Genetic Interactions With C-Terminal Domain (CTD) Kinases and the CTD of RNA Pol II Suggest a Role for *ESS1* in Transcription Initiation and Elongation in *Saccharomyces cerevisiae*

Cathy B. Wilcox,*,[1] Anne Rossettini* and Steven D. Hanes*,†,[2]

**Molecular Genetics Program, Wadsworth Center, New York State Department of Health, Albany, New York 12208 and* †*Department of Biomedical Sciences, School of Public Health, State University of New York, Albany, New York 12208*

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ABSTRACT

Ess1 is an essential prolyl isomerase that binds the C-terminal domain (CTD) of Rpb1, the large subunit of RNA polymerase II. Ess1 is proposed to control transcription by isomerizing phospho-Ser-Pro peptide bonds within the CTD repeat. To determine which step(s) in the transcription cycle might require Ess1, we examined genetic interactions between *ESS1* and genes encoding the known CTD kinases (*KIN28*, *CTK1*, *BUR1*, and *SRB10*). Although genetic interactions were identified between *ESS1* and all four kinases, the clearest interactions were with *CTK1* and *SRB10*. Reduced dosage of *CTK1* rescued the growth defect of *ess1^{ts}* mutants, while overexpression of *CTK1* enhanced the growth defects of *ess1^{ts}* mutants. Deletion of *SRB10* suppressed *ess1^{ts}* and *ess1*Δ mutants. The interactions suggest that Ess1 opposes the functions of these kinases, which are thought to function in preinitiation and elongation. Using a series of CTD substitution alleles, we also identified Ser5-Pro6 as a potential target for Ess1 isomerization within the first "half" of the CTD repeats. On the basis of the results, we suggest a model in which Ess1-directed conformational changes promote dephosphorylation of Ser5 to stimulate preinitiation complex formation and, later, to inhibit elongation.

THE expression of eukaryotic RNA polymerase II (pol II)-dependent genes is regulated at multiple levels: transcription initiation, capping, elongation, splicing, termination, and transcript cleavage (Lee and Young 2000). Each step requires accessory proteins whose activities appear to be coordinated, in part, by binding to the C-terminal domain (CTD) of the pol II large subunit, Rpb1 (Hirose and Manley 2000). How binding of these proteins to the CTD is regulated in such a way as to promote efficient transcription and mRNA processing is not understood.

The CTD is composed of multiple repeats of the consensus heptapeptide sequence YSPTSPS. In yeast, the CTD contains 26 or 27 repeats of this sequence, while in mammals the CTD contains 52 repeats (Cordon 1990). Changes in the phosphorylation state of this Ser-Pro-rich region accompany the transitions of RNA polymerase II as it moves from the promoter of a gene to its terminus (Komarnitsky *et al.* 2000). The CTD is hypophosphorylated during preinitiation complex (PIC) formation and is phosphorylated as pol II leaves the promoter and during elongation (Laybourn and Dahmus 1989). The different phosphorylation states of the CTD might act as a signal to coordinate binding and release of the multiple proteins required for individual steps of transcription.

Four potential CTD kinases have been identified, each functioning as part of a kinase-cyclin complex. These kinases are thought to act at discrete steps in transcription. Kin28-Ccl1 (Cdk7-cyclin H in humans), a component of TFIIH, facilitates promoter clearance and mRNA capping (Cismowski *et al.* 1995; Akhtar *et al.* 1996; Rodriguez *et al.* 2000). Ctk1-Ctk2, the kinase-cyclin subunits of CTDK-I, and Bur1-Bur2 (Cdk9-cyclin T in humans) are thought to promote efficient elongation (Lee and Greenleaf 1997; Majello *et al.* 1999; Murray *et al.* 2001). In addition, CTDK-I may help recruit pre-mRNA processing factors involved in 3′-end formation (Skaar and Greenleaf 2002). Srb10-Srb11 (Cdk8-cyclin C in humans), a component of the SRB/mediator, negatively regulates transcription of certain genes during exponential growth in rich media (Holstege *et al.* 1998) by inhibiting PIC formation (Hengartner *et al.* 1998) and by promoting the degradation of certain transcription activators (Chi *et al.* 2001). In addition to four CTD kinases, two CTD-specific phosphatases have been identified, Fcp1 (Archambault *et al.* 1997; Kobor *et al.* 1999) and Ssu72 (K. Shankarling and M. Hampsey, personal communication).

Changing the phosphorylation state of the CTD is one mechanism by which binding of accessory proteins to the CTD of pol II may be regulated. Another mechanism might be conformational isomerization of the CTD

[1]*Present address:* Magee-Womens Research Institute, Ovarian Cancer Center of Excellence, 204 Craft Ave., Pittsburgh, PA 15213-3054.

[2]*Corresponding author:* Wadsworth Center, 120 New Scotland Ave., Albany, NY 12208. E-mail: hanes@wadsworth.org

by enzymes called peptidyl-prolyl *cis/trans* isomerases (PPIases; Schiene and Fischer 2000). A PPIase could alter the structure of the CTD, changing the affinity of accessory proteins known to bind the CTD. One such enzyme, Ess1, which is essential for growth in yeast (Hanes *et al.* 1989), binds phospho-Ser-Pro motifs (Yaffe *et al.* 1997), such as those found within the phosphorylated CTD. Ess1 has been shown to interact physically and genetically with Rpb1 (Morris *et al.* 1999; Wu *et al.* 2000, 2001) and is thought to stimulate the binding of Fcp1, a CTD-specific phosphatase (Lin *et al.* 2002), to the CTD. Analysis of the Bye1 high-copy suppressor of *ess1* mutants led to the finding that Ess1 acts negatively in transcription elongation (Wu *et al.* 2003).

Here, we examined genetic interactions between *ESS1* and genes encoding the four known or suspected CTD kinases. The goals were to help identify those step(s) during the transcription cycle in which Ess1 might act and to identify possible target sites within the CTD that are shared between the CTD kinases and Ess1. Genetic interactions with multiple CTD kinases indicate that Ess1 is likely to act at more than one step in transcription. In addition to a negative role in elongation, our results point to a positive role for Ess1 in preinitiation complex formation. We have also discovered that $ess1^{ts}$ mutants are suppressed by serine-to-alanine mutations (YSPTAPS) in the amino-terminal half of the CTD repeats. Since Ser5-to-alanine substitution prevents phosphorylation at this position, suppression by this allele suggests that Ess1 may function by promoting dephosphorylation of Ser5 in the amino-terminal portion of the CTD.

MATERIALS AND METHODS

Yeast strains, genetic methods, and media: Yeast strains used in these experiments are listed in Table 1 and most are derived from the strain W303-1A (R. Rothstein), except YPR57, which is derived from W1021-7C and W961-5B (R. Rothstein), the *rpb1*Δ pRP112 strain (Nonet and Young 1989), and GY604 (Murray *et al.* 2001). The *ess1* mutant alleles have been described previously (Wu *et al.* 2000). Standard methods were used for transformation, mating, sporulation, and tetrad dissection (Guthrie and Fink 1991). Rich (YEPD), complete synthetic (CSM), and 5-fluoroorotic acid (5-FOA) media were prepared according to standard methods (Adams *et al.* 1997). For spot tests, cells were grown in liquid culture to an OD_{600} of 0.5–0.8 (~1–2 × 10^7 cells/ml). An estimated 2–3 μl of cells (diluted to 0.5 OD_{600} units) were spotted onto the equivalent solid media, using a multiprong device following serial 1:5 dilutions.

Gene deletions and disruptions: *KIN28*, *CTK1*, and *BUR1* gene deletions were generated by transformation of yeast strains W303-1A/B, CBW9, CBW15, and CBW32 with a polymerase chain reaction (PCR) product containing a $G418^R$ cassette flanked by ~45 bp homologous to the ends of the target open reading frame (Wach *et al.* 1994). Transformants were selected on YEPD containing 400 mg/liter geneticin (GIBCO BRL, Gaithersburg, MD). *SRB10* was disrupted (and mostly deleted) by transformation of yeast with a *Sma*I-*Pst*I restriction fragment of plasmid psrb10::TRP1 (gift of J. Dutko and R. Zitomer). This construct deletes base pairs from 300 to 1307 of the 1668-bp coding sequence of *SRB10*. The *srb10* mutant strains generated using this construct are referred to as deletions (*srb10*Δ) throughout this article. The presence of the knockouts was determined by PCR, using one primer complementary to sequence within the marker gene and another with complementarity to chromosomal DNA flanking the gene of interest. The identity of $ess1^{ts}$ alleles in tetrad segregants was determined by PCR, using oligonucleotides specific to each allele, or by DNA sequencing. Oligonucleotide sequences are available on request.

