The EMBO Journal (2004) 23, 4232–4242 | © 2004 European Molecular Biology Organization | All Rights Reserved 0261-4189/04
www.embojournal.org

THE EMBO JOURNAL

Members of the SAGA and Mediator complexes are partners of the transcription elongation factor TFIIS

Maxime Wery[1,4], Elena Shematorova[2,3,4], Benoît Van Driessche[1], Jean Vandenhaute[1], Pierre Thuriaux[2,*] and Vincent Van Mullem[1]

[1]Laboratoire de Génétique Moléculaire (URBM), Facultés Universitaires Notre-Dame de la Paix, Namur, Belgique, [2]Laboratoire de Physiogénomique, Service de Biochimie et Génétique Moléculaire, Gif-sur-Yvette Cedex, France and [3]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

TFIIS, an elongation factor encoded by *DST1* in *Saccharomyces cerevisiae*, stimulates transcript cleavage in arrested RNA polymerase II. Two components of the RNA polymerase II machinery, Med13 (Srb9) and Spt8, were isolated as two-hybrid partners of the conserved TFIIS N-terminal domain. They belong to the Cdk8 module of the Mediator and to a subform of the SAGA co-activator, respectively. Co-immunoprecipitation experiments showed that TFIIS can bind the Cdk8 module and SAGA in cell-free extracts. *spt8*Δ and *dst1*Δ mutants were sensitive to nucleotide-depleting drugs and epistatic to null mutants of the RNA polymerase II subunit Rpb9, suggesting that their elongation defects are mediated by Rpb9. *rpb9*Δ, *spt8*Δ and *dst1*Δ were lethal in cells lacking the Rpb4 subunit. The TFIIS N-terminal domain is also strictly required for viability in *rpb4*Δ, although it is not needed for binding to RNA polymerase II or for transcript cleavage. It is proposed that TFIIS and the Spt8-containing form of SAGA co-operate to rescue RNA polymerase II from unproductive elongation complexes, and that the Cdk8 module temporarily blocks transcription during transcript cleavage.

The EMBO Journal (2004) **23,** 4232–4242. doi:10.1038/sj.emboj.7600326; Published online 9 September 2004
Subject Categories: chromatin & transcription
Keywords: caffeine; mediator; mycophenolate; RNA polymerase; *Saccharomyces cerevisiae*

Introduction

All DNA-dependent RNA polymerases (Pol's) are endowed with an intrinsic ribonuclease activity that cleaves a few nucleotides from the 3′ end of the elongating transcript. This cleavage activity probably helps to backtrack elongation complexes that are out of register with the transcript 3′ end, allowing them to resume transcription (Fish and Kane (2002) and references therein). Transcript cleavage operates in bacterial and archaeal Pol's, and in the three eukaryotic enzymes (Pol I, II and III). In bacteria, it is activated by the GreA and GreB factors (Borukhov *et al*, 1993). In the eukaryotic Pol III enzyme, cleavage is catalysed by the enzyme alone and depends on its Rpc11 subunit (Chédin *et al*, 1998). In Pol II, it depends on the Rpb9 subunit (akin to Rpc11) and on a factor initially referred to as S-II in human cells (Natori *et al*, 1973; Izban and Luse, 1992; Reines, 1992) or P37 in yeast (Sawadogo *et al*, 1980), but now generally called TFIIS (Fish and Kane, 2002). Pol I also has a cleavage activity that does not depend on TFIIS (Tschochner, 1996) but may require Rpa12, a subunit paralogous to Rpb9 and Rpc11 (Nogi *et al*, 1993; Van Mullem *et al*, 2002a).

Yeasts (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*) have only one form of TFIIS, in contrast to the multiple subforms present in vertebrates (Labhart and Morgan, 1998). The corresponding null mutants (*dst1*Δ) have little or no growth defects (Archambault *et al*, 1992; Exinger and Lacroute, 1992; Nakanishi *et al*, 1992; Williams and Kane, 1996), suggesting that TFIIS is only required under specific conditions or that it is functionally redundant with other transcription factors (Davie and Kane, 2000; Lindstrom and Hartzog, 2001; Ubukata *et al*, 2003). The structure of the yeast TFIIS–Pol II complex was recently determined at a resolution of 3.8 Å and reveals its probable mode of action (Kettenberger *et al*, 2003). In short, TFIIS binds the Rpb1/Rpb9 'jaw' of Pol II and inserts into the Pol II pore, contacting the catalytic site by its highly conserved C-end bearing an invariant RSADE motif. The two carboxylic amino acids of that motif are thought to contribute to metal coordination at the level of the enzyme active site. Remarkably, the RSADE motif is present in Rpa12 and Rpc11, but not in Rpb9. This suggests that these two subunits may themselves directly contact the catalytic sites in Pol I and Pol III, as their Pol's do not require TFIIS for cleavage.

The N-terminal domain of TFIIS (approximately corresponding to its first 132 amino acids) stays on the outer surface of Pol II, where it is available for interaction with other components of the transcription complex (Kettenberger *et al*, 2003). This N-terminal part is conserved in all eukaryotes sequenced so far. It also has significant homology to the N-end of MED26 (a component of the human Mediator formerly called CRSP70 or ARC70; Ryu *et al*, 1999; Bourbon *et al*, 2004) and of the three subforms of human Elongin A (Aso *et al*, 1995; Yamazaki *et al*, 2002). This domain was found here to engage in specific interactions with Spt8 and Srb9 (now called Med13; Bourbon *et al*, 2004). Spt8 defines a subform of the SAGA co-activator (Belotserkovskaya *et al*, 2000; Pray-Grant *et al*, 2002; Sterner *et al*, 2002; Wu and Winston, 2002). Med13 (Srb9) is associated with the

*Corresponding author. Laboratoire de Physiogénomique, Service de Biochimie et Génétique Moléculaire, CEA-Saclay, Bât. 144, 91191 Gif-sur-Yvette Cedex, France. Tel.: +33 1 69 08 35 86; Fax: +33 1 69 08 47 12; E-mail: pierreth@matthieu.saclay.cea.fr
[4]These authors contributed equally to this work

Received: 21 January 2003; accepted: 21 June 2004; published online: 9 September 2004

evolutionarily conserved kinase Srb10 (Hengartner *et al*, 1998), now called Cdk8 (Bourbon *et al*, 2004). Spt8 and Med13 had not been connected so far to each other or to TFIIS, but we shall present evidence suggesting that both may be genuine partners of this factor.

Results

Spt8 and Med13 are two-hybrid partners of the N-terminal domain of TFIIS

Figure 1 presents the outcome of a two-hybrid screening using the entire TFIIS sequence fused to the $Gal4_{BD}$(1–147) domain. The corresponding pVV70 plasmid was used as a bait against a random library of yeast genomic fragments fused to the $Gal4_{AD}$(768–881) domain (Fromont-Racine *et al*, 1997; Flores *et al*, 1999). From a total of about 10^7 transformants obtained in strain Y190, 118 clones were selected by their ability to grow in the presence of 100 mM 3-aminotriazole, and were then shown to activate the *lacZ* reporter gene, as detected in a β-galactosidase assay (Figure 1C). Based on the DNA sequence of their inserts, they were allocated to 36 independent clones, defined, respectively, by 25 and 11 distinct but overlapping fragments of Spt8 and Med13. Thus, we consistently identified the same two partners and the same domains on these partners, which strongly argues for a saturating and specific two-hybrid screening.

Spt8 belongs to a subform of the yeast SAGA Pol II co-activator (Grant *et al*, 1997). This protein is essentially formed of WD40-like domains, with two acidic patches (Figure 1B). The 25 Spt8 clones isolated in the two-hybrid screening shared a 102 amino-acid segment between positions 364 and 465. This minimal TFIIS interacting domain includes one acidic patch and one of the WD40-like motifs. Med13 is a moderately conserved component of the Mediator (Borggrefe *et al*, 2002; Boube *et al*, 2002). It belongs to the Cdk8 module, where Cdk8 is a conserved kinase initially identified by its ability to phosphorylate the C-terminal domain (CTD) of the largest Pol II subunit (Hengartner *et al*, 1998). The 11 Med13 clones defined a 195 amino-acid segment (320–514) as the minimal TFIIS interacting domain. This domain is included in a region conserved in all fungal Med13 (Figure 1B). It bears no detectable similarity to the Spt8 target domain defined above.

Four domains can be recognised on the TFIIS structure and are collinear to its amino-acid sequence (Kettenberger *et al*, 2003; see also Figure 1A). The corresponding fragments were fused to the $Gal4_{BD}$(1–147) domain and tested separately against Spt8 and Med13 in a two-hybrid assay. As shown in Figure 1C, the N-terminal part of TFIIS (amino acids 1–132) is necessary and sufficient to interact with both partners. NMR data have shown that this domain is made of four closely packed α helices (Booth *et al*, 2000). They are not included in the crystal structure (Kettenberger *et al*, 2003) but are evidently exposed on the outer surface of Pol II (Figure 1A), and are thus available for interactions with other components of the transcription machinery.

The N-half of this domain (amino acids 1–74) is important for the two-hybrid interaction since its deletion abolishes the two-hybrid response (Figure 1C). However, a fragment bearing amino acids 1–74 alone failed to interact with Spt8 or Med13 (data not shown). Interestingly, this region harbours a highly conserved motif corresponding to the α2, α3 and α4 helices (Figure 1D). A Psi-Blast survey of the human genome showed that this motif is present in nine distinct gene products, including three TFIIS isoforms (Labhart and Morgan, 1998), three forms of Elongin A (Aso *et al*, 1995; Booth *et al*, 2000; Yamazaki *et al*, 2002) and the MED26 component of the Mediator (Ryu *et al*, 1999; Bourbon *et al*, 2004).

