The EMBO Journal Vol. 19 No. 14 pp. 3739–3749, 2000

Cyclophilin A and Ess1 interact with and regulate silencing by the Sin3–Rpd3 histone deacetylase

Miguel Arévalo-Rodríguez[1,2], Maria E.Cardenas[1], Xiaoyun Wu[3], Steven D.Hanes[3] and Joseph Heitman[1,2,4]

[1]Departments of Genetics, Pharmacology and Cancer Biology, Microbiology and Medicine, [2]The Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710 and [3]Molecular Genetics Program, Wadsworth Center, New York State Department of Health and Department of Biomedical Sciences, School of Public Health, State University of New York, Albany, NY 12208, USA

[4]Corresponding author
e-mail: heitm001@duke.edu

Three families of prolyl isomerases have been identified: cyclophilins, FK506-binding proteins (FKBPs) and parvulins. All 12 cyclophilins and FKBPs are dispensable for growth in yeast, whereas the one parvulin homolog, Ess1, is essential. We report here that cyclophilin A becomes essential when Ess1 function is compromised. We also show that overexpression of cyclophilin A suppresses *ess1* conditional and null mutations, and that cyclophilin A enzymatic activity is required for suppression. These results indicate that cyclophilin A and Ess1 function in parallel pathways and act on common targets by a mechanism that requires prolyl isomerization. Using genetic and biochemical approaches, we found that one of these targets is the Sin3–Rpd3 histone deacetylase complex, and that cyclophilin A increases and Ess1 decreases disruption of gene silencing by this complex. We show that conditions that favor acetylation over deacetylation suppress *ess1* mutations. Our findings support a model in which Ess1 and cyclophilin A modulate the activity of the Sin3–Rpd3 complex, and excess histone deacetylation causes mitotic arrest in *ess1* mutants.
Keywords: cyclosporin A/HDAC/parvulin/Pin1/prolyl isomerases

Introduction

Protein primary sequences suffice to direct folding *in vitro* (Anfinsen, 1973), but the situation *in vivo* is probably more complex. *In vivo*, chaperones sequester protein folding intermediates and promote spontaneous folding (Gething and Sambrook, 1992). In addition, two classes of enzyme catalyze protein folding: prolyl isomerases and protein disulfide isomerases (Fischer, 1994; Dolinski and Heitman, 1997).

The peptide bond has partial double bond character and can exist as both *cis* and *trans* isomers. For all amino acids except proline, the *trans* isomer is the preferred conformer because of steric clashes in the *cis* form. In contrast, the peptide bonds preceding proline residues are equally stable as both *cis* and *trans* isomers. In proteins with known structures, ~10% of peptidyl-prolyl bonds are in the *cis* isomer (Stewart *et al*., 1990). The ribosome is thought to synthesize the peptide bond in the *trans* isomer, and spontaneous or catalyzed isomerization yields the *cis* form (Fischer and Schmid, 1990).

Three classes of enzymes have been discovered that catalyze prolyl isomerization: cyclophilins, FK506-binding proteins (FKBPs) and parvulins (Handschumacher *et al*., 1984; Harding *et al*., 1989; Takahashi *et al*., 1989; Rahfeld *et al*., 1994). The cyclophilins and FKBPs are notable because the cyclophilin A and FKBP12 members of these families mediate the effects of the immunosuppressants cyclosporin A (CsA), FK506 and rapamycin (Heitman *et al*., 1991a; Liu *et al*., 1991; Foor *et al*., 1992). Moreover, the cyclophilins and FKBPs are large families that are ubiquitous, highly expressed and conserved from microorganisms to man. The third family of prolyl isomerases, the parvulins, is also conserved from bacteria to man. Homologs of the yeast Ess1/Ptf1 parvulin (Hanes *et al*., 1989; Hani *et al*., 1995, 1999) are present in *Drosophila* (Dodo) and humans (Pin1) (Lu *et al*., 1996; Maleszka *et al*., 1996). The human parvulin Pin1 is thought to regulate mitosis, and has an unusual substrate specificity for phospho-serine–proline peptides (Ranganathan *et al*., 1997; Yaffe *et al*., 1997; Shen *et al*., 1998; Winkler *et al*., 2000). This finding, and the fact that Pin1 and other parvulin homologs have a unique WW protein–protein interaction domain (Lu *et al*., 1999), distinguishes these enzymes from the cyclophilins and FKBPs.

The yeast *Saccharomyces cerevisiae* expresses eight different cyclophilin and four different FKBP enzymes (Dolinski *et al*., 1997). None of these 12 prolyl isomerases is essential, either alone or in combination (Dolinski *et al*., 1997). In contrast, the one yeast parvulin, Ess1, is essential (Hanes *et al*., 1989; Lu *et al*., 1996). These findings suggest that most of the yeast prolyl isomerases do not play an essential protein folding role, and may instead each have unique functions, in accord with studies in *Drosophila* and mammals revealing specific roles for the NinaA and RanBP2 cyclophilins in opsin maturation (Baker *et al*., 1994; Ferreira *et al*., 1996), and for FKBP12 as a subunit of the ryanodine receptor (Brillantes *et al*., 1994; Shou *et al*., 1998).

In the accompanying manuscript, Wu *et al*. (2000) isolated temperature-sensitive (*ts*) mutants of the Ess1 enzyme and identified multicopy genetic suppressors. Remarkably, overexpression of cyclophilin A suppresses conditional lethal mutations in Ess1. Here we have explored the molecular basis for this first example of functional overlap between the members of two different prolyl isomerase families. We present genetic and biochemical evidence that identifies one common target as components of the Sin3–Rpd3 histone deacetylase

complex (HDAC), which regulates transcriptional repression and gene silencing in yeast and mammalian cells (Vidal and Gaber, 1991; Vidal *et al*., 1991; Pazin and Kadonaga, 1997; Struhl, 1998). Our studies provide the first evidence that the functions of cyclophilins and parvulins are linked in protein folding, and provide insight into the cellular functions and substrates of this enigmatic class of protein folding enzymes.

Results

Cyclophilin A catalytic activity suppresses ess1 conditional mutations

The yeast cyclophilin A gene *CPR1* was identified as a multicopy suppressor of temperature-sensitive conditional mutations in the *ESS1* gene, which encodes an essential prolyl isomerase (Figure 1A; see also Wu *et al*., 2000). This finding provided the first link between the functions of these two classes of prolyl isomerase. Two other cyclophilins, homologs of mammalian cyclophilin 40 encoded by the yeast *CPR6* and *CPR7* genes, also weakly suppressed *ess1*^{ts} mutations (Figure 1A). The *FPR1* gene encoding FKBP12 did not (Figure 1A). These results suggest that at high concentrations the Cpr1, Cpr6 and Cpr7 cyclophilins can replace the essential function of Ess1.

To test whether the prolyl isomerase activity of cyclophilin A is required for suppression of *ess1*^{ts} mutations, we used the immunosuppressive drug CsA. At high concentrations (100 μg/ml), CsA does not affect yeast cell growth because the target of the cyclophilin–CsA complex, calcineurin, is not essential. CsA does, however, inhibit the peptidyl-prolyl isomerase (PPIase) activity of cyclophilin A; thus, if suppression of *ess1*^{ts} mutations by cyclophilin A requires enzyme activity, then CsA should abolish suppression. CsA blocked the ability of cyclophilin A (and also Cpr6 and Cpr7) to suppress, suggesting that prolyl isomerase activity is required (Figure 1B). Furthermore, several *ess1*^{ts} mutant strains were sensitive to CsA at permissive temperature (not shown), suggesting that cyclophilin A maintains viability of these strains. The FKBP12 and calcineurin inhibitor FK506 did not impair growth of *ess1*^{ts} mutants or inhibit suppression of *ess1*^{ts} mutations by cyclophilin A, indicating that the effects of CsA are not attributable to calcineurin inhibition.

Cyclophilin active site mutants were used to confirm that prolyl isomerase activity is required for genetic suppression. The H90Y cyclophilin A mutant that lacks prolyl isomerase activity (Cardenas *et al*., 1995a) failed to restore growth of *ess1*^{ts} mutants at 37°C (Figure 1A). A series of human cyclophilin A mutant enzymes with systematically reduced activity was also tested (Zydowsky *et al*., 1992; Scholz *et al*., 1999). The wild-type and mutant human cyclophilin A genes were expressed under the control of the yeast *CPR1* gene promoter and terminator from a yeast 2μ multicopy plasmid. Expression of wild-type human cyclophilin A restored growth of two *ess1*^{ts} mutants (H164R and A144T) at 37°C (Figure 1C and data not shown). The H54Q and W121A mutants, which retain 15 and 8.7% activity, respectively, partially restored growth at 37°C (Figure 1C). Cyclophilin A mutants with less activity did not support growth (Figure 1C). Thus, suppression of *ess1*^{ts} mutations is correlated with the level

