Published in final edited form as:
Mol Cell. 2006 June 9; 22(5): 633–644. doi:10.1016/j.molcel.2006.04.009.

Dephosphorylation and Genome-Wide Association of Maf1 with Pol III-Transcribed Genes during Repression

Douglas N. Roberts[1,2,3], **Boris Wilson**[1,2,3], **Jason T. Huff**[1,2,3], **Allen J. Stewart**[1,2], and **Bradley R. Cairns**[1,2,*]

[1]Howard Hughes Medical Institute

[2]Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah School of Medicine, Salt Lake City, Utah 84112

Summary

Nutrient deprivation and various stress conditions repress RNA polymerase III (Pol III) transcription in *S. cerevisiae*. The signaling pathways that relay stress and nutrient conditions converge on the conserved protein Maf1, but how Maf1 integrates environmental conditions and couples them to transcriptional repression is largely unknown. Here, we demonstrate that Maf1 is phosphorylated in favorable conditions, whereas diverse unfavorable conditions lead to rapid Maf1 dephosphorylation, nuclear localization, physical association of dephosphorylated Maf1 with Pol III, and Maf1 targeting to Pol III-transcribed genes genome wide. Furthermore, Maf1 mutants defective in full dephosphorylation display *maf1Δ* phenotypes and are compromised for both nuclear localization and Pol III association. Repression conditions also promote TFIIIB-TFIIIC interactions in crosslinked chromatin. Taken together, Maf1 appears to integrate environmental conditions and signaling pathways through its phosphorylation state, with stress leading to dephosphorylation, association with Pol III at target loci, alterations in basal factor interactions, and transcriptional repression.

Introduction

Cells couple growth to nutrient availability by regulating translational capacity and transcriptional growth programs. Nutrient deprivation and stress restrict translational capacity by repressing transcription by all three RNA polymerases. RNA polymerase I transcribes the ribosomal RNAs, RNA polymerase II transcribes the ribosomal protein genes, and RNA polymerase III (Pol III) transcribes all tRNAs and a set of noncoding RNAs important for splicing, translation, and protein transport. Conditions of nutrient deprivation or stress lead to a rapid reduction in Pol III transcription and are relayed to the Pol III machinery by several different signaling pathways (Ghavidel and Schultz, 2001; Li et al., 2000; Nierras and Warner, 1999). For unicellular eukaryotes such as *S. cerevisiae*, this process is important for optimal utilization of nutrients and survival. For higher eukaryotes, Pol III regulation is linked to cell proliferation, and several important cell cycle regulators (such as p53, Myc, and Rb) regulate Pol III (Felton-Edkins et al., 2003; Gomez-Roman et al., 2003). In addition, upregulation of Pol III transcription is a common feature of cancer cells and may contribute to their proliferative capacity (White, 2004). Though yeast lack these tumor suppressors/oncogenes, they share with higher cells a central regulator of Pol III, the Maf1 protein (Pluta et al., 2001).

*Correspondence: brad.cairns@hci.utah.edu.
[3]These authors contributed equally to this work.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, Supplemental References, seven figures, and four tables and can be found with this article online at http://www.molecule.org/cgi/content/full/22/5/633/DC1/.

The Pol III system has been defined through 20 years of elegant biochemical and genetic work (Dieci and Sentenac, 2003; Geiduschek and Kassavetis, 2001; Schramm and Hernandez, 2002). The Pol III system is highly conserved in eukaryotes and consists of three multisubunit complexes: (1) Pol III, (2) TFIIIB, which is required for transcript initiation by Pol III, and (3) TFIIIC, which is required for promoter recognition (Geiduschek and Kassavetis, 2001; Schramm and Hernandez, 2002). Pol III-transcribed promoters contain two conserved DNA sequence elements, termed A and B boxes, which are generally located within the transcribed region (Galli et al., 1981). The A and B boxes (along with the terminator) are recognized by TFIIIC, establishing TFIIIC as the promoter specificity factor (Roberts et al., 1995). TFIIIC recruits TFIIIB to Pol III promoters (Chaussivert et al., 1995). TFIIIB contains the TATA binding protein (TBP) and two additional proteins: Brf1 and Bdp1. TFIIIB and TFIIIC cooperate to recruit Pol III, though TFIIIB-Pol III interactions appear more extensive and important (Geiduschek and Kassavetis, 2001). Taken together, TFIIIC locates Pol III-transcribed genes and recruits TFIIIB, whereas TFIIIB and Pol III are essential for transcript initiation and reinitiation (Dieci and Sentenac, 1996; Kassavetis et al., 1990). For brevity, we will hereafter refer to genes transcribed by the Pol III machinery as “Pol III genes.”

The repression signals for Pol III converge on a central regulator, the Maf1 protein (Willis et al., 2004). Cells lacking Maf1 are unable to repress Pol III in response to nutrient deprivation, cell wall stress, DNA damage, or oxidative stress (Desai et al., 2005; Upadhya et al., 2002). Nutrient and stress conditions are disseminated by several signal transduction pathways, including the Pkc1 pathway (cell integrity, survival during starvation), the TOR pathway (starvation, rapamycin), and CK2 (DNA damage) (Willis et al., 2004). However, how these pathways affect Maf1 function is not understood. Maf1 is conserved in eukaryotes but lacks recognizable domains that might inform its function. Maf1 can be coprecipitated with subunits of Pol III and the Brf1 subunit of TFIIIB (Desai et al., 2005; Pluta et al., 2001). Also, recombinant Maf1 is a potent inhibitor of Pol III transcription in vitro (Desai et al., 2005). Thus, Maf1 has the genetic properties of an integrator of repression signaling and has initial biochemical connections to both Pol III and TFIIIB. However, central questions remain regarding the mechanism of signal integration and its impact on the basal machinery including: (1) how Maf1 controls repression in a signal-dependent manner and whether this occurs through posttranslational modification, (2) whether repression conditions change the cellular localization of Maf1 or the associations of Maf1 with Pol III components, and (3) if repression causes the targeting of Maf1 to Pol III genes to impose repression. This study addresses these aspects of Maf1 function and further develops the activity-occupancy relationships of Maf1 and the basal factors.

Results

Maf1 Occupies Pol III Loci, and Occupancy Increases during Repression

We utilized chromatin immunoprecipitation (ChIP) to address whether Maf1 protein interacts with genes transcribed by Pol III, and whether this interaction increases during Pol III repression imposed by nutrient deprivation. We prepared a strain encoding a Maf1 derivative bearing three copies of the HA epitope (Maf1-HA), driven by its endogenous promoter from the *MAF1* genomic locus, which fully complemented *maf1Δ* phenotypes (data not shown; [Kwapisz et al., 2002]). We sampled cultures grown in rich medium (1 × YP medium with 2% glucose) or after nutrient deprivation (0.15 × YP medium lacking glucose). Our nutrient deprivation regimen rapidly repressed Pol III transcription, lowering transcript levels to 10%–20% of normal levels within 25 min of treatment (Clarke et al. [1996], Harismendy et al. [2003], Roberts et al. [2003], and data not shown). We refer to the time period during which repression is established ($t \leq 25$ min) as acute repression and to the maintenance period as prolonged repression.

We performed ChIP of Maf1-HA and tested for occupancy at the tRNA gene *tRNA^{Lys}(CUU)G1* by multiplex and real-time quantitative PCR (qPCR) (Figures 1A and 1B). In nutrient replete conditions (t = 0), a low level of Maf1 is observed at this locus (about 2-fold enrichment; Figure 1A). Interestingly, Maf1 association with this locus increased rapidly (within 5 min) and significantly during nutrient deprivation (Figures 1A and 1B). Thus, Maf1 occupancy at *tRNA^{Lys}(CUU)G1* increases during acute repression and is maintained during prolonged repression.

To determine whether Maf1 associates in a regulated manner with all Pol III loci, we determined the occupancy of Maf1-HA genome wide during normal growth (t = 0) or after nutrient deprivation (t = 75 or 180 min) (Table S4 available in the Supplemental Data with this article online). We interrogated a genomic DNA microarray consisting of the entire yeast genome (~6200 genes) parsed into ORF (RNA polymerase II transcribed) and intergenic segments (which include the 281 Pol III-transcribed genes). For each of three experiments, Maf1 occupancy at each segment was quantified and ordered by a standard percentile rank analysis (Lieb et al., 2001). For example, the 99th percentile bin contains the 1% of intergenic segments with the highest Maf1 occupancy, whereas the 1st percentile bin bears the1%of segments with the lowest Maf1 occupancy. To determine whether Maf1 preferentially occupies Pol III-transcribed genes, we compared the mean percentile rank (MPR) of Maf1 occupancy of tRNA-adjacent intergenic segments (from the average of three independent experiments) to all intergenic segments (Figure 1C). As expected, the MPR of all intergenic segments is 49%–50%, whereas tRNA-adjacent segments have slightly higher background (MPR 59% in the untagged control) due to crosshybridization from their high sequence identity. However, under nutrient replete conditions, Maf1 shows significant enrichment at tRNA-adjacent segments (MPR to 70%).

Importantly, nutrient deprivation further increases Maf1 association, providing an MPR of 78% and 83% at t = 75 and 180 min, respectively. After subtraction of segments providing high background in the untagged control, we performed a χ^2 analysis for Maf1 occupancy at t = 0, 75, and 180. In each case, the corresponding p value was $<10^{-14}$, indicating that the enrichment for Pol III genes is highly significant. In addition to tRNA genes, Maf1 occupies the Pol III-transcribed genes *SCR1*, *SNR6*, *RPR1*, and *SNR52* under repressing conditions as well as the Pol III-occupied intergenic segment adjacent to the *UFO1* gene (Moqtaderi and Struhl, 2004; Roberts et al., 2003). Taken together, a moderate level of Maf1 associates with Pol III targets in nutrient replete conditions, shifting to a higher level of association in nutrient deprivation.