TFIIS co-purifies with Med13 and its associated Cdk8 kinase in cell-free extracts

The two-hybrid data above suggested that Med13 may be a functional partner of TFIIS. We therefore examined if the immunopurification of Med13 from cell-free extracts leads to a co-purification of TFIIS and *vice versa*. Med13::13Myc was barely detectable when introduced as a chromosomal allele, but could be readily detected when expressed from a replicative plasmid and then pulled down a significant amount of TFIIS::3HA (Figure 2A). This occurred no matter whether cells expressed Spt8 (*SPT8*$^+$) or not (*spt8*Δ). In a reciprocal experiment, TFIIS::13Myc pulled down Med13::3HA (Figure 2B).

Since Med13 belongs to the Cdk8 module of the Mediator, we also examined whether Cdk8 itself might co-purify with TFIIS. Indeed, Cdk8::13Myc co-purified with the immunoprecipitated TFIIS::3HA and TFIIS::3HA co-purified with Cdk8::13Myc (Figure 2C and D). Moreover, this co-purification was substantially reduced in a *med13*Δ context (Figure 2D). Along with our two-hybrid data, this indicates that, *in vivo*, TFIIS may associate with the Cdk8 module and that this association is, to a large extent, dependent on Med13. We note that *med13*Δ also had a minor effect on the electrophoretic mobility of TFIIS, suggesting a change in its phosphorylation pattern.

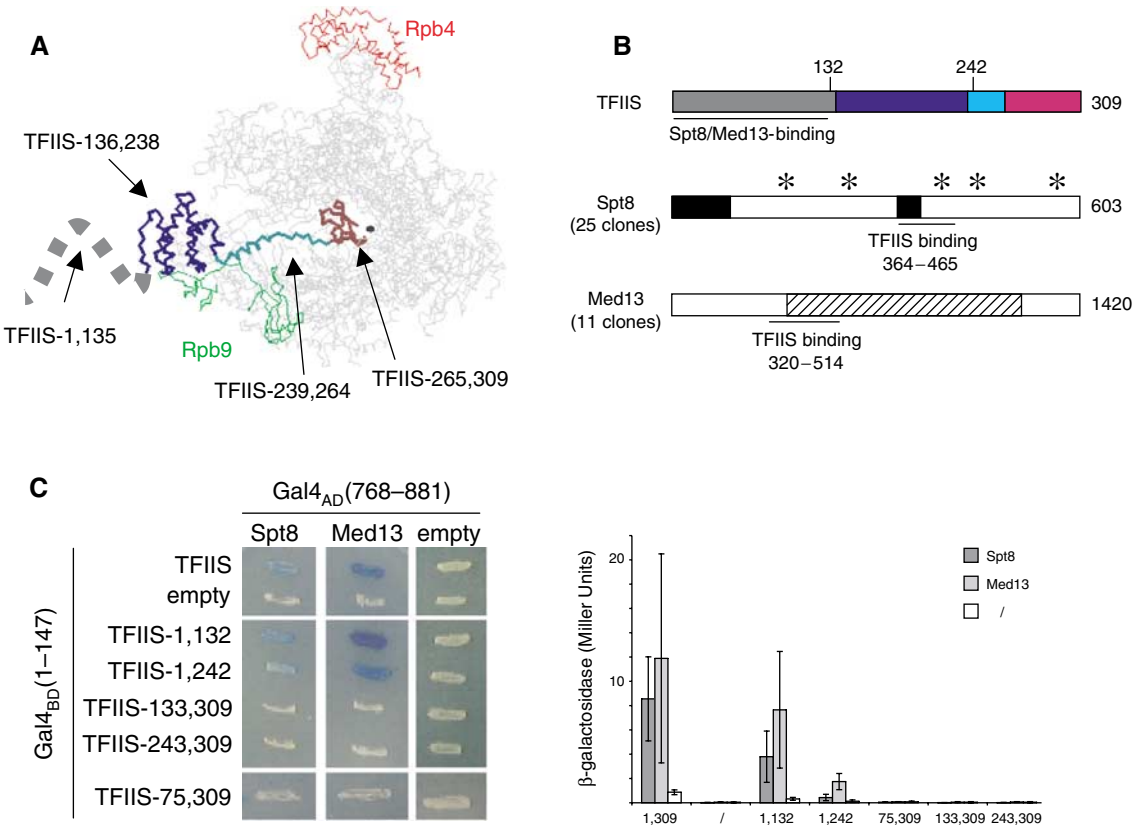
*dst1*Δ *and med13*Δ *mutants have different phenotypes*

TFIIS is encoded by the *DST1* gene. *dst1*Δ null mutants have no detectable defect except their sensitivity to 6-azauracil and mycophenolate, two nucleotide-depleting drugs that are thought to impair elongation (Exinger and Lacroute, 1992). *med13*Δ and *cdk8*Δ are not sensitive to mycophenolate and do not aggravate the sensitivity of *dst1*Δ (data not shown). Moreover, the *med13*Δ *dst1*Δ *spt8*Δ triple mutant grew like its *dst1*Δ *spt8*Δ parent, and with the same sensitivity to mycophenolate.

The only known phenotype that relates Med13 to TFIIS is their opposite effect on *rpb1* mutants with partial deletion of the Pol II CTD, such as the *rpb1*Δ*104* mutant used in this study (Allison and Ingles, 1989). Indeed, *med13*Δ and *cdk8*Δ were isolated as suppressors of such *rpb1* mutants (Liao *et al*, 1995), while *dst1*Δ is lethal in this context (Lindstrom and Hartzog, 2001). The triple mutant *dst1*Δ *med13*Δ *rpb1*Δ*104* is also lethal (data not shown), suggesting that the integrity of TFIIS may be required for the suppressor effect of *med13*Δ on Pol II CTD mutants.

TFIIS co-purifies with Spt8 and other SAGA subunits in cell-free extracts

SAGA is a Pol II co-activator bearing the Gcn5 histone acetyltransferase (Grant *et al*, 1997). It was recently shown to exist in two subforms that essentially differ by the presence or absence of the Spt8 subunit (Belotserkovskaya *et al*, 2000; Pray-Grant *et al*, 2002; Sterner *et al*, 2002; Wu and Winston, 2002). Since our two-hybrid data suggested that TFIIS may



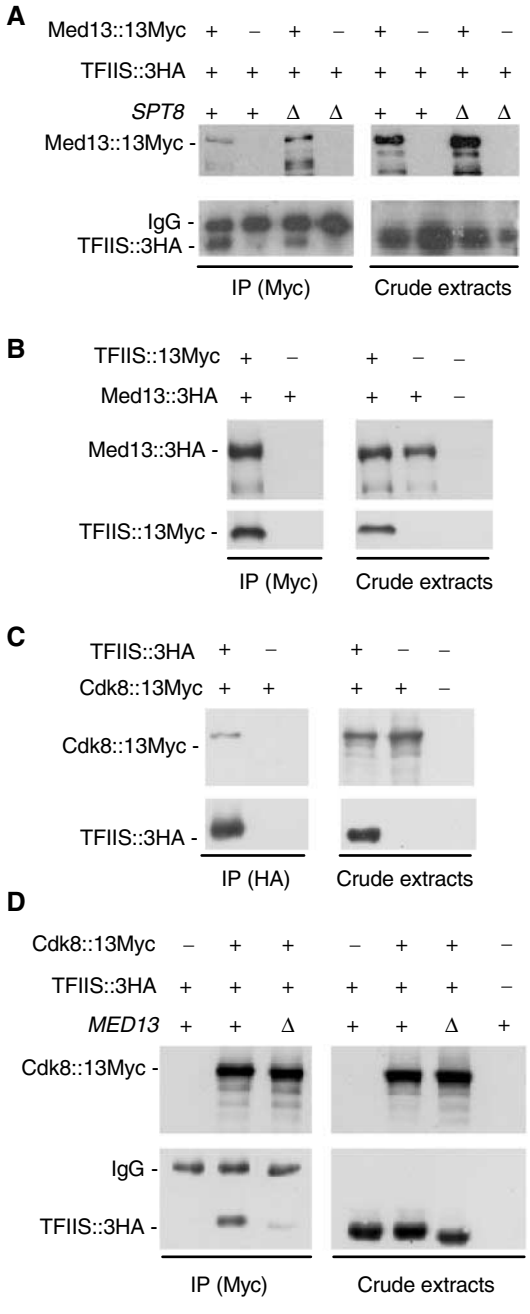
D

```
TFIIS Sc (309)     23 VLEILHVLDKEFVPTEKLLRETKVGVEVNKFKK-STNVEISKLVKKMISSWKDAINKNKRSR 83

TFIIS    (301)     24 ALDLLKEL-KNIPMTLELLQSTRIGMSVNAIRKQSTDEEVTSLAKSLIKSWKKLLDGPSTEK 84
TFIIS    (299)     27 AMDLLREL-KAMPITLHLLQSTRVGMSVNALRKQSSDEEVIALAKSLIKSWKKLLDASDAKA 87
TFIIS    (348)     26 ALDLLKKL-HSCQMSIQLLQTTRIGVAVNGVRKHCSDKEVVSLAKVLIKNWKRLLDSPGPPK 86

Elongin A1 (772)   26 LLKYLKKL-STLPITVDILAETGVGKTVNSLRKH---EHVGSFARDLVAQWKKLVPVERNAE 83
Elongin A2 (753)   27 LEKYLQKL-SALPMTADILAETGIRKTVKLLRKH---QHVGDFARDLAARWKKLVLVDRNTR 84
Elongin A3 (546)   27 LEKYLQKL-SALPMTADILAETGIRKTVKRLRKH---QHVGDFARDLAARWKKLVLVDRNTG 84

Med26 (600)        31 VLEVISSL-EKYPITKEALEETRLGKLINDVRKKTKNEELAKRAKKLLRSWQKLIEPAHQHE 91

TFIIS-like (420)  179 ALGLLTEL----QNVLELLRSTGTGMLLNVSLKQSTDEEVTSLAKSFVKSWKTLPDEPSTEK 236
TFIIS-like (208)   58 LVEALQEL-KKKIPSREVLKSTRIGHTVNKMRKHSD-SEVASLAREVYTEWKTFTEKHSNRP 118
```

