Molecular Cell, Vol. 6, 1297–1307, December, 2000, Copyright ©2000 by Cell Press

Coordinate Regulation of Yeast Ribosomal Protein Genes Is Associated with Targeted Recruitment of Esa1 Histone Acetylase

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Summary

The Esa1-containing NuA4 histone acetylase complex can interact with activation domains in vitro and stimulate transcription on reconstituted chromatin templates. In yeast cells, Esa1 is targeted to a small subset of promoters in an activator-specific manner. Esa1 is specifically recruited to ribosomal protein (RP) promoters, and this recruitment appears to require binding by Rap1 or Abf1. Esa1 is important for RP transcription, and Esa1 recruitment to RP promoters correlates with coordinate regulation of RP genes in response to growth stimuli. However, following Esa1 depletion, H4 acetylation decreases dramatically at many loci, but transcription is not generally affected. Therefore, the transcription-associated targeted recruitment of Esa1 to RP promoters occurs in a background of more global nontargeted acetylation that is itself not required for transcription.

Introduction

The acetylation of histones on conserved lysine residues within their N-terminal tails is associated with transcriptional activation (Grunstein, 1997a; Struhl, 1998). Acetylated histones, in particular acetylated H4, can facilitate access of regulatory factors to their binding sites and increase transcription on nucleosomal templates (Lee et al., 1993; Vettese-Dadey et al., 1996; Nightingale et al., 1998). The acetylation status of histone tails may also modulate the binding of nonhistone proteins to nucleosomes (Strahl and Allis, 2000). Genetic analysis indicates that histone acetylases are typically involved in transcriptional activation, whereas histone deacetylases are typically involved in transcriptional repression.

Three general models for the mechanism by which acetylases and deacetylases selectively effect gene expression have been proposed (Struhl, 1998). First, histone acetylases and deacetylases might work on a genome-wide level with little specificity for particular genomic regions. In this untargeted model, gene specificity would arise from differences among activators and repressors for binding DNA packaged in nucleosomes and from the location of binding sites with respect to nucleosomes. Second, histone acetylases and deacetylases might be selectively targeted to promoter sequences but with little specificity for individual genes. For example, the TAF130/250 histone acetylase is a component of TFIID and hence, might be present at most, and perhaps all, promoters. Third, histone acetylases and deacetylases might be specifically recruited to promoters by DNA binding activators or repressors, thereby generating localized domains of activated or repressed chromatin structure.

There is increasing evidence for specific recruitment of histone modifying activities to promoters. Chromatin immunoprecipitation experiments in yeast indicate that histone acetylation at the *HIS3* and *HO* promoters dependent on the Gcn5 histone acetylase increases upon transcriptional activation (Kuo et al., 1998; Krebs et al., 1999). In the case of *HO*, activation-specific recruitment of the Gcn5-containing SAGA complex to the promoter has been demonstrated (Cosma et al., 1999). In mammalian cells, rapid and localized hyperacetylation of histones has been observed at promoters induced by hormone treatment (Chen et al., 1999) and at the interferon-β promoter following viral infection (Parekh and Maniatis, 1999). In both cases, the p300/CBP acetylase is implicated in the acetylation. Conversely, transcriptional repression by the yeast Ume6 repressor occurs by recruitment of the Sin3-Rpd3 histone deacetylase complex, resulting in a 1–2 nucleosome domain of localized histone deacetylation (Kadosh and Struhl, 1998; Rundlett et al., 1998).

Human Tip60, *Drosophilia* MOF, and yeast Esa1 are members of a conserved family of acetylases that acetylate histone H4. Tip60 was identified as an HIV-Tat interacting protein that augments Tat-mediated transcription (Kamine et al., 1996) and is the catalytic subunit of the Tip60 complex that is involved in DNA repair and apoptosis (Ikura et al., 2000). MOF is the catalytic subunit of the MSL complex that is required for the H4 hyperacetylation associated with the 2-fold enhancement of X-linked gene transcription in *Drosophilia* males (Smith et al., 2000). MOF can activate transcription in vitro from reconstituted chromatin templates and in yeast cells if artificially targeted to a promoter (Akhtar and Becker, 2000).

Esa1 is the only yeast histone acetylase that is required for cell growth. Conditional *esa1* alleles confer a decrease in H4 acetylation at lysine 5 under restrictive conditions and display a terminal phenotype of a G_2/M arrest (Smith et al., 1998; Clarke et al., 1999). Esa1 is the catalytic subunit of the NuA4 (nucleosome acetyltransferase of histone H4) complex that acetylates histones H4 and H2A (Grant et al., 1997; Allard et al., 1999). In vitro, NuA4 can be recruited to promoters by activation domains and can stimulate activator-dependent transcription on reconstituted chromatin templates in an acetyl CoA-dependent manner (Steger et al., 1998; Utley et al., 1998; Ikeda et al., 1999; Wallberg et al., 1999; Vignali et al., 2000). In yeast cells, loss of Esa1 function results in decreased transcription of certain genes (Galarneau et al., 2000), but the molecular basis for this selective transcriptional effect is unclear. In par-

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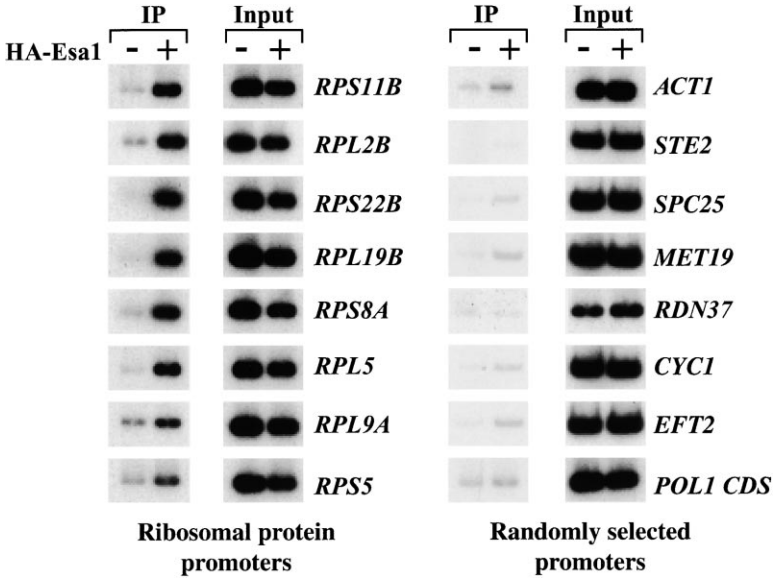

Figure 1. Identification of RP Promoters as Targets for Esa1 Recruitment

Specific recruitment of Esa1 to RP promoters. Cells containing HA_3-tagged or untagged Esa1 were grown in YPD medium and cross-linked with formaldehyde. Following immunoprecipitation, PCR was performed on total chromatin ("Input") and immunoprecipitated DNA (IP) with primers specific to various RP promoter regions, randomly selected promoters, and the *POL1* coding sequence control.

ticular, it is unknown whether Esa1 is recruited to specific promoters in vivo, and direct physiological targets of Esa1 have not been identified.