Maf1 Is a Phosphoprotein that Is Rapidly Dephosphorylated during Repression

Although the levels of Maf1 increase during prolonged repression (Figure 2A), acute repression is accompanied by only slight (at t = 10 min) or moderate (at t = 25 min) increases in protein levels. Therefore, we considered whether posttranslational modification might underlie repression by Maf1. Many of the signaling pathways that affect Pol III transcription involve phosphoregulation; however our initial attempts to monitor changes in Maf1 modification during repression using immunoblots were unsuccessful, as Maf1-HA showed no change in migration when standard whole-cell extracts were examined (data not shown). However, when we examined whole-cell extracts derived from formaldehyde- crosslinked cells (used for ChIP), Maf1-HA always migrated as a diffuse band under normal growth conditions (Figure 2A, lane 1, and Figure 2B, lane 1). Remarkably, conditions known to repress Pol III transcription caused the rapid conversion of Maf1-HA to a single faster-migrating form (Figures 2A and 2B), including nutrient deprivation, rapamycin, methane methyl sulphonate (MMS), and chlorpromazine (a cell wall stretching agent) (Figure 2B) (Upadhya et al., 2002; Zaragoza et al., 1998). Importantly, all migration differences observed strictly depended on altering the growth condition and, thus, were not caused by the crosslinking procedure itself.

A moderate amount of the diffuse slower-migrating form could be preserved if extracts were prepared rapidly in the presence of multiple phosphatase inhibitors. For example, two-dimensional analysis of Maf1-HA derived from extracts containing phosphatase inhibitors revealed a diffuse and heterogeneous set of Maf1 species that largely collapse after nutrient deprivation (Figure 2C). These results are consistent with the crosslinking procedure preserving Maf1 phosphoforms, possibly by inactivating particular phosphatases.

Two experiments establish that Maf1-HA is a phosphoprotein that is dephosphorylated during repression. First, treatment of Maf1-HA immobilized on anti-HA beads with lambda phosphatase resulted in the conversion of the slower-migrating form(s) of Maf1 to a faster-migrating species (Figure 2D). Second, in vivo labeling of Maf1 was performed, where cells (bearing Maf1-HA, or untagged) were grown in phosphate replete conditions, labeled with ^{32}P inorganic phosphate, and then subjected to rapamycin treatment for 25 min. Extracts were prepared (without crosslinking but with phosphatase inhibitors), and immunoprecipitation of Maf1-HA was performed. Interestingly, autoradiography revealed a strong diffuse band at the molecular weight of Maf1-HA (45 kDa) in extracts from untreated cells, which was highly diminished after rapamycin treatment (Figure 2E, bottom), whereas the levels of Maf1 were equivalent in the inputs and precipitates (Figure 2E, top and middle). We note that the combination of growth in phosphate replete conditions (itself a form of nutrient deprivation required for labeling), extract preparation without crosslinking, and incubation of this extract for several hours (required to perform the immunoprecipitation), greatly reduced the proportion of phosphorylated Maf1 in the extract, as a slower-migrating band is not observed in the eluate examined by immunoblot analysis (Figure 2E, middle). Thus, we are examining the small portion of phosphorylated Maf1 that remains after these treatments. Taken together, Maf1 is a phosphoprotein that is less phosphorylated in unfavorable growth conditions. For ease of discussion, we will refer to the diffuse set of species as phosphorylated Maf1 and the condensed fast-migrating species as dephosphorylated Maf1. However, it is possible that the condensed species still bears phosphorylated residues.

Pol III Interacts with Dephosphorylated Maf1

A small portion of Maf1 is coprecipitated with Pol III in extracts derived from noncrosslinked cells grown in favorable conditions (Desai et al., 2005; Pluta et al., 2001). To investigate whether this interaction changes during repression, we performed CoIPs with extracts derived from crosslinked cells (standard ChIP extracts). Our CoIP experiments were performed according to standard ChIP conditions, which involve high-stringency washes of the bound material (0.25 M NaCl, 1% Triton, and 0.1% deoxycholate). Therefore, protein-protein associations that are maintained likely depend on the crosslink. Crosslinks are reversed by extended heating in 1% SDS, allowing the separation and analysis of Maf1-associated proteins by SDS-PAGE.

We find a small portion of Maf1 associated with Pol III in ChIP inputs derived from cells grown in nutrient rich medium (Figure 3A, lane 7). However, the proportion of Maf1 associated with Pol III increased dramatically during acute repression (Figure 3A, compare lanes 7 and 8). This increased interaction was also clear in the reciprocal coprecipitation (Figure 3B, compare lanes 7 and 8). The interaction of Maf1 with Pol III during repression may also underlie the apparent increase in the abundance of Maf1 in the ChIP extracts from repressed cells (Figure 3A, compare lanes 3 and 4); Maf1 may be recruited to chromatin during repression. We note that standard ChIP extracts are enriched for chromatin relative to standard whole-cell extracts (Figure S1). In contrast to our findings with Pol III, interactions between Maf1-HA and TFIIIB (Bdp1-Myc, TBP) or TFIIIC (Tfc1-Myc) components are extremely weak or undetectable, respectively, and did not increase during repression (Figure S2 and data not shown). Thus, the proportion of Maf1 in association with Pol III increases significantly during acute repression.

Maf1 dephosphorylation and its increased association with Pol III are temporally correlated, raising the possibility that dephosphorylation might enhance Maf1 association with Pol III. To test for this, we precipitated Rpc82-Myc from ChIP extracts derived from cells grown in nutrient replete conditions (t = 0), which contain primarily phosphorylated Maf1-HA (Figure 3C, lane 1). Interestingly, only the small proportion of Maf1-HA that is dephosphorylated is precipitated with Rpc82-Myc (Figure 3C, lane 4). This is not due to dephosphorylation during the IP procedure, as an IP of the same extract with anti-HA beads under identical conditions precipitates all Maf1 species, the largest proportion of which remains diffuse and slower migrating (Figure 3C, lane 3). Importantly, the proportion of dephosphorylated Maf1 in association with Rpc82-Myc increases dramatically during nutrient deprivation (Figure 3C, compare lanes 4 and 6). Thus, dephosphorylated Maf1 interacts preferentially with Pol III, and a greater proportion associates during nutrient deprivation. Identical results are obtained when other treatments that repress Pol III are examined, such as treatment with MMS, chlorpromazine, or heat shock (Figure 3C), suggesting that Maf1 may integrate stress and nutrient status through its phosphorylation state.

maf1 Point Mutants that Are Partially Defective in Dephosphorylation Are Defective in Pol III Association

To better understand the relationship between phosphorylation and Pol III association, we made mutations in *MAF1* and examined their impact on Maf1 phosphorylation status, RNA Pol III association, and conferral of *maf1Δ* phenotypes (rapamycin sensitivity or slow growth on glycerol at 38°C). Maf1 orthologs are highly similar, and all contain three regions of high similarity (termed A, B, and C) of entirely unknown function (Figure 4A). Our strategy for mutagenesis included (1) the replacement (with alanines) of conserved serine or threonine residues throughout Maf1, or (2) replacements of conserved residues within the three known regions, which might reduce the association of phosphatases or Pol III, or (3) a combination of both types of replacements.

As expected with a broad approach, most replacements had no phenotypic effect, and within this subset we did not observe an impact on Maf1 phosphorylation status or Pol III association, when tested (Table S1). However, two alleles (*maf1-104* and *maf1-122*; Figure 4A) bearing replacements in the conserved B or C boxes, respectively, conferred effects. Both *maf1-104* and *maf1-122* conferred rapamycin sensitivity and slow growth on glycerol (Figure 4B). Interestingly, in response to nutrient deprivation, a significant proportion of these mutant Maf1 derivatives remained in the diffuse phosphorylated form and were also defective in repression-dependent association with Pol III (Figure 4C). Furthermore, both Maf1 mutants were defective in their ability to repress Pol III transcription, as assessed by pre-tRNA^{Leu3} abundance after nutrient deprivation (Figure 4D). The defects in *maf1-104* can largely be attributed to the R232H mutation in isolation, termed *maf1-124* (Figure S3). Thus, mutants that display *maf1* phenotypes are defective in full dephosphorylation and Pol III association. We speculate that these mutations may reduce Maf1 interaction with a regulatory phosphatase, with Pol III, or both.

Maf1 Localizes to the Nucleus during Repression, and Nuclear Localization Is Correlated with Dephosphorylation

We next addressed whether the phosphorylation state of Maf1 affected its cellular distribution. In nutrient replete conditions, Maf1 is distributed throughout the cell, including the nucleus. Interestingly, nutrient deprivation or rapamycin treatment led to the relocalization of the vast majority of Maf1 to the nucleus (Figure 5A and Figure S4). We note that the kinetics of redistribution appear somewhat slower than the kinetics of dephosphorylation; at treatment times (t = 30 min) when nearly full dephosphorylation of Maf1 is observed, Maf1 is largely, but not entirely, nuclear. Thus, relocalization in the nucleus occurs during acute repression but

is more extensive during prolonged repression. We then utilized our Maf1 mutants to address the relationship between dephosphorylation and nuclear localization. Interestingly, Maf1 mutants that were defective in dephosphorylation remained largely cytoplasmic during nutrient deprivation (Figures 5B, 5C, and 5D), suggesting that Maf1 dephosphorylation may promote the relocalization of Maf1 to the nucleus.

