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The Ess1 prolyl isomerase is linked to chromatin remodeling complexes and the general transcription machinery

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The Ess1/Pin1 peptidyl-prolyl isomerase (PPIase) is thought to control mitosis by binding to cell cycle regulatory proteins and altering their activity. Here we isolate temperature-sensitive *ess1* mutants and identify six multicopy suppressors that rescue their mitotic-lethal phenotype. None are cell cycle regulators. Instead, five encode proteins involved in transcription that bind DNA, modify chromatin structure or are regulatory subunits of RNA polymerase II. A sixth suppressor, cyclophilin A, is a member of a distinct family of PPIases that are targets of immunosuppressive drugs. We show that the expression of some but not all genes is decreased in *ess1* mutants, and that Ess1 interacts with the C-terminal domain (CTD) of RNA polymerase II *in vitro* and *in vivo*. The results forge a strong link between PPIases and the transcription machinery and suggest a new model for how Ess1/Pin1 controls mitosis. In this model, Ess1 binds and isomerizes the CTD of RNA polymerase II, thus altering its interaction with proteins required for transcription of essential cell cycle genes.

Keywords: CTD/mitosis/PPIase/transcription/WW domain

Introduction

Peptidyl-prolyl *cis/trans* isomerases (PPIases) catalyze the rotation about the peptide bond preceding proline, a step that can be rate-limiting for the folding of newly synthesized proteins (Hemenway and Heitman, 1993; Schmid, 1995; Fischer *et al*., 1998). PPIases are also thought to regulate the activity of mature proteins, by promoting assembly or transport of subunits (Rutherford and Zuker, 1994; Hunter, 1998; Gothel and Marahiel, 1999). PPIases can act stoichiometrically rather than catalytically, as in the processing of rhodopsin by the NinaA protein in the *Drosophila* eye (Baker *et al*., 1994) and the assembly of infectious virions of human immunodeficiency virus (Luban *et al*., 1993).

The best-studied PPIases are the cyclophilins and FKBPs, which bind the immunosuppressive drugs cyclosporin A and FK506, respectively, and block T-cell activation (Walsh *et al*., 1992; Kunz and Hall, 1993; Marks, 1996). In yeast, none of the eight cyclophilins and four FK506-binding proteins (FKBPs) is essential for growth, as shown by individual gene deletion and by deleting all 12 (Dolinski *et al*., 1997). Members of a third family, termed parvulins after an *Escherichia coli* protein of the same name, are structurally distinct from the cyclophilins and FKBPs and do not bind either cyclosporin A or FK506 (Rahfeld *et al*., 1994; Rudd *et al*., 1995). The first eukaryotic parvulin to be discovered was Ess1 (Hanes, 1988; Hani *et al*., 1995), which is essential for growth in budding yeast (Hanes *et al*., 1989).

Ess1 is highly conserved. Homologs isolated from a variety of organisms, including *Drosophila* (Dodo) and humans (Pin1), substitute for Ess1 in yeast (Lu *et al*., 1996; Maleszka *et al*., 1996). Surprisingly, complete removal of the *dodo* gene from the *Drosophila* genome yields flies that are both viable and fertile (Maleszka *et al*., 1996), suggesting that Ess1 homologs are not essential in metazoans, perhaps due to the existence of *ESS1/PIN1*-related genes such as *PIN1-like* or *hPAR14* (Campbell *et al*., 1997; Uchida *et al*., 1999). Indeed, Pin1 homozygous knockout mice are viable and show no overt phenotype (Fujimori *et al*., 1999).

Ess1 contains an N-terminal WW domain and a C-terminal PPIase domain. The WW domain is a protein–protein interaction module with a pair of invariant tryptophans found in a variety of signaling proteins that bind proline-rich peptides (Staub and Rotin, 1996; Sudol, 1996). The WW domain of Pin1 binds peptides in which phosphorylated serine or threonine precedes proline (Yaffe *et al*., 1997). The X-ray model of Pin1 shows a two-lobed structure in which the WW and PPIase domains are connected by an unstructured linker (Ranganathan *et al*., 1997).

Early studies revealed that *ess1* mutants have cell cycle defects, possibly in cytokinesis or cell separation (Hanes *et al*., 1989). Using promoter shut-off experiments in yeast, depletion of Ess1 was shown to cause mitotic arrest and nuclear fragmentation, while in human cells, treatment with *PIN1* antisense RNA results in chromatin condensation (Lu *et al*., 1996). These results, and the fact that *PIN1* was originally isolated in a two-hybrid screen with the G_2/M-specific kinase NIMA from *Aspergillus nidulans*, suggested a role in mitosis (Lu *et al*., 1996).

Biochemical interactions have been detected between Pin1 protein and mitotic phosphoproteins, including Cdc25 phosphatase and Polo-like kinase (Crenshaw *et al*., 1998; Shen *et al*., 1998). However, the functional

relevance of these interactions has not been established, as *in vivo* and genetic data are lacking. The PPIase activity of Ess1 has been demonstrated *in vitro* (Hennig *et al*., 1998); however, whether Ess1/Pin1 controls the function of cell cycle regulators by direct interaction remains an open question. Pin1 was also found to activate transcription weakly when bound to DNA via GAL4 in mammalian cells (Komuro *et al*., 1999); however, DNA-bound Ess1 in yeast does not (V.Madelian and S.D.Hanes, unpublished data). Mutations in *ESS1* (also called *PTF1*), are defective in mRNA 3′ end formation when tested with certain reporter constructs (Hani *et al*., 1999). Finally, Pin1 in *Xenopus* oocycte extracts was shown to be required for the DNA replication checkpoint (Winkler *et al*., 2000). In these experiments, Pin1 was important for regulating the G_2/M transition, but not for exit from mitosis. Thus, in different systems, Ess1/Pin1 seems to function in distinct ways.

Here, we took a genetic approach to understanding Ess1/Pin1 function. We isolated a large number of *ess1* temperature-sensitive (*ts*) mutants which indicate that both the WW and PPIase domains are critical for *in vivo* function. The mutants were used for a multicopy suppressor screen to identify proteins in the Ess1/Pin1 pathway. The suppressors identified were not cell cycle regulators, but rather proteins important for RNA polymerase II (RNA pol II) transcription and its regulation. These include YKL005C, a TFIIS-like protein; Fcp1, a C-terminal domain (CTD) phosphatase; Sap30, a member of the Sin3–Rpd3 repression complex; Cth1, a putative transcription factor; and Rpb7, a subunit of RNA pol II. We also show that Ess1 interacts genetically and physically with RNA pol II. The results establish a clear link between this essential prolyl isomerase and the general transcription machinery, and suggest that Ess1 and Pin1 act indirectly in mitosis by controlling the transcription of genes required for cell cycle progression. We also link cyclophilin A, a major target of immunosuppressive drugs, to this pathway, providing the first evidence of cross-talk among different PPIase families. A model for Ess1/Pin1 function is presented.

Results

Isolation of ts ess1 mutations

The *ESS1* gene was mutagenized *in vitro* and transformed into a host strain bearing an *ess1::URA3* disruption and an integrated *GAL1–PIN1* fusion gene. Viability of the host strain was maintained by growth in galactose-containing medium to drive expression of human *PIN1*, which substitutes for *ESS1* in yeast (Lu *et al*., 1996). To identify *ts* mutants, expression of *PIN1* was repressed by growth in glucose-containing medium, and cells were replica-plated and incubated at permissive (23°C) and non-permissive (37°C) temperatures. Using this method, 15 *ts* and three null alleles (no growth at either temperature) of *ESS1* were identified (Table I).