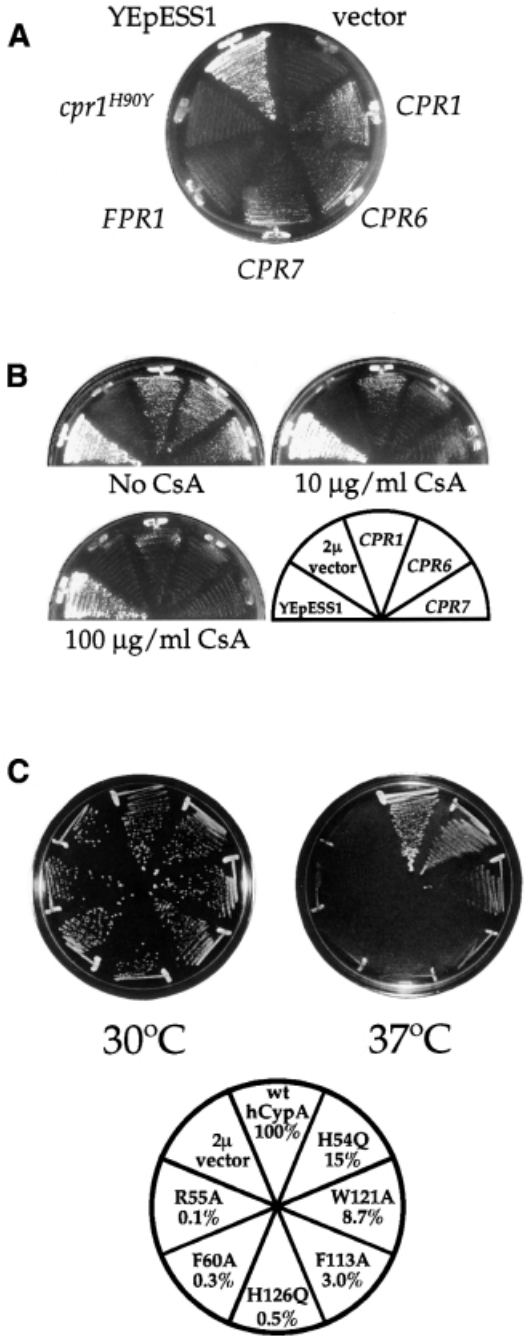

Fig. 1. Cyclophilins suppress *ess1*^{ts} mutants. (**A**) The *ess1*^{ts} mutant strain H164RW303 was transformed with 2μ plasmids expressing no protein (pRS426 vector), the cyclophilins Cpr1 (pTB3), Cpr6 (pKDw10) or Cpr7 (pKS24), FKBP12 (*FPR1*; plasmid pYJH23), the cyclophilin A active site mutant *cpr1*^{H90Y} [pCPR1(H90Y)] or Ess1 as a control (YEpESS1). Growth was for 72 h at 37°C. (**B**) Suppression of *ess1*^{ts} mutants by cyclophilin A (*CPR1*) and cyclophilin 40 homologs is CsA sensitive. *ess1*^{ts} strain H164RW303 transformed with 2μ plasmids expressing *CPR1*, *CPR6* or *CPR7* was grown on synthetic dextrose medium–uracil and then transferred to SD–Ura medium with 0, 10 or 100 μg/ml CsA and incubated for 72 h at 35°C. (**C**) Suppression of *ess1* conditional mutants by cyclophilin A requires prolyl isomerase activity. *ess1*^{ts} mutant H164RW303 was transformed with multicopy plasmids expressing wild-type or active site mutants of human cyclophilin A and growth was tested at 30 and 37°C. The wild-type cyclophilin A gene is indicated as wt hCypA, and the active site mutations and relative level of *in vitro* prolyl isomerase activity are indicated.

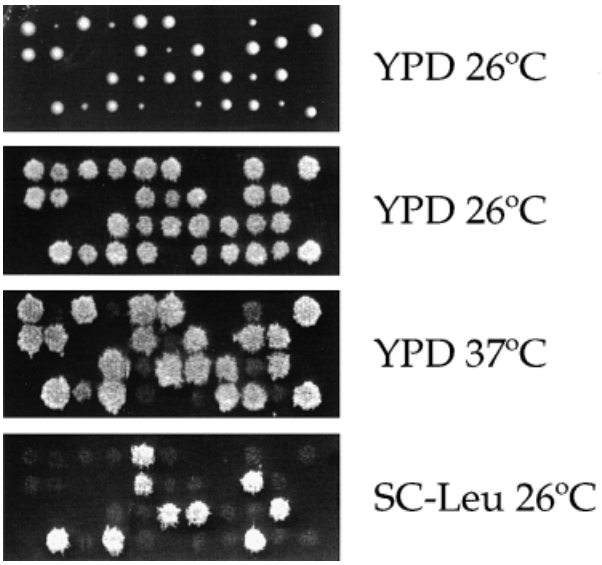

Fig. 2. Cyclophilin A and *ess1* mutations are synthetically lethal. The *ess1^{ts}/ESS1 cpr1Δ::LEU2/CPR1* diploid strain MAY3 × MH250-2c was sporulated, and tetrads were dissected and incubated on YPD medium at 26°C for 4 days. Viable meiotic segregants were replica-plated to YPD medium at 26 and 37°C to score the *ess1^{ts}* mutation, and to synthetic medium lacking leucine to score the *cpr1Δ::LEU2* cyclophilin A mutation.

of cyclophilin A prolyl isomerase activity, suggesting that the function of Ess1 and cyclophilin A requires enzymatic activity.

Cyclophilin A and FKBP12 mutations are synthetically lethal with ess1 mutations

The functional relationship between Ess1 and the 12 yeast cyclophilin and FKBP prolyl isomerases was examined using a synthetic lethal genetic approach. Specifically, 12 mutant strains each lacking one prolyl isomerase were mated to isogenic *ess1^{ts}* mutant strains. The resulting diploid strains were sporulated and tetrads analyzed to ascertain the phenotype of the double mutants.

This analysis revealed that a deletion of the cyclophilin A gene, *CPR1*, is synthetically lethal with *ess1^{ts}* mutations (Figure 2; Table I). As shown in Figure 2, when the *ess1^{ts}/ESS1 cpr1Δ/CPR1* diploid strain was sporulated and tetrads analyzed, two tetrads consisted of four viable spores, five tetrads contained three viable:one inviable spore, and four tetrads contained two viable:two inviable spores (2 PD:5 TT:4 NPD). The inviable segregants could be inferred to be *ess1^{ts} cpr1* double mutants, based on the segregation pattern of the *ess1^{ts}* mutation and the *cpr1Δ::LEU2* mutation (Leu$^+$, CsA + Li$^+$ resistant). No ts (*ess1^{ts}*) and Leu$^+$ (*cpr1Δ::LEU2*) viable spores were obtained. A plasmid expressing the cyclophilin A gene restored viability of *ess1^{ts} cpr1* double mutants (not shown). Thus, a mutation in the cyclophilin A gene, *CPR1*, is synthetically lethal with the *ess1^{ts}* mutation under conditions permissive for the *ess1^{ts}* single mutant.

A deletion mutation of the *FPR1* gene encoding FKBP12 was also synthetically lethal with *ess1^{ts}* mutations (Table I). Again, sporulation and tetrad analysis of the *ess1^{ts}/ESS1 fpr1/FPR1* strain revealed that the majority of tetrads yielded three viable and one inviable spore; the inviable spores could be inferred to be *ess1^{ts} fpr1* double mutants based on segregation of the *ess1^{ts}* and *fpr1::ADE2* mutations (rapamycin resistant), and no viable *ess1^{ts} fpr1* double mutants (ts, Ade$^+$ and rapamycin resistant) were obtained. Thus, both cyclophilin A and FKBP12 are required for viability of *ess1^{ts}* mutants.

Table I. Cyclophilin A (*cpr1*) and FKBP12 (*fpr1*) mutations are synthetically lethal with *ess1^{ts}* conditional mutations

Cross	Viable:inviable segregants		
	4:0	3:1	2:2
cpr1×ess1^{ts}	2	5	4
cpr2×	10	1	0
cpr3×	11	0	0
cpr4×	9	0	0
cpr5×	8	1	0
cpr6×	9	0	0
cpr7×	8	0	0
cpr8×	9	0	0
fpr1×ess1^{ts}	1	10	1
fpr2×	7	2	0
fpr3×	7	1	1
fpr4×	8	1	0

Data presented are for crosses with the H164R *ess1^{ts}* mutant strain MAY3; similar findings were obtained with an A144T *ess1^{ts}* mutant (MAY1). Inviable segregants in the *cpr1×ess1* cross could be inferred to be *cpr1 ess1^{ts}* double mutants (predicted to be ts and Leu$^+$). Inviable segregants in the *fpr1×ess1^{ts}* cross could be inferred to be *fpr1 ess1^{ts}* double mutants (predicted to be ts and rapamycin resistant). Inviable segregants in all other cases resulted from random spore death or failure to germinate, and *cpr ess1^{ts}* and *fpr ess1^{ts}* double mutants were viable in all of these crosses.

No other synthetic lethal interactions were observed between *ess1^{ts}* mutations and any other cyclophilin (*cpr2–cpr8*) or FKBP (*fpr2*, *fpr3* and *fpr4*) mutations (see Table I).

Cyclophilin A does not stabilize thermolabile Ess1 mutants

Cyclophilin A might restore viability of *ess1^{ts}* mutant strains by direct binding to Ess1, stabilizing or refolding the thermolabile enzyme. Ess1 protein levels were determined in strains expressing an Ess1 thermolabile mutant and cyclophilin A from a multicopy plasmid. The levels of the Ess1 H164R and A144T mutant enzymes were reduced at 37°C compared with 26°C (Figure 3A), and the A144T mutant protein was virtually undetectable at 26°C, consistent with the ts phenotype conferred by these mutant enzymes. The levels of the H164R and A144T mutant enzymes were similar in cells overexpressing cyclophilin A compared with cells containing the control plasmid (Figure 3A). Western blot confirmed that cyclophilin A was overexpressed in cells with the *CPR1* multicopy plasmid (Figure 3A). Thus, overexpression of cyclophilin A does not stabilize the Ess1 mutant enzymes.

Cyclophilin A suppresses an ess1 null mutation

Because cyclophilin A did not stabilize Ess1 ts mutant enzymes, we next considered an alternative hypothesis in which cyclophilin A suppresses *ess1* mutations by acting on a common target required for cell viability. We tested this hypothesis by determining whether overexpression of cyclophilin A suppresses an *ess1* null mutation.

