THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 280, No. 16, Issue of April 22, pp. 15510–15517, 2005
Printed in U.S.A.

Vanishingly Low Levels of Ess1 Prolyl-isomerase Activity Are Sufficient for Growth in *Saccharomyces cerevisiae**

Received for publication, October 27, 2004, and in revised form, November 23, 2004
Published, JBC Papers in Press, February 23, 2005, DOI 10.1074/jbc.M412172200

Trent R. Gemmill‡§, Xiaoyun Wu‡‖, and Steven D. Hanes‡§¶

From the ‡Wadsworth Center, New York State Department of Health and the §Department of Biomedical Sciences, School of Public Health, State University of New York, Albany, New York 12208

Ess1 is an essential peptidylprolyl-*cis/trans*-isomerase in the yeast *Saccharomyces cerevisiae*. Ess1 and its human homolog, Pin1, bind to phospho-Ser-Pro sites within proteins, including the carboxyl-terminal domain (CTD) of Rpb1, the largest subunit of RNA polymerase II (pol II). Ess1 and Pin1 are thought to control mRNA synthesis by catalyzing conformational changes in Rpb1 that affect interaction of cofactors with the pol II transcription complex. Here we have characterized wild-type and mutant Ess1 proteins *in vitro* and *in vivo*. We found that Ess1 preferentially binds and isomerizes CTD heptad-repeat (YSPTSPS) peptides that are phosphorylated on Ser5. Binding by the mutant proteins *in vitro* was essentially normal, and the proteins were largely stable *in vivo*. However, their catalytic activities were reduced >1,000-fold. These data along with results of *in vivo* titration experiments indicate that Ess1 isomerase activity is required for growth, but only at vanishingly low levels. We found that although wild-type cells contain about ~200,000 molecules of Ess1, a level of fewer than 400 molecules per cell is sufficient for growth. In contrast, higher levels of Ess1 were required for growth in the presence of certain metabolic inhibitors, suggesting that Ess1 is important for tolerance to environmental challenge.

Peptidylprolyl-*cis/trans*-isomerases (PPIases;[1] EC 5.2.1.8.) catalyze rotation about the peptide bond preceding proline (1). They are found in all organisms and are important for the folding of newly synthesized proteins, in which peptide bonds are thought to be synthesized exclusively in the *trans* conformation (2). PPIases also induce conformational changes in mature proteins, altering their structure and intermolecular interactions, thus affecting their activity (2). There are three well characterized families of PPIase, the cyclophilins, the FK506-binding proteins (FKBPs), and the parvulins (3, 4). Cyclophilins and FKBPs are the targets of immunosuppressive drugs that block T-cell activation in mammalian cells and prevent recovery from α-factor pheromone in yeast (5). Parvulin-class PPIases are structurally distinct from the cyclophilins and FKBPs and do not bind immunosuppressive drugs (6). Parvulin, an *Escherichia coli* protein, is the prototype for this family, the members of which are involved in the folding and trafficking of periplasmic and outer-membrane proteins (7).

In the yeast *Saccharomyces cerevisiae*, there are eight cyclophilins and four FKBPs, none of which are essential for growth (8). The one parvulin, Ess1, is essential, and cells that lack Ess1 arrest in mitosis and undergo nuclear fragmentation (9, 10). Ess1 homologs from *Drosophila* (Dodo) and mammals (Pin1) are functionally interchangeable in yeast but are not essential in their respective organisms (11, 12). Ess1 is essential in certain other fungi, *e.g. Candida albicans* and *Aspergillus nidulans* (13, 14), but not in others, *e.g. Schizosaccharomyces pombe* and *Cryptococcus neoformans* (15).[2] Why this PPIase is essential in some organisms and not others is unclear, but may be due to natural suppressor backgrounds in which high level expression of other PPIases, such as the cyclophilins or parvulin-like genes, can compensate for lack of Ess1 function (16, 21). Alternatively, it is possible that for some organisms, Ess1 is required under certain conditions that have not been mimicked by laboratory experiments.

Eukaryotic parvulins typically contain an amino-terminal WW domain and a carboxyl-terminal PPIase domain joined by a short linker of variable length and sequence (17). The WW domain is a protein-interaction module found in many signaling proteins (18). The WW domain of Ess1/Pin1-related proteins and the PPIase catalytic domain both bind to phospho-Ser-Pro motifs within substrate peptides, with the WW domain showing an affinity about 10-fold higher *in vitro* (19). Many proteins contain Ser-Pro motifs that are phosphorylated *in vivo*, and Pin1 in human cells is purported to regulate the activity of numerous proteins involved in the cell cycle, cancer, Alzheimer's, disease and immune function (20).

In yeast, genetic and biochemical screens identified the transcription machinery as the major *in vivo* target of Ess1 (16, 24). In particular, Ess1 binds Rpb1, the largest subunit of RNA pol II, which contains 26 carboxyl-terminal repeats of the heptad sequence YSPTSPS. Each repeat contains two potential Ess1 binding sites (underlined). The heptad repeat within the CTD of Rpb1 is known to be reversibly phosphorylated *in vivo*, and these changes in phosphorylation are correlated with distinct steps in pol II transcription (initiation, elongation, and termination) and mRNA processing (capping, 3′ cleavage, and polyadenylation (22, 23)). Our genetic analyses, and those of Hani and colleagues, have shown that Ess1 is involved in multiple stages of transcription and mRNA processing (16, 25, 26, 47).

* This work was supported in part by National Institutes of Health R01 Grant GM55108 (to S. D. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ An American Cancer Society Research Scholar. To whom correspondence should be addressed: Center for Medical Sciences, Wadsworth Center, 120 New Scotland Ave., Albany, NY12208. Tel.: 518-473-4213; Fax: 518-402-2299; E-mail: hanes@wadsworth.org.

‖ Present address: Dept. of Molecular Biology, Massachusetts General Hospital and Dept. of Genetics, Harvard Medical School, Boston, MA 02114.

[1] The abbreviations used are: PPIase, peptidylprolyl-*cis/trans*-isomerase; FKBP, FK506-binding protein; FITC, fluorescein isothiocyanate; *p*NA, *p*-nitroaniline; NOESY, nuclear Overhauser effect spectroscopy; pol, polymerase.

[2] Ren, P., Rossettini, A., Chaturvedi, V., and Hanes, S. D. (2005) *Microbiology*, in press.

We have proposed a model in which Ess1 binds and isomerizes the CTD of Rpb1, thereby affecting cofactor binding to the pol II complex (16). Studies in human cells also show an involvement of Pin1 in the control of pol II function (27, 28).

PPIases are an enigmatic group of enzymes, because their catalytic activity is not always required for their *in vivo* function. Some PPIases work stoichiometrically rather than catalytically, for example, in the processing of rhodopsin in the *Drosophila* eye and the assembly of infectious virions of HIV (29, 30). In contrast, other data support the idea that the catalytic activity is required for Pin1 and Ess1 function (16, 31, 32).

Here, we examined the *in vitro* activities of wild-type and mutant Ess1 proteins and compared their abilities to support growth *in vivo*. The binding and catalytic activity was measured using several substrate peptides, including the phosphorylated-CTD repeat. The results support the idea that the catalytic activity of Ess1 is needed for *in vivo* function. To determine how much Ess1 is required *in vivo*, we used an exquisitely sensitive hormone-dependent induction method that allowed us to titrate Ess1 concentrations over a 2- to 3-log unit range. We found that extremely low levels of Ess1 activity are sufficient to support growth under normal laboratory conditions, but that higher levels are required under more challenging growth conditions. The results suggest that the excess Ess1 may be present to allow cells to respond rapidly to environmental insults, for example, to facilitate RNA pol II-dependent induction of stress-related genes.