Figure 1 Spt8 and Med13 are TFIIS partners in a two-hybrid assay. (**A**) Spatial structure of the RNA polymerase II–TFIIS complex domain I, domain II, the inter-domain linker and domain III of TFIIS are shown in grey, blue, cyan and brown, respectively. The borders of each domain are taken from the crystal structure recently reported by Kettenberger *et al* (2003). The structure of the N-terminal domain I (positions 1–111, symbolised here by a dashed line) was solved in solution by nuclear magnetic resonance (Booth *et al*, 2000), but was not determined in association with Pol II. The Rpb4 and Rpb9 subunits of Pol II are indicated in red and green, respectively. The black sphere locates the catalytic Mg^{2+} A. This figure was prepared with the RASMOL software (www.umass.edu/microbio/rasmol/). (**B**) General organisation of Spt8, Med13 and TFIIS. TFIIS: The TFIIS domains are shown in the same code colour as in (A). A horizontal thick line denotes the minimal region supporting a two-hybrid interaction with Spt8 and Med13, based on the data shown in (C). Spt8: Stars and black boxes indicate WD40-like domains and acidic stretches, respectively. The horizontal thick line denotes the TFIIS-binding region (positions 364–465) as defined by the smallest domain common to the 25 pACT2-SPT8 clones identified by a two-hybrid screening using pVV70 as bait vector (Table II). An example of two-hybrid interaction is shown in (C). Med13: The stripped box corresponds to a region with strong homology between fungal forms of Med13. The horizontal thick line denotes the TFIIS-binding region (positions 320–514), as defined by the smallest domain common to the 11 pACT2-MED13 clones selected by two-hybrid screening. An example of two-hybrid interaction is shown in (C). (**C**) Two-hybrid interactions with TFIIS. Left panel: The complete coding sequence of TFIIS and various N-terminal or C-terminal fragments thereof were fused to the C-end of the $GAL4_{BD}$(1–147) DNA-binding domain (plasmids pVV70–pVV75, Table II) and tested for their interaction with Spt8 (plasmid pSPT8-84) and Med13 (plasmid pMED13-111). Transformants were obtained in strain Y190 and tested at 30°C for β-galactosidase activity in an overlay assay (Flores *et al*, 1999). Right panel: β-Galactosidase activity was assayed as described by Miller (1972). The average values and their standard deviation were calculated from assays performed on three independent transformants, using the same plasmid combination as in the left panel. (**D**) Conservation of the TFIIS N-terminal domain. The *S. cerevisiae* (Sc) sequence of TFIIS was compared to the current human genome. Homology search was made using the Psi-blast algorithm and improved by manual inspection. The number in brackets indicates the length of each polypeptide. The two last sequences (accession number AAH35374 and XP294568) correspond to putative gene products that are related to TFIIS but lack the invariant RSADE motif.

directly interact with Spt8, we constructed a double-mutant strain where Spt8::13Myc and TFIIS::3HA fusions were expressed from the chromosomal locus. Under these conditions, Spt8::13Myc only pulled down a barely detectable amount of TFIIS::3HA (data not shown). The expression of the two proteins from replicative vectors significantly enhanced the co-immunopurification signal, and this occurred no matter whether cells expressed Med13 (*MED13*$^+$) or not (*med13*Δ) (Figure 3A). In a reciprocal experiment, Spt8::3HA co-purified with the immunoprecipitated TFIIS::13Myc (Figure 3B). Thus, Spt8 has affinity for TFIIS in terms of two-hybrid interactions and co-purification from a yeast cell-free extract.

These data raise the question of whether Spt8 binds TFIIS in its own right or as part of the Spt8-containing form of SAGA. Figure 3C and D tentatively suggest that the latter may be true since TFIIS::3HA pulled down Gcn5::13Myc and Spt7::13Myc. Conversely, Gcn5::13Myc and Spt7::13Myc pulled down TFIIS::3HA. However, there was no clear indication that this co-purification depended on Spt8: the genetic inactivation of Spt8 reduced the co-purification, but this might be an indirect effect as there was also less TFIIS::3HA in the corresponding crude extracts (Figure 3D, compare lanes 7–8 and 9–10).



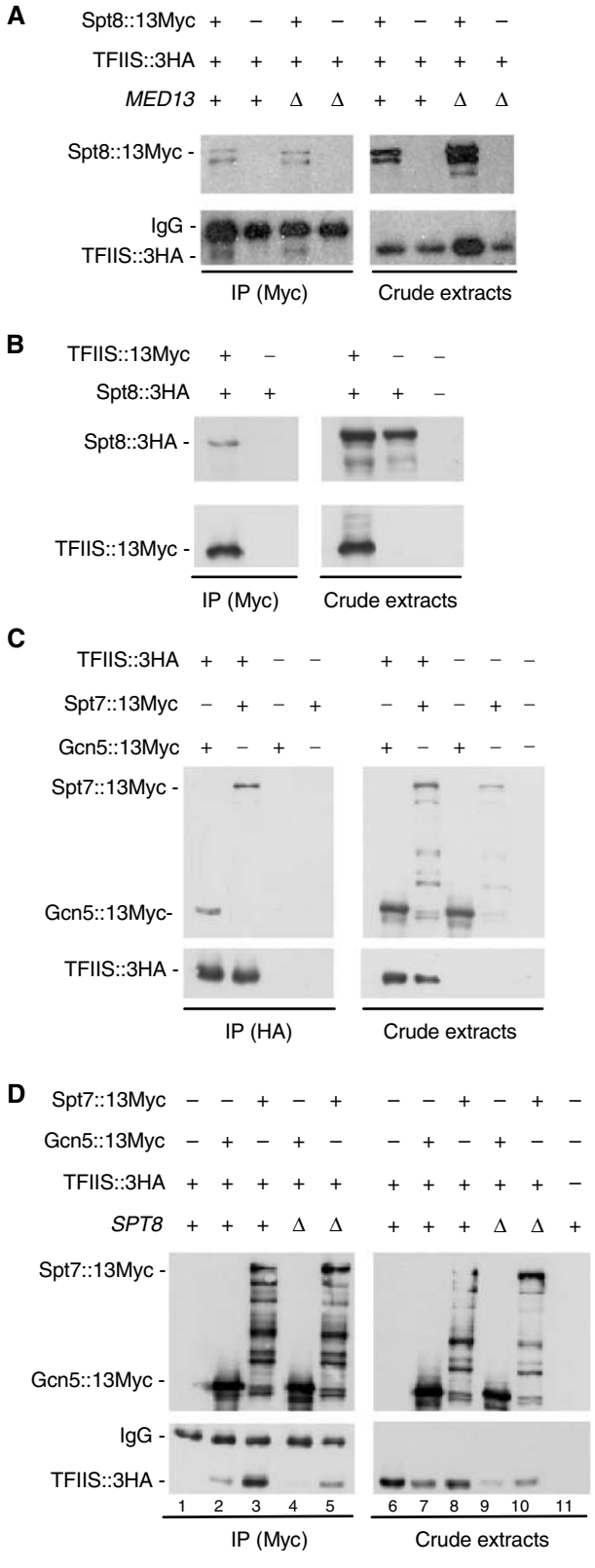
*dst1*Δ *and spt8*Δ *mutants have related phenotypes*

As already mentioned, *dst1*Δ has little or no growth defect but is sensitive to nucleotide-depleting drugs such as mycophenolate (Exinger and Lacroute, 1992; see also Figure 4A). Null mutants of several other nonessential components of the Pol II transcription machinery are also sensitive to these inhibitors (Desmoucelles *et al*, 2002). They include the *spt3*Δ, *spt7*Δ and *spt8*Δ mutants of the SAGA co-activator and the *rpb4*Δ and *rpb9*Δ mutants lacking the nonessential Pol II subunits Rpb4 or Rpb9. Figure 4A illustrates the different levels of sensitivity to mycophenolate displayed by these mutants. This drug sensitivity is not a general property of SAGA, since null mutants of Gcn5 or of the Ada2 and Ada3 subunits of SAGA (*ada2*Δ, *ada3*Δ and *gcn5*Δ) are not sensitive.

*dst1*Δ and *rpb9*Δ are epistatic, that is, the double mutant is indistinguishable from *rpb9*Δ alone in terms of growth and drug sensitivity (Figure 4A; Van Mullem *et al*, 2002b). This also holds for the *spt8*Δ *rpb9*Δ double mutant (Figure 4A and B). Remarkably, null mutants of all the other nonessential subunits of SAGA (Ada2, Ada3, Gcn5, Spt3 and Spt7) are lethal in a *rpb9*Δ context, disregarding whether they are sensitive to mycophenolate (*spt3*Δ and *spt7*Δ) or not (*ada2*Δ, *ada3*Δ and *gcn5*Δ) (Figure 4B; Van Mullem *et al*, 2002b). Thus, epistasis with *rpb9*Δ is a unique property of *spt8*Δ among SAGA null mutants.