To confirm that the *ESS1* mutations caused a *ts* growth phenotype when expressed from the genomic *ESS1* locus, several alleles (L94P, G127D, A144T and H164R) were used to replace the chromosomal *ESS1* gene. All were *ts* for growth (Figure 1A and B). An additional eight *ts* alleles were tested from centromeric plasmids in an *ess1Δ* background, and these exhibit various *ts* growth phenotypes (Figure 1B, lower panel). The arrest kinetics for all mutants are relatively slow; cells do not exhibit first cycle arrest, but instead arrest after several doublings (Figure 1A and data not shown), suggesting either that Ess1 might not be required during each cell cycle or that Ess1 target proteins, once isomerized, are stable. Cells accumulate in mitosis after 2–3 generations (4 h) at the restrictive temperature, and mitotic arrest is maximal by 8–12 h (data not shown).

Table I. Ess mutations identified in this study

Ess1mutation	Residue in Pin1	Phenotype	Location
S7P	D3	null	precedes WW
W15R	W11	*ts*	WW signature Trp
G43V	G39	*ts*[a]	WW domain
K51E	K46	*ts*[b]	linker
C62R	C57	*ts*[b]	PPIase near active site
H64P	H59	null	PPIase, catalytic[c]
L66P	L61	*ts*[a]	PPIase
S77P	S72	*ts*[a]	PPIase
L94P	I89	*ts*[a]	PPIase, α1 helix
T96P	G91	*ts*	PPIase, α1 helix
D102Y	K97	null	PPIase, α1 helix
F110S	F103	*ts*[b]	PPIase, h.p.[d]
S118L	S111	*ts*[a]	PPIase
C120R	C113	*ts*[b]	PPIase, catalytic[c]
S122P	S115	*ts*	PPIase
G127D	G120	*ts*	PPIase
A144T	A137	*ts*	PPIase
H164R	H157	*ts*	PPIase, catalytic[c]

[a]Slow growth; [b]leaky; [c]putative catalytic residue; [d]hydrophobic patch on surface.

The terminal phenotype was examined in strains in which the *ess1*^{ts} alleles were chromosomally integrated (L94P, G127D, A144T and H164R) or expressed from a centromeric plasmid (W15R). At the restrictive temperature, all the mutants undergo mitotic arrest and nuclear fragmentation within 4–8 h (Figure 1C and data not shown). These results were critical, since in earlier experiments these phenotypes appeared only after relatively long incubation times (12–24 h) after promoter shutoff (Lu *et al*., 1996), and thus might have been an indirect consequence of prolonged arrest. These results establish that *ESS1* is required for mitotic progression and that mutations in either the WW or the PPIase domain cause a similar loss-of-function phenotype.

All of the mutant proteins with the exception of Ess1(S7P) were detected in cells grown at the restrictive temperature, 37°C (Figure 1D and data not shown). Several mutant proteins (e.g. W15R, C120R and S122P) were present at near wild-type levels, while others (K51E, F110S and H164R) were reduced 2- to 10-fold. A few were reduced >50-fold at both permissive and non-permissive temperatures (A144T and L94P). Despite the large reduction, the A144T mutant shows robust growth at the permissive temperature, indicating that very low concentrations of Ess1 are sufficient for viability. These results and studies with *ESS1* promoter fusions (not shown) suggest that the reduced levels of the mutant proteins *per se* were not causing the *ts* phenotype, but that the proteins lack either binding or catalytic activity, or fail to localize correctly in the cell. The mobility of four

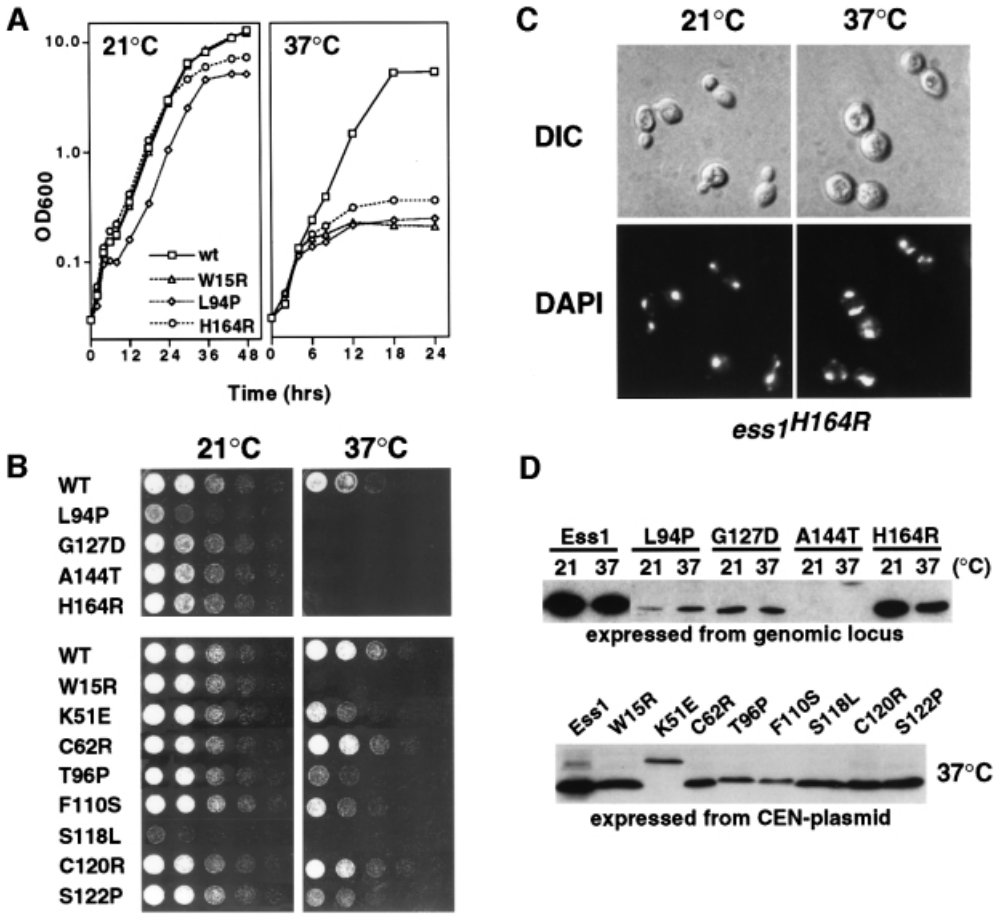

Fig. 1. Growth properties of *ess1*^{ts} mutants. (**A**) Growth of wild-type (W303-1A) and selected mutants in rich medium (YEPD) at permissive and non-permissive temperatures. The *ess1*^{W15R} allele was on a centromeric plasmid in an *ess1Δ* background. The *ess1*^{L94P} and *ess1*^{H164R} alleles were integrated into the genome. (**B**) Upper panel: growth of strains containing integrated *ess1*^{ts} alleles. Cells were grown to mid-log phase in YEPD medium, and serial 1:5 dilutions were spotted onto plates and incubated for 1–2 days at the indicated temperatures. Lower panel: growth of strains carrying plasmid-borne (CEN) copies of *ess1*^{ts} alleles in an *ess1Δ* host. Cells were grown and spotted as in the upper panel. (**C**) Mitotic arrest and nuclear fragmentation of *ess1*^{ts} mutants. Cells (*ess1*^{H164R}) were grown to mid-log phase and shifted to 37°C for 8 h. Cells were fixed, stained with DAPI, and visualized under Nomarski optics (DIC) or UV light (DAPI). (**D**) Western analysis of mutant proteins. Cells were grown at permissive temperature and shifted to 37°C for 4 h prior to harvesting. Equal amounts of total cell lysates were analyzed. Rabbit anti-Ess1 antiserum was used at a 1:4000 dilution.

proteins, K51E, A144T (Figure 1D), S77P and D102Y (not shown), was retarded for unknown reasons.