EXPERIMENTAL PROCEDURES

General—All reagents, unless otherwise specified, were from Sigma and were of ACS reagent grade or better. Aqueous solutions were prepared using 18.2 megohm resistivity water from a Barnstead Nanopure Diamond water purification system. NMR tubes were from Wilmad glass, stock number 528-PP. Yeast growth curve optical-density measurements were performed on an Amersham Biosciences Ultraspec 3000 spectrophotometer. All other spectrophotometric analyses were performed using a Beckman DU-800 spectrophotometer with Peltier temperature control.

Protein Quantitation—Concentrations of protein and tyrosine-containing peptides were measured by absorbance at 280 nM in 6 M guanidine HCl. Quantitative amino acid analysis on several samples showed no more than 6% error as compared with A_{280} values, confirming the accuracy of the method.

For estimates of *in vivo* protein concentrations, we used Western analysis. SDS-PAGE gels were prepared and run by the method of Laemmli. Western blotting onto polyvinylidene difluoride membranes (Immobilon-P, Millipore) was performed overnight in a Bio-Rad transblot apparatus using carbonate/bicarbonate/SDS buffer as suggested by the manufacturer for the transfer of proteins with large pI values. Blots were incubated with purified rabbit anti-Ess1 antibody and donkey anti-rabbit IgG-horseradish peroxidase conjugate (Amersham Biosciences) and were developed using ECL-Plus and visualized on a Typhoon 9400 variable-mode imager in fluorescence mode using 488 nm excitation and 520 nm emission wavelengths. When greater sensitivity was required, the blots were exposed to Kodak BioMax XAR film, and the signal was digitized by optical scanning and quantified using the Tnimage software.[3] To calculate intracellular protein concentrations (molecules/cell) we used the values detected by Western analysis × Avogadro's number/cell number loaded per lane.

Rabbit anti-Ess1 antisera were prepared as described previously (16). 25 ml of antiserum was adsorbed against 1 g of yeast acetone powder (protocol from T. Stevens, University of Oregon) made from an *ess1*Δ *srb10*Δ yeast strain (26), and then affinity purified on a 2-g Ess1-agarose column. Ess1-agarose was prepared from recombinant Ess1 and cyanogen bromide-activated agarose (Sigma), according to the manufacturer's instruction.

Peptides—Suc-AEPF-*p*-nitroanilide was purchased from Bachem. The remaining peptides (listed in Table I) were synthesized by the Wadsworth Center Peptide Synthesis Core facility. Phosphopeptides were synthesized using an Fmoc (*N*-(9-fluorenyl)methoxycarbonyl) precursor for phospho-serine (Nova Biochem, A22912). FITC-AEPAA was prepared by conjugation of the peptide AEPAA (Table 1) with fluorescein isothiocyanate (Isomer I; Sigma) as per the manufacturer's instruction, followed by purification of conjugated peptide by high-performance liquid chromatography on a C8 column, with a gradient of acetonitrile in 0.1% aqueous trifluoroacetic acid.

Recombinant Protein Purification—Ess1 proteins were expressed from pET-28a vectors in a Rosetta™ *E. coli* expression strain (Novagen). 750 ml of cells were grown at 37 °C to an A_{600} of 0.1, then shaken at 26 °C for 30 min, followed by the addition of 1 mM isopropyl thiogalactopyranoside and a further 2 h of incubation (at 26 °C). Cells were harvested by centrifugation, washed with water, pelleted, and frozen at −80 °C until use. The frozen cells were resuspended in 20 ml of 0.5 M NaCl, 50 μg/ml lysozyme, 5 mM Tris, pH 8.0, on ice. After standing on ice for 30 min, the mixture was sonicated, and cell debris was removed by centrifugation at 10,000 × *g* and passage through a 44-μm filter. His-tagged proteins were bound to nickel-nitriloacetic acid-agarose (Novagen) and washed as per manufacturer's instruction. Bound proteins were eluted using 0.1 M EDTA, and dialyzed against 1 mM $CaCl_2$, 1 mM β-mercaptoethanol, 0.5 M NaCl, 5 mM Tris, pH 8.0. The His tag was removed using biotinylated thrombin, which was later removed on avidin agarose. The cleaved proteins were passed again through nickel-nitriloacetic acid-agarose to remove the cleaved tag and any impurities that bound the resin. Coomassie staining of SDS-PAGE gels showed the proteins to be >95% homogeneous. Proteins were stored sterile-filtered on ice, without loss of activity, or were snap-frozen and lyophilized as described below.

Fluorescence Anisotropy—Fluorescence anisotropy measurements were performed in a PerkinElmer Life Sciences LS50B luminescence spectrophotometer, using an excitation wavelength of 488 nm and an emission wavelength of 330 nm. Measurements were performed in 20 mM Tris, pH 8.0, with a constant 0.58-μM FITC-AEPAA (F-buffer). Additions of Ess1 proteins or CTD peptides did not alter the measured fluorescence intensity of this peptide. The dissociation constant of FITC-AEPAA with Ess1 proteins (K_f) was determined by measuring the fluorescence anisotropy of 0–10 μM Ess1 proteins in F-buffer (34). Non-fluorescent CTD peptides at final concentrations of 0–1000 μM were then titrated in, to compete the FITC-AEPAA from the Ess1. An excess of the Ess1 proteins were used, to minimize the contribution of artifacts arising from the use of two fluorescent peptides bound to a single Ess1 molecule. The dissociation constant (K_d), in micromolar, was calculated as $K_d = K_f R[\mathrm{Ctd}]/([E_0] - K_f R)$, where $[E_0]$ is the total concentration of Ess1 protein in micromolar, [Ctd] is the concentration of non-fluorescent CTD-peptide in micromolar, $R = (r - r_f)/(r_b - 2r_f - r)$, r is the anisotropy of the sample, r_f is the anisotropy of FITC-AEPAA alone, and r_b is the anisotropy of completely complexed FITC-AEPAA, measured in saturating amounts of Ess1.

Prolyl-isomerase Assay—A modification of a standard chromogenic assay was used (35). Briefly, 0.05–50 nmol of enzyme in 500 μl of 10-mM Tris, pH 8.0, containing 0.2 mM substrate peptide (Suc-AEPF-*p*-nitroanilide; Bachem) was pre-warmed at 10 °C or 20 °C. 500 μl of pre-warmed 2 mg/ml chymotrypsin in 10 mM Tris, pH 8.0, was added, and the mixture was rapidly mixed by inversion. Reactions were immediately monitored at 1.1-s intervals in a Beckman DU-800 spectrophotometer, under temperature control, until the absorbance at 390 nm plateaued. By approximating the rate of prolyl isomerization to be similar for both the free and chromogenically labeled substrate peptide, we calculated the theoretical amount of *p*-nitroaniline release (*i.e.* the amount that would be released if unlabeled substrate were somehow regenerated *in situ*), using $p\mathrm{NA} = dA(A_t/A_r)$, where dA is the amount of *p*-nitroaniline released per unit time and A_t/A_r is the ratio of total substrate peptide to remaining labeled peptide. Plotting *p*NA *versus* time over the first 90% of the course of the reaction yields a straight line whose *y*-intercept is the original amount of *trans*-substrate, and whose slope is the reaction velocity in nanomoles/s. Dividing the slope (as determined by least-squares analysis, after subtraction of the rate of the uncatalyzed reaction) by the amount of enzyme yields the turnover number (k_{cat}, s^{-1}).