To identify physiological targets of Esa1, we combine chromatin immunoprecipitation and microarrays encompassing every yeast intergenic region. Specifically, we demonstrate that ribosomal protein (RP) promoters are major targets for Esa1 recruitment, thereby providing evidence for targeted recruitment of Esa1 in vivo. Nearly all the yeast RP promoters contain binding sites for the complex transcriptional regulatory factors Rap1 and Abf1, and transcription of RP genes is coordinately regulated in response to many environmental changes including nutrient availability, heat shock, and carbon source (Warner, 1999). We show that coordinate regulation of RP genes is associated with the recruitment of Esa1 to RP promoters, therefore defining an important and discrete biological role for Esa1. In addition, we show that Esa1 acetylates histone H4 on a genome-wide basis, but that this broad acetylation is not generally required for transcription. Thus, Esa1 functions in yeast cells by both untargeted and promoter-specific targeting mechanisms.

Results

Esa1 Is Specifically Recruited to RP Promoters

In order to identify genomic sequences that recruit Esa1, we adopted a nonbiased approach that combined chromatin immunoprecipitation and microarrays containing every yeast intergenic region (V. R. I. and P. O. B., submitted). Using antibodies to the HA epitope, potential Esa1 targets were identified by comparing genomic regions enriched in an $(HA)_3$-tagged Esa1 strain but not in a control strain containing untagged Esa1.

Due to the relative inefficiency of Esa1 crosslinking and the inherent background on the microarrays (see Experimental Procedures), the best signals for Esa1 recruitment were only 2- to 3-fold above background. This limitation precludes the presentation of a genome-wide profile for Esa1 association, but it permits the identification of putative Esa1 targets. We identified 30 putative Esa1 targets (defined by having signals at least 50% above background in two independent experiments) of which 16 are ribosomal protein (RP) promoters. For all six putative targets tested, Esa1 association was verified by the quantitative PCR assay; this included four RP promoters (*RPS11B*, *RPL2B*, *RPS22B*, and *RPL19B*; Figure 1) and two non-RP promoters (*TEF1* and *EPL1*; data not shown). The association of Esa1 with the *EPL1* promoter is of interest because Epl1 is a subunit of the NuA4 complex (Galarneau et al., 2000), thereby suggesting the possibility of autoregulation. Thus, the promoters identified by the microarray analysis represent true in vivo targets for Esa1, although they do not represent the complete set of Esa1 targets (see below).

To independently assess the specificity of Esa1 recruitment, we individually analyzed RP promoters, non-RP promoters, and other genomic regions that were not identified by the microarray analysis and were otherwise randomly selected. Esa1 associates with all six randomly selected RP promoters tested (Figure 1; data not shown), indicating that Esa1 associates with a high percentage of RP promoters. Depending on the RP promoter, Esa1 association is 3- to 10-fold higher than observed on numerous other genomic sequences. In contrast, 15 out of 16 randomly selected Pol II promoters (the exception being *PYK1*) and all 11 randomly selected nonpromoter regions show no evidence of Esa1 recruitment (Figure 1; data not shown). RP promoters represent 2% of all yeast promoters, and the microarray analysis suggests that roughly half of the Esa1 targets are RP promoters. This suggests that Esa1 associates with approximately 4% of yeast promoters, a value in accord with the observation that Esa1 associates with 1 out of 16 (6%) randomly selected Pol II promoters. Thus, Esa1 selectively associates with a small subset of Pol II promoters, and RP promoters represent a significant proportion of physiological Esa1 targets.

Although Esa1 selectively associates with a small subset of promoters, it appears to crosslink very weakly to

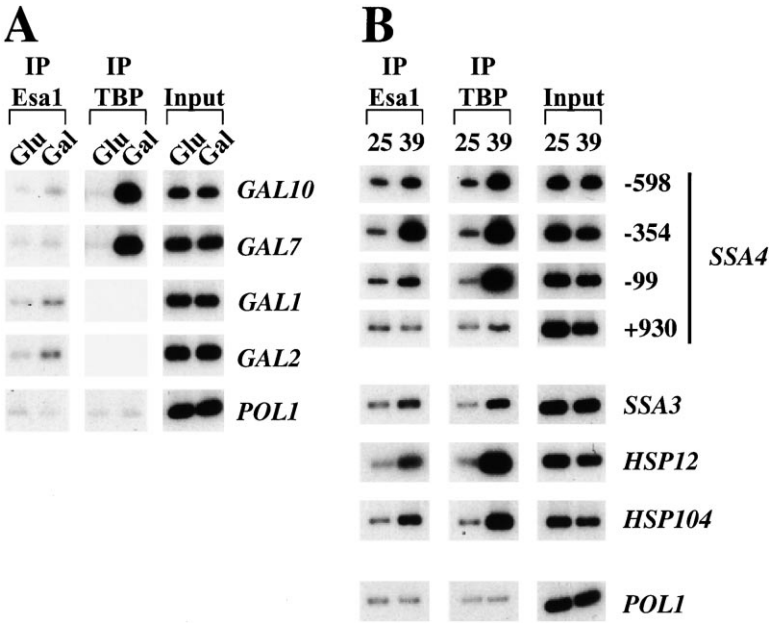

Figure 2. Esa1 Occupancy at Gal4- and Hsf1-Regulated Promoters

(A) Esa1 is not recruited to Gal4-regulated promoters. Cells containing HA_3-tagged Esa1 were grown in YP medium containing 2% glucose or 2% galactose. Immunoprecipitation was performed with HA and TBP antibodies, and Esa1 and TBP occupancy were analyzed at *GAL* gene promoters and the *POL1* coding sequence.

(B) Esa1 is recruited to Hsf1-regulated promoters following heat shock. Cells containing HA_3-tagged Esa1 were grown in synthetic complete media at 25°C, the culture was split in two, and half was subjected to a 15 min heat shock at 39°C. Esa1 and TBP occupancy were analyzed by PCR with primers to heat shock promoters and the *POL1* coding sequence. Primers spanning the *SSA4* gene (the most 5′ residue of the PCR product is indicated) were used to map the region of Esa1 binding. The Hsf1 binding site in the *SSA4* promoter is within the −354 product.

all genomic regions tested; i.e., the amounts of PCR products are slightly higher in the $(HA)_3$-tagged Esa1 strain as compared to the control strain (Figure 1). This property is not specific for Esa1 but rather is observed for every HA-tagged protein we have tested. Although we can not exclude the possibility of a nonspecific crosslinking artifact that applies to every nuclear protein, this observation suggests that Esa1 may weakly associate with chromatin in general (see below).

