mutant screen for altered sensitivity to K1 killer toxin. Genetics **163:** 875–894.

Pal, M., D. McKean and D. S. Luse, 2001 Promoter clearance by RNA polymerase II is an extended, multistep process strongly affected by sequence. Mol. Cell. Biol. **21:** 5815–5825.

Park, J. M., J. Werner, J. M. Kim, J. T. Lis and Y. J. Kim, 2001 Mediator, not holoenzyme, is directly recruited to the heat shock promoter by HSF upon heat shock. Mol. Cell **8:** 9–19.

Percipalle, P., N. Fomproix, K. Kylberg, F. Miralles, B. Bjorkroth *et al.*, 2003 An actin-ribonucleoprotein interaction is involved in transcription by RNA polymerase II. Proc. Natl. Acad. Sci. USA **100:** 6475–6480.

Pinto, I., D. E. Ware and M. Hampsey, 1992 The yeast *SUA7* gene encodes a homolog of human transcription factor TFIIB and is required for normal start site selection *in vivo*. Cell **68:** 977–988.

Piruat, J. I., and A. Aguilera, 1996 Mutations in the yeast *SRB2* general transcription factor suppress *hpr1*-induced recombination and show defects in DNA repair. Genetics **143:** 1533–1542.

Piruat, J. I., S. Chavez and A. Aguilera, 1997 The yeast *HRS1* gene is involved in positive and negative regulation of transcription and shows genetic characteristics similar to *SIN4* and *GAL11*. Genetics **147:** 1585–1594.

Plet, A., D. Eick and J. M. Blanchard, 1995 Elongation and premature termination of transcripts initiated from *c-fos* and *c-myc* promoters show dissimilar patterns. Oncogene **10:** 319–328.

Pokholok, D. K., N. M. Hannett and R. A. Young, 2002 Exchange of RNA polymerase II initiation and elongation factors during gene expression *in vivo*. Mol. Cell **9:** 799–809.

Price, D. H., A. E. Sluder and A. L. Greenleaf, 1987 Fractionation of transcription factors for RNA polymerase II from *Drosophila* Kc cell nuclear extracts. J. Biol. Chem. **262:** 3244–3255.

Qian, X., S. N. Gozani, H. Yoon, C. J. Jeon, K. Agarwal *et al.*, 1993a Novel zinc finger motif in the basal transcriptional machinery: three-dimensional NMR studies of the nucleic acid binding domain of transcriptional elongation factor TFIIS. Biochemistry **32:** 9944–9959.

Qian, X., C. Jeon, H. Yoon, K. Agarwal and M. A. Weiss, 1993b Structure of a new nucleic-acid-binding motif in eukaryotic transcriptional elongation factor TFIIS. Nature **365:** 277–279.

Rappaport, J., D. Reinberg, R. Zandomeni and R. Weinmann, 1987 Purification and functional characterization of transcription factor SII from calf thymus. Role in RNA polymerase II elongation. J. Biol. Chem. **262:** 5227–5232.

Rasmussen, E. B., and J. T. Lis, 1995 Short transcripts of the ternary complex provide insight into RNA polymerase II elongational pausing. J. Mol. Biol. **252:** 522–535.

Reeder, T. C., and D. K. Hawley, 1996 Promoter proximal sequences modulate RNA polymerase II elongation by a novel mechanism. Cell **87:** 767–777.

Reinberg, D., and R. G. Roeder, 1987 Factors involved in specific transcription by mammalian RNA polymerase II. Transcription factor IIS stimulates elongation of RNA chains. J. Biol. Chem. **262:** 3331–3337.

Reines, D., M. J. Chamberlin and C. M. Kane, 1989 Transcription elongation factor SII (TFIIS) enables RNA polymerase II to elongate through a block to transcription in a human gene in vitro. J. Biol. Chem. **264:** 10799–10809.

Reines, D., R. C. Conaway and J. W. Conaway, 1999 Mechanism and regulation of transcriptional elongation by RNA polymerase II. Curr. Opin. Cell Biol. **11:** 342–346.

Rothstein, R., 1991 Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast. Methods Enzymol. **194:** 281–301.

Rudd, M. D., M. G. Izban and D. S. Luse, 1994 The active site of RNA polymerase II participates in transcript cleavage within arrested ternary complexes. Proc. Natl. Acad. Sci. USA **91:** 8057–8061.

Sanders, S. L., J. Jennings, A. Canutescu, A. J. Link and P. A. Weil, 2002 Proteomics of the eukaryotic transcription machinery: identification of proteins associated with components of yeast TFIID by multidimensional mass spectrometry. Mol. Cell. Biol. **22:** 4723–4738.