Plasmids: The high-copy plasmid pKIN28 was made by subcloning *KIN28-HA* from pGK13 (Kimmelman *et al.* 1999) into pRS425 (2μ, *LEU2*; Christianson *et al.* 1992), using *Pst*I and *Hin*dIII. Plasmids containing $kin28^{ts16}$ and $kin28^{T162A}$ (in YCplac22; *CEN TRP1*) were from Mark Solomon (Kimmelman *et al.* 1999). *CTK1* plasmids were from Jan Jones and Arno Greenleaf. The high-copy plasmid pJYC1501 contains a *Bgl*II-*Sal*I fragment of the *CTK1* locus inserted into the same sites of YEp24 (2μ, *URA3*). pJYC1501-HA3 and pJYC1501-K212A-HA3 are derivatives that encode HA-tagged wild-type and kinase-deficient Ctk1 (K212A), respectively. pJYC1511 is a centromeric *CTK1* plasmid (*CEN HIS3*). Additional plasmids (2μ, *URA3*) encoding wild-type (YEp112CTK1WT-HA) and kinase-deficient Ctk1 (D324N; YEp112CTK1DN-HA) were from Denis Ostapenko and Mark Solomon. A high-copy *SRB10* plasmid, YEp(195)SRB10 (2μ, *URA3*), was obtained from Richard Zitomer. Plasmids bearing CTD mutations are listed in Table 4 and were from Jeff Corden. *BUR1* plasmids pGP232 (2μ, *LEU2 BUR1*), pGP162 (*CEN TRP1 BUR1*), and pGP163 (*CEN TRP1 bur1-2*) were from Gregory Prelich. YEpHESS1 has been described (Hanes *et al.* 1989).

Plasmid-loss experiments: We used a plasmid encoding *Candida albicans* Ess1, which complements *Saccharomyces cerevisiae* *ess1* mutations (Devasahayam *et al.* 2002). The *C. albicans* version was used rather than the *S. cerevisiae* version to reduce possible recombination between the plasmid-borne copy of *ESS1* and chromosomal *ess1* mutations. The *kin28 ess1* double-mutant strains were patched to solid 5-FOA − TRP medium to identify cells capable of growing after loss of pCaESS1 (2μ, *URA3*). For CTD mutant experiments, the haploid strains *rpb1*Δ pRP112 and $ess1^{H164R}$ *rpb1*Δ pRP112 were transformed with *LEU2* plasmids carrying rpb1-CTD mutations, and growth after loss of wild-type *RPB1* carried on pRP112 (*URA3*) was monitored by replica plating to 5-FOA medium.

RESULTS

Allele-specific interactions between *KIN28 and ESS1*: Kin28, an essential component of TFIIH, is thought to phosphorylate the CTD at the time of promoter escape (Hengartner *et al.* 1998). Because *ESS1* is an essential gene, we used temperature-sensitive (*ts*) alleles that allow cell growth at 25° or 30° but not at 35° or 37°. The two we used, $ess1^{H164R}$ and $ess1^{A144T}$, contain single-amino-acid substitutions in structurally distinct regions of the protein, one in the catalytic site (H164R) and one at the interface between the WW and prolyl isomerase domains (A144T; Wu *et al.* 2000). To investigate a potential genetic interaction between *ESS1* and *KIN28*, we first transformed *ess1* mutant strains with a high-copy plasmid to overexpress *KIN28*. High-copy expression of *KIN28* neither suppressed nor enhanced the *ts* lethality of either $ess1^{ts}$ allele (data not shown), nor did lowering the dos-

TABLE 1

***S. cerevisiae* strains**

Strain	Genotype	Source
W303-1A	*MAT***a** *ura3-1 trp1-1 leu2-3,112 can1-100 ade2-1 his3-11,15*	R. Rothstein
W303-1B	*MAT*α *ura3-1 trp1-1 leu2-3,112 can1-100 ade2-1 his3-11,15*	R. Rothstein
YGD-ts8W	*MAT***a** *ura3-1 leu2-3,112 trp1-1 can1-100 ade2-1 his3-11,15 ess1^{A144T}*	Wu *et al.* (2000)
YGD-ts22W	*MAT***a** *ura3-1 leu2-3,112 trp1-1 can1-100 ade2-1 his3-11,15 ess1^{H164R}*	Wu *et al.* (2000)
YPR57α	*MAT*α *ura3-1 trp1-1 can1-100 ade2-1 his3-11,15 ess1ΔHIS3* pCaESS1 (*URA3*)	P. Ren and S. Hanes
YPR57**a**	*MAT***a** *ura3-1 TRP1 can1-100 ade2-1 his3-11,15 ess1ΔHIS3* pCaESS1 (*URA3*)	P. Ren and S. Hanes
YXW-2.1	*MAT***a** *ura3-1 leu2-3,112 trp1-1 can1-100 ade2-1 his3-11,15 ess1ΔTRP1* pTPI-PIN1 (*LEU2*)	Wu *et al.* (2000)
YSH-55	*MAT***a**/α *ade2-1/ade2-1 can1-100/can1-100 his3-11,15/his3-11,15 leu2-3,112/LEU2 ura3-1/ura3-1 trp1-1/TRP1 ess1ΔHIS3/ESS1*	S. Hanes
CBW9	*MAT***a**/*MAT*α *ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/trp1-1 can1-100/can1-100 ade2-1/ade2-1 his3-11,15/his3-11,15 ess1^{H164R}/ess1^{H164R}*	Wu *et al.* (2000)
CBW15	*MAT***a**/*MAT*α *ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/trp1-1 can1-100/can1-100 ade2-1/ade2-1 his3-11,15/his3-11,15 ess1^{A144T}/ess1^{A144T}*	Wu *et al.* (2000)
CBW10	*MAT***a**/*MAT*α *ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/trp1-1 can1-100/can1-100 ade2-1/ade2-1 his3-11,15/his3-11,15 kin28ΔG418^{R}/KIN28*	This study
CBW23	*MAT***a**/*MAT*α *ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/trp1-1 can1-100/can1-100 ade2-1/ade2-1 his3-11,15/his3-11,15 kin28ΔG418^{R}/KIN28 ess1^{H164R}/ess1^{H164R}*	This study
CBW24	*MAT***a**/*MAT*α *ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/trp1-1 can1-100/can1-100 ade2-1/ade2-1 his3-11,15/his3-11,15 kin28ΔG418^{R}/KIN28 ess1^{A144T}/ess1^{A144T}*	This study
CBW25	*MAT***a**/*MAT*α *ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/trp1-1 can1-100/can1-100 ade2-1/ade2-1 his3-11,15/his3-11,15 ctk1ΔG418^{R}/CTK1*	This study
CBW26	*MAT***a**/*MAT*α *ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/trp1-1 can1-100/can1-100 ade2-1/ade2-1 his3-11,15/his3-11,15 ctk1ΔG418^{R}/CTK1 ess1^{H164R}/ess1^{H164R}*	This study
CBW27	*MAT***a**/*MAT*α *ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/trp1-1 can1-100/can1-100 ade2-1/ade2-1 his3-11,15/his3-11,15 ctk1ΔG418^{R}/CTK1 ess1^{A144T}/ess1^{A144T}*	This study
YJM1	*MAT***a** *ura3-1 trp1-1 can1-100 ade2-1 his3-11,15 srb10::TRP1*	This study
YJM2	*MAT***a** *ura3-1 trp1-1 can1-100 ade2-1 his3-11,15 ess1^{H164R} srb10::TRP1*	This study
YJM3	*MAT***a** *ura3-1 trp1-1 can1-100 ade2-1 his3-11,15 ess1^{A144T} srb10::TRP1*	This study
CBW22	*MAT***a** *ura3-1 trp1-1 can1-100 ade2-1 his3-11,15 ess1ΔHIS3 srb10::TRP1*	This study
YAR5	*MAT***a**/*MAT*α *ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/trp1-1 can1-100/can1-100 ade2-1/ade2-1 his3-11,15/his3-11,15 bur1ΔG418^{R}/BUR1 ESS1/ESS1*	This study
YAR6	*MAT***a**/*MAT*α *ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/trp1-1 can1-100/can1-100 ade2-1/ade2-1 his3-11,15/his3-11,15 bur1ΔG418^{R}/BUR1 ess1^{H164R}/ess1^{H164R}*	This study
YAR7	*MAT***a**/*MAT*α *ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/trp1-1 can1-100/can1-100 ade2-1/ade2-1 his3-11,15/his3-11,15 bur1ΔG418^{R}/BUR1 ess1^{A144T}/ess1^{A144T}*	This study
CBW32	*MAT***a**/*MAT*α *ura3-1/ura3-1 leu2-3,112/LEU2 trp1-1/trp1-1 can1-100/can1-100 ade2-1/ade2-1 his3-11/his3-11 ess1ΔHIS3/ess1ΔTRP1* pCaESS1 (*URA3*)	This study
YAR8	*MAT***a**/*MAT*α *ura3-1/ura3-1 leu2-3,112/LEU2 trp1-1/trp1-1 can1-100/can1-100 ade2-1/ade2-1 his3-11,15/his3-11,15 bur1ΔG418^{R}/BUR1 ess1ΔHIS3/ess1ΔTRP1* pCaESS1 (*URA3*)	This study
YAR15	*MAT*α *ura3-1 LEU2 trp1-1 can1-100 ade2-1 his3-11,15 bur1ΔG418^{R} ess1ΔHIS3* pbur1-2 (*TRP1*) pCaESS1 (*URA3*)	This study
GY604	*MAT*α *his3Δ250 lys2-1288 ura3-52 trp1Δ63 bur1-2*	Murray *et al.* (2001)
CBW1	*MAT***a** *ura3-1 trp1-1 leu2-3,112 can1-100 ade2-1 his3-11,15 rpb1ΔG418^{R}* pRP112 (*RPB1 URA3*)	Wu *et al.* (2000)
CBW2	*MAT***a** *ura3-1 trp1-1 leu2-3,112 can1-100 ade2-1 his3-11,15 ess1^{H164R} rpb1ΔG418^{R}* pRP112 (*RPB1 URA3*)	Wu *et al.* (2000)

age of *KIN28* in diploid strains (*kin28Δ/KIN28 ess1^{ts}/ess1^{ts}*; data not shown).