Full Dephosphorylation in Response to Nutrient Deprivation Requires Pkc1

Previous work has implicated several signal transduction pathways in Pol III repression, including the TOR and Pkc1 pathways (see Introduction). Here, we examined the requirement for particular phosphatases in mediating the dephosphorylation of Maf1 in response to nutrient deprivation. We find that mutations in most phosphatases have little or no effect (*sit4Δ*, *yvh1Δ*, *ppz1Δ*, *cdc14*^{ts}, *his2Δ*, and *msg5Δ*; data not shown). The TOR pathway phosphatase PP2A includes a regulatory subunit, Tpd3, as well as three alternative catalytic subunits: Pph21, Pph22, and Pph3. We find that cells lacking Tpd3 display significant dephosphorylation of Maf1 even in nutrient replete conditions (Figure S5A), suggesting that the activation of PP2A may lead to Maf1 dephosphorylation. This is consistent with the observation that Pol III transcription is significantly down-regulated in *tpd3Δ* cells (Willis et al., 2004). However, cells lacking Pph21 and Pph22 also display significant dephosphorylation of Maf1 in nutrient replete conditions and full dephosphorylation during nutrient deprivation (Figure S5B). This suggests that PP2A misregulation, either upregulation or downregulation, confers a stress that leads to Maf1 dephosphorylation. Here, we note that Pph3 remains to be tested during nutrient deprivation. However, a clear role for PP2A in the dephosphorylation of Maf1 in response to rapamycin treatment has been revealed through the studies of Lefebvre and colleagues (Oficjalska-Pham et al., 2006).

Interestingly, we find that cells lacking Pkc1 fail to cause Maf1 dephosphorylation in response to nutrient deprivation and that responsiveness could be restored by the addition of *PKC1* on a plasmid (Figure S5C). Thus, the activity of the Pkc1 pathway is needed for efficient Maf1 dephosphorylation in response to nutrient deprivation, suggesting that Pkc1 regulates Maf1 phosphatases during nutrient deprivation.

Spatial Resolution and Dynamics of Maf1 and the Pol III Machinery at *SCR1*

Our previous work utilized ChIP to examine the activity-occupancy relationships of certain members of the Pol III machinery at tRNA genes in vivo (Roberts et al., 2003). To extend these studies to Maf1 and to provide spatial resolution of Pol III factors, we examined *SCR1* (encoding the 522-base RNA component of the signal recognition particle), the longest Pol III-transcribed RNA in the genome (Figure 6A). We designed six qPCR amplicons (A–F) that span the upstream, transcribed, and downstream regions of *SCR1*. We then performed ChIP of Maf1, the TFIIIC members Tfc1 and Tfc6, the TFIIIB members Bdp1 and Brf1, and the Pol III members Rpc40 and Rpc82 and examined their associations. Consistent with our genome-wide analysis, Maf1-HA occupancy at *SCR1* increased significantly during repression (Figure 6B). Maf1 occupancy spans the TATA, transcription start site, and the A and B boxes and therefore does not uniquely identify Pol III as the partner for Maf1. However, this system revealed interesting associations of the basal machinery that bear on Maf1 dynamics, as described below.

Occupancy by the Pol III basal machinery of Pol III genes is exceptionally robust, up to 200-fold enrichment over control loci, providing a large dynamic range to monitor activity-occupancy relationships. For Pol III, we followed Rpc82 (dedicated to Pol III) and Rpc40 (also a member of RNA Pol I). Localization of both subunits at *SCR1* centered just after the transcription start site. Initial occupancy levels for Rpc82 were high and for Rpc40 were extremely high (note the different scales in Figure 1C). Acute repression ($t \leq 25$ min), the time

period during which repression is established (Roberts et al., 2003), is accompanied by a several-fold reduction in apparent Rpc40 association, whereas the reduction with Rpc82 was modest (Figure 6C, bottom panels). However, the occupancy levels of these two subunits at *SCR1* during repression were similar and still highly enriched: about 30- to 50-fold over the control locus. At other Pol III loci, Rpc40 and Rpc82 occupancy is typically reduced 2- to 4-fold during nutrient deprivation, but their final occupancy likewise remains very high (Roberts et al., 2003). As dephosphorylated Maf1 shows specific and increased interaction with Pol III, Maf1 occupancy at Pol III loci during repression may involve its interaction with a repressed form of Pol III (see Discussion).

For TFIIIC, both Tfc1 and Tfc6 occupied *SCR1* during active transcription and greatly increased association during acute repression, consistent with previous work at other Pol III genes (Roberts et al., 2003) and with the notion that transcription by Pol III partially displaces TFIIIC from its binding sites, located in the transcribed region. Interestingly, Tfc1 occupancy was highest at the A/B box region, whereas Tfc6 peaks at the terminator (Figure 6C, top right), consistent with in vitro experiments showing Tfc6 association with Pol III terminators (Geiduschek and Kassavetis, 2001).

Activity-occupancy relationships for TFIIIB are of particular interest as current models for repression involve Maf1 interfering with the binding of TFIIIB to Pol III genes (Desai et al., 2005; Upadhya et al., 2002). However, we find that Brf1 and Bdp1occupancy (which centered over the TATA box) remained essentially constant during acute repression (Figure 6C, middle panels). For Bdp1, we extended these analyses to include two additional tRNA genes (Figure S6), and likewise observed occupancy levels during acute repression very near their initial levels. Taken together with previous data that examined TBP and Brf1 at additional Pol II genes (Harismendy et al., 2003; Roberts et al., 2003), all three members of TFIIIB remain at Pol III genes during the acute response to nutrient deprivation. We note that prolonged repression (such as observed in stationary phase or prolonged rapamycin treatment) is accompanied by the slow release of TFIIIB from particular promoters (Harismendy et al., 2003; Roberts et al., 2003), but this release occurs long after transcriptional repression has occurred.

During acute repression, TFIIIB remains and TFIIIC occupancy increases at the Pol III loci we have tested, raising the possibility that these complexes might demonstrate increased interaction during acute repression (Roberts et al., 2003). We tested for this interaction through CoIP analysis from standard ChIP extracts. In nutrient-rich conditions, precipitation of Bdp1-Myc coprecipitated Tfc1-HA and coprecipitation increased 4- to 8-fold in nutrient deprivation (Figure S7). Likewise, reciprocal precipitation of Tfc1-HA coprecipitated Bdp1-Myc, and a 6- to 10-fold increase in coprecipitation was observed. An identical set of experiments designed to test for interactions between TFIIIC (Tfc1) and Pol III (Rpc40), as well as TFIIIB and Pol III, revealed significant coprecipitation; however, their associations did not appreciably change during acute repression (Figure S7 and data not shown). Thus, TFIIIC-TFIIIB interactions increase during acute repression, which may serve to help poise the gene for rapid reinitiation of Pol III transcription at the onset of favorable conditions.

Discussion

Whereas Pol II transcription in yeast can be repressed by many independent modes and factors, Pol III repression pathways appear to converge on a central regulator, Maf1. As Maf1 is conserved in eukaryotes, it is important to understand how it integrates repression signals, how this information is relayed to the Pol III system, and whether this involves the direct association of Maf1 with Pol III-transcribed genes.

The Regulation of Maf1 Dephosphorylation

Here, we reveal Maf1 as a phosphoprotein that is phosphorylated during favorable growth conditions and rapidly dephosphorylated in response to multiple conditions that repress Pol III, raising the possibility that Maf1 integrates cell conditions through its phosphorylation status. The diverse treatments that repress Pol III each engage a different signaling pathway (glucose, TOR, cell integrity, or DNA damage) and involve the activation of different sets of kinases and phosphatases. We note that our nutrient deprivation conditions likely involve the response to glucose deprivation, as we observe rapid Maf1 dephosphorylation even in rich media lacking glucose (data not shown). Here, we provide initial evidence for the involvement of Pkc1 in the response to nutrient deprivation. Pkc1 has previously been linked to the cell integrity pathway and survival during carbon source starvation (Willis et al., 2004). However, Pol III repression caused by rapamycin does not require Pkc1 (Willis et al., 2004), suggesting that nutrient deprivation and rapamycin converge on Maf1 via different signaling pathways. A role for PP2A in regulating Maf1 dephosphorylation is suggested by the dephosphorylation of Maf1 in *tpd3Δ* cells (Figure S5A) and strongly supported by the studies of Lefebvre and colleagues (Oficjalska-Pham et al., 2006) during rapamycin treatment. Future studies on this aspect ofMaf1 functionwill focus on identifying the additional kinases and phosphatases that mediate these diverse repression pathways and on understanding how they impact Maf1 phosphorylation status.

Maf1 Dephosphorylation Promotes Nuclear Localization and Pol III Interaction

Having observed Maf1 dephosphorylation during repression, the key question was how this modification is relayed to the Pol III system. Earlier studies revealed a small portion of Maf1 in association with Pol III under favorable growth conditions (Pluta et al., 2001) but did not examine whether this interaction was altered during repression. However, a recent study tested this and reported no increase in the interaction between Maf1 and Pol III during repression (Desai et al., 2005). In contrast, we observe a dramatic increase in the interaction between Pol III and Maf1 during repression and reveal a specificity for the dephosphorylated form of Maf1 in this interaction. Furthermore, Maf1 mutants that fail to become fully dephosphorylated during nutrient deprivation are defective in Pol III association. In addition, we show that wt Maf1 relocalizes to the nucleus during repression and that Maf1 mutants that are defective in dephosphorylation are defective for nuclear localization. Taken together, these results suggest that nutrient and stress signaling pathways utilize Maf1 dephosphorylation to regulate the proportion of Maf1 in the nucleus, and the proportion of Maf1 in association with Pol III, to relay cellular conditions to the Pol III system. These conclusions also emerge from independent work from Lefebvre and colleagues (Oficjalska-Pham et al., 2006).