NMR-based Chemical-exchange Measurements—NOESY experiments were used to measure the chemical exchange of the proline γ-carbon protons (37). The NOESY experiments were performed on 2 mM CTD-peptide samples in 99% D_2O, 25 mM sodium phosphate (pD 8.0), using a 500-MHz Bruker Avance instrument at 303 K using a 400-ms mixing time with a spectral width of 4496.4 Hz, and 256 scans. Initial studies demonstrated that the difference in decay rate between

[3] T. J. Nelson (2000) *Tnimage Scientific Image Measurement and Analysis Lab Manual*, The Johns Hopkins University, Baltimore, MD (available at entropy.brneurosci.org/tnimage).

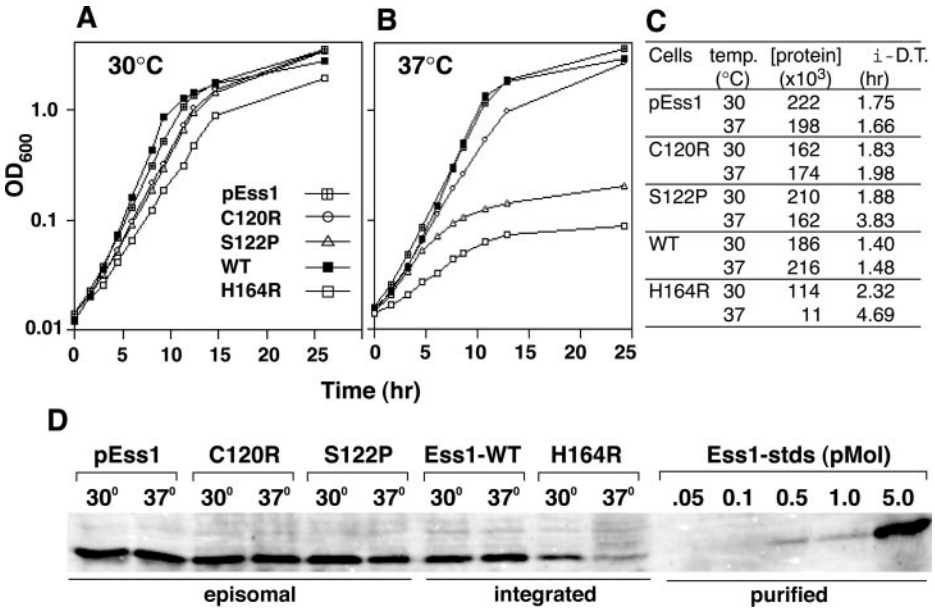

FIG. 1. **Growth rates and stability of Ess1 mutant proteins at different temperatures.** *A* and *B*, control (pEss1 and WT) and mutant (C120R, S122P, and H164R) yeast strains were grown in complete synthetic medium at the indicated temperatures. Growth was monitored by absorbance at 600 nM, and plots are representative of experiments done in triplicate. *C*, doubling times and protein concentrations (given in molecules per cell) are shown for yeast strains grown at different temperatures. The number of protein molecules per cell, *[protein]*, was calculated as described under "Experimental Procedures," and is based on Ess1 concentrations determined using data in *panel D*, below, and additional data not shown. *D*, representative Western analysis to detect Ess1 in aliquots of log-phase cells from *panels A* and *B*, above. Purified rabbit polyclonal anti-Ess1 serum was used for detection. *Lanes 1–9* each contain 1×10^6 cells. *Lane 10*, the H164R strain at 37 °C, contains 1×10^7 cells. *Lanes 11–15* contain the indicated amounts of Ess1 purified from *E. coli*.

the *cis*- and *trans*-forms of any of the proline γ-carbon protons was indistinguishable during this 400-ms mixing time. Ess1 protein samples were prepared for NMR by dialysis against 1 mM β-mercaptoethanol, 25 mM sodium phosphate, pH 8.0, and then snap-frozen, lyophilized, and stored dessicated at −80 °C. Samples were reconstituted using 99% D_2O immediately prior to the experiment. These enzyme samples were found to retain >80% of their initial activity after 16 h of experiments at 30 °C. Obtained spectra were analyzed using the NmrPipe and NmrDraw software packages (38). The peak volumes for the exchange cross-peaks were normalized against those of several diagonal peaks, to quantitate the relative percent exchanged (*P*). Because the time period measured was only 400 ms, no spontaneous exchange peaks could be detected at this temperature by this method, and the cross-peak area was always zero. The turnover number k_{cat}, in s^{-1}, was calculated as $k_{cat} = P[\text{Ctd}]/(T[E_0])$, where [Ctd] is the concentration of CTD-peptide (2 mM), *T* is the mixing time (0.4 s), and $[E_0]$ is concentration of Ess1 protein in millimolar.

Yeast Strains, Plasmid Constructs, Growth Curves, and Spot Tests—Yeast strain W303-1A (*MAT*a *ura3–1 leu2–3,112 trp1–1 can1–100 ade2–1 his3–11,15*) (source, Rod Rothstein, Columbia University) was used throughout. All mutant strains were W303-1A derivatives. The H164R strain contained an integrated copy of the mutation at the endogenous *ESS1* locus (16). The C120R, S122P, S77A, and S77E mutations were carried on an episomal *CEN* plasmid (pRS413) and were shuffled into an *ess1*Δ:*TRP1* derivative of the W303-1A strain. Therefore, in all experiments the control strain for H164R is wild-type W303-1A (WT), whereas the control for the other mutants is an *ess1*Δ pEss1 derivative of W303-1A. Fusions of *ESS1* or the H164R mutant allele to the *GAL1* promoter were done in plasmid pRS315-GAL1p (*CEN*, *LEU2*). The Gal4-ER-VP16* plasmid was obtained from Randy Morse (Wadsworth Center). For growth curve determinations, cells were grown in complete synthetic medium lacking appropriate amino acids for selection of plasmid-borne markers. β-Estradiol was prepared in 100% ethanol and was added to liquid medium just prior to growth. Cultures were grown in triplicate and the mean doubling times (*i*-D.T.) were calculated during the *initial* stages of log-phase growth, before cells reached saturation or mutant cells underwent arrest. The following formula was used: $i\text{-D.T.} = \ln(2)(T_f - T_i)/\ln(N_f/N_i)$, where N_i is the number of cells per milliliter at the initial time point T_i, and N_f is the number of cells at time point T_f. For spot tests, cells were pre-grown in liquid medium to an A_{600} of ~0.5 prior to spotting onto solid medium.

RESULTS

Quantitation of Ess1 Protein Levels in Wild-type and Mutant Strains—In previous work, several point mutations were identified in Ess1 that conferred a temperature-sensitive (*ts*) growth phenotype (16). Three of these mutations, C120R, S122P, and H164R, mapped to the PPIase active site. Two of the residues (Cys-120 and His-164) have been proposed, based on the structure of the human homolog, Pin1, to be involved in the catalytic mechanism of isomerization (39). To determine whether the catalytic activity of Ess1 is required for growth, and how much Ess1 activity is required, we first characterized the mutant proteins *in vivo*. At permissive temperature (30 °C) cells expressing the mutant proteins grow normally, whereas at non-permissive temperature (37 °C), S122P and H164R cells undergo growth arrest, whereas C120R shows modest slow growth (Fig. 1, *A* and *B*).

To determine whether the defect at 37 °C was due to degradation of the mutant proteins, we carried out Western analysis on cell extracts using anti-Ess1 antisera (Fig. 1*D*). Results show that both C120R and S122P protein levels remain essentially unchanged after the shift to 37 °C, but that H164R levels are reduced (Fig. 1*C*). Thus, degradation of the mutant proteins is not likely to be the most significant cause for the *ts*-phenotype. Instead, reversible denaturation of the mutant proteins could occur at higher temperatures, or there might be an increased cellular demand for Ess1 activity at higher temperatures that is unmet by the defective enzymes. To help to answer these questions, we characterized the mutant proteins *in vitro*.