We next tested for synthetic lethality or suppression using *kin28Δ* haploid derivatives of strain W303-1A that carried the *kin28* alleles on centromeric plasmids (*e.g.*, *kin28Δ ess1^{H164R}* pkin28^{ts16}). The *kin28* alleles used were *kin28^{ts16}*, which is temperature sensitive for growth and defective in kinase activity (Cismowski *et al.* 1995), and *kin28^{T162A}*, which has a nonphosphorylatable T-loop mutation that severely reduces its kinase activity, but does not generally affect growth (Kimmelman *et al.* 1999). Genetic interaction was tested by monitoring the ability of different *kin28 ess1* double-mutant strains to grow on 5-FOA medium after loss of a plasmid-borne copy of *ESS1* (Figure 1). In the wild-type *ESS1* background at 25°, the *kin28^{ts16}* and *kin28^{T162A}* mutations appear to retain the pCaESS1 plasmid, as detected by moderate increases in 5-FOA sensitivity compared to *KIN28* cells

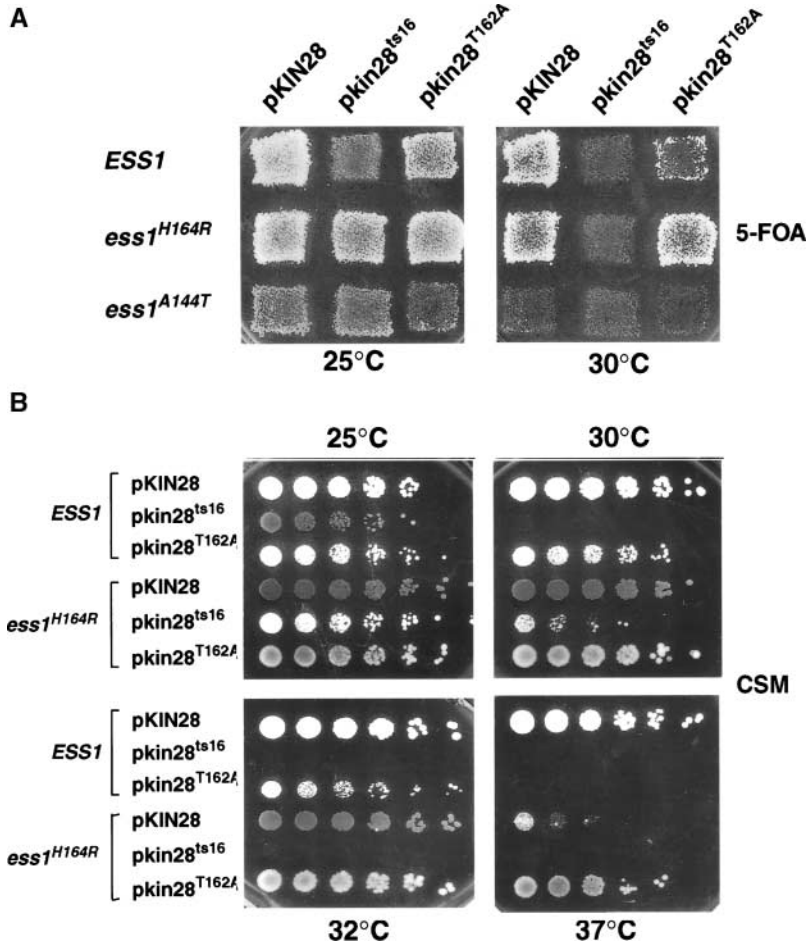

FIGURE 1.—Complex allele-specific interaction between *ess1* and *kin28* mutations. (A) The chromosomal *KIN28* gene was deleted in *ESS1* and *ess1* strains. *KIN28* function was provided by a plasmid-borne copy of *KIN28* or *kin28* as indicated. Cells were patched onto 5-FOA − trp medium and tested for the ability to lose a 2μ, *URA3*-based *ESS1* plasmid (pCaESS1) at 25° and 30°. Growth indicates ability to lose the *ESS1* plasmid. The most prominent interaction observed is between $ess1^{H164R}$ and $kin28^{T162A}$. In $kin28^{T162A}$ cells, 5-FOA resistance is maximal (*i.e.*, highest frequency of loss of pCaESS1) in an $ess1^{H164R}$ background *vs.* *ESS1* control cells. (B) *ESS1 kin28*Δ and $ess1^{H164R}$ *kin28*Δ cells bearing the indicated *KIN28* or *kin28* plasmids were spotted onto CSM − trp medium (1:5 serial dilutions) and grown for 3 days at the indicated temperatures. The $ess1^{H164R}$ mutation appears to suppress the *ts*-growth defects of both *kin28* mutants.

(Figure 1A, left, row 1). Alternatively, it is possible that the *kin28* mutant cells lost the plasmid but are slow growing (see below), giving rise to the apparent 5-FOA sensitivity. In either case, the $ess1^{H164R}$ mutation seemed to reverse this effect, facilitating loss of the pCaESS1 plasmid and subsequent growth on 5-FOA by both $kin28^{ts16}$ (at 25°) and $kin28^{T162A}$ (most visible at 30°). Results with the $ess1^{A144T}$ mutant cells were uninformative, as $ess1^{A144T}$ appeared to be generally resistant to pCaESS1 plasmid loss (or slow growing on 5-FOA), even in *KIN28* wild-type cells.

At higher temperature (30°) the effects were similar but not identical (Figure 1A, right). Resistance to 5-FOA of the $kin28^{T162A}$ $ess1^{H164R}$ cells was more pronounced compared to $kin28^{T162A}$ *ESS1* cells, indicating that the double-mutant combination is favorable, allowing *ESS1* plasmid loss. For $kin28^{ts16}$, 5-FOA resistance is no longer detected in either *ESS1* background, probably because $kin28^{ts16}$ cells grow slowly at this temperature, rather than because of a failure to lose the plasmid. From these experiments, it appears that there may be genetic interactions between *ESS1* and *KIN28*.

Genetic interaction was further examined by comparing the relative growth rates of cells that had lost the pCaESS1 plasmid on medium that lacked 5-FOA. The results indicated that $ess1^{H164R}$ suppresses the *ts*-growth defects of both $kin28^{ts16}$ and $kin28^{T162A}$ (Figure 1B). This is most easily seen at 30° for $kin28^{ts16}$, where there is partial suppression, and at 37° for $kin28^{T162A}$ (compare to *ESS1*). Independent isolates behaved similarly (data not shown). The results also show that $kin28^{T162A}$ suppressed the *ts*-growth defect of $ess1^{H164R}$ cells at 37°. Given that Kin28 is involved in transcription initiation, the results may implicate a role for Ess1 in this step. Other genetic interaction experiments between *ESS1* and *KIN28* yielded results that suggest interactions between these genes are likely to be complex and allele specific (data not shown). The simplest interpretation of our results is that Ess1 might oppose (attenuate) Kin28-dependent initiation.

Reduced *CTK1* gene dosage suppresses $ess1^{ts}$ mutations: Ctk1, another CTD kinase, has been shown to promote transcription elongation efficiency *in vitro* (LEE and GREENLEAF 1997) and is linked to 3′-end formation (CONRAD *et al.* 2000; SKAAR and GREENLEAF 2002). Ess1 has been proposed to inhibit elongation and promote termination (WU *et al.* 2003) and was recovered in a screen for mutations that cause defects in 3′-end formation (HANI *et al.* 1995). Therefore, Ess1 and Ctk1 are predicted to interact genetically, perhaps in an opposing manner.