Structure–function analysis of Ess1

Given that Ess1 and Pin1 are 46% identical in sequence and are functionally interchangeable in yeast, their structures are likely to be similar. We therefore generated a model of Ess1 based on the Pin1 structure (Ranganathan *et al*., 1997; Figure 2B). The r.m.s. deviation between the Ess1 model and Pin1 structure was <0.66 Å over the WW and PPIase domains.

The *ess1* mutations are distributed throughout the protein (Figure 2A), and selected mutations are highlighted (Figure 2B). One mutation, K51E, maps to the linker that joins the two modules, in a region that is disordered in the X-ray structure of Pin1, suggesting that the linker might become ordered upon binding substrate. Within the WW domain, mutation of the first signature tryptophan (W15R) results in a *ts* phenotype. This mutation, and G43V, demonstrate the importance of the WW domain for Ess1 mitotic function. In the A144T mutation, substitution of alanine by a polar residue probably disrupts the hydrophobic interface formed between the α4 helix of the PPIase domain and the β2/β3 loop of the WW domain, destabilizing the pocket formed between the two domains where peptide is thought to bind.

Three mutations, L94P (Figure 2B), T96P and D102Y (not shown), map to the long α1 helix in the PPIase domain that is unique to Ess1-type parvulin-class PPIases. This helix projects a hydrophobic face into the pocket between the WW and PPIase domains, and these mutations might interfere with substrate binding, causing the observed growth defect. Ranganathan *et al*. (1997) proposed that a protein substrate might bind in the WW domain pocket, wrap around Pin1 contacting a solvent-exposed hydrophobic patch consisting of residues I96, F103, M146 and L160, and loop into the PPIase active site. Consistent with this model, the F110S mutation (analogous to F103 in Pin1) would disrupt these interactions by introducing a polar residue into this hydrophobic patch.

Mutations in three of the four residues seen to interact most directly with an Ala–Pro peptide in the active site of

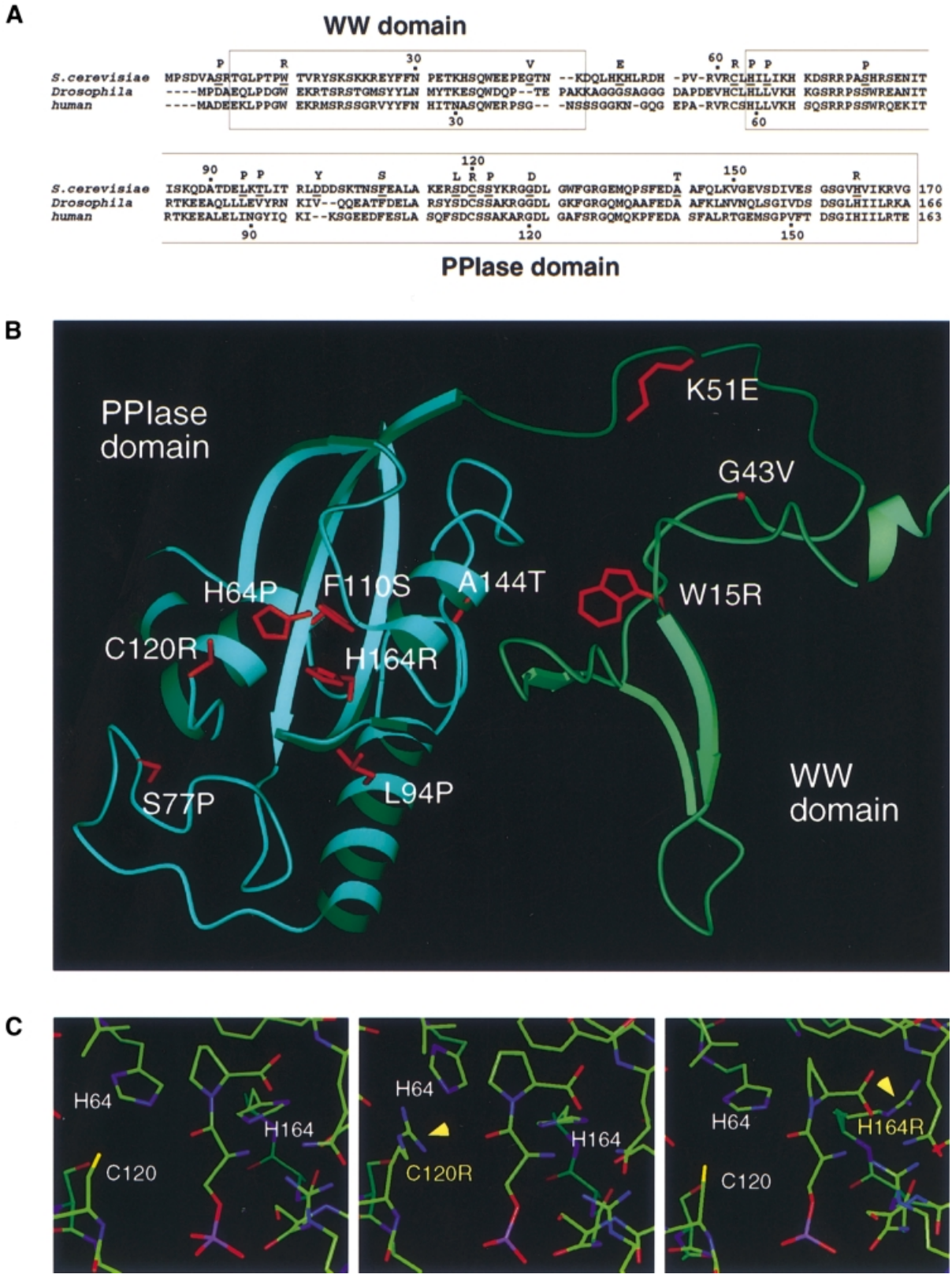

Fig. 2. Identity of *ts* and lethal substitutions in Ess1. (**A**) Alignment of yeast Ess1 and homologs from *Drosophila* (Dodo) and human (Pin1), showing the position (underlined) and identity of the substitutions. (**B**) Model of the Ess1 structure based on X-ray crystallographic data of Pin1 (Ranganathan *et al*., 1997) showing the location of selected mutations. (**C**) Model of the active site of wild-type Ess1, C120R and H164R mutants, each containing a bound phosphoserine–proline dipeptide in the *cis* configuration. Although there are minor rearrangements, none appears to preclude binding of the substrate peptide.