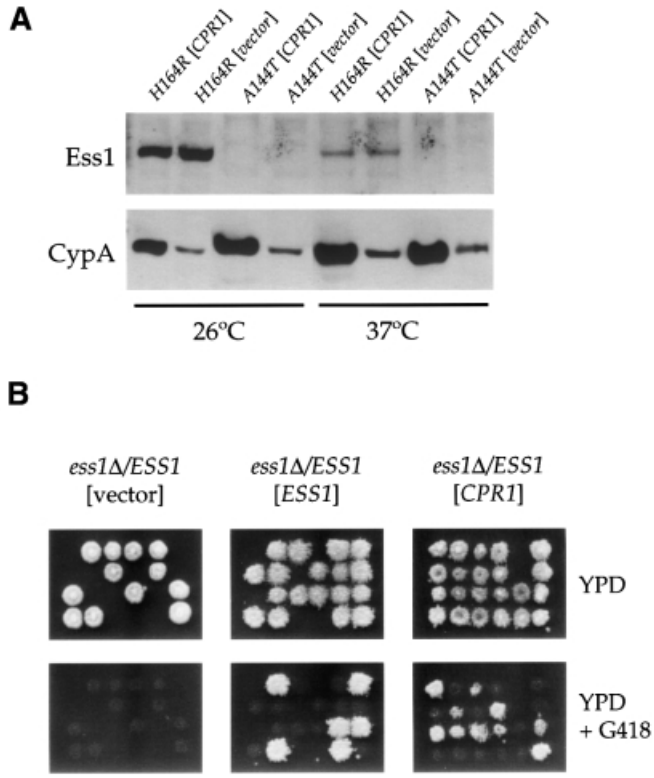

Fig. 3. Cyclophilin A does not stabilize A144T or H164R Ess1 thermolabile mutant proteins, and suppresses an *ess1* null mutation. (**A**) Cells expressing the H164R or A144T Ess1 mutant proteins (strains A144TW303 or H164RW303), and which contained a control plasmid (YEplac195 vector) or a 2μ plasmid (pTB3) overexpressing cyclophilin A (*CPR1*), were grown at 26°C until log phase, diluted and grown at 26 or 37°C for 12 h. Total protein extracts were analyzed by western blotting with antisera against Ess1 or cyclophilin A (CypA). (**B**) An *ess1Δ::G418/ESS1* diploid strain was transformed with a control 2μ plasmid (vector pRS426), or with 2μ plasmids expressing Ess1 (*ESS1*) or the cyclophilin A gene *CPR1*. Strains were sporulated, tetrads dissected and spores germinated on YPD medium at 26°C. Viable segregants were replica-plated to YPD medium containing G418 to score the *ess1Δ::G418* mutation, and also to synthetic medium lacking uracil compared with 5-FOA medium (not shown) to detect the plasmid-borne *URA3* gene.

A diploid strain was constructed in which one copy of the *ESS1* gene was deleted with the dominant G418 resistance marker. Next, 2μ multicopy plasmids lacking or expressing the *ESS1* or cyclophilin A genes were introduced into the *ess1Δ/ESS1* diploid. Overexpression of cyclophilin A restored growth in *ess1Δ* null mutants. The *ess1Δ::G418/ESS1* strain bearing the 2μ *CPR1 URA3* plasmid yielded several tetrads in which two spores produced normal colonies, and two spores produced smaller colonies with a slower growth rate, which contained the *ess1Δ::G418* mutation (Figure 3B). These viable *ess1Δ* G418-resistant colonies were all Ura^+ and 5-fluoro-orotic acid (5-FOA) sensitive (not shown), indicating that the *CPR1 URA3* plasmid expressing cyclophilin A rescues growth of strains lacking Ess1, albeit not to a wild-type level of growth. As expected, a control vector did not rescue inviable *ess1Δ* segregants whereas an *ESS1* plasmid did restore viability (Figure 3B). These results show that cyclophilin A can, in part, bypass the essential function of Ess1, and prompted us to search for a common downstream target of Ess1 and cyclophilin A.

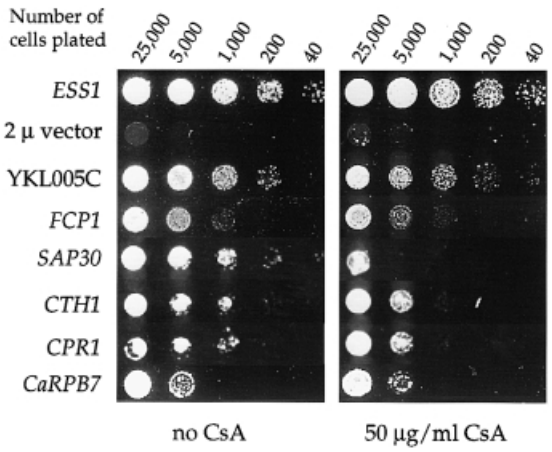

Fig. 4. Cyclosporin A blocks suppression of *ess1^{ts}* mutations by Sap30 and Cth1. An *ess1^{ts}* mutant strain (H164RW303) containing a control plasmid (vector pRS426) or plasmids expressing *ESS1* or the multicopy suppressors YKL005C, *FCP1*, *SAP30*, *CTH1*, *CPR1* or *CaRPB7* were 5-fold serially diluted, spotted on SD–Ura medium lacking (no CsA) or containing 50 μg/ml CsA, and incubated at 37°C for 3 days.

Sap30 requires cyclophilin A to suppress ess1^{ts} mutations

In addition to cyclophilin A (*CPR1*), Wu *et al.* (2000) identified five other multicopy suppressors of *ess1^{ts}* mutations, including YKL005C, Fcp1, Sap30, Cth1 and CaRpb7 (from *Candida albicans*). Three of these proteins, YKL005C, Cth1 and CaRpb7, suppress the lethal phenotype of an *ess1* null mutant (Wu *et al.*, 2000). In contrast, the Fcp1 and Sap30 proteins suppress several *ess1^{ts}* conditional alleles but not a null mutation (Wu *et al.*, 2000). To establish whether one of these proteins is a common target of Ess1 and cyclophilin A, we tested whether cyclophilin A is required for suppression of *ess1* mutations by these genes.

We first tested whether CsA inhibits suppression of the *ess1^{ts}* mutations. A concentration of 50 μg/ml CsA markedly inhibited suppression by Sap30, modestly inhibited suppression by Cth1 and cyclophilin A, and had no effect on suppression by YKL005C, Fcp1 or CaRpb7 (Figure 4). As a second test, we introduced each of the five multicopy suppressor genes into two different *ess1^{ts}/ESS1 cpr1Δ/CPR1* mutant strains (H164R and A144T), and sporulated and dissected tetrads to test whether cyclophilin A is required for suppression of *ess1^{ts}* mutations. This analysis again revealed that the same two suppressors, Sap30 and Cth1, require cyclophilin A to suppress *ess1^{ts}* mutations, whereas YKL005C, Fcp1 and CaRpb7 did not (Table II). Sap30 and Cth1 had only very modest effects on cyclophilin A mRNA and protein levels, arguing that suppression is not the result of increased cyclophilin A expression (Wu *et al.*, 2000). This suggests that Sap30 and Cth1 may be direct common targets of Ess1 and cyclophilin A. Little is known about *CTH1*, so we focused on Sap30, a component of the Sin3A–Rpd3 HDAC (Laherty *et al.*, 1998; Zhang *et al.*, 1998).

HDAC subunits Rpd3 and Sap30 are targets of cyclophilin A and Ess1

We tested the hypothesis that Ess1 and cyclophilin A physically interact with Sap30. For this purpose, His_6-tagged versions of cyclophilin A and Ess1 were purified,

Table II. Sap30 and Cth1 require cyclophilin A to suppress *ess1^{ts}* mutations

Plasmid	Suppression of *ess1 cpr1* double mutant[a]		CsA sensitivity of *ess1^{ts}* strain at 37°C[b]
Vector	no	(13%, *n* = 15)	n.d.
YEpESS1	yes	(43%, *n* = 23)	resistant
pYKL005C	yes	(31%, *n* = 26)	resistant
pFCP1	yes	(55%, *n* = 20)	resistant
pSAP30	no	(0%, *n* = 17)	sensitive
pCTH1	no	(0%, *n* = 7)	sensitive
pCPR1	yes	(50%, *n* = 4)	sensitive
pCaRPB7	yes	(33%, *n* = 12)	resistant

[a]Diploid strain *ess1^{A144T}/ESS1 cpr1Δ::LEU2/CPR1* was transformed with the multicopy suppressors or control plasmids (pRS426 vector, YEpESS1). Cells were sporulated and at least 20 tetrads were dissected. Similar data were obtained with the *ess1^{H164R}* allele (not shown). Segregants were replica-plated to medium lacking leucine at 37°C to detect those carrying the *cpr1Δ::LEU2* allele, to medium lacking uracil to detect those containing the indicated *URA3* plasmids, and to 5-FOA-medium to test the ability to lose the suppressor plasmid. 5-FOA-resistant cells are inferred to contain wild-type *ESS1* rather than *ess1^{A144T}*. The values (% suppression) are given as the percentage of cells that are viable at 37°C and are 5-FOA^S among total segregants that are Leu$^+$ (contain the *cpr1* disruption) and Ura$^+$ (contain the suppressor plasmid); *n* = total number of Leu$^+$ Ura$^+$ segregants. Maximum values would be 50% (the *ess1^{ts}/ESS1* alleles segregate 2:2).
[b]Results are for two *ess1^{ts}* strains (A144T and H164R). Sensitivity was tested on plates containing up to 100 μg/ml CsA. n.d. = not determined, since the *ess1^{ts}* mutant lacking a suppressor is inviable at 37°C.