Binding Activities of Wild-type and Mutant Ess1 Proteins—Wild-type and mutant proteins were expressed and purified from *E. coli*, and binding activity was determined using fluorescence anisotropy. Three substrate peptides were used for these assays. All are based on the CTD heptad repeat sequence (YSPTSPS) in the Rpb1 subunit of RNA pol II, but they differ in their phosphorylation at Ser-2 or Ser-5 within the repeat (Table I). Results indicate that all three mutant proteins (C120R, S122P, and H164R) bind reversibly to CTD-peptides phosphorylated on Ser-2 or Ser-5, but not to the unphosphorylated CTD-peptide (Table II). The mutant proteins have affinities close to that of the wild-type, and similar in their preference (*i.e.* pSer5 > pSer2) to that exhibited by human Pin1 (19). The results indicate that the Ess1 *ts*-mutant proteins are not defective due to lack of substrate binding, and that they are likely to be properly folded and are not generally denatured.

We also measured binding of two additional mutants, S77A and S77E. Residue Ser-77 lies at the entrance to the active site and is proposed to interact with the phosphate group of the modified Ser in substrate peptides (39). However, neither substitution affected binding significantly, and neither mutation affected cell growth rates (data not shown). In summary, all five Ess1 mutants bound phospho-Ser peptides with affinities

TABLE I
Peptide substrates used in this study

Parentheses identify the heptad repeat sequence found in the carboxyl-terminal domain of the Rpb1 subunit of RNA pol II. Bold lettering indicates the position of the phospho-Ser residue within the repeat.

Name	Sequence	Source
Chromogenic peptide	*suc*-AEPF-p-NA	Bachem
Anisotropy peptide	Fitc-AEPAA	This study
CTD	AS(YSPTSPS)YS	This study
CTD-pSer-2	AS(Y**pS**PTSPS)YS	This study
CTD-pSer-5	AS(YSPT**pS**PS)YS	This study

close to that of wild type. This is perhaps not surprising, given that most of the overall binding activity of Ess1/Pin1 proteins is due to the WW domain.

PPIase Activity of Ess1 Mutant Proteins—The enzymatic activities of the wild-type and mutant Ess1 proteins were determined using a standard chromogenic isomerase assay (Fig. 2). Wild-type, S77A, and S77E all showed significant levels of activity. However, the three *ts*-mutants S122P, C120R, and H164R were defective in this assay, showing no detectable activity at 10 °C, and barely detectable activity at 20 °C (Table III). At 20 °C the assay proceeds extremely rapidly, and low levels of activity were difficult to distinguish from background (*i.e.* relative to the blank). Assays done at 10 °C proceed more slowly, and therefore quantitative measurements for low activities were more reliable. From these data, we conclude that the *ts*-mutant proteins have activities that are <0.01% that of the wild-type enzyme.

Although the chromogenic PPIase assay is frequently used, it has several limitations. First, the synthetic peptide conjugate used in this assay is not a physiological substrate, and to date, no data exist to show how its isomerization compares to that of *bona fide* substrate such as the CTD heptad repeat. Second, although the mutant Ess1 proteins appear to be stable *in vitro* (data not shown), the effects of chymotrypsin excess, which is used in the assay, are not known. Finally, the chromogenic assay only measures the isomerization of the initial *cis*-portion of the peptide pool, ignoring most of the substrate, which is initially in the *trans*-configuration. To avoid these limitations, we used an independent (NMR) assay, described below.

Ess1 Isomerization Activity Measured by Chemical Exchange—The proline γ-carbon protons of the three CTD-peptides (Table I) were examined in two-dimensional NOESY experiments to determine the amount of chemical exchange that occurs during a 400-ms evolution time. This exchange serves to identify isomerization of the peptide bond preceding proline. As expected, no chemical exchange could be detected in the uncatalyzed reaction (no protein; Fig. 3, *upper panels*), because the rate of exchange for these bonds at 30 °C is ~1 min^{-1}, *i.e.* much shorter than the time scale of the experiment. In the presence of wild-type Ess1, the exchange rates increased markedly, and were most easily detected for the CTD-pSer5 peptide (Fig. 3, *lower panels*). Exchanges were less prominent for the CTD-pSer2 peptide and were not detectable with the unphosphorylated peptide, results that parallel those of the binding assays (Table II). These data clearly indicate that Ess1 binds and isomerizes CTD peptides, and that the order of substrate preference is pSer5 > pSer2 ≫ unphosphorylated CTD. Interestingly, an *in vivo* preference of Ess1 for Ser-5 over Ser-2 was first suggested by the results of genetic synthetic-lethality and suppression experiments using CTD and *ess1* double mutants (26).

We also analyzed isomerization of the CTD-pSer5 peptide by Ess1 mutant proteins. In agreement with the findings of the chromogenic PPIase assay, S77A and S77E both showed significant levels of activity, whereas C120R, S122P, and H164R did not (Table III). These results combined with those described in previous sections pose a significant dilemma: although the three mutant enzymes (C120R, S122P, and H164R) bind substrate reasonably well (presumably via their WW domains), all have catalytic activities that are 2–4 orders of magnitude fewer than that of the wild-type enzyme; yet they are still able to grow at the permissive temperature (30 °C).

Determining the Amount of Ess1 Activity Required for Growth in Vivo—To determine exactly how much Ess1 activity is needed to support growth in yeast, we employed a regulated-expression system that uses a Gal4-ER-VP16 transcriptional activator (40). In our experiments, addition of the hormone β-estradiol enables Gal4-ER-VP16 to activate transcription of a *pGAL1-ESS1* fusion gene (Fig. 4*A*). Protein expression is dose-dependent over a broad range of hormone concentrations (41). A low basal level of *GAL1*-driven transcription occurs in the absence of hormone, so we made use of a modified activator, Gal4-ER-VP16*, which bears a mutation (F442P) that reduces the potency of the VP16 activation domain (42), thus extending the utility of the system to very low ranges and reducing basal expression in the absence of hormone (43). Using this method we have shown that protein concentrations in yeast can be controlled in a linear fashion over nearly a 1000-fold range (41).

Cultures containing various concentrations of β-estradiol were inoculated at low density, and growth was monitored up to 25 h (Fig. 4*C*). Control cells that do not carry the inducible plasmid system were not affected by the concentrations of hormone used (data not shown). Aliquots were taken during mid-log phase growth, and cell extracts were prepared for Western analysis to measure *in vivo* protein concentrations; quantitation was done by comparison to known amounts of purified Ess1 (Fig. 4*B*, and data not shown). The results show that the concentration of wild-type Ess1 can be lowered to levels below the threshold of detection by Western analysis, and yet growth rates remained essentially normal (*e.g.* 0 hormone/no VP16 activator sample, Fig. 4, *B* and *C*). Here, cells produced an extremely low level of Ess1 (due to leakiness of the *GAL1* promoter), but this amount was sufficient for normal cell growth (Fig. 4*C*). These results indicate that fewer than 400 molecules of wild-type protein per cell are sufficient to support normal yeast cell growth under normal laboratory conditions (Fig. 4*E*). Given that wild-type cells normally contain about 200,000 molecules per cell (Fig. 1), Ess1 protein appears to be present in vast excess.

We extended this analysis to determine how much PPIase activity is required for cell growth. This was possible using the hormone-regulated system, combined with the use of a mutant, Ess1(H164R), whose *in vitro* activity was measured to be <0.01% that of wild-type Ess1 (Table III). *In vivo* titration experiments indicated that for normal, robust growth (<2-h doubling time), high hormone levels (10 nM) were needed, a level that produced about 10,000 times the amount of H164R present in wild-type levels (Fig. 4, *D* and *E*). This was not surprising, given that H164R is at least 10,000-fold less active than the wild type.