Previous work has shown that *CTK1* is not essential,

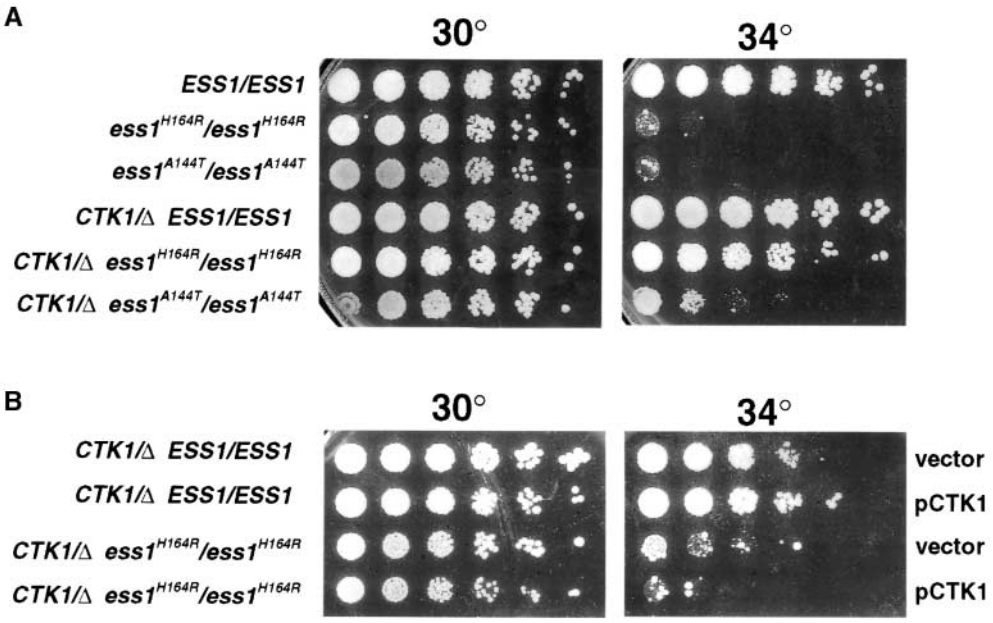

FIGURE 2.—Reduced dosage of *CTK1* suppresses the growth defect of *ess1*^{ts} cells. (A) Serial dilutions (1:5) of wild-type or isogenic *ess1*^{ts} diploids containing one or two copies of *CTK1* were spotted onto YEPD medium and grown for 2 days at 30° and 34°. *ess1*^{H164R}/*ess1*^{H164R} mutants with only one copy of *CTK1* (*CTK1*/Δ) grew nearly as well as *ESS1*/*ESS1* wild-type cells, even at 34°. (B) As a control, cells were transformed with a centromeric plasmid (pJYC1511) encoding *CTK1* or a vector control (pRS413), spotted to CSM − his medium (1:5 dilutions), and grown for 3 days. Results show that *ess1*^{H164R} mutant cells with a reduced chromosomal dosage of *CTK1* (*CTK1*/Δ) but carrying a pCTK1 plasmid were restored to temperature sensitivity (at 34°), whereas the vector control cells were not.

although *ctk1* mutants had a slow growth phenotype (LEE and GREENLEAF 1991; GIAEVER *et al.* 2002). In our strain backgound (W303-1A), however, we could not generate viable *ctk1*Δ haploid cells, even after the addition of *SSD1-v,* an allele of *SSD1* that suppresses a number of other mutations in this background (LORENZ and HEITMAN 1998). In addition, high-copy expression of *ESS1* did not suppress the *ctk1*Δ mutation (data not shown).

Instead, we worked with diploid strains in which one copy of *CTK1* was deleted. The results show that *ctk1*Δ/*CTK1* suppressed the *ts* phenotype of *ess1*^{H164R} and *ess1*^{A144T} homozygous mutants at 34° (Figure 2A). The suppression by *ctk1*Δ/*CTK1* was much stronger for the *ess1*^{H164R} mutant, whose growth is generally more robust than that of *ess1*^{A144T}. Several independently derived *ctk1*Δ mutants were used for these experiments and the results were the same (data not shown). As a control we also showed that adding back *CTK1* on a centromeric plasmid reversed this effect, rendering *ctk1*Δ/*CTK1 ess1*^{H164R}/*ess1*^{H164R} cells more temperature sensitive than vector-only controls (Figure 2B). Western blot analysis indicated that the reduced dosage of *CTK1* did not alter the expression levels of *ess1*^{ts} mutant proteins (data not shown). Decreased dosage of *CTK1* did not, however, suppress a complete deletion of *ESS1*. This was tested using a *ctk1*Δ/*CTK1 ess1*Δ/*ess1*Δ strain carrying *ESS1* on a plasmid (pCaESS1) and finding that cells were unable to lose the plasmid by plating to 5-FOA (data not shown).

The above results indicated that reduced levels of Ctk1 suppress the growth defects in *ess1*^{ts} mutants. We tested if the reciprocal was true, whether increased levels of Ctk1 might enhance the growth defects in *ess1*^{ts} mutants. Indeed, high-copy expression of *CTK1* enhanced the temperature sensitivity of both the *ess1*^{H164R} and *ess1*^{A144T} cells (Figure 3A). This effect is detected at 30° and 32° but is most prominent at 34°. Note that the plasmid used in these experiments also contains a partial open reading frame (ORF; *TGL1*). However, we do not think this ORF could have caused the observed effect because three-fifths of the coding sequence is missing. In addition, independent experiments using plasmids without this ORF present gave similar results, and an equivalent plasmid carrying a *CTK1* catalytic mutant did not produce this effect (both in Figure 3B, below).

To test whether the kinase activity of high-copy-expressed Ctk1 was required for enhancing the growth defect of *ess1*^{H164R} cells, two kinase-deficient alleles of *CTK1* were used, *ctk1*^{K212A} and *ctk1*^{D324N}. Plasmids carrying these alleles did not enhance the growth defect of *ess1*^{H164R} cells at semipermissive temperature (34°) as did wild-type control *CTK1* plasmids (Figure 3B). These results indicate that the kinase activity of Ctk1 is required for enhancing the *ts*-growth defect of *ess1*^{H164R} cells.

In summary, reduced *CTK1* dosage suppresses *ess1*^{ts} mutations, while increased *CTK1* activity enhances *ess1*^{ts} mutations, indicating that Ess1 and Ctk1 may have opposing functions during transcription. Since *CTK1* is known to stimulate elongation, these results suggest that Ess1 inhibits elongation, consistent with other recent studies (WU *et al.* 2003). In addition, since *CTK1* has also been implicated in 3′-end processing (CONRAD *et al.* 2000; SKAAR and GREENLEAF 2002), Ess1 may also be important during this step.

Genetic interactions between between *BUR1* and *ESS1*: *BUR1* encodes a kinase that may phosphorylate Ser5