Activator-Specific Recruitment of Esa1

As the microarray analysis was performed on samples obtained from cells grown under noninducing conditions, it was not suitable to examine Esa1 recruitment to promoters that respond to inducible activators. We therefore analyzed Esa1 occupancy at responsive promoters following the induction of the Gal4 and Hsf1 activators. TBP specifically associates with *GAL* promoters in cells grown in galactose when Gal4 is active but not in glucose when Gal4 is inactive (Kuras and Struhl, 1999; Li et al., 1999b) (Figure 2A). Esa1 is not associated with *GAL* promoters under either condition, showing that it is not recruited by the Gal4 activator (Figure 2A). In contrast, following activation of Hsf1 by heat shock, there is an increase in both TBP and Esa1 occupancy at Hsf1-regulated genes (Figure 2B). Mapping with primers spanning the Hsf1-regulated gene *SSA4* shows that Esa1 occupancy is strongest over the Hsf1 binding site, strongly suggesting that Hsf1 is responsible for the local recruitment of Esa1. Thus, recruitment of Esa1 to promoters appears to be activator specific.

Esa1 Recruitment Is Localized to RP Promoter Regions and Appears to Require Rap1 and/or Abf1

RP promoters typically contain two Rap1 sites and/or a single Abf1 site that are responsible for most of the transcriptional activity (Lascaris et al., 1999; Warner, 1999). As these DNA binding proteins are likely candidates to play a role in Esa1 recruitment, we mapped the binding sites for Esa1, TBP, and Rap1 using primer pairs spanning the promoter regions of two RP genes, *RPS11B* and *RPL2B* (Figure 3A). For both promoters, TBP occupancy is highest at the TATA region, and Rap1 occupancy is highest at the region containing the Rap1 binding site. The pattern of Esa1 occupancy is similar, though not identical, to that of Rap1, but it appears to spread more on both sides of the promoter regions. Although these observations do not prove that Esa1 colocalizes with Rap1 at these promoters, it is clear that Esa1 association is localized to RP promoter regions.

Computer analysis has identified putative binding sites for Rap1 or Abf1 in almost all RP promoters (Lascaris et al., 1999). Specifically, of the 137 RP promoters, 90% contain at least one Rap1 site, 20% contain at least one Abf1 site, and only two (*RPL18B* and *RPL15B*) appear to lack both Rap1 and Abf1 sites. We investigated Esa1 occupancy at RP promoters with different protein binding specificities (Figure 3B). The binding of Rap1 and Abf1 to each of the RP promoters tested was confirmed by chromatin immunoprecipitation using antibodies against Rap1 and Abf1. Esa1 is recruited to all ten RP promoters tested that contain Rap1 and/or Abf1 sites; this includes *RPL19B*, which binds only Rap1, and *RPS22B*, which binds only Abf1. Strikingly, Esa1 recruitment is not observed at the *RPL18B* promoter that binds neither Rap1 or Abf1. These observations strongly suggest that Esa1 occupancy at RPs requires either Rap1 or Abf1.

We investigated whether Rap1 is required for Esa1 recruitment by generating a derivative of the *RPS11B* promoter lacking Rap1 binding sites (Figure 3C). By introducing this Rap1-defective promoter derivative into an otherwise wild-type strain, we could simultaneously monitor Rap1 and Esa1 occupancy at wild-type and mutant *RPS11B* alleles. Loss of the Rap1 binding sites virtually eliminates Rap1 binding to the *RPS11B* promoter and significantly decreases Esa1 recruitment, such that level of Esa1 occupancy is comparable to the

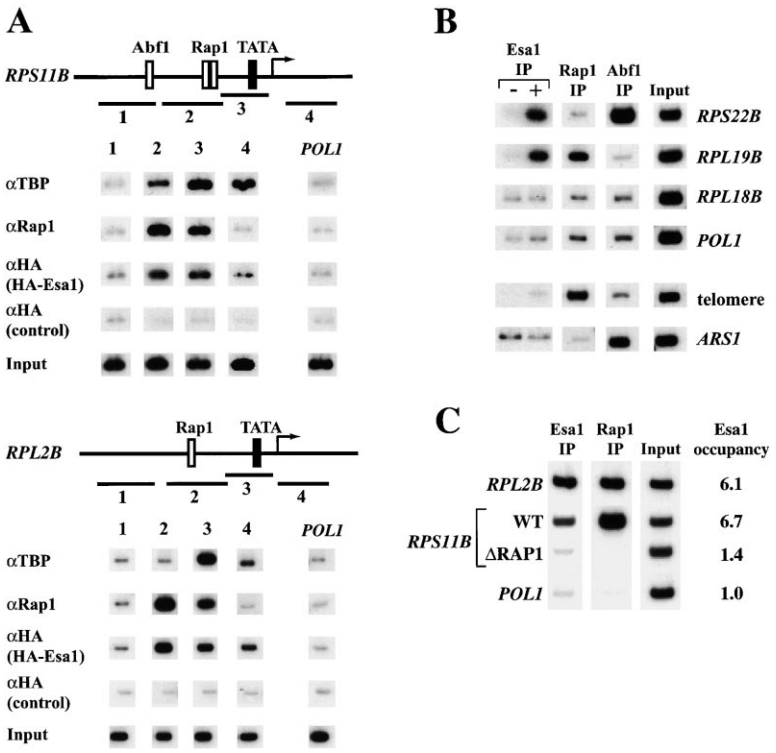

Figure 3. Esa1 Recruitment to RP Promoters Requires a Rap1 or Abf1 Binding Site

(A) Mapping of Esa1 recruitment to RP promoters. Immunoprecipitations with HA, TBP, and Rap1 antibodies were performed from cells containing HA_3-tagged or untagged Esa1. Esa1, TBP, and Rap1 recruitment was mapped by PCR with primers spanning the *RPS11B* and *RPL2B* promoters. The product numbers refer to the following sequences: *RPS11B*, (1) −887 to −569, (2) −518 to −254, (3) −276 to +14, (4) +129 to +425; *RPL2B*, (1) −1016 to −727, (2) −614 to −218, (3) −254 to −5, and (4) +27 to +325.

(B) Esa1 appears to be recruited by Rap1 and Abf1. Following immunoprecipitation with HA, Rap1, and Abf1 antibodies, occupancy was analyzed by PCR with primers to the *RPS22B* promoter (binds Abf1), *RPL19B* promoter (binds Rap1), *RPL18B* promoter (binds neither), telomere VI-R, *ARS1*, and the *POL1* coding sequence.