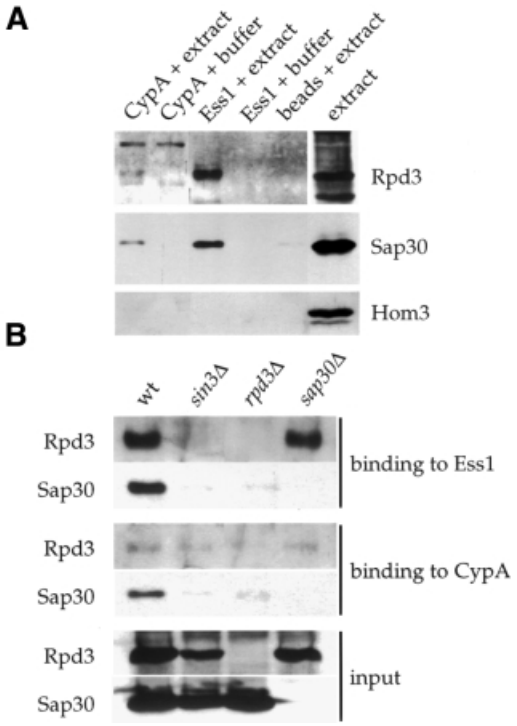

Fig. 5. Cyclophilin A and Ess1 physically interact with protein complexes containing Rpd3 and Sap30. (**A**) Yeast extracts from strain W303-1A transformed with a plasmid expressing a V5 epitope-tagged form of Sap30 (pYMR263W) were incubated with Affigel alone (beads) or coupled to cyclophilin A (CypA) or Ess1. Bound proteins were eluted and analyzed by western blotting with antisera against Rpd3 or the V5 epitope (Sap30). (**B**) Yeast extracts from wild-type (W303-1A), *sin3Δ* (MAY6) or *rpd3Δ* (MAY7) strains transformed with pYMR263W, or from an untransformed *sap30Δ* strain (MAY8), were incubated with Affigel-coupled cyclophilin A or Ess1, and the bound proteins analyzed as in (A). The lower panel shows western blot analysis of the protein extracts used in these experiments; proteins were resolved and analyzed as in (A) and (B).

coupled to Affigel beads and incubated with yeast cell extracts. Bound proteins were eluted and analyzed by western blot. As shown in Figure 5A, both Sap30 and Rpd3 interacted with Ess1 and cyclophilin A, whereas an unrelated protein, aspartokinase (Hom3), did not. In addition, no binding of Rpd3 or Sap30 was detected with control Affigel beads alone (Figure 5A). Silver staining of similar gels revealed a limited number of proteins associated with cyclophilin A or Ess1 (not shown).

To investigate further the interaction of Ess1 and cyclophilin A with the HDAC, similar binding experiments were conducted with protein extracts from yeast strains deleted for the *SIN3*, *RPD3* or *SAP30* genes. Deletion of *SIN3* disrupted the interaction of both Rpd3 and Sap30 with Ess1. Rpd3 and Sap30 were stably expressed in the *sin3Δ* mutant (Figure 5B). These findings suggest that Sin3 is a direct target of Ess1. Notably, Sin3 and Rpd3 contain multiple Ser–Pro or Thr–Pro sites that are the preferred substrate for Ess1, whereas Sap30 does not. Binding of Rpd3 to cyclophilin A was somewhat weaker but, in contrast to Ess1, did not require Sin3. This suggests that Rpd3 is a direct target of cyclophilin A, in accord with a previous report which showed that the Cpr6 and Cpr7 yeast cyclophilins bind Rpd3 *in vivo* and *in vitro* (Duina *et al*., 1996). Binding of either Ess1 or cyclophilin A to Sap30 required Rpd3, consistent with previous studies showing that mammalian Sap30 and the Rpd3 homolog HDAC1 directly interact (Zhang *et al*., 1998), and supporting models in which Rpd3 links Sin3 and Sap30, or Sap30 binds to the Sin3–Rpd3 dimer. Deletion of *SAP30* did not prevent interaction between Rpd3 and the prolyl isomerases, indicating that Sap30 occupies a peripheral position and does not mediate the Sin3–Rpd3 interaction. In summary, Ess1 and cyclophilin A specifically interact with the Sin3–Rpd3 HDAC *in vitro*.

Ess1 and cyclophilin A modulate silencing at the ribosomal DNA loci

Silencing within the rDNA array has been described in yeast as an epigenetic phenomenon involving transcriptional repression of RNA polymerase II-transcribed genes inserted in this region (Bryk *et al*., 1997; Fritze *et al*., 1997; Smith and Boeke, 1997). Recently, a general role for the yeast Sin3–Rpd3 HDAC in silencing has been

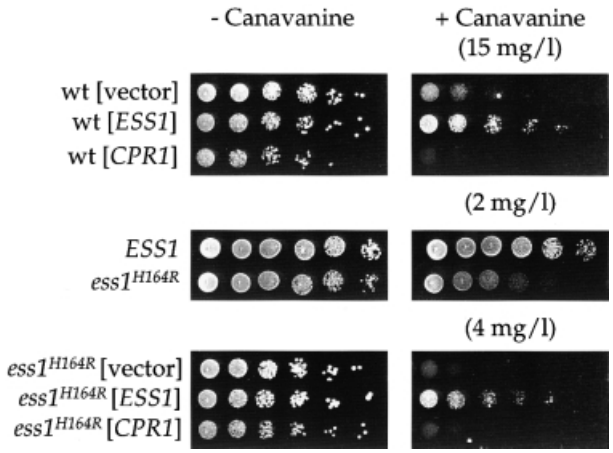

Fig. 6. Ess1 and cyclophilin A modulate silencing at the rDNA array. A wild-type (wt) yeast strain (CFY559) containing the *ADE2-CAN1* double marker integrated in the rDNA array, or isogenic *ESS1* (MAY23) and *ess1^{H164R}* (MAY22) strains, were transformed with 2μ plasmids encoding *ESS1* (YEpESS1) or *CPR1* (pTB3), or with an empty vector (YEplac195) as a control. Cells were 5-fold serially diluted, spotted on SD–Ade medium containing L-canavanine, and incubated for 48 h at 30°C.

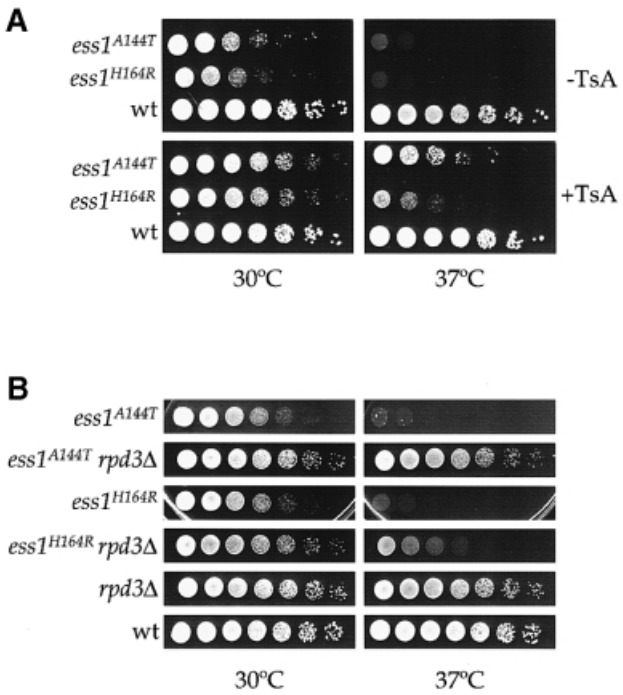

Fig. 7. Rpd3 mediates the mitotic phenotype of *ess1* conditional mutants. (**A**) Isogenic *ess1^{A144T}* (MAY1), *ess1^{H164R}* (MAY3) and *ESS1* wt (JK9-3dα) strains were 5-fold serially diluted and spotted on YPD with or without 13 μM trichostatin A (TsA) and incubated for 48 h at 30 or 37°C. (**B**) Yeast strains *ess1^{A144T}* (MAY12-7d), *ess1^{A144T} rpd3Δ* (MAY12-7b), *ess1^{H164R}* (MAY16-5c), *ess1^{H164R} rpd3Δ* (MAY16-2c), *rpd3Δ* (MAY12–5b) and wt (JK9-3dα) were 5-fold serially diluted, spotted on YPD and incubated for 48 h at 30 or 37°C.

reported, including silencing at the rDNA array; mutations in *SIN3*, *RPD3* or *SAP30* genes enhance silencing, implying that the Sin3–Rpd3 complex counteracts silencing (Smith *et al*., 1999; Sun and Hampsey, 1999).

We investigated the role of Ess1 and cyclophilin A in the activity of the Sin3–Rpd3 complex by testing their effects on expression of a genetic marker inserted in the rDNA array. Yeast strain CFY559 bearing an *ADE2–CAN1* double marker integrated in the rDNA array (Fritze *et al*., 1997) was transformed with 2μ plasmids expressing *ESS1* or *CPR1* and tested for growth in the presence of the toxic amino acid analog L-canavanine. Sensitivity to canavanine depends on the expression level of the canavanine permease encoded by the *CAN1* gene inserted in the rDNA array, and is inversely proportional to the degree of silencing. As shown in Figure 6, overexpression of Ess1 increased canavanine resistance; thus, increased Ess1 enhances silencing at the rDNA array. Substitution of the *ESS1* wild-type gene with an *ess1^{ts}* allele dramatically enhanced sensitivity to canavanine, indicating that silencing at the rDNA array is impaired when Ess1 function is compromised. The wild-type *ESS1* gene complemented the *ess1^{ts}* mutation and restored canavanine resistance (Figure 6). These results reveal a functional role for Ess1 in the modulation of transcriptional silencing and indicate that Ess1 is a negative regulator of the Sin3–Rpd3 HDAC.

Paradoxically, cyclophilin A had the opposite effect. When expressed from a multicopy plasmid, cyclophilin A increased sensitivity to canavanine (Figure 6), indicating that overexpression of cyclophilin A reduces silencing in the rDNA array, possibly by positively regulating the Sin3–Rpd3 HDAC. In agreement with this hypothesis, overexpression of cyclophilin A in the *ess1^{ts}* derivative of CFY559 did not restore resistance to canavanine (Figure 6). Taken together, these results support the hypothesis that the prolyl isomerases Ess1 and cyclophilin A functionally interact with the Sin3–Rpd3 HDAC and modulate the silencing activity of this complex.