However, when less H164R was present, cells continued to divide (Fig. 4*D*), albeit more slowly (3- to 6-h doubling time). At these hormone levels (0 or 2.5 nM), cells produced only ~15,000–200,000 molecules of H164R protein per cell (Fig. 4*E*). Using this information, we can estimate a lower limit of the amount of Ess1 activity required for minimal (slow) growth. Given that H164R has <0.01% the activity of the of wild-type enzyme, cells would require the equivalent of only 1.5–20 Ess1 molecules per cell for minimal growth (15,000 × 0.01% = 1.5; 200,000 × 0.01% = 20). This would translate into ~20–300 turnovers per second (1 Ess1 ~ 15 turnovers s^{-1}; Table III).

TABLE II
Dissociation constants (μM) for wild-type and mutant Ess1 proteins

The binding constants were determined by monitoring the change in fluorescence anisotropy of the indicated wild-type or mutant Ess1 protein bound to Fitc-AEPAA, after competition by titration with the CTD-peptides shown.

Sample	CTD	CTD-pSer-2	CTD-pSer-5
Wild-type	>3000	241.25 ± 23.28	61.01 ± 4.91
H164R	>3000	312.68 ± 7.98	90.52 ± 7.41
S122P	>3000	129.84 ± 25.89	62.58 ± 8.73
C120R	>3000	254.73 ± 2.87	169.48 ± 25.21
S77E	>3000	259.52 ± 50.12	98.69 ± 17.12
S77A	>3000	190.43 ± 50.81	54.85 ± 11.44

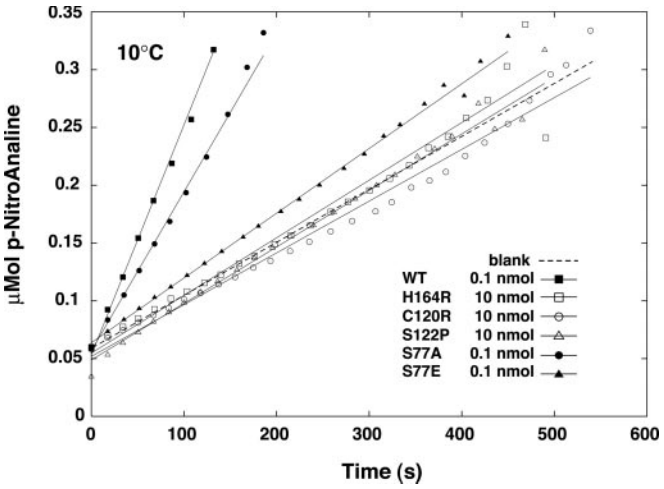

FIG. 2. **PPIase activity of Ess1 *ts*-mutants: a protease-coupled assay detects no activity.** The substrate (Suc-AEPF-p-NA) was subjected to proteolysis by chymotrypsin, which specifically releases the *p*-nitroaniline (*p*NA) from the peptide only when the *E*–P bond is in the *trans* conformation. The release of *p*NA was monitored during the assay at 10 °C by its absorbance at 390 nm, and the data were linearized as described under "Experimental Procedures." For clarity, only every 15th data point is shown; each line represents the least-squares plot whose slope was used to calculate the rate (as micromoles of *p*NA/s). Similar results were obtained when the reactions were carried out at 20 °C (Table III).

Cells with Low Ess1 Activity Are Sensitive to Inhibitors of Cell-wall Biosynthesis—The large disparity that we found between the amount of Ess1 present in normal cells (high) and the amount required for normal cell growth (low) prompted us to ask whether there were conditions under which higher levels of Ess1 are required. A modest set of media conditions and inhibitors was used to challenge the growth of wild-type and mutant cells. Included were various metabolic inhibitors and conditions of osmotic, nutritional, and mutational stress (44). Of the 23 conditions tested, we found several in which high levels of Ess1 were required for growth. For example, addition of 46 μg/ml hygromycin B did not inhibit normal cell growth, but strongly inhibited growth of H164R mutants, and cells producing very low levels of the wild-type Ess1 protein (pGAL-Ess1 with no activator present) (Fig. 5, *upper panel*). Sensitivity to hygromycin B is often taken to indicate defects in cell-wall maintenance or biogenesis (45), suggesting perhaps that high levels of Ess1 are needed for these functions. Moreover, we found that caffeine, another drug associated with cell-wall defects in yeast (45), was also a potent inhibitor (Fig. 5).

If Ess1 is important for proper maintenance of cell-wall integrity, then perhaps media conditions that stabilize the cell wall (*i.e.* help to maintain osmotic balance) would suppress the defect in H164R mutant cells at restrictive temperature. Indeed, the addition of 1.5 M sorbitol or 500 mM KCl rescued the lethal defect in H164R cells at 37 °C (Fig. 5, *lower panels*). Although these experiments do not demonstrate a direct role for Ess1 in cell wall maintenance or biogenesis, they do identify conditions under which high levels of Ess1 activity are required for growth. Several other conditions under which high levels of Ess1 were required were also identified (*e.g.* 50 mM hydroxyurea, 1 mM 5-fluorouracil, and 5 mM sodium orthovanadate; data not shown). Thus, we propose that Ess1 is maintained at high levels as a mechanism for coping with environmental stress.

DISCUSSION

In this report, we showed that under nutrient-rich, nonstress conditions, the Ess1 prolyl-isomerase is present at a level of ~200,000 molecules per cell (Fig. 1). Surprisingly, we found that fewer than 400 molecules per cell can support optimal growth in standard media (Fig. 4). Even fewer may actually suffice, but we reached the lower limit of protein expression in our system due to leakiness of the *GAL1*-promoter. Thus, Ess1 is normally present in vast excess, and the quantity sufficient for its essential function is nearly three orders of magnitude less. This is the first study in which the amount of Ess1/Pin1 required for viability in any cell type has been determined. Although our estimates of protein levels required for growth are subject to error, our finding that Ess1 is present in vast excess is not in doubt. It is known from pedigree analysis (46), tetrad analysis of heterozygous deletion strains (9), and promoter shut-off experiments (10) that cells in which Ess1 synthesis has been eliminated will survive for several generations (up to seven doublings have been observed). Our findings help explain these observations, because it would take many cell divisions to deplete Ess1 stores below the threshold required for Ess1 activity, especially if the protein was stable.

The PPIase activity of Ess1 is thought to be essential for growth, because mutations that completely remove the *ESS1* gene or substantially alter the active site are lethal (9, 10, 32). Although mutations in the WW domain that prevent substrate binding are also lethal, overexpression of the PPIase domain alone (but not the WW domain) rescues cell growth (32),[4] indicating that PPIase activity is probably necessary. The present data confirm this idea. Moreover, by comparing the enzymatic activities of wild-type and mutant proteins with their ability to support growth at various concentrations *in vivo*, we were able to estimate the enzymatic activity required for minimal growth to be about 20–300 turnovers per second, and the activity required for robust growth to be about 6000 turnovers per second (400 molecules × 15 turnovers s^{-1}). Robust growth for the H164R mutant (Fig. 4) required much more protein (3,900,000 molecules/cell × 0.0015 turnovers s^{-1}) to yield comparable activity (5850 turnovers s^{-1}). Note that these estimates are made based on *in vitro* activity against small substrate peptides, rather than against full-length proteins. Thus, the values given here may underestimate the amount of activity required.

The *ts*-mutant proteins C120R, S122P, and H164R analyzed in this study had isomerase activities below the level of detection in two different assays. However, because the mutant yeast strains bearing these mutations are viable at permissive

[4] S. D. Hanes, unpublished data.