(C) Esa1 is not recruited to a *RPS11B* allele deleted for the Rap1 binding sites. The percentage IP efficiencies (IP signal normalized to input signal) for Esa1 and Rap1 were analyzed in a strain containing both wild-type (WT) and mutant *RPS11B* alleles. Recruitment is expressed as fold over background (where background is the percentage IP efficiency at the *POL1* coding region). The mutant allele is deleted for both Rap1 binding sites, and thus PCR with the *RPS11B* primers yields two products, a longer one for the WT and a shorter one for the mutant.

background level (defined by association with the *POL1* coding sequence). Thus, Rap1 is required for Esa1 recruitment to the *RPS11B* promoter, suggesting that it is important for Esa1 association with all RP promoters containing Rap1 sites.

Rap1 and Abf1 are multifunctional proteins that can act as either activators or repressors. Aside from its role in RP activation, Rap1 is important for silencing at telomeres and at silent mating-type loci (Shore, 1994; Grunstein, 1997b). Abf1 is also important for mating-type silencing and for DNA replication at *ARS1* and other origins of replication (Newlon and Theis, 1993; Lustig, 1998). Esa1 is not associated with the right telomere of chromosome VI or the *ARS1* origin of DNA replication (Figure 3B), suggesting that recruitment of Esa1 may involve a transcriptional activation function of Rap1 and Abf1.

RP Genes Have Similar Acetylation Profiles that Are Lost Following Esa1 Depletion

The histone acetylation profiles of three RP promoters were measured by chromatin immunoprecipitation with antibodies specific to tetraacetylated H4 and a diacetylated form of H3. Acetylation levels at the various regions of the RP promoters are quantitated relative to the acetylation level of the *POL1* coding region in the identical samples (Figure 4). Acetylation levels within the *POL1* coding region are quantitatively comparable to acetylation levels within several other coding regions (J. L. R., J. Deckert, E. vom Baur, and K. S., unpublished data). All three RP genes examined show increased histone acetylation in comparison to the *POL1* coding region, and they have remarkably similar acetylation profiles. Specifically, there is a peak of H4-specific acetylation in the upstream promoter regions with relatively lower acetylation over both the 3′ coding and Rap1 binding regions and higher H4 and H3 acetylation over the TATA and 5′ coding sequences. This pattern is not observed in several other promoters (J. Deckert and K. S., unpublished data) and, hence, appears specific to RP promoters.

To assess the role of Esa1 in conferring the RP acetylation pattern, we generated a conditional Esa1 allele using the copper-inducible, double shutoff method (Moqtaderi et al., 1996). The Esa1 depletion strain grows normally in the absence of copper, but growth slows within 2 hr of the addition of 750 μM copper sulfate. The cells stop growing after 6 hr and approximately 60% display a terminal phenotype of giant and dumbbell-shaped cells (not shown) characteristic of the null phenotype observed previously (Smith et al., 1998; Clarke et al., 1999). As is often observed for cells blocked at specific stages of the cell cycle, the arrested cells are viable for at least 8 hr after copper addition. Western blotting reveals that Esa1 levels are 3% of the starting level after 2 hr of copper addition and approximately 1% after 4 hr (Figure 5A).

Following Esa1 depletion, a dramatic decrease (10- to 30-fold) in H4 acetylation is observed over the TATA regions of all three RP promoters tested (Figure 5B). Although the peak of H4 acetylation at RP promoters is

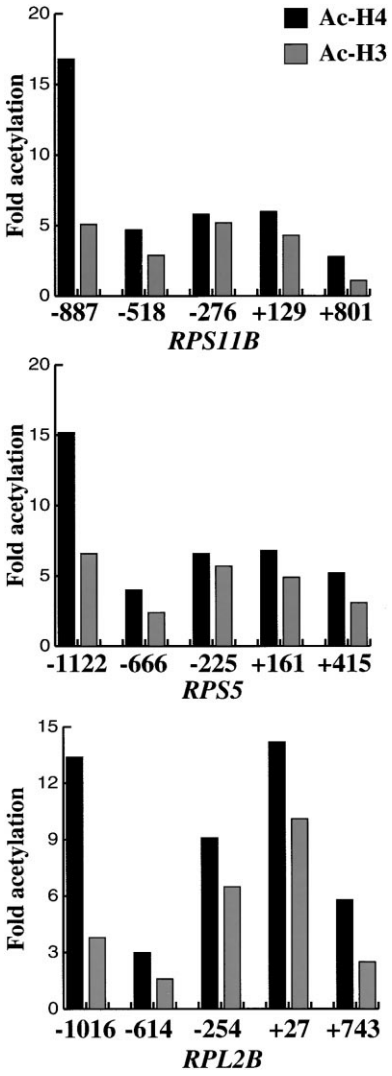

Figure 4. Acetylation Profiles of RP Genes

Immunoprecipitation was performed with antibodies to hyperacetylated H4 and diacetylated H3 followed by PCR with primers spanning across the indicated RP genes. For each PCR product, the percentage IP efficiency was calculated. Acetylation across the entire RP loci (including the coding regions) is high compared with other coding regions. Therefore, acetylation is expressed as fold over background, where background is the percentage IP efficiency at the *POL1* coding region (0.20% for H4 and 0.28% for H3). In each case, the most 5′ residue of the PCR product is indicated.

upstream of the TATA region (Figure 4), H4 acetylation is dramatically decreased (10- to 15-fold) across the entire *RPS11B* promoter region and structural gene (Figure 5C). Thus, although Esa1 recruitment to *RPS11B* is localized to the promoter (Figure 3A), Esa1-dependent acetylation spreads across the entire locus. We also observe a small (2-fold) but reproducible decrease in H3 acetylation that is centered over the TATA and 5′ coding regions. We suspect that this effect on H3 acetylation is not directly due to Esa1 because the pattern of reduced H3 acetylation differs from that of reduced H4 acetylation, and the Esa1 complex does not acetylate histone H3 in vitro on nucleosomal substrates.

Esa1 Depletion Leads to a Genome-Wide Decrease in H4 Acetylation

We examined the effect of Esa1 depletion on other regions of the genome. Loss of Esa1 leads to a dramatic decrease (6- to 15-fold) in H4 acetylation at all eight promoter regions and all four protein coding regions tested (Figure 5D). In all cases, this effect is specific to histone H4, as no significant change in H3 acetylation status was observed.

This drastic reduction of H4 acetylation is due to loss of Esa1, not to an indirect effect of cell-cycle arrest at the G_2/M transition. First, when Esa1-depletion cells are analyzed at an earlier time point (2 hr after copper addition), H4 acetylation is decreased 5- to 10-fold even though the cells are still growing (not shown). Second, cells blocked at the G_2/M transition by treatment with nocodazole show a small (typically 2-fold) decrease in both H3 and H4 acetylation (Figure 5E; note difference in scale) and hence, behave very differently than Esa1-depleted cells. Thus, although Esa1 is specifically recruited to RP promoters, Esa1 is broadly required for H4 acetylation on a genome-wide level and appears to be the major histone H4 acetylase in vivo. Conversely, although H4 acetylation at the RP genes depends on Esa1, it is not solely due to the targeted recruitment of Esa1 to the RP promoters.