Figure 2 Co-purification of TFIIS with Med13 and Cdk8 in cell-free extracts. (**A**) Co-purification of TFIIS::3HA with immunoprecipitated Med13::13Myc. Strains YPH499 (*SPT8*$^+$) and YBV61 (*spt8*Δ) were transformed with plasmids encoding the Med13::13Myc fusion (pVV226) and/or the TFIIS::3HA fusion (pVV227). Protein crude extracts were prepared as described (Van Mullem *et al*, 2002b). Med13::13Myc was immunoprecipitated by mouse monoclonal anti-Myc antibodies. Proteins (IP) were separated by SDS–PAGE, along with 10 μg of crude extracts, and revealed by Western blotting with monoclonal anti-Myc or anti-HA antibodies. The band noted IgG corresponds to the heavy chains of the mouse anti-Myc antibodies used for the immunopurification. (**B**) Co-purification of Med13::3HA with immunoprecipitated TFIIS::13Myc. Strain YPH499 was transformed with plasmids encoding the TFIIS::13Myc fusion (pVV234) and/or the Med13::3HA fusion (pVV229). Protein extraction, immunopurification and detection were as above. (**C**) Co-purification of Cdk8::13Myc with immunoprecipitated TFIIS::3HA. Strain YPH499 was transformed with plasmids encoding the TFIIS::3HA fusion (pVV228) and/or the Cdk8::13Myc (pVV233). TFIIS::3HA was immunoprecipitated by mouse monoclonal anti-HA antibodies. Protein extraction and detection were as above. (**D**) Co-purification of TFIIS::3HA with immunoprecipitated Cdk8::13Myc. Strains YPH499 (*MED13*$^+$) and YBV39 (*med13*Δ) were transformed with plasmids encoding the Cdk8::13Myc (pVV233) and/or the TFIIS::3HA fusion (pVV228). Protein extraction, immunopurification and detection were as in (A, B). No co-purification was observed when Cdk8::13Myc and TFIIS::3HA were expressed from their respective chromosomal locus (data not shown).

Crosses between *rpb4*Δ and *rpb9*Δ yield no double mutants, suggesting that these mutations are synthetic lethal (Li and Smerdon, 2002). We confirmed this interpretation by complementation tests showing that lethality is relieved in the presence of a plasmid bearing the wild-type *RPB4* or *RPB9* genes. *dst1*Δ and *spt8*Δ were also synthetic lethal with *rpb4*Δ.



In both cases, double mutants grew as microcolonies but did not form viable clones when further streaked on YPD. Moreover, the double mutants were rescued by complementation with the *RPB4* and *DST1/SPT8* wild-type genes (Figure 5). Again, this was not a general property of SAGA since *spt3*Δ and *gcn5*Δ are epistatic with *rpb4*Δ (Figure 4B). This is consistent with the fact that SAGA complexes purified from *spt3*Δ and *gcn5*Δ contain Spt8 (Sterner *et al*, 1999). In contrast, a *spt7*Δ deletion that leads to the disruption of SAGA (Sterner *et al*, 2002) is lethal in both *rpb9*Δ and *rpb4*Δ contexts.

From the data above, *dst1*Δ and *spt8*Δ behave very similarly in terms of lethality with *rpb4*Δ, epistasis to *rpb9*Δ and mycophenolate sensitivity. Yet, their physiological effects do not fully overlap. *spt8* mutants suppress *his4917*δ and are protrophic for lysine in a *LYS2–173R2* context, which reflects the less efficient transcription of solo δ and Ty1 elements in the *his4-917*δ and *LYS2-173R2* alleles (Eisenmann *et al*, 1994; Wu and Winston, 2002). These *spt* phenotypes (suppression of Ty1) are not found in *dst1*Δ and are not aggravated in a *dst1*Δ *spt8*Δ double mutant (data not shown). As shown in Figure 4C, *dst1*Δ is sensitive to caffeine (trimethylxanthine). This phenotype is shared by *rpb4*Δ and *rpb9*Δ but not by *spt8*Δ (data not shown; Sterner *et al*, 1999). Finally, *dst1*Δ aggravates mycophenolate sensitivity in *spt8*Δ (Figure 4A), indicating that the drug sensitivity of Spt8 cannot be directly mediated by its binding to TFIIS.

*The N-terminal domain of TFIIS is critical in an rpb4*Δ *context*

The N-terminal domain of TFIIS is one of the most conserved components of that polypeptide (see Figure 1D) but does not participate in Pol II binding and is not required for transcript cleavage (Agarwal *et al*, 1991; Awrey *et al*, 1998). This is consistent with the observation that a *dst1-133,309* mutant lacking this domain is not sensitive to 6-azauracil (Nakanishi *et al*, 1995; Ubukata *et al*, 2003) to mycophenolate (Figure 4D) or to caffeine (Figure 4C), and is in fact indistinguishable from wild type by all criteria examined so far. On the other hand, our two-hybrid data suggest that this N-terminal domain specifically interacts with Spt8. A deletion of that

Figure 3 Co-purification of TFIIS with components of SAGA in cell-free extracts. (**A**) Co-purification of TFIIS::3HA with immunoprecipitated Spt8::13Myc. Strains YPH499 (*MED13*$^+$) and YBV39 (*med13*Δ) were transformed with plasmids encoding the Spt8::13Myc fusion (pVV225) and/or the TFIIS::3HA fusion (pVV227). Protein extraction, immunopurification and detection were as for Figure 2A. (**B**) Co-purification of Spt8::3HA with immunoprecipitated TFIIS::13Myc. Strain YPH499 was transformed with plasmids encoding the TFIIS::13Myc fusion (pVV234) and/or the Spt8::3HA fusion (pVV230). Protein extraction, immunopurification and detection were as in Figure 2A. (**C**) Co-purification of Gcn5::13Myc and Spt7::13Myc with immunoprecipitated TFIIS::3HA. Strain YPH499 was transformed with plasmids encoding the TFIIS::3HA fusion (pVV228) and/or the Gcn5::13Myc (pVV231) or the Spt7::13Myc (pVV232) fusions. Protein extraction, immunopurification and detection were as for Figure 2C. (**D**) Co-purification of TFIIS::3HA with immunoprecipitated Gcn5::13Myc and Spt7::13Myc. Strains YPH499 (*SPT8*$^+$) and YMW220 (*spt8*Δ) were transformed with plasmids encoding the Gcn5::13Myc (pVV231) or Spt7::13Myc (pVV232) fusions and/or the TFIIS::3HA fusion (pVV228). Protein extraction, immunopurification and detection were as for Figure 2A. No co-purification was observed when Gcn5::13Myc and TFIIS::3HA were expressed from their respective chromosomal locus (data not shown).

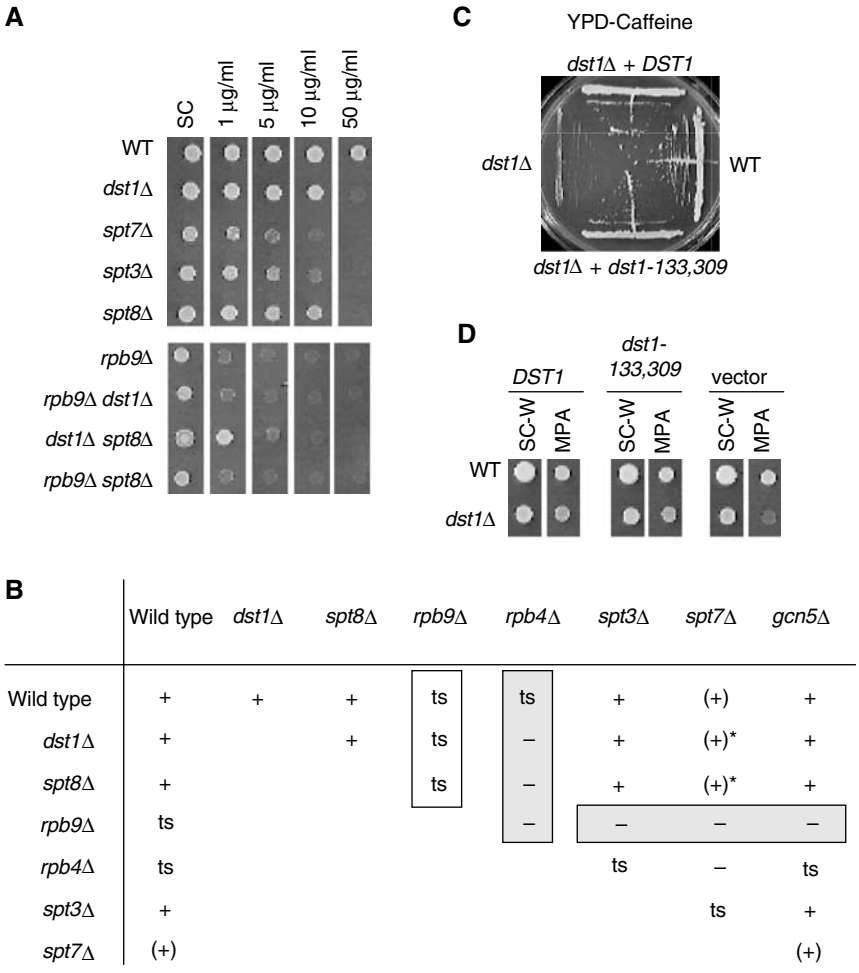


Figure 4 Phenotypic effects of *dst1*Δ and *spt8*Δ mutants. (**A**) Mycophenolate sensitivity and epistasis with *rpb9*Δ. Wild type (YPH499), *dst1*Δ (CMKy1), *spt7*Δ (Y03218), *spt3*Δ (Y04228), *spt8*Δ (Y12666), *rpb9*Δ (YVV9), *rpb9*Δ *dst1*Δ (YVV62), *dst1*Δ *spt8*Δ (YBV55) and *rpb9*Δ *spt8*Δ (YMW238) cells were spotted on SC medium without or with mycophenolate (1, 5, 10 or 50 µg/ml). Plates were incubated at 25°C for 5 days. (**B**) Recapitulation of synthetic phenotypes involving *dst1*Δ and *spt8*Δ. Symbols: +, wild type; (+), partial growth defect at 30°C; −, lethality; ts, temperature sensitivity. In each case, synthetic lethal patterns were based on the analysis of at least 15 meiotic tetrads. Stars indicate that *spt7*Δ *dst1*Δ or *spt7*Δ *spt8*Δ mutants can be temperature sensitive depending on the genetic background. This phenotype probably reflects the intervention of a third mutation present in some genetic backgrounds, but the corresponding gene has not been identified. (**C**) Caffeine sensitivity of *dst1* mutants. CMKy1 (*dst1*Δ) was transformed with pCM-DST1 (*DST1*), pCM-ΔN (*dst1-133,309*) and the empty vector pCM185. Transformants were streaked on YPD with 10 mM caffeine and incubated for 7 days at 30°C, using YPH500 (WT) as a wild-type control. (**D**) Mycophenolate sensitivity of *dst1* mutants. Wild type (DY236-6B) and CMKy1 (*dst1*Δ) were transformed with pVV80 (*DST1*) and pVV81 (*dst1-133,309*), using the empty vector (pGEN) as a control. Transformants were spotted on SC-W and SC-W with 50 µg/ml of mycophenolate. Plates were incubated at 25°C for 3 days.