TABLE III
PPIase activity ($k_{cat}s^{-1}$) of wild-type and mutant Ess1 proteins

Summary of data from samples analyzed by the chromogenic protease-coupled assay using Suc-AEPF-*p*NA (Fig. 2), and by the NMR chemical exchange method at 30 °C using CTD-peptides (Fig. 3). The turnover numbers (s^{-1}) were calculated as described under "Experimental Procedures."

Assay sample	Chromogenic				Chemical exchange by NMR			
	10 °C		20 °C		CTD	CTD pSer-2	CTD-pSer-5	
	s^{-1}	%	s^{-1}	%		s^{-1}		%
Wild-type	12.65	(100)[a]	17.21	(100)	0	2.78	17.7	(100)
H164R	0.001	(<0.01)	0.04	(0.21)	0	0.0	0.0	(0)
C120R	0.002	(0.01)	0.01	(0.05)	nd	nd	0.0	(0)
S122P	<0.001	(<0.01)	0.04	(0.23)	nd	nd	1.04	(5.9)
S77A	9.79	(77)	13.67	(79)	nd	nd	11.76	(66.4)
S77E	2.26	(18)	5.54	(32)	nd	nd	6.12	(34.6)

[a] Numbers in parentheses indicate percentage of wild-type levels.

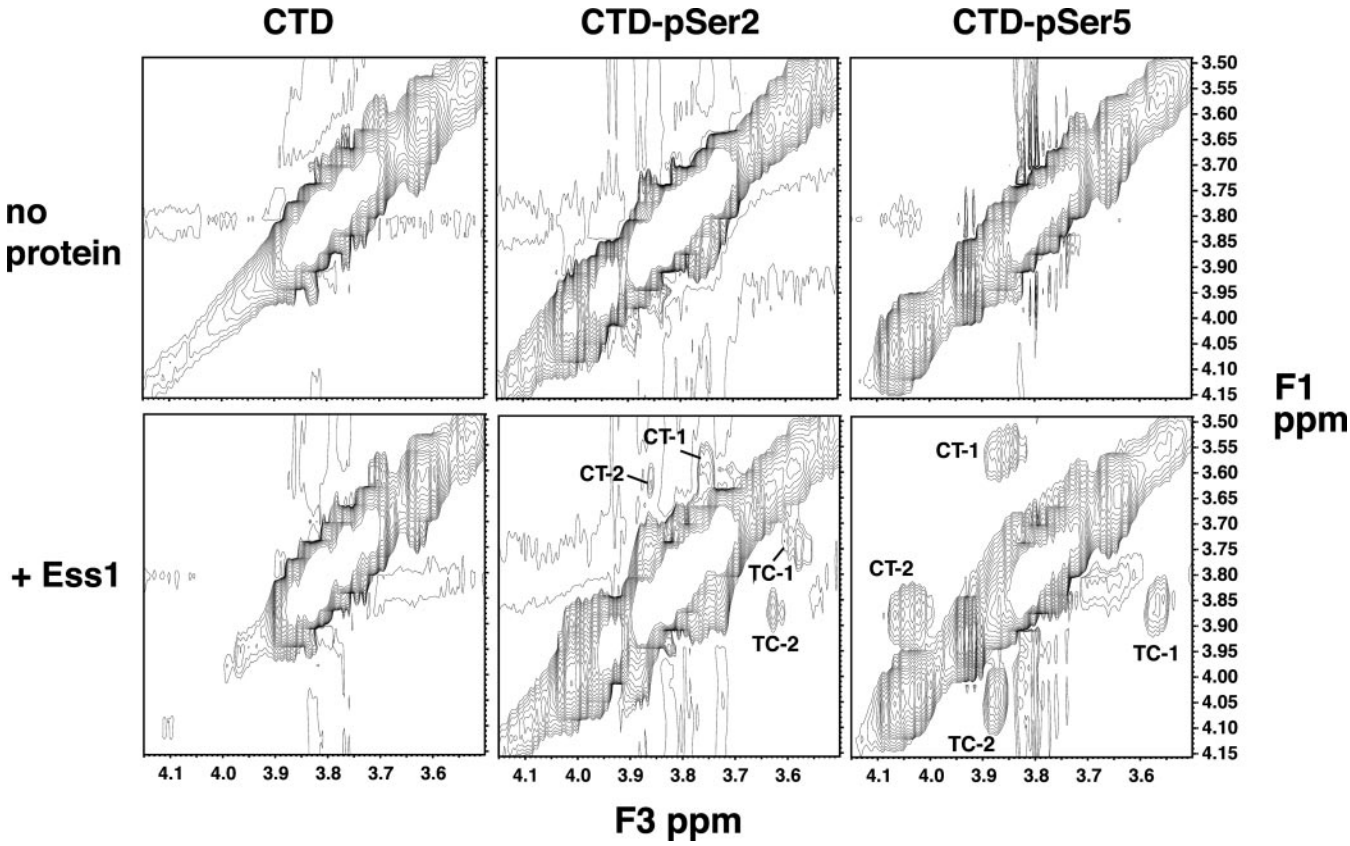

FIG. 3. **PPIase activity by NMR chemical exchange indicates that Ess1 prefers phospho-Ser-5 CTD substrates.** Three CTD-peptides (Table I) were examined, using a NOESY experiment, to quantitate the rate of chemical exchange occurring in the protons of the proline γ-carbons at 303 K (30 °C). The *upper panels* show regions surrounding these signals from samples containing only peptide. The *lower panels* show the corresponding regions from samples after the addition of 7.5 μM Ess1. Exchange peaks (CT-1, CT-2, TC-1, and TC-2) are visible between the *trans*-protons of the proline at position 5 of CTD-pSer-2 at 3.88 and 3.74 ppm and the corresponding *cis*-forms at 3.62 and 3.58 ppm, respectively. A more prominent exchange is seen between *trans*-protons in the proline at position 8 of the CTD-pSer-5 peptide at 4.05 and 3.88 ppm, and the corresponding *cis*-forms at 3.87 and 3.56 ppm, respectively. Additional experiments using mutant proteins are summarized in Table III.

temperature, the proteins are likely to have residual catalytic activity. Modeling of two of these proteins, C120R and H164R, suggested that the overall structure of the Ess1 active site would not be adversely affected by the mutation (16), but that activity should be greatly reduced, given that these residues are likely to participate in the chemistry of the reaction (39). Ser-122 is in the active site, but it does not appear to participate in catalysis (39). In addition, the three *ts*-mutant proteins did not show a measurable temperature sensitivity in PPIase activity *in vitro* (10 °C *versus* 20 °C; Table III), so we suspect that the requirement for Ess1 activity in cells, rather than the enzyme itself, is the temperature-sensitive element.

One limitation of the *GAL1*-inducible system is that it was not functional at 37 °C, the restrictive temperature of our Ess1 *ts*-mutants. Therefore, we were unable to analyze pGAL1-H164R at restrictive temperature. The lowest level of H164R production that we could generate with this system was 15,000 molecules per cell, resulting in cells that were slow growing at 30 °C (Fig. 4), but the cells did not show complete mitotic arrest as they do at 37 °C when expressing H164R under its own promoter (Fig. 1). Reduction of H164R protein to 11,000 molecules per cell, which occurs upon the shift to 37 °C (Fig. 1*C*), might be the cause of the temperature sensitivity in this strain. Or, as suggested above, cells might require more activity at higher temperatures to sustain growth (*i.e.* >15,000 H164R molecules at 37 °C, but not at 30 °C).