Esa1 Is Required for Transcription of RP Genes but Does Not Significantly Affect Transcription of Most Other Genes

The fact that Esa1 associates with the promoters of many RP genes but not with other promoters suggests that Esa1 recruitment is relevant for the regulation of RP genes. In accord with this suggestion, depletion of Esa1 leads to a significant decrease in the transcription of the three RPs examined (*RPL9A*, *RPS8A*, and *RPS11B*) (Figure 6A). This decrease in RP transcription is rapid in that it is apparent within 2 hr of copper addition. In contrast, loss of Esa1 does not affect transcription of *PGK1*, *ACT1*, *ADH1*, *GAL1*, *GAL7*, *CUP1* or *tRNAw* (Figure 6B). Thus, Esa1 is required for RP transcription, whereas it is not generally required for transcription of most other genes in vivo. After this work was completed, it was reported that Esa1 selectively affects transcription of yeast genes (Galarneau et al., 2000).

Esa1 Recruitment to RP Promoters Correlates with Coordinate Regulation of Transcription in Response to Growth Stimuli

The transcription of RP genes is regulated by cell growth rate (Warner, 1999). We therefore measured Esa1 occupancy of RP promoters following amino acid starvation and heat shock, two conditions that result in a coordinate decrease in RP gene transcription. For amino acid starvation, cells were grown in either complete medium or minimal medium containing aminotriazole, and Esa1, TBP, and Rap1 occupancy of three RP promoters was measured (Figure 7A). TBP and Esa1 occupancy at RP promoters are reduced under amino acid starvation, whereas no significant change in background was observed on the *ACT1* and *POL1* controls. Similarly, when cells were subjected to heat shock, a decrease in both TBP and Esa1 occupancy on RP promoters was ob-

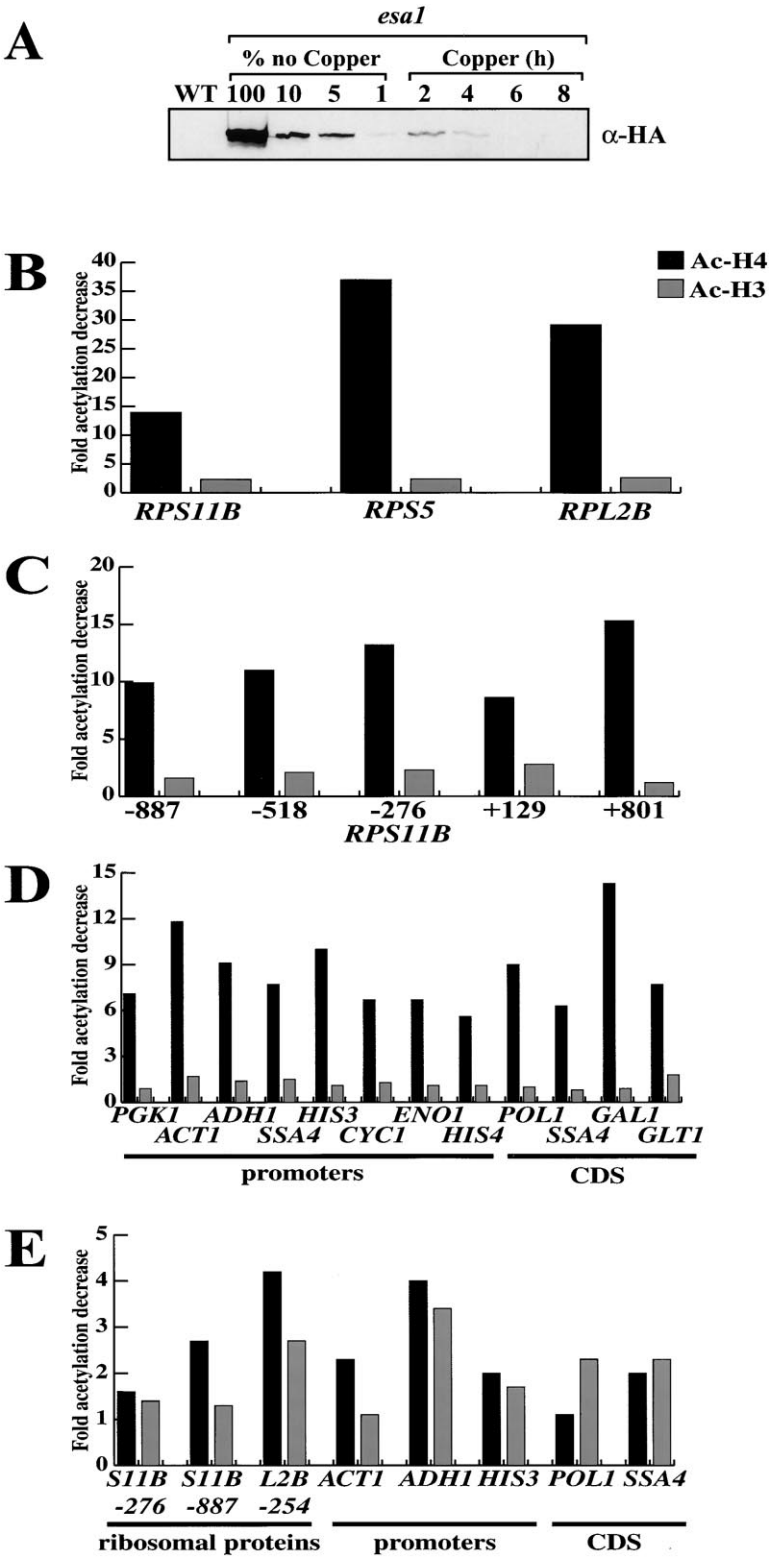

Figure 5. Analysis of Acetylation Following Esa1 Depletion

(A) Western blot analysis of Esa1 depletion. Thirty micrograms of protein from the parent strain at the zero time point and the Esa1 depletion strain at various times after addition of 750 μM copper sulfate was probed with an HA antibody. Dilutions of protein from the depletion strain at the zero time point were loaded for quantitation purposes.

(B) Effect of Esa1 depletion on acetylation at RP promoters. The Esa1 depletion and parent strains were grown in synthetic complete media plus 750 μM copper sulfate, and cross-linked chromatin was immunoprecipitated with antibodies to hyperacetylated H4 and diacetylated H3. Acetylation status was analyzed by PCR with primers to the TATA regions of RP promoters. For each PCR product, the percentage IP efficiency was calculated, and the fold decrease in acetylation following Esa1 depletion is shown.

(C) Effect of Esa1 depletion on acetylation across the *RPS11B* gene. PCR was performed with primers spanning across the *RPS11B* gene. The fold decrease in acetylation following Esa1 depletion was calculated as above.