domain is therefore expected to disrupt the Spt8–TFIIS interaction. As shown in Figure 5B, a *dst1-133,309* mutant lacking this N-terminal domain is lethal in an *rpb4*Δ context and behaves like *dst1*Δ and *spt8*Δ themselves. The same colethality was observed in *rpb4*Δ *dst1-R287Q,E291N* double mutants affecting the invariant RSADE domain of TFIIS (Figure 5C). *dst1-R287Q,E291N* is specifically defective in the transcript cleavage activity, but is not affected for Pol II binding (Ubukata *et al*, 2003). Taken together, these data show that, in the absence of Rpb4, transcription is strictly dependent on transcript cleavage and also requires a functional interaction between Spt8 and TFIIS.

Discussion

Elongating RNA polymerases that meet obstacles on the DNA template become arrested and probably displace the 3′ end of the transcript relatively to the catalytic site of polymerisation. Escape from arrest requires an RNA cleavage process that, in the case of Pol II, is strongly stimulated by the elongation factor TFIIS (Fish and Kane, 2002). Elegant structural studies by Kettenberger *et al* (2003) have shown that TFIIS binds Pol II on its Rpb9/Rpb1 jaw and that its C-terminal part reaches the internal active site of the enzyme, where the factor induces a conformational change switching Pol II to its RNA cleavage mode. However, they provide no function for the N-terminal domain of TFIIS.

This N-terminal domain forms a bulky four-helix bundle (Booth *et al*, 2000) on the periphery of the Pol II structure, approximately facing the downstream DNA (Kettenberger *et al*, 2003), and is thus available for interaction with other Pol II factors. Its physiological role has remained a mystery, but studies on the human and yeast factor have shown that it is not needed for transcript cleavage, for the binding of TFIIS

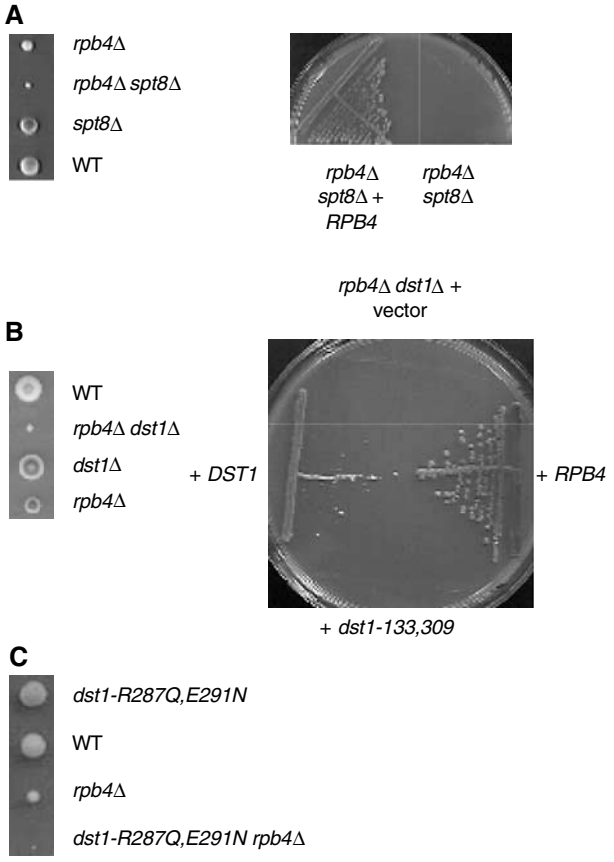


Figure 5 Spt8 and the N-terminal domain of TFIIS are critical in an *rpb4*Δcontext. (**A**) Synthetic lethality between *spt8*Δ and *rpb4*Δ. Left panel: Strains ESH13-8D (*spt8*Δ) and ESH27-15C (*rpb4*Δ) were crossed and submitted to tetrad analysis. Plates were incubated on YPD. Minute colonies corresponding to *spt8*Δ *rpb4*Δ double mutants were obtained but could not be further propagated on YPD. One tetratype is shown after 5 days at 30°C. Right panel: The cross above was repeated but diploid strains were transformed with pYX212-RPB4 (*2*μ*URA3 RPB4*) prior to sporulation. Strain ESH28-2A (*spt8*Δ *rpb4*Δ*/2*μ *URA3 RPB4*) was isolated in the meiotic offspring and then transferred on FOA to counterselect the pYX212-RPB4 plasmid. This material was then transferred to YPD and incubated for 5 days at 30°C and compared to the strain ESH28-2A as a control. (**B**) Synthetic lethality between *rpb4*Δ and mutants lacking the N-terminal domain of TFIIS. Left panel: Strain ESH1 (*dst1*Δ) was crossed to SL21-3A (*rpb4*Δ) and submitted to tetrad analysis. Plates were incubated on YPD. Minute colonies corresponding to *dst1*Δ *rpb4*Δ double mutants were obtained but have an extremely poor viability when further propagated on YPD. One tetratype is shown after 5 days at 30°C. Right panel: Strain ESH29-1B (*dst1*Δ *rpb4*Δ with the *2*μ *URA3 RPB4* plasmid pYX212-RPB4) was transformed by the *TRP1* centromeric plasmids pCM185 (vector), pCM-DST1 (*DST1*) or pCM-ΔN (*dst1-133,309*). These transformants were transferred on FOA to counterselect the pYX212-RPB4 plasmid, transferred to YPD and incubated for 5 days at 30°C, using ESH29-1B as a control (*RPB4*). Control experiments showed that pCM-ΔN complements the mycophenolate sensitivity of *dst1*Δ and is thus functional (data not shown). (**C**) Synthetic lethality between rpb4D and the *dst1-R287Q,E291N* mutant defective in the RNA cleavage activity. Strain SL21-3A (rpb4D) was crossed with D495-4D (*dst1-R287Q,E291N*) and 20 tetrads were analysed. The *dst1 rpb4*Δ double mutants invariably produced minute colonies that failed to propagate when further restreaked on YPD. One tetratype is shown after 3 days at 30°C.

to Pol II or for the stimulation of elongation *in vitro* (Agarwal *et al*, 1991; Nakanishi *et al*, 1995; Awrey *et al*, 1998). Indeed, a deletion of this domain could not so far be associated to any growth phenotype in *S. cerevisiae*. In mammals, this domain has a clear homology to the N-terminal region of the transcription factors MED26 and Elongin A (Aso *et al*, 1995; Ryu *et al*, 1999), and it is hard to believe that this evolutionary conservation is not associated with some specific function.

We report here that the N-terminal domain of TFIIS interacts with Spt8 and Med13. Both are well-defined components of the Pol II transcription machinery, but were so far not connected to each other or to TFIIS. Med13 belongs to the Cdk8 kinase module of the Mediator. The two-hybrid interaction between Med13 and TFIIS is further supported by co-immunoprecipitation data showing that TFIIS co-purified with Med13 and its associated Cdk8 kinase. Moreover, co-purification with Cdk8 was substantially reduced in a *med13*Δ mutant. In yeast and human cells, GST::TFIIS fusions pull down a Pol II holoenzyme that contains Cdk8 (Pan *et al*, 1997; Hirst *et al*, 1999). Furthermore, this only requires the N-terminal part of the human TFIIS (positions 1–103). Taken together, these data support the idea that TFIIS binds the Mediator at the level of its Cdk8 module, by a specific interaction between Med13 and the N-terminal part of TFIIS. Since Cdk8 inhibits transcription via its CTD kinase activity (Hengartner *et al*, 1998), it may facilitate the TFIIS-dependent cleaving process by temporarily holding back elongation (Figure 6A). Alternatively, the kinase could phosphorylate the N-end of TFIIS, as the latter is phosphorylated in human cells (Horikoshi *et al*, 1985; Agarwal *et al*, 1991).

Spt8 defines a recently discovered subform of the SAGA Pol II co-activator (Belotserkovskaya *et al*, 2000; Pray-Grant *et al*, 2002; Sterner *et al*, 2002; Wu and Winston, 2002). Along with our two-hybrid data, the co-immunopurification of TFIIS with Spt8, Gcn5 and Spt7 argues for a direct interaction between TFIIS and the Spt8-containing form of SAGA. However, the genetic inactivation of Spt8 did not impair the co-purification of TFIIS with Gcn5 and Spt7. One possibility is that SAGA binds the Pol II/TFIIS transcription complex by additional contact points. In this context, we note that Spt7 is a two-hybrid partner of the Rpb9 subunit of Pol II (M Werner and P Thuriaux, unpublished observation).