The vanishingly low levels of Ess1 found to be sufficient for growth have several important implications. First, because fewer than 400 molecules of Ess1 support strong growth, it is unlikely that Ess1 is important for transcription of all pol

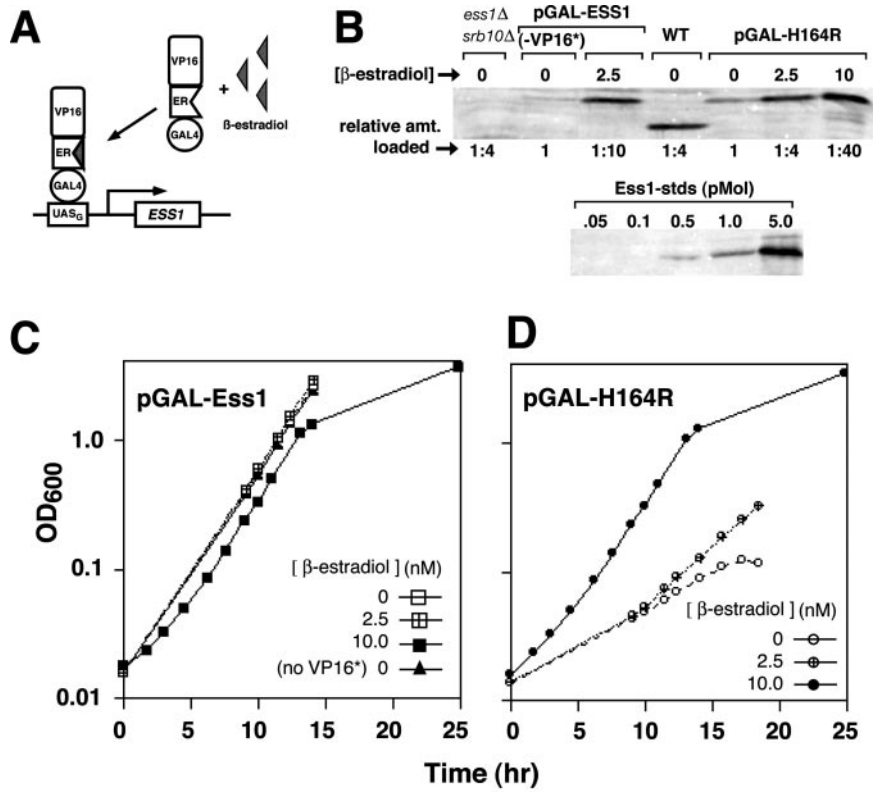

FIG. 4. **Use of a regulated expression system to determine lower limits of Ess1 required for yeast cell growth.** *A*, schematic of the expression system. Wild-type *ESS1* or the *H164R* coding sequences were placed under the control of the *GAL1* promoter (UAS_G) and were expressed from an episomal plasmid in an *ess1*Δ strain. A Gal4-ER-VP16* fusion construct was used to induce expression of the Ess1 or H164R proteins by the addition of β-estradiol. *B*, representative Western blot, probed with anti-Ess1 antibody, used to quantitate the levels of Ess1 in cells exposed to 0, 2.5, or 10 nM β-estradiol. pGAL-ESS1 and pGAL-H164R cells also contained the Gal4-ER-VP16* activator, except as indicated. Control samples are from an *ess1*Δ *srb10*Δ strain, which produces no Ess1 protein, or from wild-type (*WT*) cells. The *GAL1*-driven Ess1 and H164R proteins contain an amino-terminal HA tag and thus migrate with a lower mobility than does the wild-type protein. Ess1 standards (purified from *E. coli*) were run on the same gel and are shown below. *C* and *D*, growth at 30 °C of β-estradiol-treated *ess1*Δ cells carrying the indicated pGAL-ESS1 or pGAL-H164R plasmid. Cells also carried the Gal4-ER-VP16* activator plasmid except as indicated. *E*, summary of growth rate (i-D.T.) from *panels C* and *D* above, and Ess1 concentration as *[protein]*, in molecules/cell (as in Fig. 1). Protein concentrations were calculated from Western data in *panel B*, above. Note that trace amounts of Ess1 are still expressed (*E*) and that cells can still grow (*C*), even in the absence of the Gal4-ER-VP16* activator protein; this is presumably due to leakiness of the *GAL1*-promoter under these conditions (2% glucose).

E

Plasmid (*ess1*Δ)	VP16* act.	β-es nM	[protein] (x10^3)	i-D.T. (hr)
pGAL-Ess1	-	0	0.368	1.95
pGAL-Ess1	+	0	<1	1.80
	+	2.5	493	1.83
	+	10.0	nd	1.83
pGAL-H164R	+	0	15	5.57
	+	2.5	199	3.37
	+	10.0	3,950	1.88

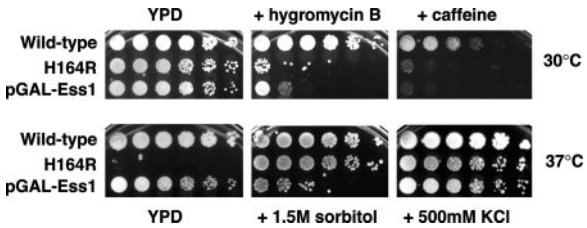

FIG. 5. **High levels of Ess1 activity are required to support growth under stress.** Wild-type, H164R, or *ess1*Δ cells carrying a pGAL-Ess1 construct (but no Gal4-ER-VP16* activator protein) were grown to mid-log phase in YPD, and serial dilutions (1:5) were spotted onto YPD, or YPD containing the indicated agent. Cells were grown for 2–4 days at the indicated temperature. *Upper panels*, hygromycin B was used at 46 μg/ml, and caffeine at 8 mM; the results indicate severe growth inhibition. *Lower panels*, addition of sorbitol or high salt (KCl) suppresses the *ts*-growth defect of H164R cells.

II-transcribed genes. Instead, it is likely that only a subset of genes are highly dependent upon Ess1 under normal growth conditions. This is consistent with results of preliminary microarray analysis that showed a change in the expression of only about 5% (~300) of yeast genes, upon depletion of Ess1 activity.[5] The number of Ess1 molecules required is also substantially lower than the estimated number of total RNA pol II complexes in actively growing cells (~16,000 (33)).

A second implication is that cells maintain high levels of Ess1 so as to withstand potential environmental challenge. This is based on the finding that, whereas low levels of Ess1 were sufficient for growth under normal conditions, cells did not grow under conditions of stress (Fig. 5). Thus, high Ess1 levels may permit cells to respond rapidly to changing conditions, for example, by facilitating transcriptional induction of pol II-dependent stress-response genes.

Finally, our study showed that Ess1 can isomerize peptides carrying the phospho-CTD repeat sequence found in Rpb1, which Ess1 is known to bind and interact with genetically (16, 21). The ~6-fold preference for phospho-Ser-5 over phospho-Ser-2 is consistent with genetic results showing that a Ser-5 CTD mutation (Ser-5 to Ala within amino-terminal CTD repeats), but not a Ser-2 mutation, could suppress the H164R mutation, thereby allowing growth at 37 °C (26). The results are also consistent with binding by Pin1 (19) and may help to explain the preferential effects of Pin1 on the phosphorylation state of Ser-5, over that of Ser-2, of the CTD (27, 36).

Acknowledgments—We are indebted to David LeMaster and Lynn McNaughton of the Wadsworth Center (WC) Structural Biology NMR Core Facility for assistance in the NMR-based assays, and Robert MacColl and Leslie Eisele of the WC Biochemistry Core for assistance with fluorescence anisotropy assays. We also thank Li-Ming Changchien and Frank Maley of the WC Amino Acid Analysis and Protein Sequencing Core for peptide and protein sequence analysis

[5] M. Arévalo-Rodríguez, J. Heitman, X. Wu, and S. D. Hanes, unpublished data.

and quantitative amino acid analyses, and Charles Hauer and Robert Stack of the WC Mass Spectrometry Core for protein sequencing and mass determinations. DNA sequencing and oligonucleotide synthesis were performed by the WC Molecular Genetics Core. We also thank James Dias and the WC Peptide Synthesis Core for synthetic peptides, and Robert Trimble for comments on the manuscript.