(D) Esa1 depletion leads to a global decrease in H4 acetylation. PCR was performed with primers for eight randomly selected promoters and four coding sequences. The fold decrease in acetylation following Esa1 depletion was calculated.

(E) Nocodazole-induced G_2/M arrest leads to a distinct pattern of acetylation changes. The parent strain was grown in YPD, the culture was split in two, and nocodazole was added to half. Cells were arrested for 150 min until 93% of the cells were arrested with a large bud. Both asynchronus and G_2/M arrested cells were cross-linked and immunoprecipitated with antibodies to acetylated H4/H3. Percentage IP efficiency was calculated, and the data are presented as fold decrease in acetylation in nocodazole-treated (G_2/M arrested) cells compared to untreated (asynchronous) cells.

served (Figure 7B). Importantly, there was no change in Rap1 occupancy at RP promoters following amino acid starvation, and there was no change or a slight but reproducible increase in the case of *RPL19B* following heat shock. Thus, coordinate and growth-regulated transcription of RP genes is associated with recruitment of Esa1.

Discussion

Esa1 Is Specifically Recruited to Promoters In Vivo in an Activator-Specific Manner

In this study, we identify RP and heat shock promoters as physiological targets for Esa1 recruitment. Recruitment of Esa1 to RP and heat shock genes is promoter

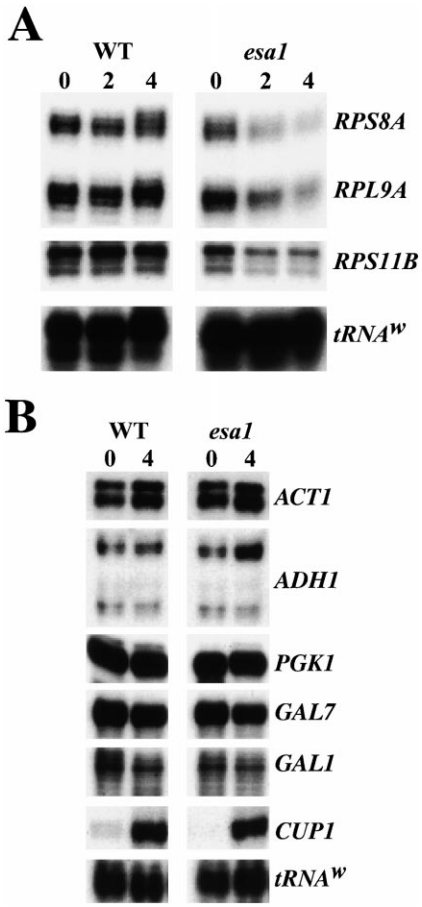

Figure 6. Esa1 Depletion Leads to a Specific Decrease in RP Gene Transcription

(A) Depletion of Esa1 leads to a decrease in RP transcription. RP transcription was measured by S1 nuclease protection assays on RNA isolated from the Esa1 depletion and parental strains at 0, 2, and 4 hr after the addition of copper.

(B) Depletion of Esa1 does not generally affect transcription. The *esa1* and parent strains were grown in synthetic complete media with 2% glucose as carbon source (2% galactose for analysis of Gal4 activated transcription). Transcription was analyzed both prior to and 4 hr after copper addition. The *CUP1* gene is induced by copper and, therefore, little transcription of this gene is seen prior to copper addition.

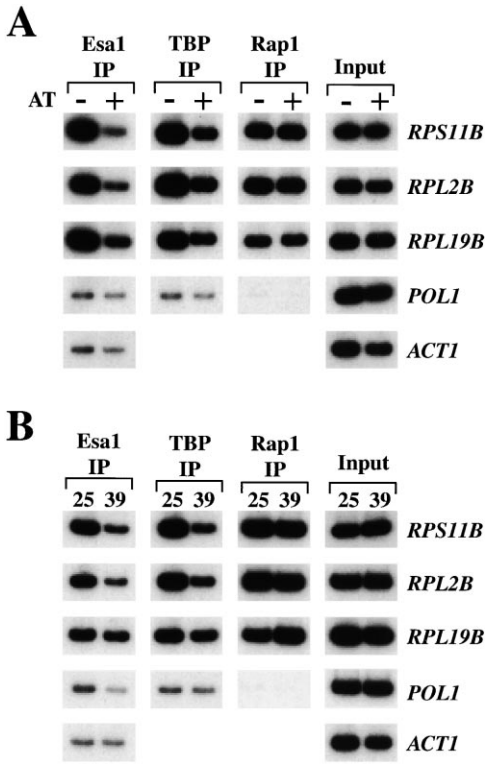

Figure 7. Esa1 Occupancy at RP Promoters Following Amino Acid Starvation or Heat Shock

(A) Esa1 occupancy at RP promoters following amino acid starvation. Cells containing HA_3-tagged Esa1 were grown initially in synthetic complete media. Half of the cells were transferred to minimal media supplemented with required amino acids and 10 mM aminotriazole (AT) and grown for 4 hr. Immunoprecipitation was performed with HA, TBP, and Rap1 antibodies, and occupancy was analyzed by PCR.

(B) Esa1 occupancy at RP promoters following heat shock. Cells containing HA_3-tagged Esa1 were grown in synthetic complete media at 25°C, the culture was split in two, and half was subjected to a 15 min heat shock at 39°C. Occupancy was analyzed as in (A).

specific, mapping to the binding sites for the Hsf1 and Rap1 activators. It is highly likely that these activators are involved in the recruitment, and this has been demonstrated for Rap1 in the case of the *RPS11B* promoter. Although not directly examined, it seems likely that Esa1 is recruited to RP and heat shock genes as part of the NuA4 complex because this complex can enhance transcription on nucleosomal templates in vitro (Steger et al., 1998; Utley et al., 1998; Ikeda et al., 1999; Wallberg et al., 1999; Vignali et al., 2000). Our observation that Esa1 is recruited by Rap1 and Hsf1 but not by Gal4 or by activators at the randomly selected promoters in Figure 1 provides evidence of activator-specific recruitment of a histone acetylase to endogenous genes in vivo. Thus, although NuA4 interacts with acidic activators in vitro (Utley et al., 1998; Ikeda et al., 1999), mechanisms of selectivity must exist in vivo that provide activator specificity. Such specificity was implied in previous work showing that the STAT1, RAR, and CREB activators require different histone acetylase activities to activate transcription (Korzus et al., 1998). Microarray analysis and analysis of randomly selected promoters suggests that Esa1 associates with a small subset (roughly 5%) of yeast promoters under standard growth conditions. RP promoters represent a significant proportion (roughly half) of such Esa1-associated promoters under these conditions.