Spt8 and TFIIS are not normally required for transcription in *S. cerevisiae*, since *spt8*Δ and *dst1*Δ null mutants have little or no effect on growth, and since a deletion of the Spt8-interacting N-terminal domain of TFIIS (*dst1-133,309*) is indistinguishable from wild type. Remarkably, both factors are essential in *rpb4*Δcells lacking the Rpb4 subunit of Pol II. This is not a general property of SAGA mutants since *gcn5*Δ and *spt3*Δ mutants, for example, are viable in an *rpb4*Δ context. *gcn5*Δ lacks the histone acetylase of SAGA but, like *spt3*Δ, still produces an Spt8-containing form of SAGA (Sterner *et al*, 2002; Wu and Winston, 2002). In the case of TFIIS, the lethality of *rpb4*Δ *dst1-R287Q,E291N* (where *dst1-R287Q,E291N* affects the invariant RSADE motif and specifically lacks the cleavage activity, see Ubukata *et al*, 2003) proves that transcript cleavage is critical in the absence of Rpb4. The fact that this lethality extends to the *dst1-133,309* mutant deleted for the N-terminal domain of TFIIS strongly suggests that the interaction with Spt8 is also needed in an Rpb4-less context.

*spt8*Δ and *dst1*Δ are epistatic with *rpb9*Δ mutants lacking Rpb9, the other nonessential Pol II subunit (Van Mullem *et al* (2002b) and this study). Again, this is not a general property of SAGA mutants since *gcn5*Δ *rpb9*Δ double mutants are lethal, suggesting that the histone acetylase activity of

SAGA is critical in an Rpb9-less context. Thus, Rpb4 and Rpb9 are not directly involved in RNA polymerisation but may stabilise two distinct conformations of Pol II. The absence of Rpb9 makes Pol II incompetent for transcript cleavage (as shown by Awrey *et al*, 1998), and we speculate that the absence of Rpb4 would instead make Pol II strictly dependent on transcript cleavage. Both conditions would lead to slow growth and to a strong sensitivity to nucleotide-depleting drug, but for different reasons. For example, Pol II molecules lacking Rpb4 could be prone to transcription accidents, obliging them to resort to cleavage. This would account for the strict dependency of *rpb4*Δ on Rpb9 and TFIIS, since both are required for RNA cleavage (Awrey *et al*, 1997). As sketched out in Figure 6B, the ability to recruit the Spt8-containing form of SAGA via the N-terminal of TFIIS may also be essential under these conditions. This may not be restricted to the elongating Pol II, as TFIIS binds the promoter and coding part of *GAL1* (Pokholok *et al*, 2002; D Prather, E Larschan and F Winston, personal communication). SAGA itself is recruited to the promoter (Cosma *et al*, 1999; Bhaumik and Green, 2001; Larschan and Winston, 2001). This, however, need not apply to the Spt8-containing form, and an elongation role of SAGA is indeed suggested by the sensitivity of *spt3*Δ, *spt7*Δ and *spt8*Δ to nucleotide-depleting drugs (Desmoucelles *et al*, 2002).

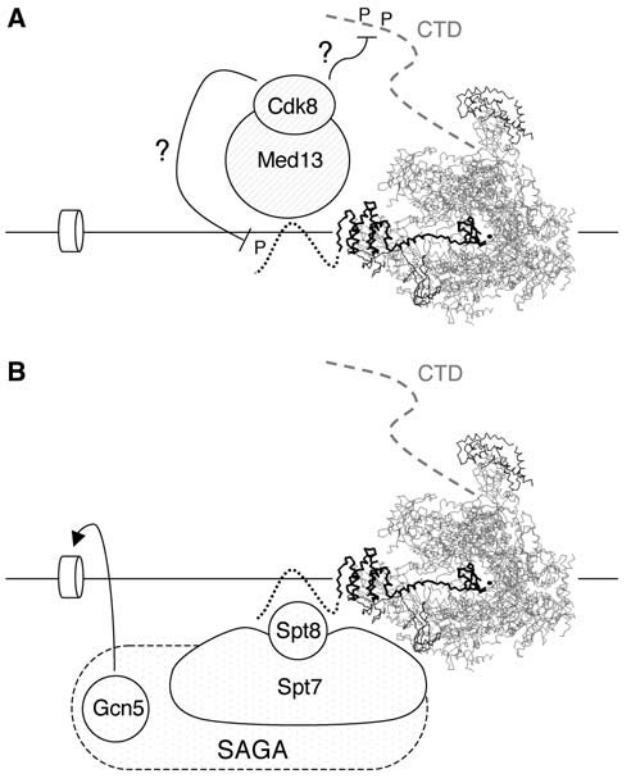


Figure 6 Model of the interactions relating arrested Pol II, TFIIS, SAGA and the Cdk8 module. (**A**) Interaction between arrested Pol II, TFIIS and the Cdk8 module. The schematic representation of the Pol II/TFIIS complex is as in Figure 1A. The TFIIS and Med13 interaction recruits the Cdk8 module on an arrested Pol II/TFIIS complex. The Cdk8 kinase may facilitate the TFIIS-dependent cleaving process by temporarily holding back elongation via inhibitory phosphorylation (marked by 'P') on the CTD. Cdk8 may also phosphorylate TFIIS on its N-terminal domain. (**B**) Interaction between arrested Pol II, TFIIS and the Spt8-containing form of SAGA. Pol II may become frequently arrested during elongation. Transcript cleavage is then required to resume transcription, in a way that depends on TFIIS and Rpb9. The N-terminal domain of TFIIS (dashed line) binds Spt8 and recruits the Spt8-containing form of SAGA. Based on genetic evidence, this is proposed to be essential in the absence of Rpb4, but does not depend on the Gcn5 histone acetylase. However, Gcn5 may optimise elongation, as suggested by the synthetic lethality between *rpb9*Δ and *gcn5*Δ.

Materials and methods

Strains

Yeast strains were constructed by standard meiotic crosses and transformations (Table I). Most of the mutant strains were full deletions with a KanMX4 insertion (Euroscarf: http://www.uni-frankfurt.de/fb15/mikro/euroscarf/). In crosses involving the KanMX4 marker in the two parental strains, double mutant segregants were isolated from a nonparental ditype tetrad, and confirmed by PCR. Y14279 (*med13*Δ) and Y12666 (*spt8*Δ) derive from the BY4742 strain (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*). Y04411 (*dst1*Δ), Y04228 (*spt3*Δ), Y03218 (*spt7*Δ) and Y02666 *(spt8Δ)* derive from BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*). The other parental strains used were CMKy1 (Davie and Kane, 2000), FY1093 (*spt7*Δ; Wu and Winston, 2002) RPO21-Δ104 (Allison and Ingles, 1989), MC11-1 (Choder and Young, 1993), OG30-4C (*gcn5*Δ::*HIS3*) and YVV9 (*rpb9*Δ; Van Mullem *et al*, 2002b), SL21 (Shpakovski *et al*, 2000), YPH499 and YPH500 (Sikorski and Hieter, 1989). Mt8 is a *dst1-R287Q, E291N* double-mutant defective in the RNA cleavage activity (Ubukata *et al*, 2003). DY236-6B is a *MATa trp1Δ63* strain obtained by crossing FY67 (Winston *et al*, 1995) to YPH500. Strain Y190 (*MATa gal4 gal80 his3 trp1-901 ade2-1 ura3-52 leu2-2,112 CYH1^{R}URA3*::*GAL1*::*lacZ LYS2*::*GAL4(UAS)*::*HIS3*) was used as host in two-hybrid tests (Flores *et al*, 1999). In this strain, *HIS3* and *lacZ* are used as reporter genes of the two-hybrid interaction.

Table I Yeast strains

Strain	Genotype	Origin
D485-4D	*MATa ade2-1 leu2-Δ1 lys2-801 ura3-52 dst1-R287Q,E291N*	Mt8 × YPH500
ESH1	*MATa ade2-1 his3Δ200 leu2Δ1 lys2-801 trp1Δ63 ura3-52 dst1*::*hisG*	Spontaneous subclone of CMKy1
ESH9-2A	*MATα his3 leu2 met15Δ0 trp1Δ63 ura3 dst1Δ*::*KanMX4*	YPH500 × Y04411
ESH13-8D	*MATα ade2-1 his3 leu2 met15Δ0 trp1Δ63 ura3 spt8Δ*::*KanMX4*	SL21-3A × Y02666
ESH27-15C	*MATa ade2-1 his3 leu2 trp1 ura3 rpb4Δ*::*HIS3*	RPO21-Δ104 × MC11-1
ESH28-2A	*MATα ade2-1 his3 leu2 trp1 ura3 spt8Δ*::*KanMX4 rpb4Δ*::*HIS3 /2*μ *URA3 RPB4*	ESH13-8D × ESH27-15C with pYX-RPB4
ESH29-1B	*MATa ade2-1 his3 leu2 trp1 ura3 dst1Δ*::*KanMX4 rpb4Δ*::*HIS3 /2*μ *URA3 RPB4*	ESH9-2A × ESH27-15C with pYX-RPB4
SL21-3A	*MATα ade2-1 his3Δ200 leu2Δ1 lys2-801 trp1Δ63 rpb4Δ*::*URA3(Kl)*	Segregant of SL21
YBV39	*MATα ade2-1 his3 leu2 lys2 trp1Δ63 ura3 med13Δ*::*KanMX4*	CMKy1 × Y14279
YBV55	*MATα ade2-1 his3Δ leu2Δ lys2 ura3 spt8Δ*::*KanMX4 dst1Δ*::*hisG-URA3-hisG*	CMKy1 × Y12666
YBV61	*MATα ade2-1 his3Δ leu2Δ lys2 trp1Δ63 ura3 spt8Δ*::*KanMX4*	CMKy1 × Y12666
YMW135	*MATα his3Δ1 leu2Δ0 lys2Δ0 trp1Δ63 spt8Δ*::*KanMX4*	Y12666 × DY236-6b
YMW220	*MATa his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 spt8Δ*::*KanMX4*	Y03218 × YMW135
YMW238	*MATa ade2-1 his3 leu2 lys2 ura3 rpb9Δ*::*HIS3 spt8Δ*::*KanMX4*	YVV9 × Y12666
YVV62	*MATα ade2-1 his3Δ200 leu2 lys2 trp1Δ63 ura3-52 rpb9Δ*::*HIS3 dst1Δ*::*hisG-URA3-hisG*	YVV9 × CMKy1