Santisteban, M. S., T. Kalashnikova and M. M. Smith, 2000 Histone H2A.Z regulates transcription and is partially redundant with nucleosome remodeling complexes. Cell **103:** 411–422.

Santos-Rosa, H., and A. Aguilera, 1995 Isolation and genetic analysis of extragenic suppressors of the hyper-deletion phenotype of the *Saccharomyces cerevisiae hpr1*Δ mutation. Genetics **139:** 57–66.

Santos-Rosa, H., B. Clever, W. D. Heyer and A. Aguilera, 1996 The yeast *HRS1* gene encodes a polyglutamine-rich nuclear protein required for spontaneous and *hpr1*-induced deletions between direct repeats. Genetics **142:** 705–716.

Sarkar, S., A. K. Azad and A. K. Hopper, 1999 Nuclear tRNA aminoacylation and its role in nuclear export of endogenous tRNAs in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **96:** 14366–14371.

Sawadogo, M., A. Sentenac and P. Fromageot, 1980 Interaction of a new polypeptide with yeast RNA polymerase B. J. Biol. Chem. **255:** 12–15.

Sawadogo, M., B. Lescure, A. Sentenac and P. Fromageot, 1981 Native deoxyribonucleic acid transcription by yeast RNA polymerase–P37 complex. Biochemistry **20:** 3542–3547.

Scafe, C., D. Chao, J. Lopes, J. P. Hirsch, S. Henry *et al.*, 1990 RNA polymerase II C-terminal repeat influences response to transcriptional enhancer signals. Nature **347:** 491–494.

Schiestl, R. H., and R. D. Gietz, 1989 High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. Curr. Genet. **16:** 339–346.

Schneider, E. E., T. Albert, D. A. Wolf and D. Eick, 1999 Regulation of *c-myc* and immunoglobulin kappa gene transcription by promoter-proximal pausing of RNA polymerase II. Curr. Top. Microbiol. Immunol. **246:** 225–231.

Schott, D., T. Huffaker and A. Bretscher, 2002 Microfilaments and microtubules: the news from yeast. Curr. Opin. Microbiol. **5:** 564–574.

Sekimizu, K., Y. Nakanishi, D. Mizuno and S. Natori, 1979 Purification and preparation of antibody to RNA polymerase II stimulatory factors from Ehrlich ascites tumor cells. Biochemistry **18:** 1582–1588.

Sen, R., H. Nagai and N. Shimamoto, 2001 Conformational switching of *Escherichia coli* RNA polymerase-promoter binary complex is facilitated by elongation factor GreA and GreB. Genes Cells **6:** 389–401.

Shaw, R. J., and D. Reines, 2000 Saccharomyces cerevisiae transcription elongation mutants are defective in *PUR5* induction in response to nucleotide depletion. Mol. Cell. Biol. **20:** 7427–7437.

Sherman, F., G. R. Fink and J. B. Hicks, 1986 *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Shilatifard, A., 1998 Factors regulating the transcriptional elongation activity of RNA polymerase II. FASEB J. **12:** 1437–1446.

Shilatifard, A., R. C. Conaway and J. W. Conaway, 2003 The RNA polymerase II elongation complex. Annu. Rev. Biochem. **72:** 693–715.

Shu, Y., H. Yang, E. Hallberg and R. Hallberg, 1997 Molecular genetic analysis of Rts1p, a B′ regulatory subunit of *Saccharomyces cerevisiae* protein phosphatase 2A. Mol. Cell. Biol. **17:** 3242–3253.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:** 19–27.

Stirling, D. A., K. A. Welch and M. J. Stark, 1994 Interaction with calmodulin is required for the function of Spc110p, an essential component of the yeast spindle pole body. EMBO J. **13:** 4329–4342.

Strobl, L. J., and D. Eick, 1992 Hold back of RNA polymerase II at the transcription start site mediates down-regulation of *c-myc in vivo*. EMBO J. **11:** 3307–3314.

Sugino, A., J. Nitiss and M. A. Resnick, 1988 ATP-independent DNA strand transfer catalyzed by protein(s) from meiotic cells of the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **85:** 3683–3687.

Svejstrup, J. Q., 2002 Chromatin elongation factors. Curr. Opin. Genet. Dev. **12:** 156–161.

Svejstrup, J. Q., Y. Li, J. Fellows, A. Gnatt, S. Bjorklund *et al.*, 1997 Evidence for a mediator cycle at the initiation of transcription. Proc. Natl. Acad. Sci. USA **94:** 6075–6078.