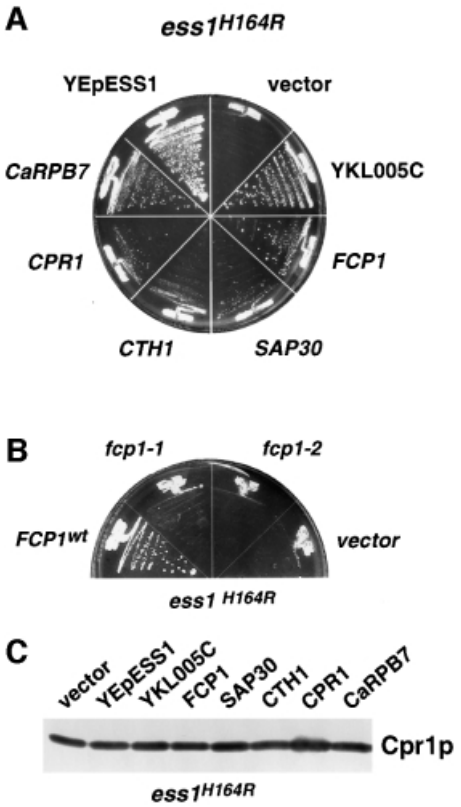

Fig. 3. High-copy suppressors of the lethal *ess1* mutant phenotype. (**A**) Suppression of *ess1^{H164R}* host cells at 37°C by six different high-copy plasmids carrying the indicated genes. Control plasmids were YEpESS1 or an empty vector (pRS426). *CaRPB7* is from *Candida albicans*. Cells were streaked onto medium that lacked uracil and incubated at 37°C for 5 days. (**B**) Fcp1 phosphatase activity is required for suppression of *ess1^{ts}* mutants. Suppression of *ess1^{H164R}* mutant cells at 37°C was tested using high-copy plasmids (2μ; *TRP1*) encoding wild-type (*FCP1*) or mutants alleles (*fcp1-1* and *fcp1-2*) that lack CTD phosphatase activity (Kobor *et al*., 1999). (**C**) Western analysis to detect cyclophilin A (Cpr1) in whole-cell lysates from *ess1^{H164R}* mutant cells carrying the indicated suppressors, or control plasmids, YEpESS1 or pRS426. Cells were grown at permissive temperature and shifted to 37°C for 4 h. Rabbit anti-cyclophilin A serum was used at a 1:3000 dilution.

Pin1 (H59, C113, S154 and H157; Ranganathan *et al*., 1997) were identified in our screen (H64P, C120R and H164R; Table I). As expected, mutation of H64, whose counterpart in Pin1 is thought to catalyze isomerization, results in a null phenotype. In contrast, the C120R mutation in Ess1 is not lethal, but confers a *ts* phenotype. Arginine modeled at this position does not preclude binding of a phosphorylated Ser–Pro dipeptide (Figure 2C); however, it would not allow formation of the covalent intermediate proposed for C113 in Pin1 (Ranganathan *et al*., 1997). Thus, Ess1 might catalyze isomerization by a mechanism independent of this cysteine intermediate.

Finally, the Ess1 H164R mutant is *ts* for growth. The equivalent histidine (H159) in Pin1 stabilizes the covalent intermediate formed with substrate peptide. Indeed, arginine modeled at this position does not disrupt the overall architecture of the active site (Figure 2C) and the guanido group of arginine might partially substitute for the imido nitrogen of histidine, allowing sufficient isomerase activity at permissive temperature.

Table II. Multicopy suppressors of *ess1^{ts}* mutants

Suppressor	Protein/similarities	Suppress *ess1*Δ?	Knockout phenotype
YKL005C	TFIIS-like	yes	viable
FCP1	CTD phosphatase	no	lethal
YMR263W	SAP30, HDAC complex	no	viable
CTH1	human Tis11, txn factor	yes	viable
CPR1	cyclophilin A	yes	viable
CaRPB7	*Candida albicans* Rpb7	yes	n.d.

The references for the knockouts are as follows: YKL005C (this study), *FCP1* (Archambault *et al*., 1997), YMR263W (Zhang *et al*., 1998), *CTH1* (Thompson *et al*., 1996) and *CPR1* (Davis *et al*., 1992). n.d., not determined.

Multicopy suppressors of ess1^{ts} mutants suggest a role in transcription

To identify proteins in the Ess1 pathway, we searched for yeast genes that, when present on multicopy plasmids, suppress the lethal phenotype of *ess1^{ts}* mutants at 37°C. Five suppressors were identified (Figure 3) plus an additional one identified in unrelated experiments (see below). All six suppress at least three different *ess1^{ts}* mutants: H164R, A144T and L94P. Four of them, YKL005C, *CTH1*, *CPR1* and *CaRPB7*, suppressed a complete deletion of *ESS1* (*ess1Δ*) (Table II), suggesting that they act downstream of Ess1 or in a parallel pathway.

The suppressor most commonly isolated (11 times) was the yeast open reading frame YKL005C, which predicts a 68 kDa protein with 40% similarity to the *Drosophila* and human transcription elongation factor TFIIS over about one-third of the protein (amino acids 177–354). The YKL005C protein also contains a PHD finger (amino acids 72–133), a cysteine-rich motif (Aasland *et al*., 1995) found in Polycomb and Trithorax group proteins, which, in *Drosophila* participates in global chromatin-mediated regulation of homeotic genes (Paro, 1990; Tamkun, 1995). Based on these similarities, the YKL005C protein might be important for transcription activation (or elongation) by chromatin remodeling.

Another suppressor, YMR263W, which encodes a homolog of human Sap30, a component of the Sin3A–Rpd3 histone deacetylase complex (HDAC), is recruited to promoters by sequence-specific DNA-binding proteins where it represses transcription (Zhang *et al*., 1998). In the accompanying paper (Arévalo-Rodríguez *et al*., 2000), Ess1 was shown to bind Sap30 indirectly via other members of this complex (Sin3–Rpd3) and to antagonize HDAC activity. Both Sin3 and Rpd3 are Ser/Thr–Pro rich, suggesting that they might be targets of Ess1-dependent isomerization. Suppression of *ess1* mutants by Sap30 is likely to result from a reduction in Sin3–Rpd3 HDAC activity by a titration effect.

Two suppressors are essential components of the RNA pol II complex itself. The first, *FCP1*, encodes a CTD phosphatase (Archambault *et al*., 1998; Kobor *et al*., 1999). Dephosphorylation of the CTD is required for recycling of RNA pol II for re-initiation, and is associated with paused polymerase complexes, which require re-phosphorylation to resume elongation (Dahmus, 1996). Suppression of *ess1^{ts}* mutants by *FCP1*, and the observed interaction between Ess1 and the CTD (see below) suggest

that isomerization of the CTD by Ess1 might promote CTD dephosphorylation by Fcp1. In *ess1* mutants, high concentrations of Fcp1 would overcome the need for isomerization, allowing dephosphorylation of the CTD. According to this model, the phosphatase activity of Fcp1 should be required for suppression. Indeed, two *fcp1* mutants that fail to dephosphorylate the RNA pol II CTD *in vivo* (Kobor *et al*., 1999) do not suppress *ess1^{ts}* mutants (Figure 3B).

The second suppressor that is a component of the RNA pol II complex is Rpb7, a σ factor-like protein that associates with the holoenzyme during stress, such as starvation and heat shock, and is required for initiation (McKune *et al*., 1993; Khazak *et al*., 1995). The version of Rpb7 we identified is encoded by a gene from the pathogenic fungus *Candida albicans* (G.Devasahayam, V.Chaturvedi and S.D.Hanes, unpublished). Curiously, neither the *Saccharomyces cerevisiae* nor the human *RPB7* genes suppressed *ess1^{ts}* mutants in our assays (not shown). Perhaps this is because yeast Rpb7 requires Rpb4 for function (McKune *et al*., 1993), or because Rpb7 from *C.albicans* functions more efficiently at 37°C, which is favorable for *C.albicans*.

The last transcription-related suppressor is *CTH1*, which encodes a CCCH-type Zn^{2+} finger protein that is a homolog of the mammalian serum-induced protein Tis11 (Varnum *et al*., 1991). Tis11 activates transcription (Ma and Herschman, 1995), but was also shown to translocate to the cytoplasm, bind AU-rich sequences in the 3′ ends of mRNA and promote mRNA degradation (Carballo *et al*., 1998). When overexpressed, however, Tis11 stabilizes tumor necrosis factor-α (TNF-α) mRNA (Lai *et al*., 1999). Overexpression of *CTH1* in yeast might stabilize mRNAs important for cell cycle progression, thereby obviating the need for Ess1 to promote transcription, or compensating for defects in 3′ end formation (Hani *et al*., 1999).