Maf1 Associates with Pol III-Transcribed Genes during Repression

A key mechanistic question is whether Maf1 impacts the Pol III system via a direct association with Pol III-transcribed genes. Here, we show that Maf1 associates with Pol III-transcribed genes and displays enhanced association during repression, suggesting that direct physical interaction with Pol III genes is an important attribute of repression by Maf1 (Figure 7). Our results contrast with a recent study (Desai et al., 2005) that did not observe Maf1 at one particular tRNA gene (*SUP54 tRNA*^{Leu}), tested by the ChIP approach. This may be reconciled by considering the moderate efficiency with which Maf1 can be localized by ChIP at certain tRNA genes. However, as we examined Maf1 localization genome wide, we were able to examine the entire set of Pol III-transcribed genes (281 loci) as a class, which clearly revealed the presence of Maf1 and its enrichment at Pol III loci during repression.

Occupancy of Maf1 and the Pol III Machinery at Pol III Loci during Repression

Nutrient deprivation reduces the apparent occupancy of Pol III at *SCR1* and other Pol III loci (Harismendy et al., 2003; Roberts et al., 2003). However, we show that Pol III occupancy still remains very high in comparison to control loci. A common interpretation of ChIP data is that the signal obtained in a ChIP experiment and occupancy of the template examined are identical parameters that scale linearly. This interpretation may generally apply when considering a DNA binding protein that binds in one mode to a single available site. However, for factors with different modes of DNA interaction, the measured occupancy may in part reflect the mode/extent of DNA interaction. For example, an RNA polymerase can engage a DNA locus in one of many modes (closed, open, abortively initiating, elongating, etc.) or simply be linked to the region via protein tethering. Thus, an alternative explanation for the reductions in Pol III ChIP efficiency is that a significant fraction of inactive Pol III still remains at repressed targets, but transcriptional inactivity reduces interactions between Pol III and target DNA. For example, repressed Pol III might be tethered to Pol III loci through interactions with TFIIIB and/or TFIIIC during acute repression. Importantly, the presence of Pol III at repressed loci provides an explanation for Maf1 association dynamics described below.

Models for Maf1 in Conferring Pol III Repression

Previous models for Maf1 action involved Maf1 preventing the assembly of TFIIIB at Pol III promoters and also interfering with the recruitment of Pol III to promoters (Desai et al., 2005). This model would predict that repressed Pol III genes would lack TFIIIB and Pol III in vivo. However, our ChIP studies show that all three components of TFIIIB remain fully associated with Pol III genes during acute repression, when repression is established. Furthermore, although there is a moderate reduction in Pol III association during acute repression at certain genes (as described above), Pol III occupancy still remains quite high. Thus, our results argue that virtually all of TFIIIB, and a significant proportion of Pol III, is retained at Pol III loci during acute repression in vivo, during the critical phase when transcriptional repression is established. Previous in vitro studies have clearly shown that Maf1 can interfere with the ability of TFIIIB to form new complexes at Pol III promoters and that this likely involves the inactivation of a component of TFIIIB (likely Brf1) (Desai et al., 2005; Upadhya et al., 2002). These results are easily accommodated in the revised model, described below.

Our observations suggest the following speculative model for Maf1 in Pol III system regulation (Figure 7). During normal growth conditions, only a small portion of Maf1 is dephosphorylated, which attenuates the Pol III transcriptome through association with a small proportion of Pol III, explaining why Pol III transcription increases moderately in *maf1Δ* cells. Diverse stress conditions lead to the rapid dephosphorylation of Maf1, greatly increasing the proportion of Maf1 molecules in the nucleus and enabling its association with Pol III; this interaction underlies the increased occupancy of Maf1 at Pol III genes during repression. Transcriptional repression reduces the crosslinking efficiency of Pol III to the DNA. However, the large increase in Maf1-Pol III protein-protein interactions compensates for the reduction in Pol III-DNA interactions, providing the observed increase in Maf1 occupancy at Pol III genes during repression. We suggest that the ability of Maf1 to prevent the assembly of new TFIIIB complexes (Desai et al., 2005;Upadhya et al., 2002) is an attribute of prolonged repression, useful for maintaining the repressed state, but is not an attribute of acute repression, during which TFIIIB remains bound to Pol III promoters.

In conclusion, our work reveals several steps in the mechanism of Pol III regulation: Maf1 functions as an integrator of environmental conditions and signaling pathways through its phosphorylation state, with stress leading to dephosphorylation, increased nuclear localization,

association with Pol III, targeting to Pol III genes, and alterations in basal factor interactions that together lead to transcriptional repression.

Experimental Procedures

Strain Construction

All *S. cerevisiae* strains are S288C background (unless otherwise indicated), with genotypes in Table S2. Subunits of Pol III (Rpc40 and Rpc82), TFIIIB (Bdp1 and Brf1), TFIIIC (Tfc1 and Tfc6), and Maf1 were tagged at their C termini with either 13 copies of the Myc epitope or three copies of the hemagglutinin (HA) epitope through integration at their genomic locus.

Growth Conditions and Extract Preparations

Initital growth: rich medium (1 × YP or synthetic complete medium lacking uracil with 2% glucose) grown toOD_{600} 0.7 at 30°C (t = 0). Nutrient deprivation: cells were collected and resuspended in 0.15×YP lacking glucose or 0.15× synthetic complete media lacking uracil and glucose and grown at 30°C for the time indicated. Drug additions: 100 nM rapamycin, 0.08% (w/v) methyl methanesulfonate (MMS), and 250 μM chlorpromazine (CPZ). Extracts from crosslinked cells (for CoIP and ChIP): cells were grown in rich media to OD_{600} 0.7, crosslinked overnight with 1% formaldehyde, washed twice in TBS, and pelleted. Pellets were lysed in ChIP lysis buffer (140 mM NaCl, 50mMHEPES, 1mMEDTA, 1%Triton X-100, and 0.1% sodium deoxycholate with protease inhibitors) by mechanical bead disruption. Lysed extracts were centrifuged, and the pellet (enriched for chromatin and membrane fractions) was retained, whereas the supernatant (enriched for cytoplasm/nucleoplasm) was discarded. The pellet was then resuspended in ChIP lysis buffer, sonicated (to release the chromatin), and recentrifuged (to remove the membrane fraction), and the supernatant was collected as the chromatin-enriched fraction. Extracts (noncrosslinked) and CoIP conditions (for both crosslinked and noncrosslinked extracts) were performed by standard methods, with details provided in the Supplemental Experimental Procedures.

ChIP and qPCR

ChIP and genome-wide ChIP were performed as described previously (Roberts et al., 2003), with modifications described in the Supplemental Experimental Procedures. The Maf1 occupancy dataset is in Table S4. qPCR was performed as described previously (Roberts et al., 2003). Primers can be found in Table S3. Occupancy in ChIP samples was determined by dividing the relative abundance of a particular region (*SCR1* A–F or $tRNA^{Lys}[CUU]G1$) by the relative abundance of a control region (*TRA1* ORF).

Preparation of Extracts and Immunoprecipitations

Noncrosslinked extracts (for 2D-gel electrophoresis and radiolabeling experiments): cells were grown in selective media to an OD_{600} of 0.7, and after centrifugation, pellets were immediately frozen in liquid nitrogen. Pellets were thawed and washed one time in breaking buffer (50mM Tris-HCl [pH 7.5], 5mMEDTA, 12% glycerol, 0.1% Triton X-100, 250 mM NaCl, 0.5 mM DTT, protease inhibitors, and phosphatase inhibitors [2× Phosphatase Inhibitor Cocktail Set 1, Calbiochem + 0.2 mM Sodium Flouride and 0.2 mM β-Glycerophosphate]). Cells were lysed in breaking buffer by mechanical bead disruption, and after centrifugation, the supernatant was collected.

For CoIPs, extracts from crosslinked cells (600 μg) were added to 1.0×10^7 Pan Mouse IgG Dynabeads (Dynal Biotech, preincubated with BSA and either 2 μg anti-HA [12CA5] or 1.3 μg anti-Myc [9E11, Genetex]) for 4 hr at 4°C. The beads were washed four times with ChIP lysis buffer (250 mM NaCl). Samples were eluted directly into SDS sample buffer and

incubated for 15 min at 90°C before running on SDS-PAGE. Antibodies for Western detection were either polyclonal anti-HA (Abcam 9110) or monoclonal anti-Myc (9E10). In Figure 4, extract (800 μg) was incubated with 4.0×10^7 Pan Mouse IgG Dynabeads in low-salt IP buffer (see 2D-gel electrophoresis above) for 7 hr at 4°C and washed three times in high-salt IP buffer (see 2D-gel electrophoresis above). For determining phosphorylation status of Maf1 in association with Pol III (Figure 3C), 1.0×10^7 beads of HA or Myc Pan mouse IgG Dynabeads (as above) were incubated with extract (600 μg) from crosslinked YBC2077 in ChIP lysis buffer (w/250 mM NaCl) for 4 hr at 4°C. Beads were washed three times with ChIP lysis buffer (w/250 mM NaCl), one time with ChIP lysis buffer (w/375 mM NaCl), and eluted in SDS-PAGE sample buffer, immunoblotted, and probed with polyclonal anti-HA (Abcam 9110).