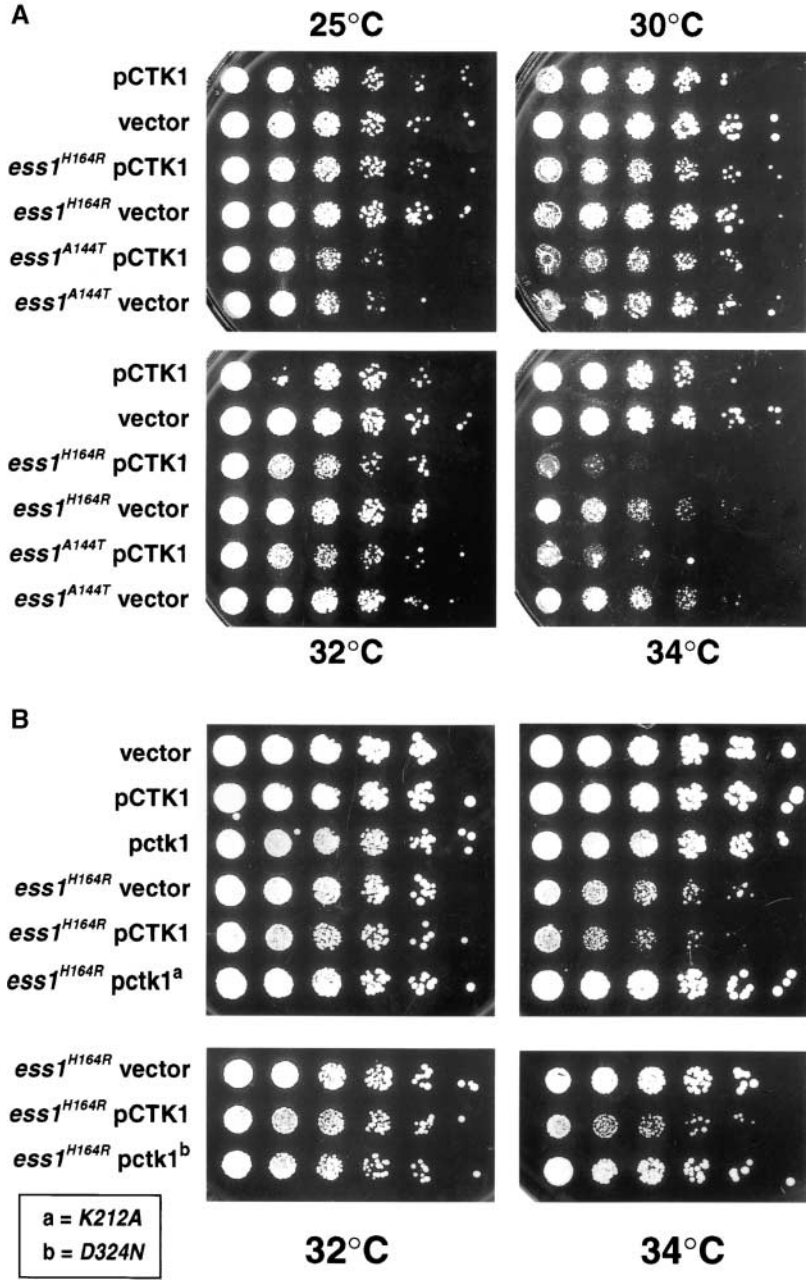

FIGURE 3.—Overexpression of kinase-active *CTK1* enhances the growth defect of $ess1^{ts}$ cells. (A) Serial dilutions (1:5) of wild-type or isogenic $ess1^{ts}$ haploid cells containing vector alone or a high-copy *CTK1* plasmid were spotted onto CSM − ura medium and grown for 3 days at the indicated temperatures. Both $ess1^{H164R}$ and $ess1^{A144T}$ mutants bearing the pCTK1 plasmid grew slower than cells bearing the control vector; this is particularly visible at 32° and 34°. (B) Cells were treated as in A except that plasmids encoding a kinase-deficient mutant were used, and the cells were grown and spotted on CSM − trp medium. Top, growth of $ess1^{H164R}$ cells expresssing the control wild-type *CTK1*, but not the kinase-deficient $ctk1^{K212A}$, is inhibited at semirestrictive temperature (34°). Bottom, similar to the top except mutant allele $ctk1^{D324N}$ and a *CTK1* control plasmid were used.

residues within the CTD repeat and, like *CTK1*, acts positively to promote transcription elongation (MURRAY *et al.* 2001). Other studies confirm a role in elongation, but suggest that Bur1 kinase may have targets other than the CTD (KEOGH *et al.* 2003).

We tested for genetic interaction between *BUR1* and *ESS1*, using a variety of experiments. High-copy *BUR1* expression did not enhance or suppress the *ts*-growth defect of $ess1^{H164R}$ (or $ess1^{A144T}$) mutant cells at various temperatures (25°, 30°, 34°, and 37°), nor did a reduction in *BUR1* dosage, for example, in a *bur1*Δ/*BUR1* $ess1^{H164R}/ess1^{H164R}$ diploid strain (data not shown). In addition, *bur1*Δ/*BUR1 ess1*Δ/*ess1*Δ mutant cells were inviable upon loss of an *ESS1*-containing (*URA3*) plasmid as indicated by the failure to grow on 5-FOA medium (data not shown). Thus, neither overexpression nor reduced dosage of *BUR1* suppressed (or enhanced) *ess1* mutations. We also generated a *bur1*Δ *ess1*Δ haploid mutant bearing a plasmid-borne copy of the *bur1-2* allele (see below) and an *ESS1* plasmid (pCaESS1). This strain could not lose the pCaESS1 plasmid, suggesting there is no suppression of *ess1*Δ by *bur1-2* (data not shown).

Segregation analysis was then used to test whether *ess1 bur1* double mutants are synthetic lethal. Because both *BUR1* and *ESS1* are essential, we used the *bur1-2* slow-growth allele (MURRAY *et al.* 2001), combined with the $ess1^{H164R}$ *ts* allele. The results are shown in Table 2. For controls, we analyzed the *bur1-2* and $ess1^{H164R}$ single mutants. As previously demonstrated (MURRAY *et al.* 2001), *bur1-2/BUR1-2* diploids gave rise to four viable spores,

TABLE 2

Segregation analysis of *bur1 ess1* double mutants

Relevant genotype	Cross	No. of tetrads	Viable spores per tetrad[a]				
			0	1	2	3	4
bur1-2/BUR1	GY604 × W3031A	17	0	0	1	2	14
ess1^{H164R}/ESS1	W3031B × YGDts22W	19	1	1	1	4	12[b]
bur1-2/BUR1 ess1^{H164R}/ESS1	GY604 × YGDts22W	47	0	0	3	18	26

[a] Tetrads were dissected on YEPD plates and incubated 5–6 days at 25° (rows 1 and 3) or at 30° (row 2).
[b] X. Wu and S. D. Hanes (unpublished data).

and these showed 2:2 segregation for mild slow growth (Figure 4). As expected, *ess1^{H164R}/ESS1* diploids gave rise to four viable spores at the permissive temperature, which grew equally well. In contrast, *bur1-2/BUR1-2 ess1^{H164R}/ESS1* double mutants gave rise to a large number of tetrads bearing fewer than four viable spores. This result is consistent with two unlinked genes interacting to produce a synthetic enhancement of their individual growth defects (see also Figure 4 legend). However, if the double-mutant combination was always lethal, we would have expected far fewer than half the tetrads to yield four viable spores. Instead, slightly more than half the tetrads yielded four viable spores. This result suggests that the double mutants are most likely to be very slow growing (*e.g.*, tiny colonies in tetrads 1, 4, and 7 in Figure 4), but that these mutants show some variability in their growth phenotype, ranging from inviability (*e.g.*, dead spores in tetrads 2, 5, and 6; Figure 4) to slow/moderate growth (small/medium colony in tetrad 3; Figure 4).

The slow-growing (and nonviable) colonies are inferred to be *bur1-2 ess1^{H164R}* double mutants. For *ess1^{H164R}*, *ts* growth at 37° was monitored and segregation patterns were consistent with the above inference. From these experiments, we conclude that *bur1-2* and *ess1^{H164R}* mutations exhibit synthetic lethality or synthetic slow growth.

BUR1 has previously been shown to interact with other genes involved in elongation (*SPT4/5*), as well as with RNA pol II itself and a CTD phosphatase (*FCP1*; Murray *et al.* 2001). Because *ESS1* also interacts with *SPT4/5* (Wu *et al.* 2003), as well as with RNA pol II and *FCP1* (Wu *et al.* 2000; see below), it was perhaps expected that we would detect interactions between *BUR1* and *ESS1*. The genetic interaction observed between *ESS1* and *BUR1* is consistent with a role for *ESS1* in elongation. However, in contrast to results with *CTK1*, the *BUR1* results seem to indicate a positive role for Ess1 in elongation.

Disruption of *SRB10* suppresses *ess1^{ts}* and *ess1*Δ mutations: Phosphorylation of the CTD by Srb10 before PIC formation has been shown to inhibit transcription (Hengartner *et al.* 1998). We previously reported interactions between *ESS1* and *RPB1* that implicate Ess1 as a positive regulator of transcription (Wu *et al.* 2000). This suggested that any interaction between Ess1 and Srb10 would be antagonistic. Since *SRB10* is not essential (Liao *et al.* 1995), we deleted *SRB10* (*srb10*Δ) in wild-type and *ess1^{ts}* haploid strains to determine whether there is a genetic interaction with *ESS1*. As expected, the *srb10*Δ *ess1^{H164R}* and *srb10*Δ *ess1^{A144T}* double-mutant strains grew at permissive temperatures (30° and 32°; Figure 5). However, the double mutants also grew at the semipermissive temperature (34°), and one of the mutants, *srb10*Δ *ess1^{H164R}*, grew at the restrictive temperature (37°). These results indicate that the *srb10*Δ mutation suppresses the growth defect of *ess1^{ts}* mutants.

The *srb10* mutation also suppressed a complete deletion of *ESS1*. This was shown by deleting *SRB10* in a haploid *ess1*Δ strain bearing a pCaESS1 plasmid and then curing cells of the plasmid (data not shown). These *srb10*Δ *ess1*Δ cells grew at 30°, 32°, and 34° (Figure 5). However, due to the possibility that suppressors might have arisen during the plasmid-loss procedure, we confirmed this result using standard segregation analysis with diploid cells of the following genotype: *srb10*Δ*/SRB10 ess1*Δ*/ESS1*. Suppression of the *ess1*Δ mutation by the unlinked *srb10*Δ mutation should alter the normal 2:2 viable:inviable segregation pattern observed for *ESS1* disruption (Hanes *et al.* 1989), generating 3:1 and 4:0 segregation patterns and allowing recovery of viable His$^+$ segregants (*i.e.*, that contain the *ess1*Δ deletion). Indeed, 3:1 and 4:0 segregation was observed (Table 3), indicating that *srb10*Δ suppresses the *ess1*Δ mutation. As expected, all the viable His$^+$ segregants (*ess1*Δ::*HIS3*) obtained were also Trp$^+$, indicating that the *srb10* mutation (*srb10*Δ::*TRP1*) was also present. Viability was lower at 30° than at 25°, consistent with our observations that the requirement for *ESS1* is stricter at higher temperatures (X. Wu, C. B. Wilcox and S. D. Hanes, unpublished results).