Rpd3 histone deacetylase activity mediates mitotic arrest in ess1^{ts} mutants

Our results suggest that expression of genes normally controlled by the Sin3–Rpd3 histone deacetylase may be altered in *ess1* mutants. Anomalous expression of one or more of these genes may cause the mitotic arrest exhibited by conditional *ess1* mutants at non-permissive temperature. We tested the proposal that the Sin3–Rpd3 complex is a target of negative regulation by Ess1 by testing whether reducing Rpd3 deacetylase activity suppresses *ess1^{ts}* mutations.

Trichostatin A (TsA), an inhibitor of the Rpd3 HDAC, partially suppressed the ts phenotype of *ess1* mutants, supporting the hypothesis that the mitotic arrest of the *ess1* mutants is, at least in part, caused by an aberrant increase in the Sin3–Rpd3 HDAC activity. To test this model further, we evaluated the role of Rpd3 in the *ess1* phenotype by a genetic approach. The G418 resistance marker was used to delete one copy of *RPD3* in two *ess1^{ts}/ESS1* strains, which were then sporulated to analyze the phenotype of *ess1^{ts} rpd3Δ* double mutants. Both diploid strains produced a large proportion of tetrads exhibiting 3:1 or 4:0 segregation of temperature-resistant:temperature-sensitive spores. Interestingly, none of the temperature-sensitive segregants was *rpd3Δ::G418*, indicating that deletion of *RPD3* suppresses the *ess1* mutations (not shown). Two temperature-resistant *rpd3Δ* segregants (Figure 7B) were shown by backcrosses to the wild-type parental strain to be *ess1^{ts} rpd3Δ* double

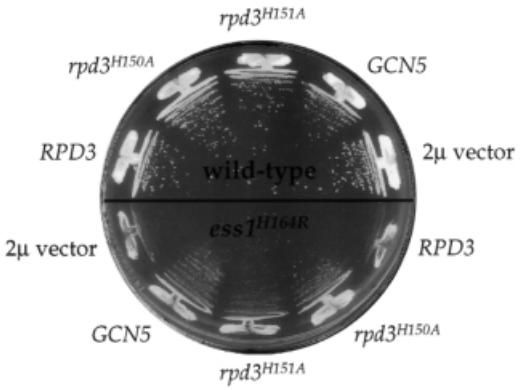

Fig. 8. Overexpression of Gcn5 or dominant-negative alleles of *RPD3* suppresses an *ess1* conditional mutation. Temperature-sensitive *ess1* strain H164RW303 ($ess1^{H164R}$) or wild-type strain W303-1A was transformed with 2μ plasmids expressing *RPD3* (YEplac112-RPD3), $rpd3^{H150A}$ (YEplac112-RPD3-H150A), $rpd3^{H151A}$ (YEplac112-RPD3-H151A) or *GCN5* (Yep10PGK-exp-ScGCN5), or with an empty plasmid, and tested for growth at 35°C.

mutants, confirming that the *rpd3Δ* mutation suppresses *ess1* mutations.

We further analyzed the function of Rpd3 in *ess1* mutants by a complementary genetic approach. Yeast Rpd3 mutants have been characterized that are devoid of catalytic activity and that have dominant-negative activity in *RPD3* wild-type strains (Kadosh and Struhl, 1998). Overexpression of two such dominant-negative Rpd3 mutants, $rpd3^{H150A}$ and $rpd3^{H151A}$ (Kadosh and Struhl, 1998), partially suppressed the ts phenotype, indicating that reducing Rpd3 activity rescues *ess1* conditional mutants (Figure 8).

Finally, we asked whether overexpression of the yeast histone acetyltransferase Gcn5 could suppress *ess1* mutations. Gcn5 and Rpd3 are known to play opposing roles in transcriptional regulation of the *HO* gene (Perez-Martin and Johnson, 1998). Overexpression of Gcn5 partially restored growth of an $ess1^{ts}$ mutant strain at 37°C (Figure 8).

Taken together, these results support the hypothesis that excess histone deacetylase activity of Rpd3 causes misregulation of mitotic genes, and is responsible, at least in part, for the cell cycle arrest of *ess1* mutants.

Discussion

Cyclophilin A mediates inhibition of calcineurin by the immunosuppressive drug CsA, but the cellular functions of cyclophilin A have remained elusive. In the studies reported here, we have found that cyclophilin A is a multicopy suppressor of conditional mutations in the one essential prolyl isomerase in yeast, the parvulin Ess1. The cyclophilin A inhibitor CsA blocks suppression, and CsA also inhibits growth of $ess1^{ts}$ mutant strains under permissive growth conditions. Cyclophilin A active site mutants fail to suppress. Moreover, mutations in the cyclophilin A gene *CPR1*, or in the FKBP12 gene *FPR1*, are synthetically lethal with conditional *ess1* mutations. Cyclophilin A does not suppress by promoting folding or stability of the thermolabile Ess1 mutant enzymes, and overexpression of cyclophilin A also restores viability in strains in which the *ESS1* gene has been deleted, indicating that cyclophilin A can bypass the normal cellular requirement for Ess1. This finding suggests that cyclophilin A and Ess1 can act on common targets required for mitosis, and that prolyl isomerase activity is linked to their essential function.

RNA polymerase II as a target of Ess1

In several recent reports (Albert *et al*., 1999; Morris *et al*., 1999) and the accompanying manuscript by Wu *et al*. (2000) the C-terminal domain (CTD) of RNA polymerase II has been identified as a direct target of Ess1. Wu *et al*. (2000) failed to find any evidence that cyclophilin A associates with the CTD. High affinity binding of Ess1 to the CTD involves the Ess1 WW domain and not the prolyl isomerase domain (Morris *et al*., 1999), providing an explanation for why cyclophilin A, which lacks the WW domain, might not interact.

Ess1 and cyclophilin A have common targets

In the accompanying manuscript, Wu *et al*. (2000) identify multicopy suppressors of *ess1* mutations. We show that two suppressors, Cth1 and Sap30, require cyclophilin A to restore viability in *ess1* mutants and may represent common targets of cyclophilin A and Ess1.

Sap30 is found in a complex with the Sin3 and Rpd3 proteins, which are components of the yeast HDAC (Laherty *et al*., 1998; Zhang *et al*., 1998). While Sap30 lacks serine-prolyl or threonine-prolyl sequences that are the known substrates of Ess1, both Sin3 and Rpd3 have multiple Ser–Pro or Thr–Pro sites. Thus, one plausible model is that Ess1 interacts with Sin3 or Rpd3 to catalyze isomerization events required for protein folding and assembly of the HDAC. In accord with this model, our biochemical studies revealed that interaction of Ess1 with the Sin3–Rpd3 HDAC is mediated by Sin3. Interaction between cyclophilin A and Sap30 was disrupted by deletion of *SIN3*, although weak binding to Rpd3 was still detected, indicating that interaction between cyclophilin A and Rpd3 can be Sin3 independent. Most interestingly, the yeast cyclophilin 40 homologs Cpr6 and Cpr7 were identified originally in a two-hybrid screen with the Rpd3 subunit of histone deacetylase and shown to interact directly with Rpd3 *in vitro* and *in vivo* in the yeast two-hybrid assay (Duina *et al*., 1996). Our findings suggest that cyclophilin A, as well as Cpr6 and Cpr7, functionally and physically interacts with Rpd3.

Histone deacetylases play a central role in both repression and activation of gene expression. The *RPD3* and *SIN3* genes positively and negatively regulate transcription of many genes (Vidal and Gaber, 1991; Vidal *et al*., 1991). The DNA-binding protein Ume6 represses transcription by recruiting the Sin3–Rpd3 complex to target promoters (Kadosh and Struhl, 1997). Recruitment of the Sin3–Rpd3 complex to reporter promoters via LexA hybrids represses transcription (Wang and Stillman, 1993; Kadosh and Struhl, 1997), suggesting a general role for Sin3 and Rpd3 as co-repressors. The Sin3–Rpd3 HDAC fulfills a different role in gene silencing. In yeast, gene silencing prevents expression of the cryptic mating loci (*HM*) (reviewed in Kingston *et al*., 1996) and represses transcription of reporter genes inserted near telomeres (Gottschling *et al*., 1990) or in the rDNA tandem array

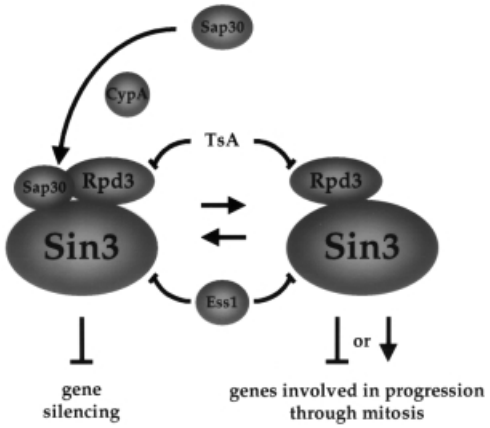

Fig. 9. Model for the mechanism of suppression of *ess1* mutations by CypA and Sap30. In this model, Ess1 is a negative modulator of the Sin3–Rpd3 complex, which in turn functions as a global transcriptional regulator. In *ess1* mutants, hyperactive Sin3–Rpd3 complexes deregulate expression of genes involved in cell cycle control, causing mitotic arrest. Cyclophilin A (CypA) recruits Sap30 to the complex, reduces activity with mitotic targets and suppresses cell cycle arrest of *ess1* mutants.