Table II Plasmids

Plasmid	Yeast genes	Backbone vector
pCM-DST1	*CEN TRP1 ptetO$_7$::DST1*	pCM185
pCM-ΔN	*CEN TRP1 ptetO$_7$::dst1-133,309*	pCM185
pSPT8-84	*2 μ LEU2 Gal4$_{AD}$(768–881)::spt8-334,520*	pACT2
PMED13-111	*2 μ LEU2 Gal4$_{AD}$(768–881)::med13-106,669*	pACT2
pVV70	*2 μ TRP1 Gal4$_{BD}$(1–147)::DST1*	pGBT9
pVV71	*2 μ TRP1 Gal4$_{BD}$(1–147)::dst1-1,132*	pGBT9
pVV72	*2 μ TRP1 Gal4$_{BD}$(1–147)::dst1-1,242*	pGBT9
pVV73	*2 μ TRP1 Gal4$_{BD}$(1–147)::dst1-75,309*	pGBT9
pVV74	*2 μ TRP1 Gal4$_{BD}$(1–147)::dst1-133,309*	pGBT9
pVV75	*2 μ TRP1 Gal4$_{BD}$(1–147)::dst1-243,309*	pGBT9
pVV80	*2 μ TRP1 pPGK::DST1*	pGEN
pVV81	*2 μ TRP1 pPGK::dst1-133,309*	pGEN
pVV225	*2 μ TRP1 pPGK::SPT8::13Myc*	pVV203
pVV226	*2 μ TRP1 pPGK::MED13::13Myc*	pVV203
pVV227	*2 μ URA3 pPGK::DST1::3HA*	pVV215
pVV228	*2 μ TRP1 pPGK::DST1::3HA*	pVV201
pVV229	*2 μ TRP1 pPGK::MED13::3HA*	pVV201
pVV230	*2 μ TRP1 pPGK::SPT8::3HA*	pVV201
pVV231	*2 μ URA3 pPGK::GCN5::13Myc*	pVV217
pVV232	*2 μ URA3 pPGK::SPT7::13Myc*	pVV217
pVV233	*2 μ URA3 pPGK::CDK8::13Myc*	pVV217
pVV234	*2 μ URA3 pPGK::DST1::13Myc*	pVV217
pYX212-RPB4	*2 μ URA3 pTPI1::RPB4*	pYX212

Growth media

YPD and SC are standard complete or synthetic growth media with 2% glucose. YPD-Caffeine contains caffeine at 10 mM. SC-W is tryptophan omission media. MPA contains mycophenolate (Sigma) freshly dissolved in methanol (10 mg/ml), added to SC or SC-W. FOA (Boeke *et al*, 1984) is SC with 0.1% of 5-fluoro orotic acid (Toronto Research Chemicals). *Spt* phenotypes were assessed by their ability to restore histidine prototrophy in *his4-917δ* and to generate lysine auxotrophy in a *LYS2-173R2* context (Wu and Winston, 2002).

Plasmids

Plasmids (Table II) were prepared by standard subcloning or by the GATEWAY™ technique (Walhout *et al*, 2000), except for pSPT8-84 and pMED13-111, which were isolated from a random library of genomic fragments (about 0.7 kb) fused to the Gal4$_{AD}$(768–881). Constructs based on polymerase chain reaction (PCR) were sequenced to avoid spurious mutations generated by the amplification process. pVV70–pVV75 are derivatives of pGBT9 with full-length or partly deleted forms of *DST1* fused to the Gal4$_{BD}$(1–147) domain, constructed by directional cloning between *Sma*I and *Bam*HI. Their expression was tested by Western blotting assay using anti-Gal4$_{BD}$(1–147) antibodies (Clontech). pVV80 (2 μ *TRP1 DST1*) was constructed by cloning the *DST1 Sma*I–*Sal*I insert from pVV70 into the multicopy expression vector pGEN (Shpakovski *et al*, 1995). pVV81 (2 μ *TRP1 dst1-133,309*) was obtained by directional cloning of a PCR-amplified *Bam*HI–*Cla*I *dst1-133,309* insert into the pGEN. pCM-DST1 and pCM-ΔN were obtained by subcloning the *Bam*HI–*Mlu*I *DST1* and *dst1-133,309* inserts of pVV80 and pVV81 into pCM185 (Gari *et al*, 1997). PYX212-RPB4 was constructed by cloning a PCR-amplified *RPB4* coding sequence between the *Nco*I and *Sal*I sites of the multicopy expression vector pYX212 (Yeast R&D Systems). pVV225–pVV234 were constructed using the GATEWAY™ technique (Walhout *et al*, 2000). Briefly, the *SPT8*, *GCN5*, *SPT7*, *MED13*, *CDK8* and *DST1* coding sequences were PCR-amplified without their termination codon from oligonucleotides ending with attB1 and attB2 sites. Entry clones were generated by *in vitro* recombination with the attP1 and attP2 sites of the pDONR™201 vector (Invitrogen). Inserts were sequenced and then subcloned using LR reactions into appropriate pVV201, pVV203, pVV215 or pVV217 pGEN-derived vectors (Van Mullem *et al*, 2003).

Immunopurification

Immunopurification was carried out as previously described (Van Mullem *et al*, 2002b), starting from 1 mg of yeast crude extract and using about 0.8 μg of mouse monoclonal anti-Myc (9E10 from Babco) or anti-HA (12CA5) antibody. Beads were washed three times for 5 min in a modified IP buffer (20 mM HEPES, pH 7.5, 0.5 mM EDTA, 500 mM NaCl, 1 mM dithiothreitol, 20% glycerol, 0.1% Triton X-100). Immunoprecipitated proteins were eluted, heated for 10 min at 95°C, separated by SDS–PAGE and revealed by monoclonal anti-HA or anti-Myc antibodies (Babco) using the ECL™ Western Blotting Detection kit (Amersham).

Acknowledgements

We thank André Sentenac for his kind support and Michel Werner for helpful comments during this work. Drs Fred Winston, Caroline Kane and Toshiyuki Nakanishi provided useful mutant strains, Audrey Suleau constructed plasmid pYX212-RPB4 and Séverine Coddens helped in immunopurification experiments. We also thank an anonymous referee for illuminating critics of an earlier version of this paper. This work was partly supported by the Direction des Relations Internationales (CEA). MW was supported by short-term fellowships of EMBO and FEBS. ES was supported by a Young Scientist Fellowship from INTAS, by the Russian Federation of Young Scientists and by research programmes from the Russian Academy of Sciences and the Russian Foundation of Basic Research. BVD and VVM were supported by the Belgian Fonds pour la Recherche dans l'Industrie et l'Agriculture and Fonds National de la Recherche Scientifique, respectively.

References

Agarwal K, Baek KH, Jeon CJ, Miyamoto K, Ueno A, Yoon HS (1991) Stimulation of transcript elongation requires both the zinc finger and RNA polymerase II binding domains of human TFIIS. *Biochemistry* **30:** 7842–7851

Allison LA, Ingles CJ (1989) Mutations in RNA polymerase II enhance or suppress mutations in gal4. *Proc Natl Acad Sci USA* **86:** 2794–2798

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

Aso T, Lane WS, Conaway JC, Conaway RC (1995) Elongin (SIII): a multisubunit regulator of elongation by RNA polymerase II. *Science* **269:** 1439–1443

Awrey DE, Shimasaki N, Koth C, Weilbaecher R, Olmsted V, Kazanis S, Shan X, Arellano J, Arrowsmith CH, Kane CM, Edwards AM (1998) Yeast transcript elongation factor (TFIIS), structure and function. II: RNA polymerase binding, transcript cleavage, and read-through. *J Biol Chem* **273:** 22595–22605

Awrey DE, Weilbaecher RG, Hemming SA, Orlicky SM, Kane CM, Edwards AM (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

Belotserkovskaya R, Sterner DE, Deng M, Sayre MH, Lieberman PM, Berger SL (2000) Inhibition of TATA-binding protein function by SAGA subunits Spt3 and Spt8 at Gcn4-activated promoters. *Mol Cell Biol* **20:** 634–647

Bhaumik SR, Green MR (2001) SAGA is an essential *in vivo* target of the yeast acidic activator Gal4p. *Genes Dev* **15:** 1935–1945

Boeke JD, Lacroute F, Fink GR (1984) A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. *Mol Gen Genet* **197:** 345–346

Booth V, Koth CM, Edwards AM, Arrowsmith CH (2000) Structure of a conserved domain common to the transcription factors TFIIS, elongin A, and CRSP70. *J Biol Chem* **275:** 31266–31268

Borggrefe T, Davis R, Erdjument-Bromage H, Tempst P, Kornberg RD (2002) A complex of the srb8, -9, -10, and -11 transcriptional regulatory proteins from yeast. *J Biol Chem* **277:** 44202–44207

Borukhov S, Sagitov V, Goldfarb A (1993) Transcript cleavage factors from *E. coli*. *Cell* **72:** 459–466

Boube M, Joulia L, Cribbs DL, Bourbon HM (2002) Evidence for a Mediator of RNA polymerase II transcriptional regulation conserved from yeast to man. *Cell* **110:** 143–151