Of the 57 double-mutant tetrads dissected, however, only 16 showed 3:1 segregation (28%), and 3 showed 4:0 segregation (5%) rather than the expected ~67 and ~17%, respectively. In addition, of the Trp$^+$ segregants (*srb10*Δ::*TRP1*), only 23% (rather than 50%) were His$^+$ (*ess1*Δ::*HIS3*). Random spore inviability may not be the cause, since neither *ess1*Δ*/ESS1* nor *srb10*Δ*/SRB10* single mutants showed spore-viability problems (Table 3). These results indicate that another gene might be required or

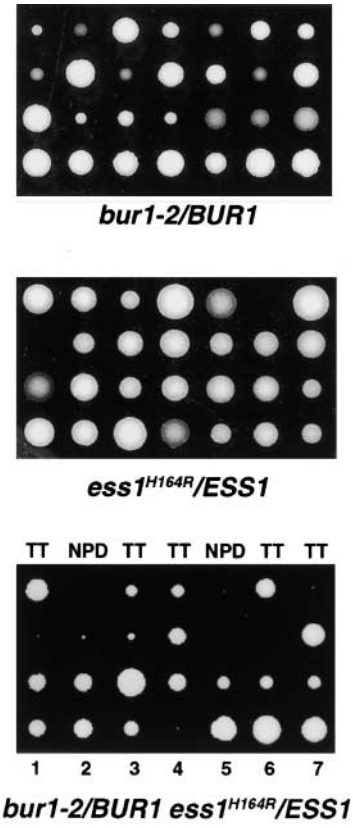

FIGURE 4.—Segregation analysis of *bur1 ess1* double mutants indicates a synthetic growth defect. Tetrads were dissected as described in Table 2. Tetrads were verified using independent markers. The darker color that is visible for some colonies is due to segregation of the *ade2-1* allele. Very small and nongrowing colonies (bottom) are likely to be *bur1-2 ess1^{H164R}* double mutants. For two unlinked mutations, we expected segregation patterns of 1:1:4 for PD:NPD:TT. If we assume that *bur1-2 ess1^{H164R}* double mutants are extremely slow growing (or dead), then the following colony phenotypes would be expected for segregants of the double-mutant cross: parental ditype (PD), 2 large, 2 medium; nonparental ditype (NPD), 2 large, 2 small (or dead); tetratype (TT), 2 large, 1 medium, 1 small (or dead). Among the 26 tetrads that yielded four viable spores (data from Table 2) the ditypes were scored as follows: 5:3:18 for PD:NPD:TT. This ratio is close to the expected 1:1:4, supporting the assumption that *bur1-2 ess1^{H164R}* double mutants are extremely slow growing or dead (in the case of 3:1 viable:inviable tetrads).

that suppression by *srb10*Δ is not fully penetrant. It is also possible that *ess1*Δ *srb10*Δ double-mutant spores have germination defects. In any case, the results show that, in certain backgrounds, the deletion of *SRB10* can relieve cells of their requirement for *ESS1*. Given that Srb10 inhibits PIC formation, it seems likely that Ess1 stimulates PIC formation.

While deletion of *SRB10* suppressed the phenotype of *ess1*Δ mutants, deletion of *ESS1* did not suppress the *srb10*Δ mutant phenotype, that of slow growth on galactose-containing medium. If anything, *ess1*Δ *srb10*Δ double mutants grew slower than *srb10*Δ single mutants on rich or synthetic media containing galactose as the carbon source (data not shown). We also tested whether high-copy expression of *SRB10* enhanced or suppressed growth defects caused by *ess1^{ts}* mutations at restrictive temperature. No effects were detected (data not shown), as might be expected if Ess1 acts downstream of Srb10 (see DISCUSSION).

A CTD half-substitution allele that suppresses *ess1* mutants: The above results revealed genetic interactions between *ESS1* and genes encoding CTD-modifying enzymes. This prompted us to investigate possible direct genetic interactions between *ESS1* and the CTD. In otherwise wild-type yeast, the CTD can be truncated to 10 YSPTSPS repeats with no apparent effect on growth, and 8 repeats is sufficient for viability at 30°, but cells are cold sensitive, while mutants bearing more severe CTD truncation alleles are inviable at any temperature (NONET and YOUNG 1989; WEST and CORDEN 1995). These defects are suppressed, to a certain extent, by mutations in *s*uppressor of *R*NA pol *B* (*SRB*) genes (NONET and YOUNG 1989), including *SRB10* (THOMPSON *et al.* 1993; LIAO *et al.* 1995; HENGARTNER *et al.* 1998). Phenotypically, deletion of *SRB10* "lengthens" the CTD by several repeats, augmenting Rpb1 function (HENGARTNER *et al.* 1998). Our previous work suggested that *ess1* mutations do just the opposite, compromising Rpb1 function and sensitizing cells to the effects of truncated CTD alleles, *i.e.*, effectively "shortening" the CTD (WU *et al.* 2000).

Here, we examined genetic interactions between *ess1* mutations and various "half-substitution" CTD truncation/subsitution alleles. The CTDs are truncated so they contain only 10–14 heptad repeats rather than the usual 26–27, and they carry substitutions of Ser2 or Ser5 within the repeat to either Ala or Glu. However, these substitutions are restricted to either the "first half" or the "second half" (amino- or carboxy-terminal ends) of the CTD (see Table 4). In this way, we hoped to delineate the importance of first-half *vs.* second-half CTD repeats, as well as distinguishing the effects of Ser2 *vs.* Ser5 substitutions. Previous analysis of CTD half-substitution mutants indicated that serines at the same position (*e.g.*, Ser5) within different heptad repeats may have distinct roles (WEST and CORDEN 1995; FONG and BENTLEY 2001). This is supported by the finding that suppressors of second-half mutations do not necessarily suppress first-half mutations (YURYEV and CORDEN 1996). Here, we tested whether the CTD half-substitution mutants could replace wild-type *RPB1* in an *ess1^{ts}* background.

Plasmids expressing a CTD half mutant, wild-type *RPB1*, or an empty vector were transformed into *rpb1*Δ *ESS1* and *rpb1*Δ *ess1^{H164R}* strains carrying *RPB1* on a *URA3*-containing plasmid. Complementation of the *rpb1*Δ mutation was measured by patching cells to 5-FOA medium to detect *RPB1* plasmid loss (Figure 6). This experiment allowed us to compare the ability of different CTD alleles to function in *ESS1 vs. ess1^{ts}* cells at the permissive temperature (30°) to identify possible synthetic-lethal interactions.

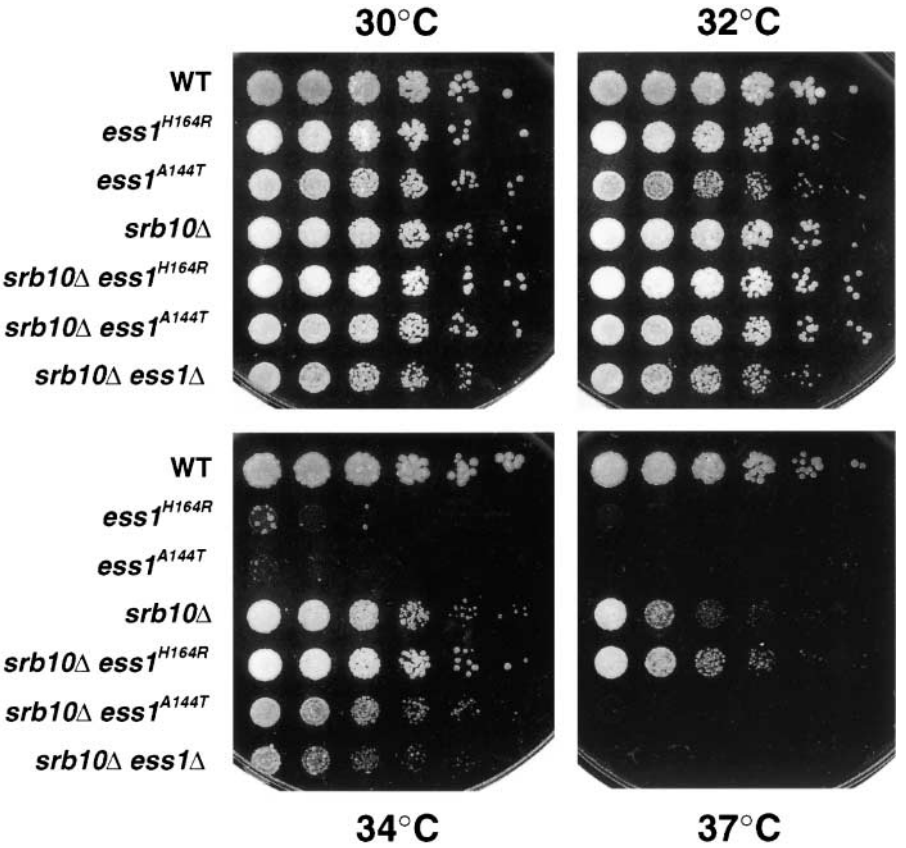

FIGURE 5.—Deletion of *SRB10* suppresses *ess1*^{ts} and *ess1*Δ mutants. Serial dilutions (1:5) of wild-type or *ess1*^{ts} cells containing *SRB10* or *srb10*Δ or an *ess1*Δ *srb10*Δ double deletion strain were spotted onto YEPD medium and grown for 2 days at the indicated temperatures. The *srb10* deletion alone resulted in only a slight *ts*-growth defect. Deletion of *SRB10* allowed growth of the *ess1*^{H164R} strains at restrictive temperature (37°) and of the *ess1*Δ strain at temperatures up to 34°.