REFERENCES

1. Schmid, F. X., Mayr, L. M., Mucke, M., and Schonbrunner, E. R. (1993) *Adv. Protein Chem.* **44,** 25–66
2. Rutherford, S. L., and Zuker, C. S. (1994) *Cell* **79,** 1129–1132
3. Dolinski, K., and Heitman, J. (1997) in *Guidebook to Molecular Chaperones and Protein Folding Catalysis* (Gething, M.-J., ed) pp. 359–369, A Sambrook and Tooze Publication at Oxford University Press, Oxford
4. Arevalo-Rodriguez, M., Wu, X., Hanes, S. D., and Heitman, J. (2004) *Front. Biosci.* **9,** 2420–2446
5. Kunz, J., and Hall, M. N. (1993) *Trends Biochem. Sci.* **18,** 334–338
6. Rahfeld, J. U., Schierhorn, A., Mann, K., and Fischer, G. (1994) *FEBS Lett.* **343,** 65–69
7. Rudd, K. E., Sofia, H. J., Koonin, E. V., Plunkett, G., 3rd, Lazar, S., and Rouviere, P. E. (1995) *Trends Biochem. Sci.* **20,** 12–14
8. Dolinski, K., Muir, S., Cardenas, M., and Heitman, J. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 13093–13098
9. Hanes, S. D., Shank, P. R., and Bostian, K. A. (1989) *Yeast* **5,** 55–72
10. Lu, K., Hanes, S. D., and Hunter, T. (1996) *Nature* **380,** 544–547
11. Maleszka, R., Hanes, S. D., Hackett, R. L., de Couet, H. G., and Miklos, G. L. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 447–451
12. Fujimori, F., Takahashi, K., Uchida, C., and Uchida, T. (1999) *Biochem. Biophys. Res. Commun.* **265,** 658–663
13. Devasahayam, G., Chaturvedi, V., and Hanes, S. D. (2002) *Genetics* **160,** 37–48
14. Joseph, J. D., Daigle, S. N., and Means, A. R. (2004) *J. Biol. Chem.* **279,** 32373–32384
15. Huang, H. K., Forsburg, S. L., John, U. P., O'Connell, M. J., and Hunter, T. (2001) *J. Cell Sci.* **114,** 3779–3788
16. Wu, X., Wilcox, C. B., Devasahayam, G., Hackett, R. L., Arevalo-Rodriguez, M., Cardenas, M. E., Heitman, J., and Hanes, S. D. (2000) *EMBO J.* **19,** 3727–3738
17. Hani, J., Stumpf, G., and Domdey, H. (1995) *FEBS Lett.* **365,** 198–202
18. Sudol, M. (1996) *Prog. Biophys. Mol. Biol.* **65,** 113–132
19. Verdecia, M. A., Bowman, M. E., Lu, K. P., Hunter, T., and Noel, J. P. (2000) *Nat. Struct. Biol.* **7,** 639–643
20. Lu, K. P., Liou, Y. C., and Vincent, I. (2003) *BioEssays* **25,** 174–181
21. Fujimori, F., Gunji, W., Kikuchi, J., Mogi, T., Ohashi, Y., Makino, T., Oyama, A., Okuhara, K., Uchida, T., and Murakami, Y. (2001) *Biochem. Biophys. Res. Commun.* **289,** 181–190
22. Lin, P. S., Tremeau-Bravard, A., and Dahmus, M. E. (2003) *Chem. Rec.* **3,** 235–245
23. Bentley, D. (2002) *Curr. Opin. Cell Biol.* **14,** 336–342
24. Morris, D. P., Phatnani, H. P., and Greenleaf, A. L. (1999) *J. Biol. Chem.* **274,** 31583–31587
25. Wu, X., Rossettini, A., and Hanes, S. D. (2003) *Genetics* **165,** 1687–1702
26. Wilcox, C. B., Rossettini, A., and Hanes, S. D. (2004) *Genetics* **167,** 93–105
27. Xu, Y. X., Hirose, Y., Zhou, X. Z., Lu, K. P., and Manley, J. L. (2003) *Genes Dev.* **17,** 2765–2776
28. Palancade, B., Marshall, N. F., Tremeau-Bravard, A., Bensaude, O., Dahmus, M. E., and Dubois, M. F. (2004) *J. Mol. Biol.* **335,** 415–424
29. Luban, J., Bossolt, K. L., Franke, E. K., Kalpana, G. V., and Goff, S. P. (1993) *Cell* **73,** 1067–1078
30. Baker, E. K., Colley, N. J., and Zuker, C. S. (1994) *EMBO J.* **13,** 4886–4895
31. Fischer, G., Tradler, T., and Zarnt, T. (1998) *FEBS Lett.* **426,** 17–20
32. Zhou, X. Z., Kops, O., Werner, A., Lu, P. J., Shen, M., Stoller, G., Kullertz, G., Stark, M., Fischer, G., and Lu, K. P. (2000) *Mol. Cell* **6,** 873–883
33. Koleski, A. J., and Young, A. Y. (1994) *Nature* **368,** 466–469
34. Lakowicz, J.R. (1999) *Principles of Fluorescence Spectroscopy 2nd edition,* pp. 291–313, Plenum Publishing Corp., New York
35. Fischer, G., Bang, H., and Mech, C. (1984) *Biomed. Biochim. Acta* **43,** 1101–1111
36. Kops, O., Zhou, X. Z., and Lu, K. P. (2002) *FEBS Lett.* **513,** 305–311
37. Kern, D., Drakenberg, T., Wikstrom, M., Forsen, S., Bang, H., and Fischer, G. (1993) *FEBS Lett.* **323,** 198–202
38. Delaglio, F., Grzesiek, S., Vuister, G. W., Zhu, G., Pfeifer, J., and Bax, A. (1995) *J. Biomol. NMR* **6,** 277–293
39. Ranganathan, R., Lu, K. P., Hunter, T., and Noel, J. P. (1997) *Cell* **89,** 875–886
40. Louvion, J. F., Havaux-Copf, B., and Picard, D. (1993) *Gene* (*Amst.*) **131,** 129–134
41. Burz, D. S., Rivera-Pomar, R., Jackle, H., and Hanes, S. D. (1998) *EMBO J.* **17,** 5998–6009
42. Regier, J. L., Shen, F., and Triezenberg, S. J. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 883–887
43. Stafford, G. A., and Morse, R. H. (1998) *J. Biol. Chem.* **273,** 34240–34246
44. Hampsey, M. (1997) *Yeast* **13,** 1099–1133
45. Klis, F. M., Mol, P., Hellingwerf, K., and Brul, S. (2002) *FEMS Microbiol. Rev.* **26,** 239–256
46. Hanes, S. D. (1988) *Isolation, Sequence and Mutational Analysis of ESS1, a Gene Essential for Growth in Saccharomyces cerevisiae.* Ph.D. thesis, Brown University, Providence, RI
47. Hani, J., Schelbert, B., Bernhardt, A., Domdey, H., Fischer, G., Wiebauer, K., and Rahfeld, J. U. (1999) *J. Biol. Chem.* **274,** 108–116

Molecular Basis of Cell and Developmental Biology:

Vanishingly Low Levels of Ess1 Prolyl-isomerase Activity Are Sufficient for Growth in *Saccharomyces cerevisiae*

Trent R. Gemmill, Xiaoyun Wu and Steven D. Hanes

J. Biol. Chem. 2005, 280:15510-15517.
doi: 10.1074/jbc.M412172200 originally published online February 23, 2005