Two-Dimensional Gel Electrophoresis of Maf1

Whole-cell extract (800 μg) was incubated with 2.0×10^7 Pan mouse IgG Dynabeads (Dynal Biotech, preincubated with BSA and 2 μg anti-HA [12CA5]) for 4 hr at 4°C in low-salt IP buffer (50 mM Tris-HCL, 1 mM EDTA, 10% glycerol, 0.05% Tween 20, 100 mM NaCl, 0.5 mM DTT, protease inhibitors, and phosphatase inhibitors [see extract preparation]). Beads were washed three times with high-salt IP buffer (same as low-salt IP buffer except 250 mM NaCl) and resuspended in urea sample buffer + resolyte (40 mM Tris-HCL [pH 7.5], 8Murea, 4%CHAPs, 2%Bio-lyte 3–10 Ampholyte, and bromophenol blue). Samples were then subjected to either isoelectric focusing (using Readystrip IPG strips pH 3–10 [Biorad]) or SDS-PAGE.

Radiolabeling Maf1

Cultures of YBC2077 were grown to OD_{600} 0.5 before ^{32}P was added for 40 min, and whole-cell extracts prepared (as above). Radiolabeled extract (500 μg) was incubated with 2.0×10^7 Pan mouse IgG Dynabeads for 5 hr at 4°C. Pellets were washed four times with high-salt IP buffer (50 mM Tris-HCL, 1 mM EDTA, 10% glycerol, 0.05% Tween 20, 500 mM NaCl, 0.5 mM DTT, protease inhibitors, and phosphatase inhibitors) and incubated for an additional 4.5 hr in wash buffer. Beads were resuspended in SDS sample buffer, and the supernatant was separated on a 7.5% acrylamide gel.

Phosphatase Treatment of Maf1

Crosslinked extract (100 μg) was incubated with 1.0×10^7 Pan mouse IgG Dynabeads (Dynal Biotech, preincubated with BSA and 2 μg anti-HA [12CA5]) for 4 hr at 4°C. Beads were washed twice with ChIP lysis buffer containing 250 mM NaCl, once with ChIP lysis buffer containing 375 mM NaCl, and once with LiCl wash buffer (10 mM Tris-HCl [pH 8.0], 250 mM LiCl, 0.5% NP-40, 0.5% sodium deoxycholate, and 1mMEDTA). Beads were washed and then equilibrated in 45 μl Lambda protein phosphatase buffer (50 mM HEPES, 100mM NaCl, 0.1mMEGTA, 2mM DTT, 0.01% Brij 35, 2 mM$MnCl^2$, pH 7.5 at 25°C). Lambda phosphatase (800 U, NEB) was added at 30°C for 30 min, washed once with ChIP lysis buffer (375 mM NaCl), and resuspended in sample buffer for SDS-PAGE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Brian Dalley, Qian Yang, and Adrienne Tew for processing genome-wide ChIP slides, Jason Lieb for genomics advice, Kevin Struhl for strains, Magdalena Boguta for the Maf1-HA plasmid, Derick Holt for statistical analysis, and Issam Aldiri for isolation of the *maf1-122* mutation. We thank Don Ayer, Craig Kaplan, and Tim Parnell for comments on the manuscript. Wethank David Virshup for the *tpd3Δ* strain and Yu Jiang for the *pph21Δ pph22Δ* strain. We thank O. Lefebvre and colleagues for helpful discussions. This work was supported by GM60415 (to B.R.C)

for support of A.J.S and B.W.; CA24014 and the Huntsman Cancer Institute for microarray and core facilities; 5 T32 DK007115 (to J. Kushner) for support of D.N.R.; and Howard Hughes Medical Institute (for support of J.T.H., reagents, and genomics resources).

References

Chaussivert N, Conesa C, Shaaban S, Sentenac A. Complex interactions between yeast TFIIIB and TFIIIC. J Biol Chem 1995;270:15353–15358. [PubMed: 7797524]

Clarke EM, Peterson CL, Brainard AV, Riggs DL. Regulation of the RNA polymerase I and III transcription systems in response to growth conditions. J Biol Chem 1996;271:22189–22195. [PubMed: 8703032]

Desai N, Lee J, Upadhya R, Chu Y, Moir RD, Willis IM. Two steps in Maf1-dependent repression of transcription by RNA polymerase III. J Biol Chem 2005;280:6455–6462. [PubMed: 15590667]

Dieci G, Sentenac A. Facilitated recycling pathway for RNA polymerase III. Cell 1996;84:245–252. [PubMed: 8565070]

Dieci G, Sentenac A. Detours and shortcuts to transcription reinitiation. Trends Biochem Sci 2003;28:202–209. [PubMed: 12713904]

Felton-Edkins ZA, Kenneth NS, Brown TR, Daly NL, Gomez-Roman N, Grandori C, Eisenman RN, White RJ. Direct regulation of RNA polymerase III transcription by RB, p53 and c-Myc. Cell Cycle 2003;2:181–184. [PubMed: 12734418]

Galli G, Hofstetter H, Birnstiel ML. Two conserved sequence blocks within eukaryotic tRNA genes are major promoter elements. Nature 1981;294:626–631. [PubMed: 7312050]

Geiduschek EP, Kassavetis GA. The RNA polymerase III transcription apparatus. J Mol Biol 2001;310:1–26. [PubMed: 11419933]

Ghavidel A, Schultz MC. TATA binding protein-associated CK2 transduces DNA damage signals to the RNA polymerase III transcriptional machinery. Cell 2001;106:575–584. [PubMed: 11551505]

Gomez-Roman N, Grandori C, Eisenman RN, White RJ. Direct activation of RNA polymerase III transcription by c-Myc. Nature 2003;421:290–294. [PubMed: 12529648]

Harismendy O, Gendrel CG, Soularue P, Gidrol X, Sentenac A, Werner M, Lefebvre O. Genome-wide location of yeast RNA polymerase III transcription machinery. EMBO J 2003;22:4738–4747. [PubMed: 12970186]

Kassavetis GA, Braun BR, Nguyen LH, Geiduschek EP. S. cerevisiae TFIIIB is the transcription initiation factor proper of RNA polymerase III, while TFIIIA and TFIIIC are assembly factors. Cell 1990;60:235–245. [PubMed: 2404611]

Kwapisz M, Smagowicz WJ, Oficjalska D, Hatin I, Rousset JP, Zoladek T, Boguta M. Up-regulation of tRNA bio-synthesis affects translational readthrough in maf1-delta mutant of Saccharomyces cerevisiae. Curr Genet 2002;42:147–152. [PubMed: 12491008]

Li Y, Moir RD, Sethy-Coraci IK, Warner JR, Willis IM. Repression of ribosome and tRNA synthesis in secretion-defective cells is signaled by a novel branch of the cell integrity pathway. Mol Cell Biol 2000;20:3843–3851. [PubMed: 10805727]

Lieb JD, Liu X, Botstein D, Brown PO. Promoter-specific binding of Rap1 revealed by genome-wide maps of protein- DNA association. Nat Genet 2001;28:327–334. [PubMed: 11455386]

Moqtaderi Z, Struhl K. Genome-wide occupancy profile of the RNA polymerase III machinery in Saccharomyces cerevisiae reveals loci with incomplete transcription complexes. Mol Cell Biol 2004;24:4118–4127. [PubMed: 15121834]

Nierras CR, Warner JR. Protein kinase C enables the regulatory circuit that connects membrane synthesis to ribosome synthesis in Saccharomyces cerevisiae. J Biol Chem 1999;274:13235–13241. [PubMed: 10224082]

Oficjalska-Pham D, Harismendy O, Smagowicz WJ, Gonzalez de Peredo A, Boguta M, Sentenac A, Lefebvre O. General repression of RNA polymerase III transcription is triggered by protein phosphatase type 2A-mediated dephosphorylation of Maf1. Mol Cell 2006;22(this issue):623–632. [PubMed: 16762835]

Pluta K, Lefebvre O, Martin NC, Smagowicz WJ, Stanford DR, Ellis SR, Hopper AK, Sentenac A, Boguta M. Maf1p, a negative effector of RNA polymerase III in Saccharomyces cerevisiae. Mol Cell Biol 2001;21:5031–5040. [PubMed: 11438659]

Roberts DN, Stewart AJ, Huff JT, Cairns BR. The RNA polymerase III transcriptome revealed by genome-wide localization and activity-occupancy relationships. Proc Natl Acad Sci USA 2003;100:14695–14700. [PubMed: 14634212]

Roberts S, Colbert T, Hahn S. TFIIIC determines RNA polymerase III specificity at the TATA-containing yeast U6 promoter. Genes Dev 1995;9:832–842. [PubMed: 7705660]

Schramm L, Hernandez N. Recruitment of RNA polymerase III to its target promoters. Genes Dev 2002;16:2593–2620. [PubMed: 12381659]

Upadhya R, Lee J, Willis IM. Maf1 is an essential mediator of diverse signals that repress RNA polymerase III transcription. Mol Cell 2002;10:1489–1494. [PubMed: 12504022]

White RJ. RNA polymerase III transcription–a battleground for tumour suppressors and oncogenes. Eur J Cancer 2004;40:21–27. [PubMed: 14687785]

Willis IM, Desai N, Upadhya R. Signaling repression of transcription by RNA polymerase III in yeast. Prog Nucleic Acid Res Mol Biol 2004;77:323–353. [PubMed: 15196897]

Zaragoza D, Ghavidel A, Heitman J, Schultz MC. Rapamycin induces the G0 program of transcriptional repression in yeast by interfering with the TOR signaling pathway. Mol Cell Biol 1998;18:4463–4470. [PubMed: 9671456]