Consistent with published observations (WEST and CORDEN 1995), all four of the serine-to-alanine mutants substituted for *RPB1* in the control *ESS1* strain (Figure 6, bottom left, rows 3, 4, 7, and 8). In the *ess1*^{H164R} mutant strain, however, only the Ser5-to-alanine substitutions complemented the *rpb1*Δ mutation, allowing cell growth (bottom right, rows 4 and 8), whereas Ser2-to-alanine substitutions did not (rows 3 and 7). These results identify a synthetic-lethal interaction between *ess1*^{H164R} and Ser2, but not Ser5 mutations. Similar results were obtained with plasmid-loss experiments using liquid cultures (data not shown). The lack of sensitivity between *ess1*^{H164R} and the Ser5-to-alanine mutation (*i.e.*, mutating both is no worse than mutating either one alone) points to Ser5 as a possible direct target of Ess1 (see also below).

Mutation of Ser2 or Ser5 to glutamic acid in the first half of the CTD (Figure 6, bottom, rows 5 and 6) did not support growth in either *ESS1* or *ess1*^{H164R} cells. While the inability of these mutants to complement *rpb1*Δ may indicate that dephosphorylation of Ser2 and Ser5 in the first half of the CTD is essential for viability, this result is relatively uninformative with respect to the role of

TABLE 3

Deletion of *srb10* suppresses *ess1* deletion mutants

			Viable spores per tetrad				
Relevant genotype (strain name)	Temperature	No. of tetrads	0	1	2	3	4
*ess1*Δ::*HIS3/ESS1*[a] (YSH-55)	25°	19	1	1	16	1	0
	30°	24	0	3	21	0	0
*srb10*Δ::*TRP1/SRB10* (YJM1 × W303-1B)	25°	19	0	0	0	1	18
	30°	15	0	0	0	2	13
*srb10*Δ::*TRP1/SRB10 ess1*Δ::*HIS3/ESS1*[b]	25°	21	0	1	11	6	3
(YJM1 × YPR57α)	30°	36	1	3	22	10	0

[a] All viable segregants are His^-.
[b] Of the 18 viable segregants that are His^+, all are Trp^+.

TABLE 4

CTD mutant plasmids used

Plasmid	Description[a]	Original name[b]	Complements *rpb1*Δ?[c]
$S2A_4ctd_7$	$(YAPTSPS)_4(YSPTSPS)_7$	$pY1A^2(4)WT(7)$	Yes
$S5A_5ctd_7$	$(YSPTAPS)_5(YSPTSPS)_7$	$pY1A^5(5)WT(7)$	Yes
$S2E_5ctd_7$	$(YSPTEPS)_5(YSPTSPS)_7$	$pY1E^2(5)WT(7)$	No[d]
$S5E_5ctd_7$	$(YSPTEPS)_6(YSPTSPS)_7$	$pY1E^5(5)WT(7)$	No
ctd_4S2A_6	$(YSPTSPS)_4(YAPTSPS)_6$	$pY1WT(4)A^2(6)$	Yes
ctd_7S5A_7	$(YSPTSPS)_7(YSPTAPS)_7$	$pY1WT(7)A^5(7)$	Yes
ctd_6S2E_7	$(YSPTSPS)_6(YEPTSPS)_7$	$pY1WT(6)E^2(7)$	No
ctd_6S5E_7	$(YSPTSPS)_6(YSPTEPS)_7$	$pY1WT(6)E^5(7)$	No

[a] Underline identifies position of substitution.
[b] West and Corden (1995).
[c] Data are from West and Corden (1995), except for $S2E_5ctd_7$ and ctd_6S5E_7 (this study).
[d] Reported as viable in West and Corden (1995).

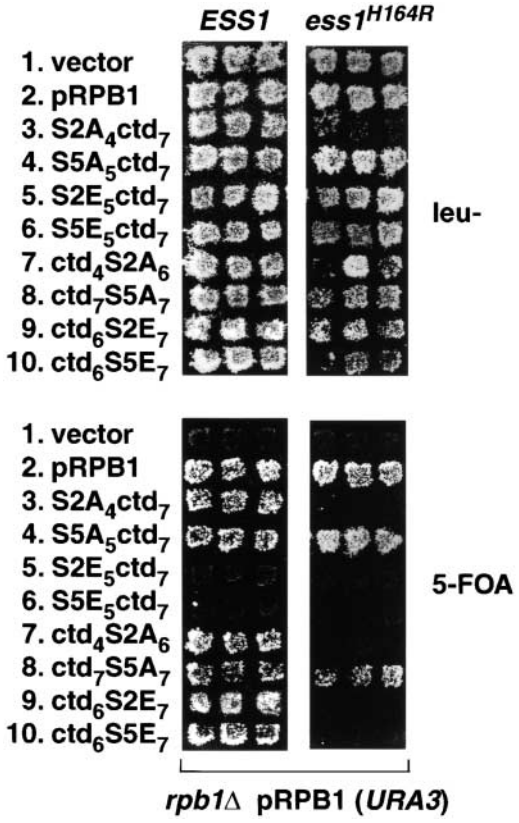

Figure 6.—Synthetic-lethal interactions between *ess1*^{H164R} and specific CTD mutations. The indicated plasmids (rows 1–9) were transformed into *ESS1 rpb1*Δ pRPB1 or *ess1*^{H164R} *rpb1*Δ pRPB1 strains. Three individual transformants for each plasmid were replica plated to leu$^-$ control medium and to 5-FOA medium to detect loss of the pRPB1 plasmid (2μ, *URA3*). Cells were incubated at 30° for 4 days. Three CTD mutant plasmids were unable to complement *rpb1*Δ in the *ess1*^{H164R} mutant strain as detected by the inability to grow on 5-FOA *vs.* the control leu$^-$ medium (right side; rows 3, 7, and 9). Failure to grow on 5-FOA medium indicates synthetic lethality between these three mutations and *ess1*^{H164R}.

Ess1. However, mutation of Ser2 or Ser5 to glutamic acid in the second half of the CTD (rows 9 and 10) did support growth in the *ESS1* cells, but not in *ess1*^{H164R} cells (*i.e.*, they are synthetic lethal). This result is consistent with the idea that *ess1* mutations sensitize cells to second-half mutations, as if Ess1 and these residues of the CTD function in the same pathway but at different steps.

These results suggest that Ess1 targets a subdomain within the CTD (Ser5, first half). For example, Ess1-dependent isomerization might promote dephosphorylation of Ser5 in the first half of the CTD. If true, then a Ser5-to-alanine substitution (which mimics the dephosphorylated form of Ser5) in the first half of the CTD might relieve the requirement for Ess1, whereas a Ser5-to-alanine substitution in the second half of the CTD would not, nor would a substitution of Ser2 to alanine. This is exactly what we observed (Figure 7); a Ser5 first-half mutation ($S5A_5ctd_7$) suppressed the temperature-sensitive growth defect of *ess1*^{H164R} cells (and *ess1*^{A144T}, data not shown) at 34°, whereas a Ser5 second-half mutation (ctd_7S5A_7) and a Ser2 first-half mutation ($S2A_4ctd_7$) did not. We could not test Ser5-to-glutamic acid substitutions (which mimic the phophorylated forms) because they do not support cell growth in an *rpb1*Δ background (Figure 6). The suppression of *ess1*^{ts} mutations by $S5A_5ctd_7$ suggests that Ess1 binding to the CTD promotes dephosphorylation of first-half Ser5 residues, perhaps by isomerization of Ser5-Pro6 dipeptide bonds. This could block the action of CTD kinases on Ser5 or expose phospho-Ser5 to the action of a CTD phosphatase.