Finally, we identified *CPR1*, which encodes the prolyl isomerase cyclophilin A, as a suppressor of *ess1^{ts}* mutants. This was a surprise given that the substrate specificities of parvulin-class PPIases (Ess1) and cyclophilins are distinct (Hennig *et al*., 1998), and that cyclophilin A is cytoplasmic and does not contain a WW domain, which is thought to be critical for Ess1/Pin1 binding to target proteins (Lu *et al*., 1999; Morris *et al*., 1999). The PPIase activity of cyclophilin A is required for this suppression (Arévalo-Rodríguez *et al*., 2000).

It is possible that the five suppressors implicated in transcription suppress *ess1^{ts}* mutations by increasing expression of cyclophilin A. To test this idea, northern and western analyses were used to measure steady-state levels of *CPR1* mRNA and protein in *ess1^{ts}* mutants carrying each of the multicopy suppressors. *CPR1* mRNA and protein levels were not significantly altered (Figures 6B and 3C). More importantly, three of the five genes (*YKL005C*, *FCP1* and *CaRPB7*) suppressed *ess1^{ts}* mutants in strains in which *CPR1* is disrupted (Arévalo-Rodríguez *et al*., 2000). These results indicate that suppression is not due to increased transcription of *CPR1*.

What do the suppressors of *ess1* have in common? With the exception of cyclophilin A, all are likely to function during the various stages of transcription. Work from many laboratories has shown that all these stages, including initiation, elongation, termination and mRNA processing, require the CTD of RNA pol II large subunit (Rpb1) (Carlson, 1997; Corden and Patturajan, 1997). Results presented below suggest that the suppressors work by overcoming defects in CTD function resulting from lack of Ess1.

Ess1 interacts physically with the CTD of RNA polymerase II

The CTD of RNA pol II carries multiple copies (26 in yeast; 52 in humans) of the heptapeptide YSPTSPS, or close variants. Previous work showed that Pin1 binds to peptides that contain phosphorylated Ser–Pro motifs (Yaffe *et al*., 1997). Both Ser2 and Ser5 within the YSPTSPS motif are known to be phosphorylated *in vivo* (Dahmus, 1996; Yuryev and Corden, 1996; Ho and Shuman, 1999), resulting in two Pin1-binding sites per repeat. We tested whether Ess1 interacts with the CTD using a two-hybrid assay. Activation-tagged Ess1 interacts strongly with a LexA fusion protein carrying nine repeats of the CTD heptapeptide motif, whereas control proteins did not (Figure 4A and data not shown).

Ess1 also interacted with the CTD *in vitro* as shown by affinity pull-down assays. Ess1-conjugated beads pull down LexA–CTD (but not LexA alone) from yeast cell extracts, whereas beads conjugated with cyclophilin A or FKBP12 did not (Figure 4B). These results show that Ess1 interacts with the CTD, but do not distinguish whether this interaction is direct or indirect. Far-western analysis indicates that this interaction is direct. Proteins from whole-cell extracts were fractionated on SDS–polyacrylamide gels, transferred to nitrocellulose and incubated with purified His-tagged Ess1. Filters were washed and reacted with antibodies to Ess1. The major Ess1-reactive band co-migrates with RNA pol II (Figure 4C). Control blots using antibodies to Ess1 or RNA pol II show that anti-Ess1 serum does not cross-react with RNA pol II. Since the antibodies used against pol II are specific for the phospho-CTD form of the enzyme, these results suggest that Ess1 interacts with the phosphorylated form (II0) of RNA pol II. Biochemical interaction between Ess1 and the phospho-CTD of RNA pol II has also been reported by Morris *et al*. (1999). Analogous experiments with cyclophilin A failed to detect an interaction with RNA pol II (Figure 4C).

ESS1 interacts genetically with RNA polymerase II and SRB2

If the interaction between Ess1 and the CTD of RNA pol II is biologically meaningful, then we should detect genetic interactions between *ESS1* and *RPB1*, the gene encoding the large subunit of RNA pol II. To test this, three approaches were used. First, we deleted one copy of the *RPB1* gene in isogenic wild-type and *ess1^{ts}* mutant diploid strains. These strains were tested for growth at permissive temperature. As shown in Figure 5A, homozygous *ess1* mutant cells bearing one copy of *RPB1* (*ess1^{H164R}*/*ess1^{H164R} RPB1/rpb1Δ*) were severely growth inhibited compared with control strains. This growth defect is not seen in cells with either one or two copies of wild-type *ESS1* (Figure 5A and data not shown). Thus, *ess1^{ts}* mutations are synthetically lethal with *RPB1* heterozygous mutations.

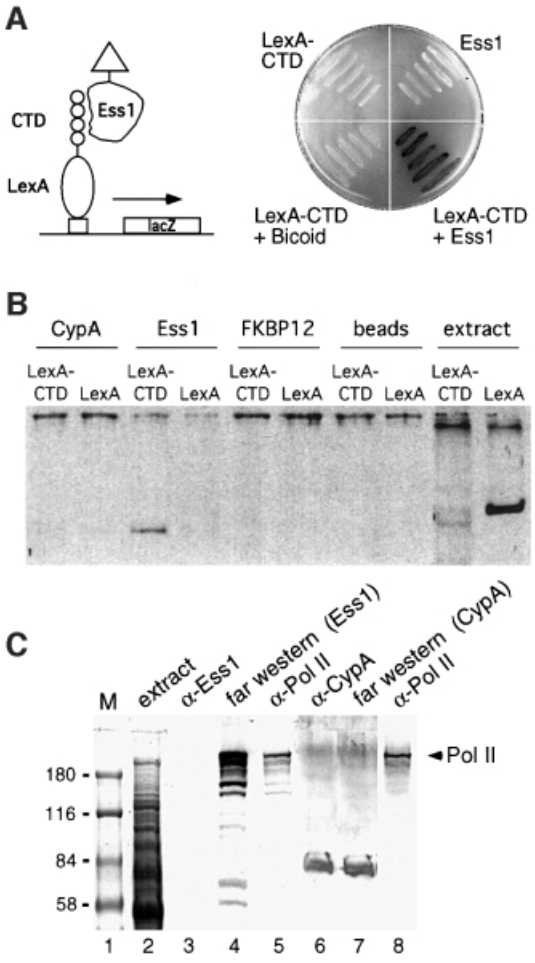

Fig. 4. Ess1 interacts with the CTD of RNA pol II *in vivo* and *in vitro*. (**A**) A schematic of the two-hybrid experiment is shown on the left. Yeast cells expressing the indicated proteins were streaked on an X-gal-containing plate and incubated overnight at 30°C (right). The $LexA_{1–87}$–CTD bait protein contains nine repeats of the YSPTSPS motif and interacts with activation domain-tagged Ess1 but not with the control protein, Bicoid. Neither $LexA_{1–87}$–CTD nor Ess1 alone activates transcription. (**B**) Ess1, but not cyclophilin A, interacts with $LexA_{1–87}$–CTD *in vitro*. Cyclophilin A- (CypA), Ess1- or FKBP12-conjugated affinity beads or control beads were reacted with total protein extracts from cells expressing either a $LexA_{1–87}$–CTD fusion protein or intact $LexA_{1–202}$. Bound proteins were eluted and analyzed by western blotting with LexA antisera. Extract denotes whole-cell lysates not reacted with affinity beads. (**C**) Far-western analysis shows that Ess1 reacts with a protein that co-migrates with hyperphosphorylated RNA pol II. Lanes 1 and 2 are marker proteins and Coomassie-stained total yeast extracts, respectively. Lanes 4 and 7 are far-western samples using His-tagged Ess1 or CypA proteins as probes, and detected using the cognate antibodies. Lanes 3, 5, 6 and 8 are standard western blots reacted with the indicated antisera.

