CK2-mediated phosphorylation of general repressor Maf1 triggers RNA polymerase III activation

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Abstract

Maf1 protein is a global negative regulator of Pol III transcription conserved from yeast to man. We report that phosphorylation of Maf1 by casein kinase II (CK2), a highly evolutionarily conserved eukaryotic kinase, is required for efficient Pol III transcription. Both recombinant human and yeast CK2 were able to phosphorylate purified human or yeast Maf1, indicating that Maf1 can be a direct substrate of CK2. Upon transfer of *Saccharomyces cerevisiae* from repressive to favorable growth conditions, CK2 activity is required for the release of Maf1 from Pol III bound to a tRNA gene and for subsequent activation of tRNA transcription. In a yeast strain lacking Maf1, CK2 inhibition showed no effect on tRNA synthesis, confirming that CK2 activates Pol III via Maf1. Additionally, CK2 was found to associate with tRNA genes, and this association is enhanced in absence of Maf1, especially under repressive conditions. These results corroborate the previously reported TFIIIB-CK2 interaction and indicate an important role of CK2-mediated Maf1 phosphorylation in triggering Pol III activation.

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Introduction

RNA polymerase (Pol) III is responsible for the transcription of some 300 different genes in yeast (class III genes), mostly tRNA genes (1-3). In-depth analyses of the yeast Pol III transcription system have revealed a cascade of protein-DNA and protein-protein interactions leading to the recruitment of Pol III to its target tRNA genes: binding of the six-subunit TFIIIC factor to the intragenic promoter, TFIIIC-directed recruitment and assembly of the three subunits of TFIIIB (TBP, Brf1 and Bdp1) and subsequent recruitment of the 17-subunit Pol III enzyme (4). High rate of tRNA transcription is achieved through many rounds of re-initiation by Pol III on stable DNA-bound complexes of the initiation factor TFIIIB (5, 6)

Pol III is under control of the general negative regulator Maf1 (7, 8), which binds to Pol III-specific subcomplex C82/34/31 that is required for transcription initiation (9). In the repressive complex Maf1 impairs recruitment of Pol III to a complex of promoter DNA with the initiation factors TFIIB and thus prevents closed-complex formation (6, 9). Maf1 is essential for repressing Pol III transcription in yeast and mediates several signaling pathways (10). In addition to the down-regulation that occurs normally in the stationary phase, Pol III repression accompanying starvation, respiratory growth as well oxidative and replication stress also requires Maf1 (11-13). Maf1 inhibits Pol III transcription via a mechanism that depends on the dephosphorylation and nuclear accumulation of Maf1 followed by its physical association with Pol III at Pol III-transcribed genes genome-wide (8, 14). In contrast Maf1 phosphorylation occurs in favorable growth conditions and is linked to cytoplasmic localization of Maf1 (8, 15).

Maf1 was recently found to be phosphorylated by protein kinases PKA (16, 17), Sch9 (18-20) and TORC1 (21), but participation of other kinases in Maf1 regulation is conceivable. First, phosphorylation by PKA prevents Maf1 nuclear import (17) but does not seem to be involved in direct regulation of Maf1 activity by carbon source or nutrient availability (11, 19). Second, tRNA transcription levels were not significantly changed either by Sch9- or TORC1-dependent phosphorylation of Maf1 (18, 19, 21). Finally, mutating the potential phosphorylation sites for Sch9 and PKA still allowed regulation of Pol III indicating another critical factor (18, 19). The results presented here point to CK2 as a possible missing kinase.

Casein kinase 2 (CK2), a vital, pleiotropic and highly conserved serine/threonine phosphotransferase is involved in transcription-directed signaling, gene control, and cell cycle regulation and has been implicated in critical cellular processes such as proliferation, apoptosis, differentiation, and transformation (22). CK2 is mostly present as a tetramer composed of two catalytic and two regulatory subunits (in yeast Cka1/2 and Ckb1/2, respectively). The regulatory subunits stimulate the catalytic subunits, stabilize the CK2 heterotetramer, and act as a scaffold for specific kinase partners (23) The enzyme is expressed constitutively at a modest level, is predominantly in the nucleus, where it is a component of several chromatin-associated complexes, and is predicted to phosphorylate a broad spectrum of nuclear as well as cytoplasmic proteins involved in multiple aspects of gene expression (24).