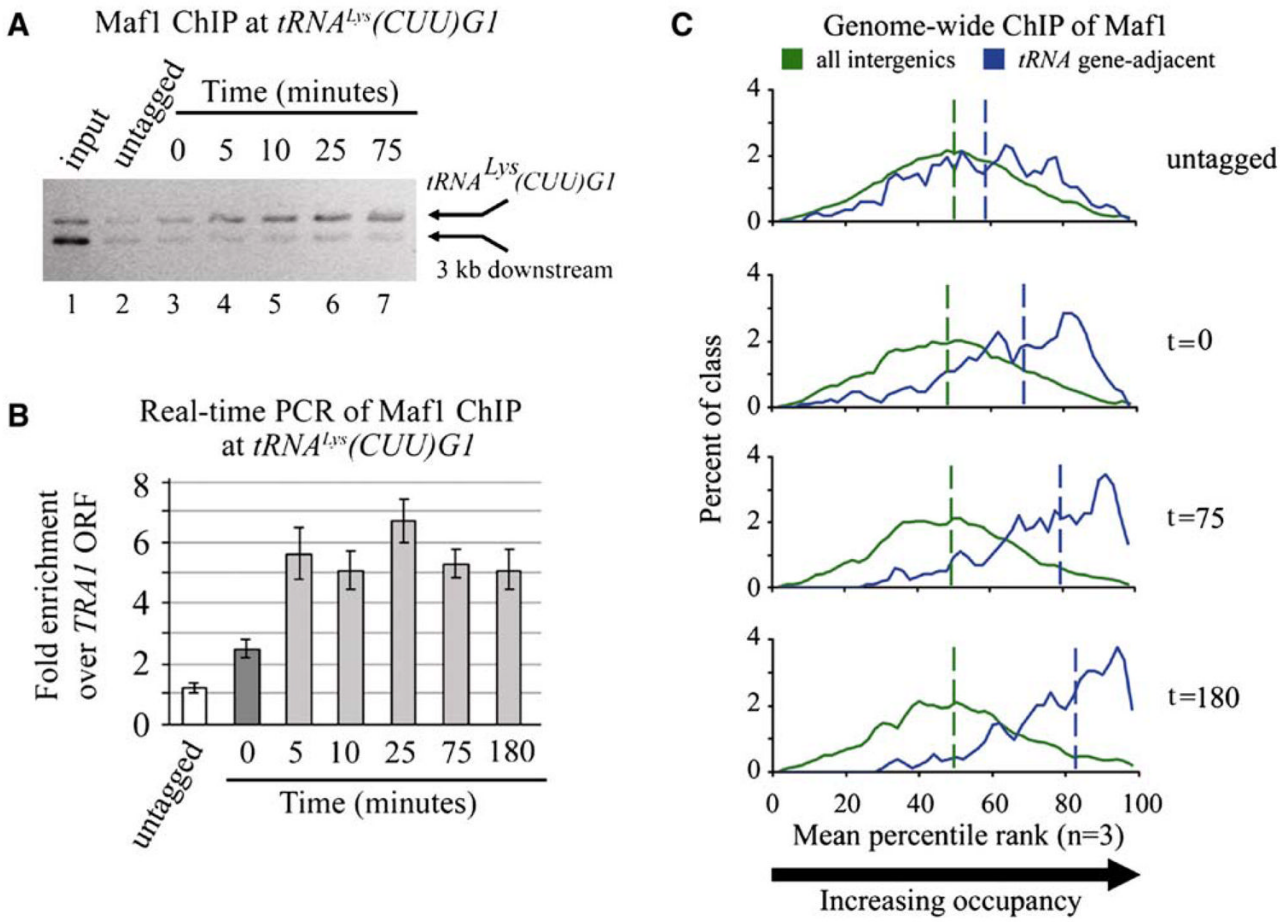

Figure 1.

Maf1 Occupancy of Pol III-Transcribed Genes Increases during Repression

(A) Maf1 occupancy of *tRNA^{Lys}(CUU)G1*. ChIP of untagged strain (FT3) and Maf1-HA strain YBC2062 in exponential growth (t = 0) or after nutrient deprivation (t = 5, 10, 25, and 75). Amplicons: the upper band spans *tRNA^{Lys}(CUU)G1*, and the lower a region of the Pol II-transcribed *MDS3* ORF, located about 3 kb downstream.

(B) Real-time qPCR quantification of *tRNA^{Lys}(CUU)G1* occupancy. Values are the average of three quantifications ± one SD.

(C) Genome-wide occupancy of Maf1. ChIP was performed on an untagged strain (FT3) in exponential growth or on the Maf1-HA strain YBC2062 in exponential growth (t = 0) or after nutrient deprivation (t = 75 and 180). The mean percentile rank (MPR) of three independent experiments is presented. The vertical dashed line shows the median MPR for each class: either all intergenics (black) or the subset of intergenics directly adjacent to tRNA genes (gray). The unbroken lines are the 5-bin moving average of the percent of spots for each class, derived from one percentile-sized bins.

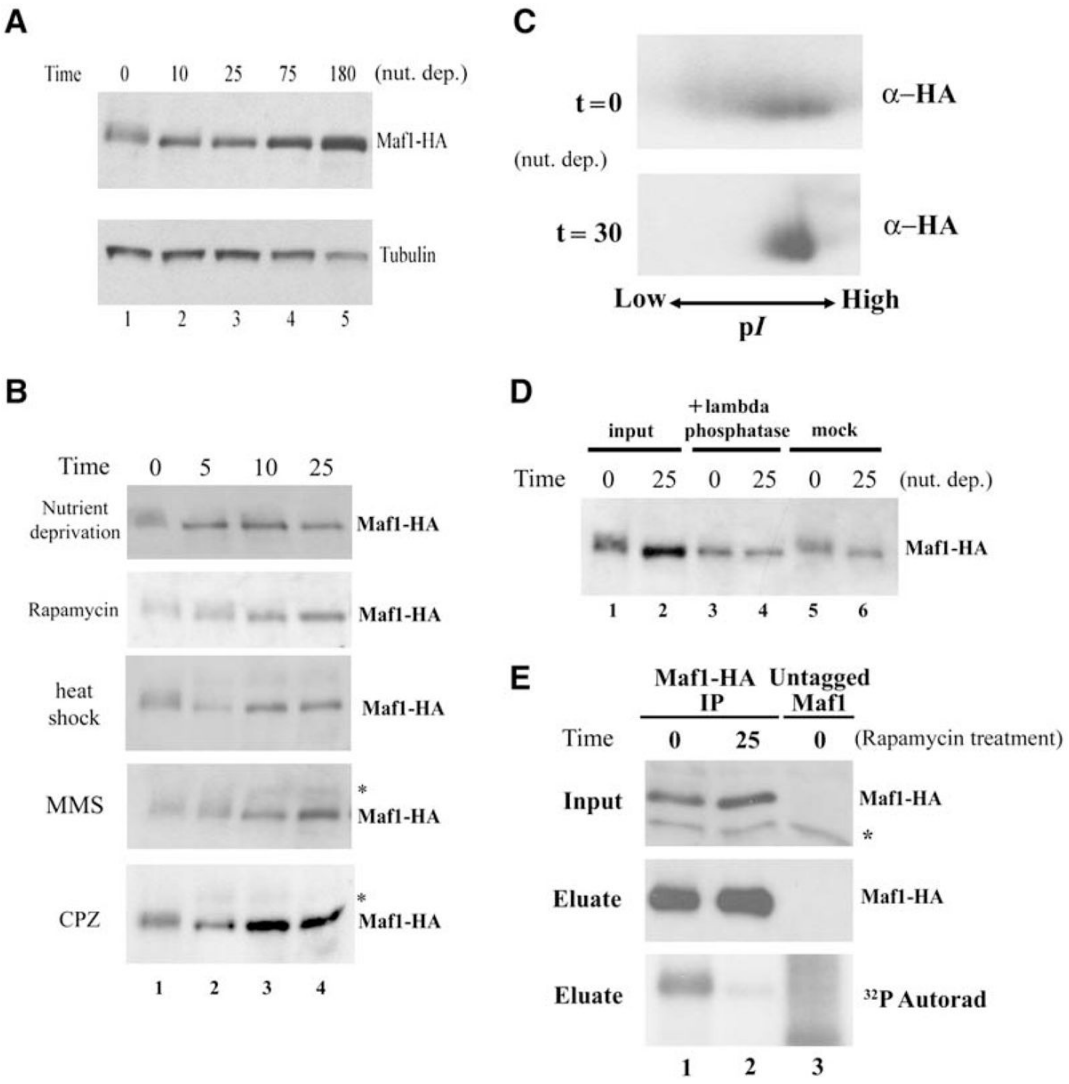

Figure 2.

Nutrient Deprivation or Stress Confers Maf1 Dephosphorylation

(A) Maf1-HA protein levels during the nutrient deprivation timecourse. A Western blot of whole-cell extracts (25 μg) from cells (YBC2062) crosslinked with 1% formaldehyde was probed with anti-HA or anti-tubulin antibodies.

(B) Maf1 migration is altered after nutrient deprivation, rapamycin treatment (100 nM), MMS treatment (0.08% w/v), or chlorpromazine (CPZ, 250 μM). A Western blot of whole-cell extracts (25 μg) from crosslinked cells (YBC2077) was probed with anti-HA antibody. A very weak, slower-migrating form of Maf1-HA is observed after some treatments (asterisk).

(C) Maf1 phosphorylation monitored by 2D-gel electrophoresis. Maf1-HA was immunoprecipitated from extracts derived from exponentially growing cultures (t = 0) or after nutrient deprivation for 30 min (t = 30), and separated (1) by isoelectric focusing and (2) by SDS-PAGE. Immunoblots were probed with anti-HA antibody.