(Bryk *et al*., 1997; Fritze *et al*., 1997; Smith and Boeke, 1997). The Sin3–Rpd3 complex is, paradoxically, required for disruption of silencing at these loci (De Rubertis *et al*., 1996; Rundlett *et al*., 1996; Kadosh and Struhl, 1998; Magnaghi-Jaulin *et al*., 1998; Sun and Hampsey, 1999; Smith *et al*., 1999).

We studied the influence of Ess1 and cyclophilin A on silencing at the rDNA array. Both prolyl isomerases modulate the expression of an RNA polymerase II-transcribed reporter gene inserted in this region, although with opposite effects; Ess1 enhances silencing, whereas cyclophilin A inhibits silencing. We propose that Ess1 and cyclophilin A influence silencing by physically interacting with the Sin3–Rpd3 complex (Figure 9).

Rpd3 mediates mitotic arrest of ess1 conditional mutants

We have shown that deletion of *RPD3* partially restores viability of *ess1^{ts}* mutants (Figure 7B). Suppression by *RPD3* deletion was not inhibited by CsA and did not increase cyclophilin A levels, indicating that cyclophilin A is not required (not shown). Overexpression of dominant-negative Rpd3 active site mutants, the histone acetyltransferase Gcn5 (Figure 8) or treatment with the HDAC inhibitor TsA (Figure 7A), also suppressed *ess1^{ts}* mutations. In addition, *ess1^{ts}* mutant strains show a phenotype consistent with an altered function of the Sin3–Rpd3 complex (decreased silencing at the rDNA locus). Taken together, these findings support a model in which Ess1 inhibits Rpd3 function, and *ess1* mutants have increased Rpd3 histone deacetylase activity that results in mitotic arrest.

What is the connection between Rpd3 and the cell cycle arrest exhibited by *ess1* mutants? In mammalian cells, the histone deacetylase inhibitors TsA and trapoxin induce G_1 and G_2 phase cell cycle arrest, indicating an involvement of histone deacetylase activity in cell cycle progression (Yoshida *et al*., 1995). Deletion of *RPD3* increases the life span in yeast, suggesting a correlation between aging and rDNA silencing (Kim *et al*., 1999). Loss of silencing at the rDNA array observed in the *ess1^{ts}* mutant is not likely to be the cause of the mitotic arrest, because deletion of the *SIN3* or *SAP30* genes, both of which are required for disruption of silencing, did not rescue *ess1^{ts}* mutants (data not shown). Moreover, overexpression of Sap30 suppresses *ess1^{ts}* mutations.

What is the mechanism by which Sap30 suppresses *ess1^{ts}* mutations? One possibility is that overexpression of Sap30 diverts Rpd3 from its cellular targets involved in cell cycle arrest. It has been found that yeast Sap30 can repress transcription in a *sin3Δ* mutant, suggesting that Sap30 can interact with and recruit Rpd3 directly to a promoter in a Sin3-independent manner (Zhang *et al*., 1998). Taken together, these findings support a model in which overexpression of Sap30 recruits Rpd3 to function in silencing, relieving Rpd3 repression of downstream target genes that are required for mitotic progression (see Figure 9).

Regulation of Sin3–Rpd3 HDAC by Ess1 and cyclophilin A

Sap30 requires cyclophilin A for suppression (Figure 4; Table II). Overexpression of cyclophilin A decreases silencing at the rDNA loci, indicating that cyclophilin A counteracts this function of Ess1. However, cyclophilin A also suppresses *ess1* mutations. Our proposal is that both prolyl isomerases catalyze protein conformational changes required for the assembly or activity of the Sin3–Rpd3 complex. Our biochemical studies are consistent with a model in which Ess1 directly interacts with the Sin3 component of HDAC, whereas cyclophilin A interacts directly with Rpd3. In this model, Ess1 functions to down-regulate the histone deacetylase activity of Rpd3; the decreased silencing observed in *ess1* mutants then results from deregulated, hyperactive Sin3–Rpd3 complexes. In the model depicted in Figure 9, cyclophilin A catalyzes conformational changes in Rpd3 and/or Sap30 that recruit Sap30 to the Rpd3–Sin3 complex and regulate silencing. Overexpression of cyclophilin A (or of Sap30, in a cyclophilin A-dependent fashion) drives the equilibrium towards the formation of a Sin3–Rpd3–Sap30 complex that is still competent to disrupt silencing (Figure 6), but that has a reduced capacity to interact with downstream targets mediating cell cycle arrest (Figure 9). This model is consistent with that proposed in the accompanying manuscript (Wu *et al*., 2000) in which mitotic arrest in *ess1* mutants is the result of misregulation of genes caused by aberrant RNA polymerase II transcription. Further studies will be required to examine in detail the physical interactions of Ess1 and cyclophilin A with the Sin3–Rpd3 HDAC, and to examine how Ess1 and cyclophilin regulate histone deacetylase activity.

Materials and methods

Yeast strains

Yeast strains used in this work are listed in Table III. Strain MAYX47-4b is a product of a cross of strains JK9-3da and KDY27-7b. Strain MAYX48-6d is from a cross of strains JK9-3da and KDY28. Strain MAY1 was obtained from strain JK9-3dα by introduction of the *ess1-A144T* mutation using plasmid YIpess1^{A144T} (Wu *et al*., 2000) via two-step gene replacement. For this purpose, strain JK9-3dα was first transformed with this plasmid linearized with *Bst*XI, excision of the

Table III. Yeast strains

Strain	Genotype	Reference
JK9-3da	*MAT***a** *his4 HMLa leu2-3, 112 rme1 trp1 ura3-52*	Heitman *et al*. (1991a)
JK9-3dα	*MAT*α (JK9-3da)	Heitman *et al*. (1991a)
MH250-2c	*cpr1Δ::LEU2* (JK9-3da)	Cardenas *et al*. (1994)
MAYX47-4b	*cpr2Δ::TRP1* (JK9-3da)	this study
MAYX48-6d	*cpr3::HIS3* (JK9-3da)	this study
KDY9	*cpr4Δ::URA3* (JK9-3da)	Dolinski *et al*. (1997)
KDY19	*cpr5Δ::LEU2* (JK9-3da)	Dolinski *et al*. (1997)
KDY46	*cpr6::G418* (JK9-3da)	Dolinski *et al*. (1997)
KDY65	*cpr7Δ::G418* (JK9-3da)	Dolinski *et al*. (1997)
SMY98-1	*cpr8Δ::G418* (JK9-3da)	Dolinski *et al*. (1997)
JHY2-1c	*fpr1::ADE2* (JK9-3da)	Cardenas *et al*. (1994)
KDY61	*fpr2Δ::URA3* (JK9-3da)	Dolinski *et al*. (1997)
KDY86-6a	*fpr3Δ::URA3* (JK9-3da)	Dolinski *et al*. (1997)
KDY54	*fpr4Δ::G418* (JK9-3da)	Dolinski *et al*. (1997)
KDY27-7b	*MAT*α *cpr2Δ::TRP1 cpr4Δ::URA3* (JK9-3da)	this study
KDY28	*MAT*α *cpr3::HIS3 cpr4Δ::URA3 cpr5Δ::LEU2* (JK9-3da)	this study
W303-1A	*MAT***a** *ura3-1 leu2-3, 112 trp1-1 can1-100 ade2-1 his3-11,15 [PSI+]*	R.Rothstein
A144TW303	*ess1-A144T* (W303-1A)	S.Hanes
H164RW303	*ess1-H164R* (W303-1A)	S.Hanes
MAY1	*ess1-A144T* (JK9-3dα)	this study
MAY3	*ess1-H164R* (JK9-3dα)	this study
MAY5	*CPR1/cpr1Δ::LEU2 ESS1/ess1Δ::G418* (JK9-3da/α)	this study
MAY6	*sin3Δ::G418* (W303-1A)	this study
MAY7	*rpd3Δ::G418* (W303-1A)	this study
MAY8	*sap30Δ::G418* (W303-1A)	this study
MAY12	*ESS1/ess1-A144T RPD3/rpd3Δ::G418* (JK9-3da/α)	this study
MAY16	*ESS1/ess1-H164R RPD3/rpd3Δ::G418* (JK9-3da/α)	this study
MAY12-5b	*MAT***a** *rpd3Δ::G418* (JK9-3da)	this study
MAY12-7b	*MAT***a** *ess1-A144T rpd3Δ::G418* (JK9-3da)	this study
MAY12-7d	*MAT*α *ess1-A144T* (JK9-3da)	this study
MAY16-2c	*MAT***a** *ess1-H164R rpd3Δ::G418* (JK9-3da)	this study
MAY16-5c	*MAT***a** *ess1-H164R* (JK9-3da)	this study
CFY559	*MAT***a** *ade2Δ::hisG can1Δ::hisG his4 leu2 lys2 pfx1 tyr1-2 ura3-52rDNA::pCAR1 (CAN1-ADE2)*	Fritze *et al*. (1997)
MAY22	*ess1-H164R* (CFY559)	this study
MAY23	*ESS1* (CFY559)	this study
MAY22	*ess1-H164R* (CFY559)	this study

plasmid was selected on 5-FOA at 24°C and ts colonies were selected. Strain MAY3 was obtained in a similar way using plasmid YIpess1^{H164R}. MAY22 was obtained from CFY559 in a similar way; MAY23 is a wild-type *ESS1*, 5-FOA-resistant strain obtained from the same YIpess1^{H164R} transformant as MAY22. Strain MAY5 was derived from strain JK9-3da/α by replacing the entire open reading frame (ORF) of one *ESS1* allele with *kanMX* (Wach *et al*., 1994). MAY6 was obtained from W303-1A by replacing the entire *SIN3* ORF with *kanMX*. MAY7 was obtained from W303-1A by replacing the entire *RPD3* ORF with *kanMX*. Strains MAY12 and MAY16 were obtained in a similar fashion from diploids *ess1^{A144T}/ESS1* and *ess1^{H164R}/ESS1*, respectively, by replacing one allele of RPD3 with *kanMX*. MAY8 was obtained from W303-1A by replacing the entire *SAP30* ORF with *kanMX*. Gene disruptions were all verified by PCR. MAY12-5b, MAY12-7b and MAY12-7d are meiotic products of MAY12. MAY16-2c and MAY16-5c are meiotic products of MAY16.