It has been shown previously that CK2 is a positive regulator of Pol III transcription in yeast and humans. CK2 is stably associated with TFIIIB (25, 26) and phosphorylates subunits TBP in yeast, and Brf1 and Bdp1 in humans (26, 27). CK2 activity is required for assembly of Pol III transcription initiation complex on gene promoters in human cells (26, 28). Surprisingly, phosphorylation of Bdp1 by CK2 decreases Pol III activity during mitosis in human cells (29).

We previously demonstrated that nuclear localization of Maf1 is not sufficient for Pol III repression but must be accompanied by Maf1 dephosphorylation to ensure interaction with Pol III and inhibition of tRNA transcription (15). We also concluded that phosphorylation of Maf1 is essential for its export out of the nucleus. We thus postulated the existence of a nuclear kinase which would phosphorylate Maf1, thereby breaking its interaction with the Pol III complex and allowing activation of tRNA transcription. Here we show that CK2 is the postulated kinase involved in this process.

Results

Maf1 is phosphorylated by CK2 kinase. CK2 is the evolutionarily conserved kinase involved in numerous pathways regulating gene expression. We reasoned that it might also be involved in Maf1 regulation. To determine effect of CK2 inactivation on Maf1 phosphorylation state *in vivo*, we took

advantage of yeast strains with decreased catalytic activity of CK2 (30). In wild-type (wt) cells grown in glucose-containing medium, two phosphorylated forms of Maf1 are observed in SDS-PAGE as slower migrating additional bands. This pattern of Maf1 phosphorylation was variably altered in the single *cka1-Δ* and double *cka1-Δ cka2^{ts}* mutants. Inhibition of CK2 activity following shift to an elevated temperature resulted in lack of Maf1 phosphorylation of Maf1 in *cka1-Δ cka2^{ts}* cells unlike in wt or *cka1-Δ* strains where we observed no change or only a slight decrease in Maf1 phosphorylation, respectively (Fig. 1).

Because active CK2 have been shown to bind directly TBP subunit of TFIIIB (Ghadivel and Schultz, 2001), we used co-immunoprecipitation experiments to test whether Maf1 was able to interact with CK2 catalytic subunit. Crude extracts were prepared from Cka2-HA tagged cells and control wild type cells with untagged Cka2. Cells were grown in glucose medium, transferred to pre-warmed glycerol medium and harvested after 3 hours. HA-tagged Cka2 was immunoprecipitated from cell extracts with magnetic beads coated with anti-HA antibodies and the immunoprecipitates were examined for the presence of Maf1 by immunoblotting. As shown in Figure 2, Cka2 was efficiently immunopurified from crude extracts (compare lane 1 with lane 2 and lane 3 with lane 4) and hyperphosphorylated form Maf1 was selectively co-immunoprecipitated with HA-tagged Cka2 from glucose cells (compare lane 5 with control lane 7). Maf1 was also detectable in Cka2-HA immune complexes from glycerol cells (compare lane 6 with control lane 8). This result indicates that Maf1 and Cka2, catalytic subunit of CK2, interact with each other.

Next we asked if Maf1 could be directly phosphorylated by CK2 *in vitro*. Recombinant *S. cerevisiae* Maf1 expressed in *Escherichia coli* was incubated with [γ^{32}P]-ATP and purified yeast TAP tagged CK2. Analysis of the reaction outcome by SDS-PAGE and autoradiography indicated that Maf1 was phosphorylated (Fig. 3). Consistent with the idea that CK2 is responsible for the observed phosphorylation of recombinant Maf1, incorporation of [γ^{32}P]-ATP was completely blocked by tetrabromobenzotriazol (TBBt), a specific CK2 inhibitor, (31).