Takizawa, P. A., A. Sil, J. R. Swedlow, I. Herskowitz and R. D. Vale, 1997 Actin-dependent localization of an RNA encoding a cell-fate determinant in yeast. Nature **389:** 90–93.

Tang, H., Y. Liu, L. Madabusi and D. S. Gilmour, 2000 Promoter-proximal pausing on the *hsp70* promoter in *Drosophila melanogaster* depends on the upstream regulator. Mol. Cell. Biol. **20:** 2569–2580.

Thompson, C. M., A. J. Koleske, D. M. Chao and R. A. Young, 1993 A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. Cell **73:** 1361–1375.

Tong, A. H., M. Evangelista, A. B. Parsons, H. Xu, G. D. Bader *et al.*, 2001 Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science **294:** 2364–2368.

Ueno, K., K. Sekimizu, D. Mizuno and S. Natori, 1979 Antibody against a stimulatory factor of RNA polymerase II inhibits nuclear RNA synthesis. Nature **277:** 145–146.

Ujvari, A., M. Pal and D. S. Luse, 2002 RNA polymerase II transcription complexes may become arrested if the nascent RNA is shortened to less than 50 nucleotides. J. Biol. Chem. **277:** 32527–32537.

Uptain, S. M., C. M. Kane and M. J. Chamberlin, 1997 Basic mechanisms of transcript elongation and its regulation. Annu. Rev. Biochem. **66:** 117–172.

Vainberg, I. E., S. A. Lewis, H. Rommelaere, C. Ampe, J. Vandekerckhove *et al.*, 1998 Prefoldin, a chaperone that delivers unfolded proteins to cytosolic chaperonin. Cell **93:** 863–873.

Van Mullem, V., M. Wery, M. Werner, J. Vandenhaute and P. Thuriaux, 2002 The Rpb9 subunit of RNA polymerase II binds transcription factor TFIIE and interferes with the SAGA and elongator histone acetyltransferases. J. Biol. Chem. **277:** 10220–10225.

Wach, A., A. Brachat, R. Pohlmann and P. Philippsen, 1994 New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. Yeast **10:** 1793–1808.

Wada, T., T. Takagi, Y. Yamaguchi, A. Ferdous, T. Imai *et al.*, 1998 DSIF, a novel transcription elongation factor that regulates RNA polymerase II processivity, is composed of human Spt4 and Spt5 homologs. Genes Dev. **12:** 343–356.

Waldherr, M., A. Ragnini, B. Jank, R. Teply, G. Wiesenberger *et al.*, 1993 A multitude of suppressors of group II intron-splicing defects in yeast. Curr. Genet. **24:** 301–306.

Weeks, J. R., S. E. Hardin, J. Shen, J. M. Lee and A. L. Greenleaf, 1993 Locus-specific variation in phosphorylation state of RNA polymerase II *in vivo*: correlations with gene activity and transcript processing. Genes Dev. **7:** 2329–2344.

Weilbaecher, R. G., D. E. Awrey, A. M. Edwards and C. M. Kane, 2003 Intrinsic transcript cleavage in yeast RNA polymerase II elongation complexes. J. Biol. Chem. **278:** 24189–24199.

Wind, M., and D. Reines, 2000 Transcription elongation factor SII. BioEssays **22:** 327–336.

Wind-Rotolo, M., and D. Reines, 2001 Analysis of gene induction and arrest site transcription in yeast with mutations in the transcription elongation machinery. J. Biol. Chem. **276:** 11531–11538.

Wu, J., D. E. Awrey, A. M. Edwards, J. Archambault and J. D. Friesen, 1996 In vitro characterization of mutant yeast RNA polymerase II with reduced binding for elongation factor TFIIS. Proc. Natl. Acad. Sci. USA **93:** 11552–11557.

Yankulov, K., J. Blau, T. Purton, S. Roberts and D. L. Bentley, 1994 Transcriptional elongation by RNA polymerase II is stimulated by transactivators. Cell **77:** 749–759.

Yarm, F., I. Sagot and D. Pellman, 2001 The social life of actin and microtubules: interaction versus cooperation. Curr. Opin. Microbiol. **4:** 696–702.

Zabrocki, P., C. Van Hoof, J. Goris, J. M. Thevelein, J. Winderickx *et al.*, 2002 Protein phosphatase 2A on track for nutrient-induced signalling in yeast. Mol. Microbiol. **43:** 835–842.

Zawel, L., K. P. Kumar and D. Reinberg, 1995 Recycling of the general transcription factors during RNA polymerase II transcription. Genes Dev. **9:** 1479–1490.

Communicating editor: F. Winston