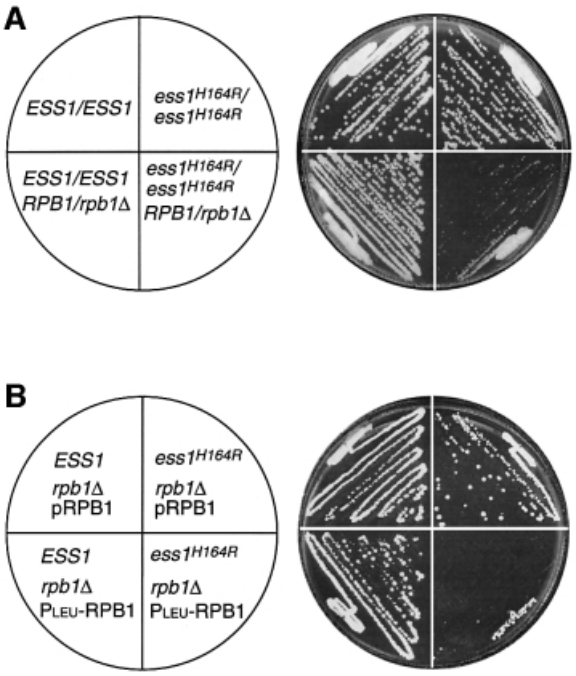

Fig. 5. *ESS1* shows a genetic interaction with *RPB1*. (**A**) The $ess1^{H164R}$ mutant is synthetically lethal with a reduced dosage of the *RPB1* gene. Cells of the indicated genotype (W303-1A background) were streaked to rich medium and grown for 3 days at permissive temperature (30°C). (**B**) The $ess1^{H164R}$ mutant is synthetically lethal with reduced levels of *RPB1* expression. Cells of the indicated genotype (W303-1A background) were streaked to selective medium and grown for 3 days at permissive temperature (25°C). pRPB1 is a *CEN-ARS* (*URA3*) plasmid that expresses the large subunit of RNA pol II from its natural promoter. P_{LEU}-*RPB1* is a *CEN-ARS* (*TRP1*) plasmid (also known as pLEU-RPO21; Archambault *et al*., 1996) that expresses the large subunit of RNA pol II from the repressible *LEU2* promoter. The plate shown contains 2 mM leucine, threonine and isoleucine, which represses the P_{LEU} promoter, driving low-level expression of *RPB1*.

Secondly, we tested the sensitivity of $ess1^{ts}$ haploid cells to lowered levels of *RPB1* expression using a repressible promoter strategy (Kobor *et al*., 1999). Strains were generated in which *RPB1* was deleted from the chromosome in wild-type and $ess1^{ts}$ cells. Since *RPB1* is an essential gene, viability was maintained by a plasmid-borne copy of *RPB1*. However, when $ess1^{ts}$ mutant cells carrying the plasmid P_{LEU}*RPB1* were shifted to repressing conditions (2 mM leucine) where *RPB1* expression is low, a severe growth defect was observed (Figure 5B). Wild-type cells were only modestly affected, and $ess1^{ts}$ cells bearing a control plasmid that expresses *RPB1* from its own promoter (pRPB1) showed no growth defects (Figure 5B).

Thirdly, we found that $ess1^{ts}$ mutants are hypersensitive to the dominant-negative effects of CTD truncation mutants. This was discovered in efforts to test whether $ess1^{ts}$ mutations enhance (or suppress) the phenotypes reported for CTD truncations and substitutions (West and Corden, 1995). Attempts to generate the appropriate strains were thwarted because introduction of CTD mutants proved toxic in $ess1^{ts}$ cells at permissive temperature, even though they have a normal chromosomal *RPB1* gene (Table III, upper). This toxicity was reversed by co-transformation with wild-type *ESS1* (Table III, lower). The simplest explanation is that RNA pol II function is already compromised in *ess1* mutants, and therefore cells are hypersensitive to the dominant-negative effects of CTD mutations. Note that a similar dominant-negative effect was reported for some *srb* mutants (Yuryev and Corden, 1996), which were originally isolated as suppressors of CTD mutants, and are known to play important roles in RNA pol II function (Thompson *et al*., 1993).

The above experiments suggest that Ess1 positively regulates RNA pol II function. If so, then *ESS1* mutations should be synthetically lethal with mutations in other

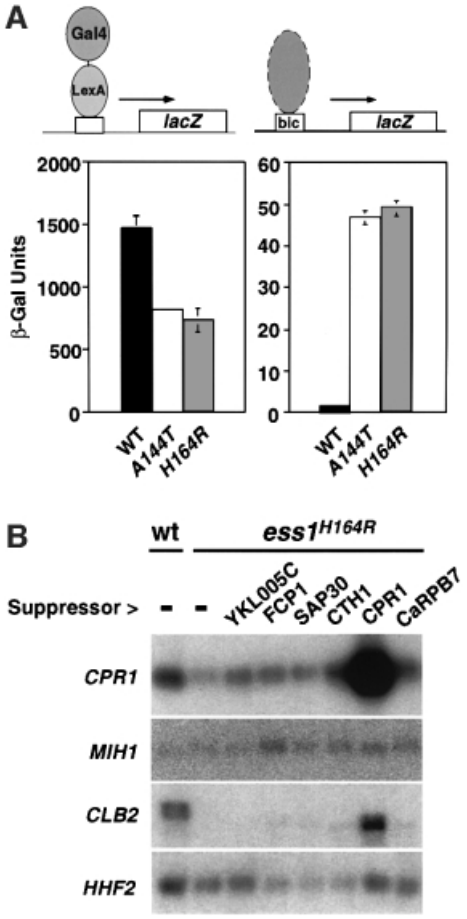

Fig. 6. Selective effects on transcription in *ess1* mutant cells. (**A**) *LacZ* reporter gene expression in yeast. Left: wild-type or *ess1*^{ts} mutant yeast were transformed with a plasmid expressing LexA–Gal4 (pSH17-4; Hanes and Brent, 1989), and a 4×-LexA operator-*lacZ* reporter construct (pSH18-8; S.D.Hanes and R.Brent, unpublished) at 23°C. β-galactosidase activity was measured in liquid cultures of five independent isolates. Similar results were obtained at 37°C (not shown). Right: cells were transformed with a Bicoid-site *lacZ* reporter (pWZ11-1; Zhu and Hanes, 2000) and treated as above, except that cells were incubated at 35°C. The differences between wild-type and the *ess1* mutants were less pronounced at 23°C (not shown). (**B**) Northern analysis of genes encoding mitotic regulators in *ess1*^{H164R} mutant cells. Wild-type cells or *ess1*^{H164R} mutant cells with control vector pRS426 (–) or the indicated multicopy suppressors were grown in selective medium and shifted to 37°C for 4 h prior to harvesting. A 6 μg aliquot of total RNA was loaded per lane. Duplicate blots were hybridized with random primed ^{32}P-labeled probes for *CPR1* (cyclophilin A) and *MIH1* (Cdc25 phosphatase), or *CLB2* (cyclin B) and *HHF2* (histone H4).