DISCUSSION

In this article, we present genetic evidence that Ess1 interacts with all four known CTD kinases, indicating that it is likely to act at multiple stages of the transcription cycle. The clearest genetic interactions indicate that Ess1 opposes the effects of Ctk1 and Srb10. Ess1 may also oppose Kin28 and work positively with Bur1. The

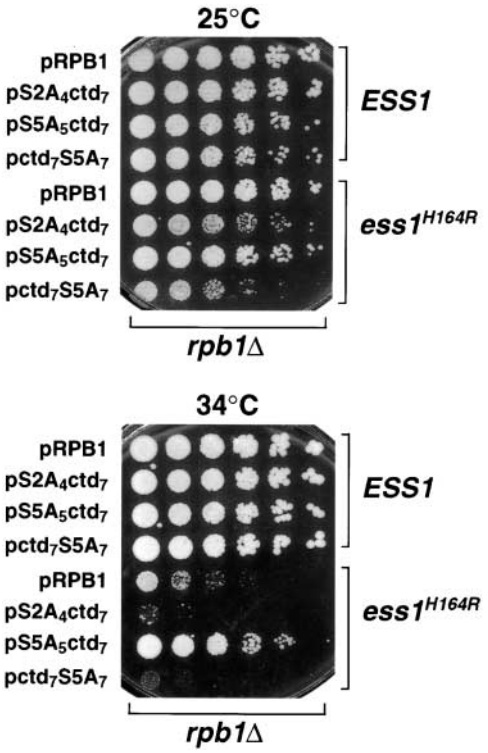

FIGURE 7.—Ser5 in the first half of the CTD may be the target of Ess1 within the CTD. *ESS1 rpb1*Δ or *ess1*^{H164R} *rpb1*Δ strains carrying plasmids (*CEN LEU2*) encoding wild-type *RPB1* or CTD mutants with the indicated serine-to-alanine substitutions were spotted (1:5 serial dilutions) onto YEPD medium and grown for 2 days at 25° or 34°. Ser5-to-alanine substitution in the first half of the CTD (pS5A$_5$ctd$_7$) suppressed the temperature sensitivity of *ess1*^{H164R}, allowing growth at 34°.

TABLE 5

Summary of genetic interactions between CTD kinases and the CTD with *ESS1*

Mutation or overexpression	Effect on growth defect of *ess1*^{ts} mutations
kin28	Suppress[a]
KIN28↑[b]	No effect
*ctk1*Δ/*CTK1*	Suppress
CTK1↑	Enhance
*srb10*Δ	Suppress[c]
SRB10↑	No effect
bur1-2	Enhance[d]
BUR1↑	No effect
CTD S2A (first half only)	Enhance[e,f]
CTD S2A (second half only)	Enhance[e]
CTD S5A (first half only)	Suppress[f]
CTD S5A (second half only)	Enhance[f]
CTD S2E (second half only)	Enhance[e]
CTD S5E (second half only)	Enhance[e]

[a] Allele-specific interaction.
[b] Arrows (↑) indicate high-copy expression.
[c] Also suppresses *ess1*Δ.
[d] Apparent synthetic slow-growth/lethality.
[e] Based on 5-FOA plasmid-loss assay (Figure 6).
[f] Based on growth of double mutant (Figure 7).

types of genetic interactions observed (summarized in Table 5), combined with the known substrate preference of Ess1/Pin1 prolylisomerases for phospho-Ser-Pro motifs, suggest a model for Ess1 function. In this model, Ess1 binds the CTD after the CTD is phosphorylated by Srb10. Ess1 then catalyzes a conformational change in the CTD that promotes dephosphorylation by CTD-specific phosphatases, such as Fcp1 and Ssu72. This dephosphorylation would reverse the negative effects of Srb10 and stimulate PIC formation. Ess1 would also be required later in the transcription process, possibly during promoter clearance (Kin28 step), but more likely for elongation and termination/3′-end formation (see below). Here, Ess1 may act by helping Bur1-dependent elongation and later by antagonizing the effects of Ctk1, which promotes elongation. Thus, our model suggests that Ess1 and CTD kinases work together to coordinate multiple steps in transcription and that both covalent (phosphorylation) and noncovalent (isomerization) modifications of the CTD are crucial to this process.

Ess1 acts positively in transcription initiation: In this study, we observed genetic interactions between *ESS1* and *SRB10*, which regulates the formation of the preinitiation complex (HENGARTNER *et al.* 1998). Deletion of *SRB10* suppressed both *ts* mutants and a complete deletion of *ESS1*. Thus, when Srb10's negative effect on PIC formation is removed, Ess1 is no longer needed, suggesting that the normal function of Ess1, which occurs genetically downstream of Srb10, is to overcome the effects of Srb10 and stimulate PIC formation. Because Srb10 phosphorylates the CTD, we presume that Ess1 isomerization promotes CTD dephosphorylation by making the phospho-CTD a better conformational substrate for a CTD-phosphatase. In addition, genetic interactions observed with *KIN28* suggest that Ess1 may also play a role later during initiation and promoter clearance, although these interactions appear to indicate a negative role for Ess1 during these stages.

Ess1 may inhibit elongation and promote 3′-end formation: Previous work has implicated Ess1 in 3′-end pre-mRNA processing by an unknown mechanism (HANI *et al.* 1995). Here, we provide genetic data that might help explain this finding. We found that reducing the dosage of *CTK1* suppresses the lethality of *ess1*^{ts} mutations at restrictive temperature, indicating that Ess1 and Ctk1 have opposing functions. Ctk1 has been shown to increase elongation efficiency and may play a role in 3′-end processing (LEE and GREENLEAF 1997; SKAAR and GREENLEAF 2002), presumably by phosphorylating the CTD. Since Ctk1 acts positively in elongation, the results point to a negative role for Ess1 in elongation, consistent with the findings of another recent study (WU *et al.* 2003). We propose the following scenario: Ess1 binds the phospho-CTD on the elongating polymerase and catalyzes an isomerization that in turn promotes dephosphorylation

by Fcp1 or Ssu72. By doing so, Ess1 would oppose the pro-elongation action of Ctk1 and perhaps enhance the binding of pre-mRNA processing factors to the CTD, thus stimulating 3′-end processing.

Isomerization by Ess1 likely promotes the dephosphorylation of the first half of the CTD at serine 5: Results of complementation tests with Ser2 and Ser5 substitutions (Figure 6) and the finding that the CTD mutation, $S5A_5ctd_7$, suppresses *ess1*^{ts} mutants (Figure 7) imply that the mutation of Ser5 to alanine (S5A) in the first half of the CTD compensates for the loss of Ess1. Together with genetic results showing that Ess1 opposes the actions of at least two CTD kinases (Srb10 and Ctk1), these data suggest that one role of Ess1 isomerization is to prevent phosphorylation of Ser5 or to promote its dephosphorylation. Therefore, when Ess1 function is compromised, Ser5 would be inappropriately phosphorylated, preventing PIC formation and, in later steps, interfering with proper elongation and 3′-end formation.

While this model nicely fits our data, Srb10 and Ctk1 have been shown to phosphorylate the CTD on Ser2 to a greater degree than that on Ser5 (Hengartner *et al.* 1998; Patturajan *et al.* 1999; Cho *et al.* 2001; Ramanathan *et al.* 2001; S. Buratowski and M. Keogh, personal communication). At this point, it is not clear how to explain this difference. There are many possibilities. One is that these kinases also phosphorylate Ser5 to a minor degree, but that these modifications have large effects on Rpb1 function. A second possibility is that Ess1 acts indirectly to promote dephosphorylation of Ser5 (in the first half of the CTD) and that this mechanism might involve another CTD kinase, such as Kin28 or Bur1, which are known to be important for phosphorylation of Ser5. For example, Ser2 phosphorylation by Srb10 (or Ctk1) might stimulate Ess1 binding to Ser2-Pro3 and subsequent isomerization of the CTD. The resulting conformational change might reduce Ser5 phosphorylation (by Kin28 or Bur1) or increase dephosphorylation by Ssu72 phosphatase, which seems to have a preference for phospho-Ser5 (K. Shankarling and M. Hampsey, personal communication). This might explain the observation that S2A mutations are synthetic lethal (same pathway, different step) with the *ess1*^{H164R} mutation (Figure 6, rows 3 and 7), whereas an S5A mutation suppresses *ess1*^{H164R} (Figure 7), suggesting Ser5 might be the functional target.

Finally, it is possible that the phosphorylation/dephosphorylation states of Ser2 and Ser5 are coupled. For example, phosphorylation at Ser2 might stimulate phosphorylation at Ser5, perhaps by a processive mechanism involving one or more kinases. Thus, in the absence of Srb10 or Ctk1 function, Ser2 would not be phosphorylated, causing loss of Ser5 phosphorylation, thereby reducing or eliminating the requirement for Ess1. In this scenario Ess1 would normally act on Ser5-Pro6.

Conclusions: Ess1 was originally proposed to regulate cell division at mitosis. This was based primarily on the mitotic defects observed in yeast *ess1* mutants (Hanes *et al.* 1989; Lu *et al.* 1996). Its human counterpart, Pin1, was proposed to control mitosis in human cells by isomerization of mitotic phosphoproteins such as Cdc25 (Crenshaw *et al.* 1998; Shen *et al.* 1998). However, our previous work (Arévalo-Rodríguez *et al.* 2000; Wu *et al.* 2000, 2001) suggested an alternative model, one in which Ess1 (and Pin1) controls the transcription of genes necessary for mitosis, and the mitotic-arrest phenotype in *ess1* mutant cells is an indirect consequence of the loss of Ess1 function. Work presented here supports a transcription-based model for mitotic regulation in which Ess1 interacts with the CTD of RNA polymerase II and along with CTD kinases controls the binding of transcription and mRNA processing cofactors. Loss of proper transcriptional control in *ess1* mutants may trigger mitotic arrest pathways. We suggest this model might hold true in other organisms, including humans.

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