Implications for Esa1 Function

Esa1 occupancy at RP promoters correlates with the level and coordinate regulation of transcription, implying that recruitment to RP promoters is a specific and biologically important function of Esa1. However, Esa1 is not generally required for transcription in vivo, as many genes are unaffected by Esa1 depletion. Esa1 might conceivably play a role in the transcription of these genes if Esa1 is redundant with some other function. For example, if histone acetylation at promoters is required for transcription, and acetylation of either H4 or H3 is sufficient, an H3 histone acetylase could compen-

sate for the loss of Esa1. Alternatively, modification of chromatin structure may simply not be important in the regulation of transcription of many genes in yeast, a notion supported by the observation that expression of 75% of genes is not significantly altered by depletion of histone H4 and nucleosome loss (Wyrick et al., 1999).

Although Esa1 is essential for viability (Smith et al., 1998; Clarke et al., 1999), and many RP genes are essential for cell growth, we doubt that RP transcription is the essential role of Esa1. Specifically, loss of Esa1 causes a G_2/M arrest, whereas loss of RP transcription should lead to a general growth defect. Although Esa1 is clearly important for RP transcription and coordinate regulation, we suspect that there is enough Esa1-independent transcription of RP genes to permit cell growth. One explanation for the essential role of Esa1 is that Esa1 is required for transcription of genes involved in passage through the G_2/M transition. An alternative but not mutually exclusive explanation is that the essential role of Esa1 might be independent of transcription. Deletion of Esa1 leads to a G_2/M cycle arrest that is dependent on the *RAD9* DNA damage checkpoint (Smith et al., 1998; Clarke et al., 1999). Incompletely or inappropriately acetylated chromatin following replication may be recognized as damage and thus lead to a cell cycle arrest. Interestingly, mutations in the H4 N-terminal tail lysines also lead to G_2/M delays that are *RAD9* dependent (Kayne et al., 1988; Megee et al., 1995), and depletion of the entire histone H4 leads to a G_2 arrest and block in chromosomal segregation (Kim et al., 1988).

Esa1-Dependent Acetylation

Depletion of Esa1 leads to a dramatic and widespread decrease in H4 acetylation at all genomic regions studied. Together with the observation that *esa1* temperature-sensitive alleles confer reduced H4 acetylation as assayed by Western blot (Clarke et al., 1999), these results imply that Esa1 is the major H4 acetylase in yeast. We cannot exclude that Esa1 may have other substrates, particularly because nonhistone targets have been identified for other acetylases (Kouzarides, 2000).

Although the three RP promoters examined have very similar acetylation profiles, we do not understand why the peak of H4 acetylation does not correspond with the localization of Esa1. However, targeting of the NuA4 complex to a specific site within nucleosome arrays in vitro generates a broad domain of histone acetylation (Vignali et al., 2000). If Rap1-dependent targeted recruitment of Esa1 in vivo generates a comparably broad domain of increased H4 acetylation, this could explain the peak of H4 acetylation that is considerably upstream of the Rap1 binding site. To account for the relatively lower level of H4 acetylation that occurs in the vicinity of the Rap1 site and core promoter region, we suggest that transcription-related events (e.g., transcription itself, assembly of the Pol II complex, or recruitment of other chromatin modifying activities) might block Esa1 function or independently affect chromatin structure or the acetylation. In any event, the unexpected lack of correspondence between Esa1 association and H4 acetylation prompts the suggestion that the positive transcriptional function of Esa1 might not simply be acetylation of histone H4, but rather might involve acetylation of a nonhistone substrate or some acetylation-independent function of the NuA4 complex.

Implications for the Coordinate Regulation of RP Transcription

Our results indicate that the coordinate regulation of RP genes in response to various conditions is associated with Esa1 recruitment. Specifically, the decrease in RP transcription that occurs under conditions of heat shock or amino acid starvation is accompanied by a corresponding decrease in Esa1 occupancy at RP promoters. Rap1 is required for Esa1 recruitment to the *RPS11B* promoter and is likely to be important for recruitment to other RP promoters with Rap1 sites. Thus, in addition to recruiting a silencing complex containing the Sir histone deacetylase to telomeres and silent mating-type loci (Grunstein, 1997b; Imai et al., 2000), Rap1 appears to recruit an activator complex containing Esa1 histone acetylase to promoters. Recruitment of acetylase activity provides a possible mechanism for previously proposed roles for Rap1 in increasing accessibility of other factors to chromatin (Devlin et al., 1991; Drazinic et al., 1996) and for Rap1 sites acting as boundary elements preventing the spread of heterochromatin (Bi and Broach, 1999).

The Rap1 sites of RP genes are primarily responsible to confer regulation by amino acid starvation (Moehle and Hinnebusch, 1991) and other growth-related changes (Klein and Struhl, 1994; Li et al., 1999a; Warner, 1999). Under these conditions, Rap1 DNA binding activity in cell-free extracts is unaffected (Moehle and Hinnebusch, 1991; Klein and Struhl, 1994). Our observation that Rap1 occupancy of RP promoters does not change following amino acid starvation or heat shock suggests that coordinate regulation of RP genes is due to a change in the cofactors recruited by Rap1. Transcriptional inhibition in response to amino acid starvation or heat shock could result from a decreased interaction between Rap1 and the Esa1 complex, perhaps due to a posttranslational modification of Rap1 or a component of the Esa1 complex. Protein kinase A is implicated in RP transcriptional regulation in response to carbon and nitrogen availability (Klein and Struhl, 1994; Neuman-Silberberg et al., 1995) and hence, is a candidate for mediating a signal to Rap1 or Abf1.

Specific Recruitment versus Global Effects

Although Esa1 is specifically recruited to RP and heat shock promoters, it also plays a global role in acetylation of histone H4. Depletion of Esa1 leads to a dramatic decrease in H4 acetylation at all genomic regions tested, yet Esa1 does not appear to be recruited to *ACT1*, *CYC1*, or *POL1*, and Esa1 depletion has no affect on transcription of many genes (Galarneau et al., 2000; Figure 6). Therefore, specific targeting of Esa1 and coordinate regulation of RP promoters appears to occur in a background of nontargeted histone acetylation that is not generally required for transcription.

Although promoter-specific targeting of histone acetylases and deacetylases and the creation of localized domains of chromatin structure have been emphasized, there is clear evidence that these enzymatic activities

have untargeted genome-wide effects. In yeast, a global increase in both H3 and H4 acetylation occurs at S-phase (Krebs et al., 1999). In addition, Western blots of histones isolated from cells individually deleted for the deacetylases Rpd3, Hda1, or Hos3 show an increase in histone acetylation (Rundlett et al., 1996; Carmen et al., 1999). Conversely, cells lacking either Gcn5 (Zhang et al., 1998; Krebs et al., 1999; Kuo et al., 2000 [this issue of *Molecular Cell*]) or Esa1 function (Clarke et al., 1999; this work) show a decrease in bulk histone acetylation. Such gross changes in acetylation indicate that these enzymes cannot simply be acting at a few targeted promoters but are likely to also play more genome-wide roles.