Table III. *Ess1* mutant strains are hypersensitive to CTD truncation alleles

	Vector	pRPB1	pWT0	pWT9	pA5(15)
Wild type	+++	+++	+++	+++	+++
H164R	++	++	–	–	–
A144T	++	++	–	–	–
Wild type + pESS1	+++	+++	+++	+++	+++
H164R + pESS1	++	++	++	++	++
A144T + pESS1	++	+	++	++	++

Wild-type or *ess1*^{ts} mutants (H164R, A144T) were transformed with equal amounts (~0.2–0.5 μg) of plasmids encoding wild-type or mutant forms of RNA pol II large subunit and grown for 3 days at 30°C. Strains in the bottom half of the table were co-transformed with pESS1 (*CEN*, *HIS3*), which encodes wild-type *ESS1*. Transformation efficiency is summarized as follows: +++ (>10 000); ++ (1001–10 000); + (100–1000); – (<100). Plasmid pRPB1 expresses wild-type Rpb1. A total of eight different CTD mutant plasmids was tested and the results were identical. Representative data for three mutants are shown. Plasmids pWT0, pWT7, pWT8 and pWT9 express truncated RNA pol II derivatives bearing zero, seven, eight or nine copies of the wild-type heptapeptide repeat (YSPTSPS), respectively. Plasmids pA2(18), pE2(15), pA5(15) and pE5(18) express RNA pol II bearing the indicated number of repeats in which Ser2 or Ser5 of the heptad repeat is mutated to alanine (A) or glutamic acid (E). The *RPB1* plasmids have been described (West *et al*., 1995; Yuryev *et al*., 1996).

Table IV. *Ess1* mutants are synthetically lethal with *srb2* mutants

Relevant genotype	5-FOA^{R}colonies/ total	*CaESS1* plasmid loss[a]
ESS1 SRB2	742/10 992	68
ESS1 srb2Δ	620/7480	83
ess1^{H164R} *SRB2*	286/25 050	11
ess1^{H164R} *srb2Δ*	0/39 690	0

Strains of the indicated genotype carrying a functional *ESS1* gene on a CEN-based *URA3* plasmid were grown overnight in the absence of selection (in YEPD) and plated to 5-FOA-containing medium. All growth was at permissive temperature (30°C). 5-FOA^{R} colonies were scored versus the total number of colony-forming units (c.f.u.) as measured by duplicate platings to YEPD. The fraction of 5-FOA^{R} colonies reveals the frequency of plasmid loss ([a]), which is given per 1000 c.f.u. The *C.albicans ESS1* gene (*CaESS1*), which is functionally homologous to *S.cerevisiae ESS1* (G.Devasahayam, V.Chaturvedi and S.D.Hanes, unpublished), was used to prevent unwanted recombination of the plasmid-borne copy of *ESS1* and the chromosomal *ess1*^{H164R} allele.

genes that positively regulate RNA pol II, such as *SRB2*, which encodes a component of the mediator complex that interacts with the CTD (Thompson *et al*., 1993). Indeed, this is true, as shown using a plasmid loss assay in which double mutant cells (*ess1*^{ts} *srb2Δ*) were unable to lose an *ESS1*-containing plasmid even at permissive temperature (Table IV). This result provides additional genetic linkage between *ESS1* and the transcription machinery.

Ess1 mutants show selective effects on gene transcription

Genetic and physical evidence indicate that Ess1 functions in transcription. If true, then the expression of some or all genes might be affected in *ess1* mutants. We therefore measured expression levels of *lacZ* reporter genes in wild-type and *ess1* mutant cells. The results indicate that one reporter, whose expression was driven by the LexA–GAL4 activator, is dependent on Ess1. Mutant cells show an ~2-fold reduction in β-galactosidase activity (Figure 6A, left). This effect was not due to a change in the relative amounts of intracellular LexA–GAL4 protein as detected by immunoblotting (not shown). In contrast, another reporter, which carried upstream Bicoid-binding sites, was stimulated nearly 40-fold in *ess1* mutants (Figure 6A, right), suggesting that Ess1 helps keep this gene silent. Activation of this reporter was by an endogenous yeast activator, since Bicoid was not expressed in these experiments. The results show that different genes have different requirements for Ess1.

We also examined the expression of two G_2/M-specific genes in wild-type and *ess1*^{ts} mutant cells grown at the restrictive temperature (Figure 6B). Relative to the control gene *HHF2* (histone H4), the expression of mitotic regulator *MIH1* (*cdc25* phosphatase) was not changed significantly in *ess1* mutant cells, whereas *CLB2* (cyclin B) levels were dramatically reduced. With the exception of *CPR1*, the multicopy suppressors did not restore *CLB2* expression to wild-type levels. These results suggest that Ess1 is important for the expression of a subset of genes, but not all genes. Note that in budding yeast *CLB2* is not essential (Fitch *et al*., 1992), so it is unlikely that this reduction alone is responsible for the mitotic arrest observed in *ess1* mutants.

Discussion

The results presented here provide genetic and biochemical evidence that Ess1, a parvulin-class PPIase, interacts with the transcription machinery and that this interaction is essential for cell viability. Isomerization of the CTD provides a mechanism by which Ess1 might regulate the interaction of RNA pol II with accessory proteins required for initiation, elongation, termination and splicing. Genetic suppressors of *ess1* mutants encode proteins involved in several of these processes, suggesting that Ess1 function might be required at multiple steps in transcription.

This work is consistent with the observations by Hani *et al*. (1995, 1999) that *ess1* (*ptf1*) mutants are defective in mRNA processing. However, this work does not support the current model that Ess1/Pin1 proteins control mitosis via direct interaction with cell cycle regulatory proteins, and suggests either that Ess1/Pin1 proteins are multifunctional, or that biochemical interactions with mitotic regulatory proteins might not be physiologically relevant. Instead, we propose that Ess1 and the mammalian homolog Pin1 are important for transcription of genes whose products can be rate-limiting for mitosis in eukaryotic cells. Using whole-genome approaches available in *S.cerevisiae*, it should be possible to determine the identity of those target genes.

Both the WW and PPIase domains are necessary for mitotic function

Our mutational analyses provide insight into the function of eukaryotic parvulin-class prolyl isomerases. Several of the mutants support predictions made based on the structural model of Pin1. However, some of the results were unexpected. For example, two of the three predicted PPIase catalytic residues could be replaced (C120R and H164R) without abolishing the mitotic activity of Ess1 at permissive temperature. This was not expected given their absolute conservation among Ess1/Pin1 homologs, and their observed interaction with the substrate peptide in the co-crystal. These results suggest that isomerization occurs by a mechanism different from that previously proposed (Ranganathan *et al*., 1997), or that efficient isomerization is not required for mitotic function under some conditions.

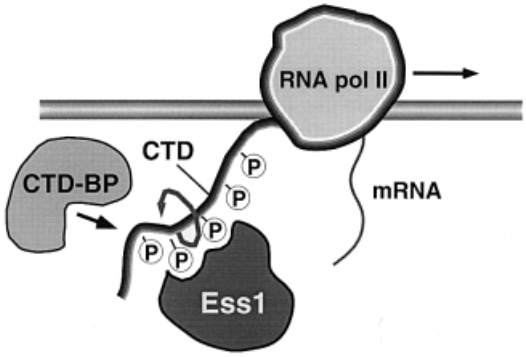

Fig. 7. Model for the action of Ess1 in transcription. Ess1 binds the phosphorylated CTD of RNA pol II, catalyzing its isomerization, thus acting as a regulatory switch for loading of proteins required for initiation, elongation, termination and 3′ end formation. In this model, Ess1 coordinates the sequential steps of transcription by changing the three-dimensional structure of the CTD, altering the affinity of protein–CTD interactions. Binding of Ess1 to the CTD would be regulated by phosphorylation–dephosphorylation by CTD kinases and CTD phosphatases (e.g. Fcp1). Ess1 might instead work stoichiometrically; Ess1 would sterically block (or promote) binding of RNA pol II-associated proteins to the phosphorylated CTD.