Plasmids

YIpess1^{ts} plasmids used for integration of the *ess1^{ts}* alleles A144T and H164R are described in Wu *et al*. (2000). Plasmid pTB3 (2μ *CPR1 URA3*) has been described (Cardenas *et al*., 1995b). Plasmid pCPR1(H90Y) (2μ *CPR1^{H90Y} URA3*) is a derivative of pRS426 that carries an H90Y allele of *CPR1* that was PCR-amplified from mutant strain TB24-M141 (Cardenas *et al*., 1995a). Human wild-type and mutant cyclophilin A genes under the control of the 5′- and 3′-untranslated regions of the yeast cyclophilin A *CPR1* gene were kindly provided by Jeremy Luban and were subcloned into the YEplac195 vector (2μ *URA3*), giving rise to plasmids phCypA (wild-type cyclophilin A), phCypA(H54Q), phCypA(R55A), phCypA(F60A), phCypA(F113A), phCypA(W121A) and phCypA(H126Q), used for the overexpression suppression studies described here. Plasmids for *CPR6/7* were pKDw10 (2μ *CPR6 URA3*) and pKS24 (2μ *CPR7 URA3*) (Dolinski *et al*., 1998). Plasmid pYJH23 (Heitman *et al*., 1991b) has a 2 kb *Eco*RI fragment containing the *FPR1* gene cloned in the *Eco*RI site of pSEY8 (2μ *URA3*). Plasmid YEpESS1 (2μ *ESS1 URA3*) has a *Bam*HI fragment containing the *ESS1* gene cloned in the *Bam*HI site of YEp24. Plasmids containing *ess1^{ts}* suppressor genes *CTH1*, *SAP30*, *YKL005C*, *FCP1* and *CaRPB7* are described in Wu *et al*. (2000). The 2μ plasmid pYMR263W, expressing a V5 epitope-tagged version of yeast Sap30 under control of the *GAL1* promoter, was from Invitrogen. Plasmid YEplac112-RPD3 was described in Kadosh and Struhl (1997). Plasmids YEplac112-RPD3-H150A and YEplac112-RPD3-H151A were described in Kadosh and Struhl (1998). Plasmid Yep10PGK-exp-ScGCN5 (2μ, *GCN5*, *TRP1*) was kindly provided by Kevin Struhl. Plasmid pET-ESS1.1 derived from pET28a (Novagen), encodes a His$_6$-tagged version of Ess1.

Binding to Sap30 and Rpd3

His$_6$-FKBP12 and His$_6$-cyclophilin A were purified as described (Cardenas *et al*., 1994, 1995a). Purification of His$_6$-Ess1p was carried out in the same way, using plasmid pET-ESS1.1. The mutant *Escherichia coli* strain RY3041 (from Ry Young) lacking the non-essential histidine-rich slyD peptidyl-prolyl isomerase was used to produce His$_6$-tagged proteins (Roof *et al*., 1997). Yeast strains transformed with plasmid pYMR263W (V5-Sap30) were grown in SD–Ura medium to an OD$_{600}$ of 0.7, transferred to SGal–ura medium, and incubated for 12 h to induce Sap30 expression. Whole-cell protein extracts were prepared by glass bead disruption in a lysis buffer containing 20 mM HEPES pH 7.9, 150 mM NaCl, 10 mM $MgCl_2$, 10% glycerol, 0.2% Tween-20 and a cocktail of protease inhibitors consisting of 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 1 μg/ml pepstatin, 1 mM benzamidine, 100 μM leupeptin, 1% trasylol and 1 μg/ml TPCK. Affinity chromatography assays were performed with His$_6$-cyclophilin A or His$_6$-Ess1 coupled to

Affigel 10 (Bio-Rad) as described (Cardenas *et al*., 1994) using the lysis buffer described above to wash PPIase-coupled beads after incubation with cell extracts. Bound proteins were resolved by SDS–PAGE in a 12% acrylamide gel, transferred to an Immobilon PVDF membrane (Immun-Blot; Bio-Rad) and analyzed by western blotting. Sap30 and Rpd3 were detected with anti-V5 monoclonal antibody (Invitrogen) or a rabbit polyclonal antiserum specific for Rpd3 (Santa Cruz). Rabbit polyclonal antibodies against cyclophilin A and Ess1 were as described (Cardenas *et al*., 1995a; Wu *et al*., 2000).

Acknowledgements

We thank Ry Young, Jeremy Luban, Rochelle Esposito, Kevin Struhl, Carl Falco, Kara Dolinski and Rick Gaber for reagents. These studies were supported in part by R01 grant AI39115 (NIAID) to J.H. and M.E.C., K01 award CA77075 (NCI) to M.E.C. and ROI grant GM55108 (NIH) to S.D.H. J.H. is an associate investigator of the Howard Hughes Medical Institute.

References

Albert,A., Lavoie,S. and Vincent,M. (1999) A hyperphosphorylated form of RNA polymerase II is the major interphase antigen of the phosphoprotein antibody MPM-2 and interacts with the peptidyl-prolyl isomerase Pin1. *J. Cell Sci.*, **112**, 2493–2500.

Anfinsen,C.B. (1973) Principles that govern the folding of protein chains. *Science*, **181**, 223–230.

Baker,E.K., Colley,N.J. and Zuker,C.S. (1994) The cyclophilin homolog NinaA functions as a chaperone, forming a stable complex *in vivo* with its protein target rhodopsin. *EMBO J.*, **13**, 4886–4895.

Brillantes,A.-M.B. *et al*. (1994) Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. *Cell*, **77**, 513–523.

Bryk,M., Banerjee,M., Murphy,M., Knudsen,K.E., Garfinkel,D.J. and Curcio,M.J. (1997) Transcriptional silencing of Ty1 elements in the *RDN1* locus of yeast. *Genes Dev.*, **11**, 255–269.

Cardenas,M.E., Hemenway,C., Muir,R.S., Ye,R., Fiorentino,D. and Heitman,J. (1994) Immunophilins interact with calcineurin in the absence of exogenous immunosuppressive ligands. *EMBO J.*, **13**, 5944–5957.

Cardenas,M.E., Lim,E. and Heitman,J. (1995a) Mutations that perturb cyclophilin A ligand binding pocket confer cyclosporin A resistance in *Saccharomyces cerevisiae*. *J. Biol. Chem.*, **270**, 20997–21002.

Cardenas,M.E., Muir,R.S., Breuder,T. and Heitman,J. (1995b) Targets of immunophilin–immunosuppressant complexes are distinct highly conserved regions of calcineurin A. *EMBO J.*, **14**, 2772–2783.

De Rubertis,F., Kadosh,D., Henchoz,S., Pauli,D., Reuter,G., Struhl,K. and Spierer,P. (1996) The histone deacetylase RPD3 counteracts genomic silencing in *Drosophila* and yeast. *Nature*, **384**, 589–591.

Dolinski,K. and Heitman,J. (1997) Peptidyl-prolyl isomerases (PPIases). In Tooze,S. (ed.), *Guidebook to Molecular Chaperones and Protein Folding Catalysts*. Oxford University Press, Oxford, UK, pp. 359–369.

Dolinski,K., Muir,R.S., Cardenas,M.E. and Heitman,J. (1997) All cyclophilins and FK506 binding proteins are, individually and collectively, dispensable for viability in *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA*, **94**, 13093–13098.

Dolinski,K.J., Cardenas,M.E. and Heitman,J. (1998) *CNS1* encodes an essential p60/Stil homolog in *Saccharomyces cerevisiae* that suppresses cyclophilin 40 mutations and interacts with Hsp90. *Mol. Cell. Biol.*, **18**, 7344–7352.

Duina,A.A., Marsh,J.A. and Gaber,R.F. (1996) Identification of two CyP-40-like cyclophilins in *Saccharomyces cerevisiae*, one of which is required for normal growth. *Yeast*, **12**, 943–952.

Ferreira,P.A., Nakayama,T.A., Pak,W.L. and Travis,G.H. (1996) Cyclophilin-related protein RanBP2 acts as chaperone for red/green opsin. *Nature*, **383**, 637–640.

Fischer,G. (1994) Peptidyl-prolyl *cis/trans* isomerases and their effectors. *Angew. Chem. Int. Ed. Engl.*, **33**, 1415–1436.

Fischer,G. and Schmid,F.X. (1990) The mechanism of protein folding. Implications of *in vitro* refolding models for *de novo* protein folding and translocation in the cell. *Biochemistry*, **29**, 2205–2212.

Foor,F., Parent,S.A., Morin,N., Dahl,A.M., Ramadan,N., Chrebet,G., Bostian,K.A. and Nielsen,J.B. (1992) Calcineurin mediates inhibition by FK506 and cyclosporin of recovery from α-factor arrest in yeast. *Nature*, **360**, 682–684.

Fritze,C.E., Verschueren,K., Strich,R. and Esposito,R.E. (1997) Direct evidence for *SIR2* modulation of chromatin structure in yeast rDNA. *EMBO J.*, **16**, 6495–6509.

Gething,M.-J. and Sambrook,J. (1992) Protein folding in the cell. *Nature*, **355**, 33–45.

Gottschling,D.E., Aparicio,O.M., Billington,B.L. and Zakian,V.A. (1990) Position effect at *S.cerevisiae* telomeres: reversible repression of Pol II transcription. *Cell*, **63**, 751–762.