(D) Maf1 is a phosphoprotein. Maf1-HA from crosslinked extracts: input (lanes 1 and 2), or captured on beads and treated with (lanes 3 and 4) or without (lanes 5 and 6) lambda phosphatase (see Experimental Procedures). Samples were separated by SDS-PAGE, blotted, and probed with anti-HA antibody.

(E) Radiolabeling reveals dephosphorylation of Maf1 after rapamycin treatment. Labeled Maf1-HA was immunoprecipitated from extracts (see Experimental Procedures) separated by SDS-PAGE and examined by autoradiography.

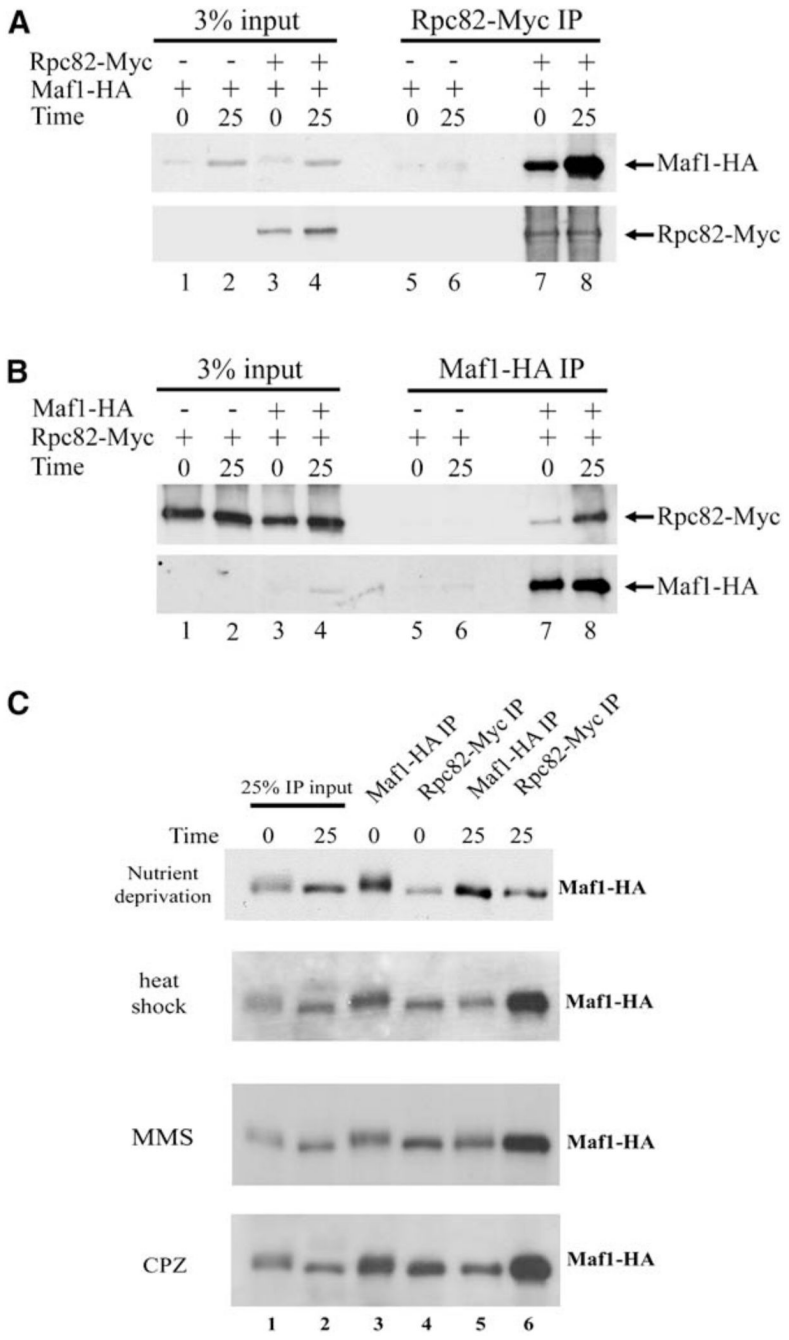

Figure 3.

Dephosphorylated Maf1 Interacts with Pol III during Repression

(A and B) CoIP with anti-Myc ([A], Rpc82-Myc IP) or anti-HA ([B], Maf1-HA IP) immunoblotted for Maf1-HA or Rpc82-Myc as indicated at right. The presence (+) or absence (−) of an epitope tag on the subunit is indicated. Time indicates exponential growth in nutrientrich media (0: lanes 1, 3, 5, and 7) or 25 min in nutrient deprivation (25: lanes 2, 4, 6, and 8). Strains: YBC2062, (A), lanes 1, 2, 5, and 6; YBC2072, (B), lanes 1, 2, 5, and 6; and YBC2077, (A) and (B), lanes 3, 4, 7, and 8.

(C) Pol III specifically interacts with the dephosphorylated form of Maf1. A strain bearing Rpc82-Myc and Maf1-HA (YBC2077) was grown exponentially (t = 0) and subjected to the

treatments as described in Figure 2 for 25 min. Crosslinked extracts were directly loaded (lanes 1 and 2) or subjected to immunoprecipitation with either anti-HA (lanes 3 and 5) or anti-Myc (lanes 4 and 6) antibodies. Samples were immunoblotted with anti-HA.

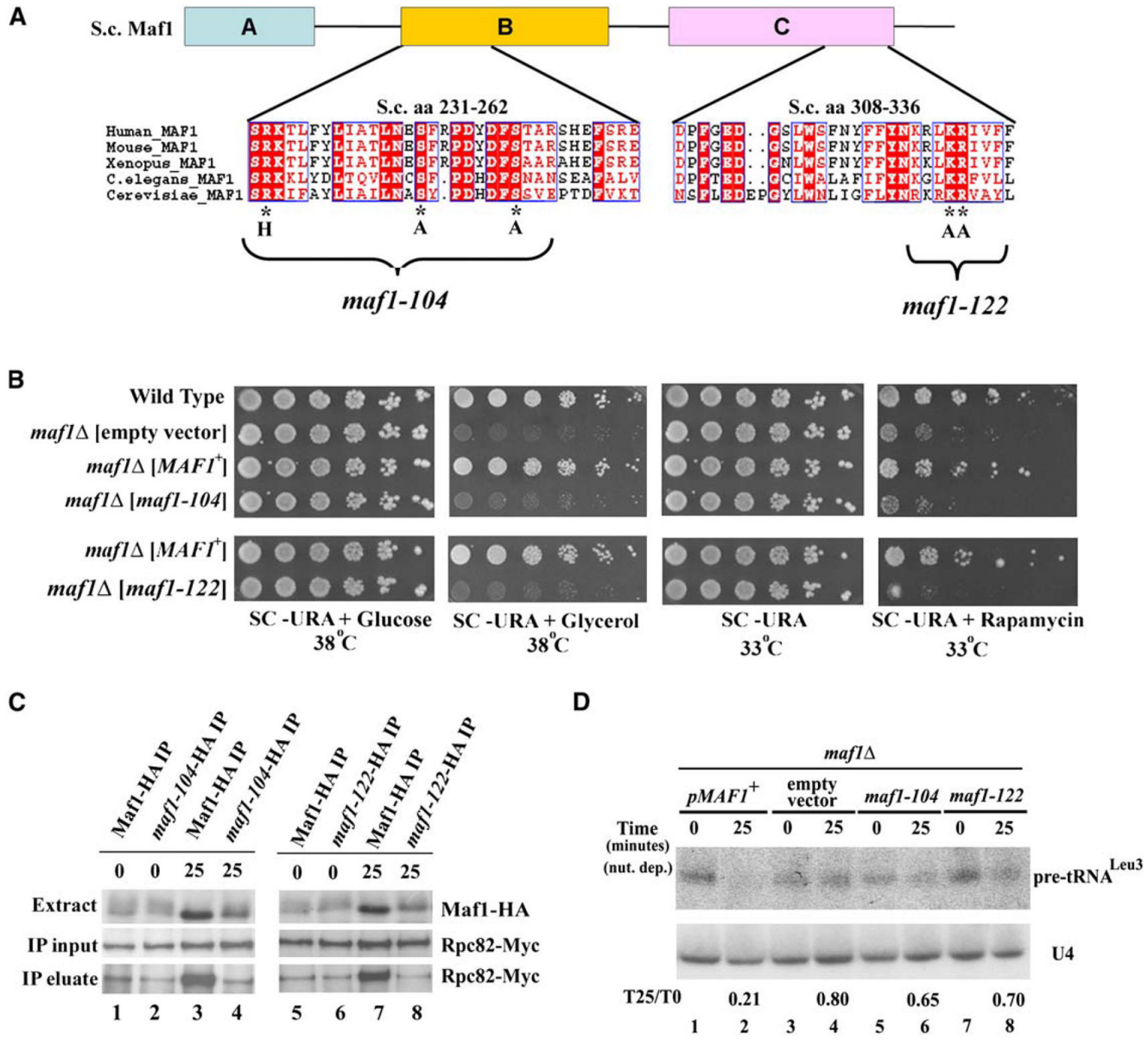

Figure 4.

Maf1 Mutant Analysis

(A) Location of *maf1* mutations in the conserved B and C boxes.

(B)Growth of *maf1* mutants are monitored on medium harboring glycerol as the sole carbon source at 38°C and on medium containing rapamycin (35 nM).