Bourbon HM, Aguilera A, Ansari AZ, Asturias FJ, Berk AJ, Bjorklund S, Blackwell TK, Borggrefe T, Carey M, Carlson M, Conaway JW, Conaway RC, Emmons SW, Fondell JD, Freedman LP, Fukasawa T, Gustafsson CM, Han M, He X, Herman PK, Hinnebusch AG, Holmberg S, Holstege FC, Jaehning JA, Kim YJ, Kuras L, Leutz A, Lis JT, Meisterernest M, Naar AM, Nasmyth K, Parvin JD, Ptashne M, Reinberg D, Ronne H, Sadowski I, Sakurai H, Sipiczki M, Sternberg PW, Stillman DJ, Strich R, Struhl K, Svejstrup JQ, Tuck S, Winston F, Roeder RG, Kornberg RD (2004) A Unified Nomenclature for Protein Subunits of Mediator Complexes Linking Transcriptional Regulators to RNA Polymerase II. *Mol Cell* **14:** 553–557

Chédin S, Riva M, Schultz P, Sentenac A, Carles C (1998) The RNA cleavage activity of RNA polymerase III is mediated by an essential TFIIS-like subunit and is important for transcription termination. *Genes Dev* **12:** 3857–3871

Choder M, Young RA (1993) A portion of RNA polymerase II molecules has a component essential for stress responses and stress survival. *Mol Cell Biol* **13:** 6984–6991

Cosma MP, Tanaka T, Nasmyth K (1999) Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter. *Cell* **97:** 299–311

Davie JK, Kane CM (2000) Genetic interactions between TFIIS and the Swi–Snf chromatin-remodeling complex. *Mol Cell Biol* **20:** 5960–5973

Desmoucelles C, Pinson B, Saint-Marc C, Daignan-Fornier B (2002) Screening the yeast 'disruptome' for mutants affecting resistance to the immunosuppressive drug, mycophenolic acid. *J Biol Chem* **277:** 27036–27044

Eisenmann DM, Chapon C, Roberts SM, Dollard C, Winston F (1994) The *Saccharomyces cerevisiae* SPT8 gene encodes a very acidic protein that is functionally related to SPT3 and TATA-binding protein. *Genetics* **137:** 647–657

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

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

Flores A, Briand JF, Boschiero C, Gadal O, Andrau JC, Rubbi L, Van Mullem V, Goussot M, Marck C, Carles C, Thuriaux P, Sentenac A, Werner M (1999) A protein–protein interaction map of yeast RNA polymerase III. *Proc Natl Acad Sci USA* **96:** 7815–7820

Fromont-Racine M, Rain JC, Legrain P (1997) Toward a functional analysis of the yeast genome through exhaustive two-hybrid screens. *Nat Genet* **16:** 277–282

Gari E, Piedrafita L, Aldea M, Herrero E (1997) A set of vectors with tetracycline-regulatable promoter system for modulated gene expression in *Saccharomyces cerevisiae*. *Yeast* **13:** 837–848

Grant PA, Duggan L, Cote J, Roberts SM, Brownell JE, Candau R, Ohba R, Owen-Hughes T, Allis CD, Winston F, Berger SL, Workman JL (1997) Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes Dev* **11:** 1640–1650

Hengartner CJ, Myer VE, Liao SM, Wilson CJ, Koh SS, Young RA (1998) Temporal regulation of RNA polymerase II by Srb10 and Kin28 cyclin-dependent kinases. *Mol Cell* **2:** 43–53

Hirst M, Kobor MS, Kuriakose N, Greenblatt J, Sadowski I (1999) GAL4 is regulated by the RNA polymerase II holoenzyme-associated cyclin-dependent protein kinase SRB10/CDK8. *Mol Cell* **3:** 673–678

Horikoshi M, Sekimizu K, Hirashima S, Mitsuhashi Y, Natori S (1985) Structural relationships of the three stimulatory factors of RNA polymerase II from Ehrlich ascites tumor cells. *J Biol Chem* **260:** 5739–5744

Izban MG, Luse DS (1992) The RNA polymerase II ternary complex cleaves the nascent transcript in a 3′–5′ direction in the presence of elongation factor SII. *Genes Dev* **6:** 1342–1356

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

Labhart P, Morgan GT (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

Larschan E, Winston F (2001) The *S. cerevisiae* SAGA complex functions *in vivo* as a coactivator for transcriptional activation by Gal4. *Genes Dev* **15:** 1946–1956

Liao SM, Zhang J, Jeffery DA, Koleske AJ, Thompson CM, Chao DM, Viljoen M, Van Vuuren H, Young RA (1995) A kinase–cyclin pair in the RNA polymerase II holoenzyme. *Nature* **374:** 193–196

Lindstrom DL, Hartzog GA (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

Li S, Smerdon MJ (2002) Rpb4 and Rpb9 mediate subpathways of transcription-coupled DNA repair in *Saccharomyces cerevisiae*. *EMBO J* **21:** 5921–5929

Miller JH (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor, New York, USA: Cold Spring Harbor Laboratory

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

Nakanishi T, Shimoaraiso M, Kubo T, Natori S (1995) Structure–function relationship of yeast S-II in terms of stimulation of RNA polymerase II, arrest relief, and suppression of 6-azauracil sensitivity. *J Biol Chem* **270:** 8991–8995

Natori S, Takeuchi K, Takahashi K, Mizuno D (1973) DNA dependent RNA polymerase from Ehrlich ascites tumor cells. II. Factors stimulating the activity of RNA polymerase II. *J Biochem (Tokyo)* **73:** 879–888

Nogi Y, Yano R, Dodd J, Carles C, Nomura M (1993) Gene RRN4 in *Saccharomyces cerevisiae* encodes the 12.2 subunit of RNA polymerase I and is essential only at high temperature. *Mol Cell Biol* **13:** 114–122

Pan G, Aso T, Greenblatt J (1997) Interaction of elongation factors TFIIS and elongin A with a human RNA polymerase II holoenzyme capable of promoter-specific initiation and responsive to transcriptional activators. *J Biol Chem* **272:** 24563–24571

Pokholok DK, Hannett NM, Young RA (2002) Exchange of RNA polymerase II initiation and elongation factors during gene expression *in vivo*. *Mol Cell* **9:** 799–809

Pray-Grant MG, Schieltz D, McMahon SJ, Wood JM, Kennedy EL, Cook RG, Workman JL, Yates III JR, Grant PA (2002) The novel SLIK histone acetyltransferase complex functions in the yeast retrograde response pathway. *Mol Cell Biol* **22:** 8774–8786

Reines D (1992) Elongation factor-dependent transcript shortening by template-engaged RNA polymerase II. *J Biol Chem* **267:** 3795–3800

Ryu S, Zhou S, Ladurner AG, Tjian R (1999) The transcriptional cofactor complex CRSP is required for activity of the enhancer-binding protein Sp1. *Nature* **397:** 446–450

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

Shpakovski GV, Acker J, Wintzerith M, Lacroix JF, Thuriaux P, Vigneron M (1995) Four subunits shared by the three classes of RNA polymerases are functionally interchangeable between *Homo sapiens* and *Saccharomyces cerevisiae*. *Mol Cell Biol* **15:** 4702–4710

Shpakovski GV, Gadal O, Labarre-Mariotte S, Lebedenko EN, Miklos I, Sakurai H, Proshkin SA, Van Mullem V, Ishihama A, Thuriaux P (2000) Functional conservation of RNA polymerase II in fission and budding yeasts. *J Mol Biol* **295:** 1119–1127

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

Sterner DE, Belotserkovskaya R, Berger SL (2002) SALSA, a variant of yeast SAGA, contains truncated Spt7, which correlates with activated transcription. *Proc Natl Acad Sci USA* **99:** 11622–11627

Sterner DE, Grant PA, Roberts SM, Duggan LJ, Belotserkovskaya R, Pacella LA, Winston F, Workman JL, Berger SL (1999) Functional organization of the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation, and TATA-binding protein interaction. *Mol Cell Biol* **19:** 86–98

Tschochner H (1996) A novel RNA polymerase I-dependent RNase activity that shortens nascent transcripts from the 3′ end. *Proc Natl Acad Sci USA* **93:** 12914–12919

Ubukata T, Shimizu T, Adachi N, Sekimizu K, Nakanishi T (2003) Cleavage, but not read-through, stimulation activity is responsible for three biologic functions of transcription elongation factor S-II. *J Biol Chem* **278:** 8580–8585

Van Mullem V, Landrieux E, Vandenhaute J, Thuriaux P (2002a) Rpa12p, a conserved RNA polymerase I subunit with two functional domains. *Mol Microbiol* **43:** 1105–1113

Van Mullem V, Wery M, De Bolle X, Vandenhaute J (2003) Construction of a set of *Saccharomyces cerevisiae* vectors designed for recombinational cloning. *Yeast* **20:** 739–746

Van Mullem V, Wery M, Werner M, Vandenhaute J, Thuriaux P (2002b) The Rpb9 subunit of RNA polymerase II binds TFIIE and functionally interacts with the SAGA and Elongator factors. *J Biol Chem* **277:** 10220–10225

Walhout AJ, Temple GF, Brasch MA, Hartley JL, Lorson MA, van den Heuvel S, Vidal M (2000) GATEWAY recombinational cloning: application to the cloning of large numbers of open reading frames or ORFeomes. *Methods Enzymol* **328:** 575–592

Williams LA, Kane CM (1996) Isolation and characterization of *Schizosaccharomyces pombe* gene encoding transcript elongation factor TFIIS. *Yeast* **12:** 227–236

Winston F, Dollard C, Ricuperco-Hovasse S (1995) Construction of a set of convenient *Saccharomyces cerevisiae* strains that are isogenic to S288C. *Yeast* **11:** 53–55

Wu PY, Winston F (2002) Analysis of Spt7 function in the *Saccharomyces cerevisiae* SAGA coactivator complex. *Mol Cell Biol* **22:** 5367–5379

Yamazaki K, Guo L, Sugahara K, Zhang C, Enzan H, Nakabeppu Y, Kitajima S, Aso T (2002) Identification and biochemical characterization of a novel transcription elongation factor, Elongin A3. *J Biol Chem* **277:** 26444–26451