Ess1 suppressors and interactions with RNA pol II suggest a role in transcription

All of the multicopy suppressors of *ess1*^{ts} mutants except one are known or suspected components of the large complex known as the transcriptosome. This finding and our demonstration that Ess1 interacts genetically and physically with RNA pol II suggest a model depicted in Figure 7.

In this model, Ess1 would act catalytically to isomerize the CTD of RNA pol II, altering its structure to promote the binding or dissociation of CTD-interacting proteins that function in the sequential steps of transcription and mRNA processing. The signal for Ess1–CTD interaction would be phosphorylation at serines 2 and/or 5 of the heptad repeat, thereby increasing its affinity for the WW domain of Ess1, which would effectively tether the PPIase domain to the CTD. Isomerization of the CTD would then trigger dephosphorylation by Fcp1, allowing other proteins to bind to (or dissociate from) the CTD. Interaction of Ess1 with the CTD might explain why Hani *et al*. (1999) observed mRNA 3′-processing defects in *ess1* mutants; proteins involved in 3′-processing are likely to require interaction with the CTD.

In our model, Fcp1 would act after Ess1 on the CTD. Consistent with this idea, we find that whereas high-copy expression of *FCP1* suppresses *ess1* mutants (Figure 3), the reverse is not true. High-copy expression of *ESS1* did not suppress *fcp1* mutants (data not shown), indicating that *FCP1* is genetically downstream of *ESS1*.

Ess1 might not be the only prolyl isomerase that associates with RNA pol II via the CTD. A set of conserved proteins called CASPs (CTD-associated SR-like proteins), which are thought to bind mRNA and to be important for mRNA processing, has been shown to interact with the CTD. One of these proteins, mammalian CASP10, contains an N-terminal cyclophilin-like domain, shown to have PPIase activity *in vitro* (Bourquin *et al*., 1997). As yet, no regulatory activity on RNA pol II by CASP10 has been described. A recent report found an

interaction between human Pin1 and the CTD, although no functional significance was established (Albert *et al*., 1999).

A role for Ess1 in chromatin remodeling and cell cycle progression

It is well established that chromatin remodeling by histone deacetylation is important for control of cell cycle genes (Brehm *et al*., 1998). The results presented here and in Arévalo-Rodríguez *et al*. (2000) also connect Ess1 to the Sin3–Rpd3 HDAC, and suggest that Ess1 antagonizes HDAC activity. Loss of Ess1 function would increase deacetylase activity and repression of cell cycle genes, leading to mitotic arrest. Interestingly, yeast mutants lacking an essential protein called Esa1 show a mitotic arrest phenotype very similar to that seen in *ess1* mutants (Clarke *et al*., 1999), and Esa1 was shown to be a histone acetyl-transferase (HAT). Thus, in both *ess1* and *esa1* mutants, histone deacetylation would be favored over acetylation, perhaps leading to similar misregulation of genes required for mitosis.

Parvulin- and cyclophilin-class PPIases act in concert to promote mitosis

In yeast, as in other eukaryotes, the natural targets of the cyclophilins have been difficult to identify, because mutations of their genes, alone or in combination, have little or no phenotype (Dolinski *et al*., 1997). Here, we identify the major cytoplasmic cyclophilin, cyclophilin A, as a suppressor of *ess1* mutants. No cyclophilins have previously been implicated in essential cell cycle activities. The simplest interpretation of our results is that overexpression of cyclophilin A suppresses *ess1* mutants, because at higher concentrations it binds certain Ess1 substrates non-specifically. Since overexpression of cyclophilin A suppresses *ess1* deletion mutants, it suggests that cyclophilin A and Ess1 act in parallel pathways and bind to common targets, such as Sin3–Rpd3 HDAC, as shown in the accompanying paper (Arévalo-Rodríguez *et al*., 2000). The idea is supported by the fact that $ess1^{ts}$ *cpr1Δ* double mutants are synthetic lethal at permissive temperature and that $ess1^{ts}$ mutants are cyclosporin A sensitive (Arévalo-Rodríguez *et al*., 2000; X.Wu and S.D.Hanes, unpublished data). Thus, in addition to linking the parvulin-class Ess1/Pin1 prolyl isomerase to transcriptional regulatory mechanisms, these studies provide the first evidence for cross-talk among different families of prolyl isomerases.

Materials and methods

Plasmids and strains

DNA manipulations were performed according to standard procedures (Ausubel *et al*., 1987). Details of plasmid construction and gene knockouts of *YKL005C*, *RPB1* and *SRB2* are available in the Supplementary data or upon request. Chromosomal integration of $ess1^{ts}$ mutations into strain W303-1A (*MAT***a** *ura3-1 leu2-3,112 trp1-1 can1-100 ade2-1 his3-11,15 [psi+]*; R.Rothstein) was carried out by integration/excision using $YIpEss1^{ts}$ plasmids. The presence of the $ess1^{ts}$ alleles in the genome was confirmed by phenotype and by PCR amplification and DNA sequencing.

Isolation of ts ess1 mutants

ESS1 was mutagenized by error-prone PCR and transformed into yeast using gapped-plasmid methodology (Muhlrad *et al*., 1992) (see Supplementary data). From a total of 6600 transformants, 15 *ts* alleles bearing single substitutions were identified.

Molecular modeling

Sequence alignment and initial homology modeling of Ess1, from the X-ray crystal structure of Pin1 (Ranganathan *et al*., 1997) with a bound Ser–Pro dipeptide, were carried out using Look (Molecular Applications Group), with additional modeling using Insight II and Discover 3 (MSI). Energy minimizations were performed as described in the Supplementary data.

Identification of multicopy suppressors

Yeast strain YGD-ts22W, a W303-1A derivative bearing the $ess1^{H164R}$ allele, was transformed with a yeast genomic library and suppressors identified by selecting for Ura^+ transformants at 37°C. From an estimated 2×10^5 Ura^+ transformants, 156 clones grew at 37°C. To confirm that the suppression was plasmid linked, cells were replica-plated to 0.1% 5-fluoro-orotic acid (5-FOA) at 37°C. From the 113 $5\text{-}FOA^S$ clones, plasmids were rescued and restriction analysis and PCR showed that 80 carried inserts, 25 of which were *ESS1*. Eighteen of the remaining clones suppressed when retested, and by DNA sequencing they fell into five classes. Subcloning and retransformation identified the gene responsible for the suppression. Suppression of an *ess1* deletion mutant was tested by tetrad dissection and plasmid curing, and the results concurred.

Interaction of Ess1 with the CTD

The two-hybrid assays were performed as described (Gyuris *et al*., 1993) using pSH18-34, which contains eight LexA operators driving *GAL1-lacZ* (S.D.Hanes and R.Brent, unpublished). Expression of the $LexA_{1–87}$–CTD was verified by western blotting using anti-LexA antisera (R.Brent). Details of the affinity pull-down experiments and far-western analysis are available in the Supplementary data. For far-western analysis, anti-phospho-CTD antibodies were a gift of Arno Greenleaf.

Supplementary data

Supplementary data to this paper are available at *The EMBO Journal* online.

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