(C) Phosphorylation and Pol III association are monitored in *maf1* mutants. Crosslinked extracts were either loaded directly or subjected to immunoprecipitation with anti-HA. Inputs and eluates are probed with anti-Myc and anti-HA antibodies.

(D) Northern analysis of $tRNA^{Leu3}$ transcription during nutrient deprivation. Values are the ratio of expression in nutrient replete conditions (t = 0) over the expression during acute repression (t = 25 min).

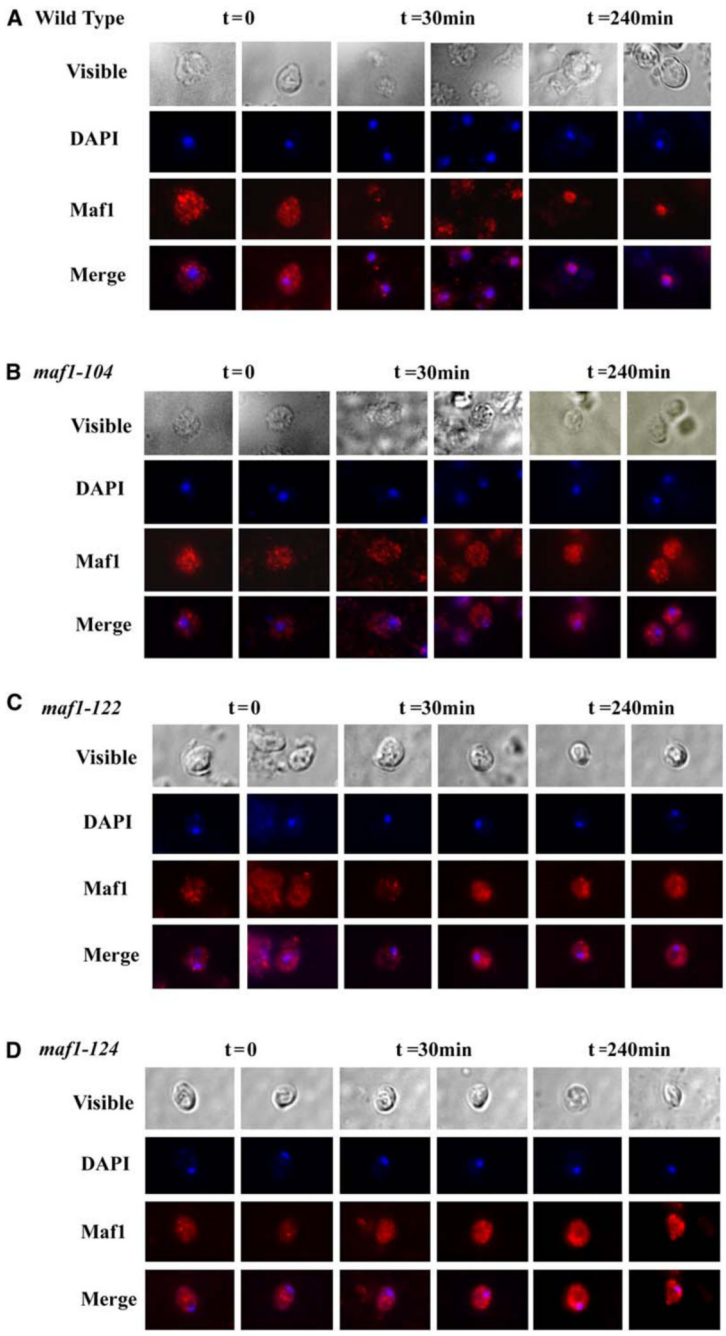

Figure 5.

Relocalization of Maf1 to the Nucleus during Nutrient Deprivation

Localization of Maf1-HA and mutant derivatives during nutrient deprivation for 0, 30, or 240 min. Maf1-HA, red (FITC); DNA (nucleus), blue (DAPI). Merged, overlays of the Maf1 and DAPI images.

(A) Localization of wt Maf1.

(B) Localization of *maf1-104*.

(C) Localization of *maf1-122*.

(D) Localization of *maf1-124*.

Strains: (A), YBC2026 [p1863]; (B), YBC2026 [p1841]; (C), YBC2026 [p1954]; and (D), YBC2026 [p2153]. For relocalization during rapamycin treatment, see Figure S4.

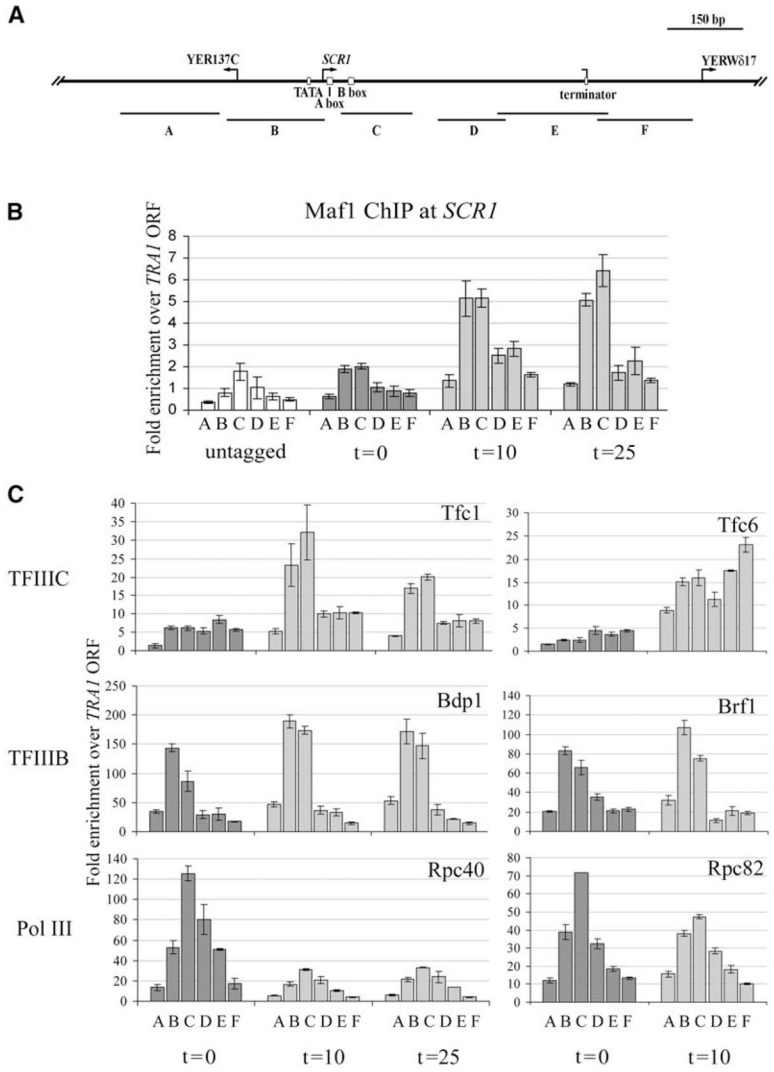

Figure 6.

Tiling of *SCR1* Localizes Maf1 and the Basal Transcription Machinery during Acute Repression

(A) Amplicons (A–F) used, placed on the physical map of the *SCR1* region (7).

(B and C) qPCR quantification of factor occupancy ([B], Maf1-HA; [C], Tfc1-HA, Tfc6-HA, Bdp1-Myc, Brf1-HA, Rpc40-HA, and Rpc82- HA) at *SCR1* tiles during active transcription (exponential growth: t = 0; dark gray) or acute repression (nutrient deprivation: t = 10; light gray). Values are the average of three quantifications ± one SD.

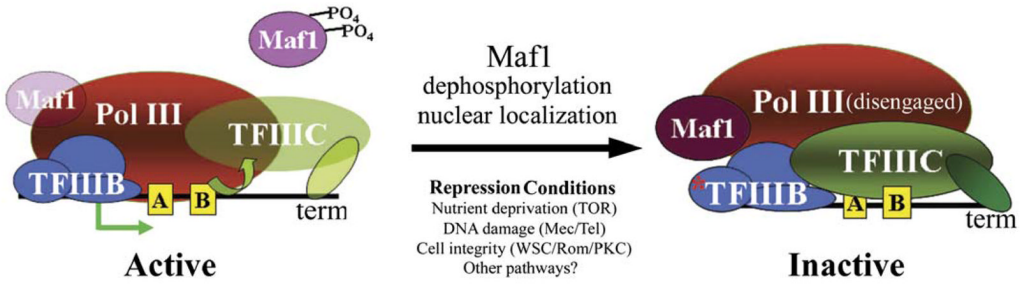

Figure 7.

A Model for Pol III Repression

In favorable growth conditions, the Pol III system (Pol III, TFIIIB, and TFIIIC) forms an initiation complex and commits to RNA synthesis (Active). During elongation, active Pol III may displace (arrow) TFIIIC from the A/B box (semiopaque TFIIIC). TFIIIC may be fully ejected from the template, or remain tethered to the locus, either through interactions with the terminator (via Tfc6) or other members of the Pol III system. In exponential growth, Maf1 is largely phosphorylated, attenuating a small portion of Pol III (semiopaque Maf1). Nutrient deprivation or stress promotes Maf1 dephosphorylation, increasing the proportion of Maf1 molecules in association with Pol III and Pol III targets (Inactive, opaque Maf1). This association attenuates Pol III targets by an unknown mechanism. Pol III inactivity lowers its effective interaction (ChIP) with target DNA, allowing full TFIIIC occupancy. During the acute phase of repression, the interaction between TFIIIC and TFIIIB increases. However TFIIIB slowly releases from certain Pol III targets during prolonged repression (data not shown). Maf1 may directly inhibit Pol III and/or promote TFIIIB inactivation (red asterisk).