Handschumacher,R.E., Harding,M.W., Rice,J. and Drugge,R.J. (1984) Cyclophilin: a specific cytosolic binding protein for cyclosporin A. *Science*, **226**, 544–547.

Hanes,S.D., Shank,P.R. and Bostian,K.A. (1989) Sequence and mutational analysis of *ESS1*, a gene essential for growth in *Saccharomyces cerevisiae*. *Yeast*, **5**, 55–72.

Hani,J., Stumpf,G. and Domdey,H. (1995) *PTF1* encodes an essential protein in *Saccharomyces cerevisiae*, which shows strong homology with a new putative family of PPIases. *FEBS Lett.*, **365**, 198–202.

Hani,J., Schelbert,B., Bernhardt,A., Domdey,H., Fischer,G., Wiebauer,K. and Rahfeld,J. (1999) Mutations in a peptidylprolyl-*cis/trans*-isomerase gene lead to a defect in 3′-end formation of a pre-mRNA in *Saccharomyces cerevisiae*. *J. Biol. Chem.*, **274**, 108–116.

Harding,M.W., Galat,A., Uehling,D.E. and Schreiber,S.L. (1989) A receptor for the immunosuppressant FK506 is a *cis–trans* peptidyl-prolyl isomerase. *Nature*, **341**, 758–760.

Heitman,J., Movva,N.R. and Hall,M.N. (1991a) Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science*, **253**, 905–909.

Heitman,J., Movva,N.R., Hiestand,P.C. and Hall,M.N. (1991b) FK506-binding protein proline rotamase is a target for the immunosuppressive agent FK506 in *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA*, **88**, 1948–1952.

Kadosh,D. and Struhl,K. (1997) Repression by Ume6 involves recruitment of a complex containing Sin3 corepressor and Rpd3 histone deacetylase to target promoters. *Cell*, **89**, 365–371.

Kadosh,D. and Struhl,K. (1998) Histone deacetylase activity of Rpd3 is important for transcriptional repression *in vivo*. *Genes Dev.*, **12**, 797–805.

Kim,S., Benguria,A., Lai,C.Y. and Jazwinski,S.M. (1999) Modulation of life-span by histone deacetylase genes in *Saccharomyces cerevisiae*. *Mol. Biol. Cell*, **10**, 3125–3136.

Kingston,R.E., Bunker,C.A. and Imbalzano,A.N. (1996) Repression and activation by multiprotein complexes that alter chromatin structure. *Genes Dev.*, **10**, 905–920.

Laherty,C.D. *et al*. (1998) SAP30, a component of the mSin3 corepressor complex involved in N-CoR-mediated repression by specific transcription factors. *Mol. Cell*, **2**, 33–42.

Liu,J., Farmer,J.D., Lane,W.S., Friedman,J., Weissman,I. and Schreiber,S.L. (1991) Calcineurin is a common target of cyclophilin–cyclosporin A and FKBP–FK506 complexes. *Cell*, **66**, 807–815.

Lu,K.P., Hanes,S.D. and Hunter,T. (1996) A human peptidyl-prolyl isomerase essential for regulation of mitosis. *Nature*, **380**, 544–547.

Lu,P.-J., Zhou,X.Z., Shen,M. and Lu,K.P. (1999) Function of WW domains as phosphoserine- or phosphothreonine-binding modules. *Science*, **283**, 1325–1328.

Magnaghi-Jaulin,L., Groisman,R., Naguibneva,I., Robin,P., Lorain,S., Villian,J.P.L., Troalen,F., Trouche,D. and Harel-Bellan,A. (1998) Retinoblastoma protein represses transcription by recruiting a histone deacetylase. *Nature*, **391**, 601–605.

Maleszka,R., Hanes,S.D., Hackett,R.L., Couet,H.G.D. and Miklos,G.L.G. (1996) The *Drosophila melanogaster dodo* (*dod*) gene, conserved in humans, is functionally interchangeable with the *ESS1* cell division gene of *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA*, **93**, 447–451.

Morris,D.P., Phatnani,H.P. and Greenleaf,A.L. (1999) Phospho-carboxyl-terminal domain binding and the role of prolyl isomerase in pre-mRNA 3′-end formation. *J. Biol. Chem.*, **274**, 31583–31587.

Pazin,M.J. and Kadonaga,J.T. (1997) What's up and down with histone deacetylation and transcription? *Cell*, **89**, 325–328.

Perez-Martin,J. and Johnson,A.D. (1998) Mutations in chromatin components suppress a defect of Gcn5 protein in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **18**, 1049–1054.

Rahfeld,J.-U., Rucknagel,K.P., Schelbert,B., Ludwig,B., Hacker,J., Mann,K. and Fischer,G. (1994) Confirmation of the existence of a third family among peptidyl-prolyl *cis/trans* isomerase. Amino acid sequence and recombinant production of parvulin. *FEBS Lett.*, **352**, 180–184.

Ranganathan,R., Lu,K.P., Hunter,T. and Noel,J.P. (1997) Structural and functional analysis of the mitotic rotamase Pin1 suggests substrate recognition is phosphorylation dependent. *Cell*, **89**, 875–886.

Roof,W.D., Fang,H.Q., Young,K.D., Sun,J. and Young,R. (1997) Mutational analysis of *slyD*, an *Escherichia coli* gene encoding a protein of the FKBP immunophilin family. *Mol. Microbiol.*, **25**, 1031–1046.

Rundlett,S.E., Carmen,A.A., Kobayashi,R., Bavykin,S., Turner,B.M. and Grunstein,M. (1996) HDA1 and RPD3 are members of distinct yeast histone deacetylase complexes that regulate silencing and transcription. *Proc. Natl Acad. Sci. USA*, **93**, 14503–14508.

Scholz,C., Maier,P., Dolinski,K., Heitman,J. and Schmid,F.X. (1999) R73A and H144Q mutants of the yeast mitochondrial cyclophilin Cpr3 exhibit a low prolyl isomerase activity in both peptide and protein-folding assays. *FEBS Lett.*, **443**, 367–369.

Shen,M., Stukenberg,P.T., Kirschner,M.W. and Lu,K.P. (1998) The essential mitotic peptidyl-prolyl isomerase Pin1 binds and regulates mitosis-specific phosphoproteins. *Genes Dev.*, **12**, 706–720.

Shou,W. *et al*. (1998) Cardiac defects and altered ryanodine receptor function in mice lacking FKBP12. *Nature*, **391**, 489–492.

Smith,J.S. and Boeke,J.D. (1997) An unusual form of transcriptional silencing in yeast ribosomal DNA. *Genes Dev.*, **11**, 241–254.

Smith,J.S., Caputo,E. and Boeke,J.D. (1999) A genetic screen for ribosomal DNA silencing defects identifies multiple DNA replication and chromatin-modulating factors. *Mol. Cell. Biol.*, **19**, 3184–3197.

Stewart,D.E., Sarkar,A. and Wampler,J.E. (1990) Occurrence and role of *cis* peptide bonds in protein structures. *J. Mol. Biol.*, **214**, 253–260.

Struhl,K. (1998) Histone acetylation and transcriptional regulatory mechanisms. *Genes Dev.*, **12**, 599–606.

Sun,Z.-W. and Hampsey,M. (1999) A general requirement for the Sin3–Rpd3 histone deacetylase complex in regulating silencing in *Saccharomyces cerevisiae*. *Genetics*, **152**, 921–932.

Takahashi,N., Hayano,T. and Suzuki,M. (1989) Peptidyl-prolyl *cis–trans* isomerase is the cyclosporin A-binding protein cyclophilin. *Nature*, **337**, 473–475.

Vidal,M. and Gaber,R.F. (1991) *RPD3* encodes a second factor required to achieve maximum positive and negative transcriptional states in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **11**, 6317–6327.

Vidal,M., Strich,R., Esposito,R.E. and Gaber,R.F. (1991) *RPD1 (SIN3/UME4)* is required for maximal activation and repression of diverse yeast genes. *Mol. Cell. Biol.*, **11**, 6306–6316.

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

Wang,H. and Stillman,D.J. (1993) Transcriptional repression in *Saccharomyces cerevisiae* by a SIN3–LexA fusion protein. *Mol. Cell. Biol.*, **13**, 1805–1814.

Winkler,K.E., Swenson,K.I., Kornbluth,S. and Means,A.R. (2000) Requirement of the prolyl isomerase Pin1 for the replication checkpoint. *Science*, **287**, 1644–1647.

Wu,X., Wilcox,C.B., Devasahayam,G., Hackett,R.L., Arévalo-Rodríguez,M., Cardenas,M.E., Heitman,J. and Hanes,S.D. (2000) The Ess1 prolyl isomerase is linked to chromatin remodeling complexes and the general transcription machinery. *EMBO J.*, **19**, 3727–3738.

Yaffe,M.B. *et al*. (1997) Sequence-specific and phosphorylation-dependent proline isomerization: a potential mitotic regulatory mechanism. *Science*, **278**, 1957–1960.

Yoshida,M., Horinouchi,S. and Beppu,T. (1995) Trichostatin A and trapoxin: novel chemical probes for the role of histone acetylation in chromatin structure and function. *BioEssays*, **17**, 423–430.

Zhang,Y., Sun,Z.W., Iratni,R., Erdjument-Bromage,H., Tempst,P., Hampsey,M. and Reinberg,D. (1998) SAP30, a novel protein conserved between human and yeast, is a component of a histone deacetylase complex. *Mol. Cell*, **1**, 1021–1031.

Zydowsky,L.D., Etzkorn,F.A., Chang,H.Y., Ferguson,S.B., Stolz,L.A., Ho,S.I. and Walsh,C.T. (1992) Active site mutants of human cyclophilin A separate peptidyl-prolyl isomerase activity from cyclosporin A binding and calcineurin inhibition. *Protein Sci.*, **1**, 1092–1099.

Recieved November 2, 1999; revised and accepted May 30, 2000