We suggest that these untargeted enzymatic activities serve to maintain a genome-wide balance between histone acetylation and deacetylation that is largely (but not completely) nonspecific for transcription of individual genes. At certain promoters, targeted recruitment of histone acetylases and deacetylases and concomitant generation of localized domains of modified histones occurs in this global background, thereby leading to changes in transcription. Upon cessation of the environmental or developmental signals for altered gene regulation, the genes return to the ground acetylation state by virtue of the untargeted histone acetylases and deacetylases. In addition, the observation that the peak of H4 acetylation does not coincide with Esa1 localization at the promoter suggests that targeted recruitment of Esa1 might also affect transcription by acetylation of a nonhistone substrate and/or by an acetylation-independent mechanism of the NuA4 complex. In any event, Esa1 functions through both the untargeted and gene-specific recruitment models described in the Introduction, and it plays a direct role in overall chromatin structure and promoter-specific activation.

Experimental Procedures

DNA Constructs, Yeast Strains, and Growth Conditions

To generate the tagged Esa1 strain, *ESA1* noncoding sequence (−500 to ATG), three HA epitopes, a three glycine linker, and *ESA1* sequence from +2 to +444 were cloned in-frame to each other in ylplac211. The plasmid was linearized at an SpeI site within the *ESA1* coding sequence and introduced into the strain FY1351 that is isogenic to S288C (*MATa*, *leu2Δ0*, *lys2Δ0, ura3Δ0*, a gift from Fred Winston). Two-step gene replacement yielded a strain with HA_3-*ESA1* under its endogenous promoter as the sole copy of *ESA1.*

The Esa1 depletion strain was constructed in the double shutoff parent strain ZMY117 (isogenic to ZMY60 [Moqtaderi et al., 1996] except for *leu2*::*PET56*). *ESA1* sequence (+1 to +444) was fused in-frame to an *ANB1*-driven ubiquitin-arginine-lacI-HA_3 N-terminal cassette. Promoter sequences from *ESA1* (−628 to –1) were introduced in-frame and upstream of the cassette. The resulting plasmid was linearized at a SpeI site within the *ESA1* sequence and introduced into ZMY117. Following integration at the *ESA1* locus, the short 5′ fragment of *ESA1* under its endogenous promoter was removed by a loop out between the two *ESA1* promoter sequences. The resulting strain contains full-length *ESA1* under the *ANB1* cassette as its only copy of *ESA1*.

The *RPS11B* mutant was generated by cloning *RPS11B* noncoding sequences (−987 to −307) in-frame with *RPS11B* −278 to +1042 in yCplac33. Sequences from –306 to –279 (containing the two Rap1 binding sites) were replaced by a Xba1 restriction site. The resulting plasmid was transformed into the HA_3-Esa1 strain to yield a strain containing both wild type and mutant promoters for *RPS11B*.

Strains were grown under standard conditions in either YP, synthetic complete, or minimal media. To arrest cells at G_2/M, nocodazole (Sigma) was added directly to the medium to a final concentration of 15 μg/ml. Cells were arrested for 2–3 hr until greater than 90% of cells were arrested with a large bud as determined by examination under the microscope.

Chromatin Immunoprecipitation

Chromatin preparation, immunoprecipitation, and quantitative PCR were performed exactly as described previously (Kuras and Struhl, 1999), with the exception that the salt concentration was not adjusted for the IPs (therefore performed in 150 mM NaCl). The following antibodies were used: α-HA (12CA5 ascites fluid or F7, Santa Cruz), α-TBP (gift from Laurie Stargell), α-Rap1 (yC-19, Santa Cruz), α-Abf1 (yC-20, Santa Cruz), α-acetylated H4 (against H4 acetylated at K5, 8, 12, and 16, Upstate Biotechnology), and α-acetylated H3 (against H3 acetylated at K9 and 14, Upstate Biotechnology). Each immunoprecipitation was performed from at least two independent chromatin preparations. A titration of each template was performed to determine the linear range for each combination of primers and DNA.

Microarray Analysis

Chromatin from HA_3-Esa1 and untagged cells was immunoprecipitated with HA antibodies, and amplification of the immunoprecipitated material and hybridization to microarrays performed as described elsewhere (V. R. I. and P. O. B., submitted). The maximal crosslinking efficiency for Esa1 (0.3%, which is observed on the *RPS11B* and *RPL2B* promoters) is 10-fold lower than the crosslinking efficiency for TBP at these same promoters. Because of this modest Esa1 crosslinking efficiency and the relatively large size (500–1000 bp) of the intergenic regions on the arrays, the best signals for Esa1 recruitment were only 2- to 3-fold above background.

Putative Esa1 targets were identified from the microarray analysis in two ways. First, as defined by having signals of 50% over the background in two independent experiments; this criterion identifies 30 Esa1 targets of which 16 are RP promoters. Second, as defined by having an average signal of 2-fold over background in the two independent experiments; there are 16 Esa1 targets of which 11 are RP promoters. For all cases tested by the quantitative PCR assay, we confirmed that the promoters identified by the microarray analysis were truly targets for Esa1.

Transcriptional Analysis

The copper-inducible double shutoff method has been described previously (Moqtaderi et al., 1996). The Esa1 depletion and control strains were cultured in synthetic complete media to an OD_{600} of approximately 0.3, and copper sulfate was added to a final concentration of 750 μM to initiate depletion of Esa1. Total cellular RNA was isolated at various times after the addition of copper, and the transcript levels for various genes were determined by S1 nuclease protection assays on 20–40 μg of RNA (Iyer and Struhl, 1996).

Western Blotting

Whole-cell extracts from the Esa1 depletion and parent strains were prepared at various times after the addition of copper sulfate. Cells were lysed with glass beads in RIPA buffer, and 30 μg of protein was probed with an α-HA antibody (12CA5 ascites fluid) by standard techniques.

Acknowledgments

We thank Elmar vom Baur and Jutta Deckert for discussions about unpublished data on histone acetylation, Elmar vom Baur for construction of the *ANB1*-ubiquitin-*ESA1* plasmid, Zarmik Moqtaderi for help with obtaining the *RPS11B*-ΔRAP1 strain, Peter Kosa for help with the microarray experiment, Vera Cherkasova for help with microscopy, Danesh Moazed for telomere PCR primers, Laurie Stargell for the αTBP antibody, Fred Winston for the FY1351 strain, and Simon Boulton for critical reading of the manuscript. This work was supported by postdoctoral fellowships from EMBO and the Human

Frontier Science Program to J. L. R. and by research grants to K. S. from the National Institutes of Health (GM 30186 and GM 53720).

Received June 28, 2000; revised October 16, 2000.

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