To extend the results obtained using recombinant Maf1, we studied phosphorylation of yeast Maf1 and human Maf1 with highly purified human CK2. Human recombinant Maf1, expressed in insect cells, was mixed with purified human CK2 alpha catalytic subunit in the presence of [γ^{32}P]-ATP resulting in successful phosphorylation (Fig. S1). Notably, human CK2 also phosphorylated yeast Maf1. Therefore the process of Maf1 phosphorylation by CK2 is evolutionarily conserved.

Having established that CK2 phosphorylates Maf1 *in vitro*, we next asked what the specific phosphorylation sites are. The mass-spectrometry analysis of CK2-phosphorylated recombinant yeast Maf1 revealed phosphorylation of serine S388 and at least one phosphorylation site located in serine tract S159-162 (Table S1, repeated experiments 1-3). Importantly, parallel mass spectrometry analysis of Maf1 immunopurified from cells exponentially growing in glucose identified

phosphorylation of both one and two serines within S159-162 tract (Table S1, experiments 4-6). Unfortunately in this analysis S388-containing peptide was not detected thus we were not able to confirm modification at this site. Beside S159-162 sites assigned presumably to CK2, several PKA/Sch9–specific phosphorylation sites were also identified in Maf1 from glucose cells confirming results published previously (17, 19) (Table S1, repeated experiments 4-6). All possible phosphorylation sites detected in CK2-phosphorylated recombinant Maf1 were inactivated resulting in 5StA mutant (S159A, S160A, S161A, S162A, S388A). Without a doubt Maf1 phosphorylation *in vivo* was not observed in 5StA mutant (Fig. 4). Interestingly, concurrent western analysis of 1StA (S388A) shows no effect on Maf1 phosphorylation while analysis of 4StA (S159A, S160A, S161A, S162A) mutant revealed differently migrating forms of Maf1 (Fig. 4). Thereby contribution of both, S159-162 and S388 CK2- phosphorylation sites seems to be similarly important for Maf1 function. Notably, the lack of Maf1 phosphorylation in 5StA mutant seems not to be an effect of protein miss-folding since it complemented growth phenotype of *maf1-Δ* (Fig. S2A). Moreover, inactivation of CK2-phosphorylation sites in Maf1 somewhat decreased Pol III activity (Fig. S2B).

CK2 is necessary for regulation of Maf1 activity by carbon source. As shown previously, Maf1 is phosphorylated in response to a shift from respiratory conditions to fermentative growth (15). To examine a possible role of CK2 in Maf1 phosphorylation in response to carbon source, we used the single *cka1-Δ* and double *cka1-Δ cka2^{ts}* mutants. Cells exponentially growing in a rich glucose medium (YPD) were transferred to a nonfermentable glycerol medium (YPGly) at 30°C then back to the glucose medium and incubated at 37^{o}C. Gradual phosphorylation of Maf1 at 37^{o}C was observed in the control, but phosphorylation was less efficient in *cka1-Δ* and did not occur in the *cka1-Δ cka2^{ts}* mutant cells (Fig. 5A). Maf1 phosphorylation was also inhibited when wild-type cells were transferred from YPGly to YPD supplemented with TBBt (Fig. 5B). Altogether these results suggest participation of CK2 in Maf1 phosphorylation during the switch between respiration and fermentation.

Noteworthy, TBBt only modestly affected Maf1 phosphorylation in cells growing exponentially in YPD (Fig. S3A). Differences observed between the effects of TBBt and mutations in catalytic subunits of CK2 on Maf1 phosphorylation in cells exponentially growing in YPD (Fig. S3 A and B) raised further ideas. First, deletion of a single catalytic subunit of CK2 did not significantly change Maf1 phosphorylation (Fig. 1 and Fig. S3B), indicating a functional redundancy of the two catalytic subunits. Second, specificity of TBBt towards the Cka1 and Cka2 catalytic subunits varied; TBBt precluded phosphorylation of Maf1 only in *cka2-Δ* cells (Fig. S3B), suggesting that Cka1 is more sensitive to this inhibitor.

The correlation of Maf1 phosphorylation and export to the cytoplasm observed previously raised the question of whether a decrease of CK2 activity would concomitantly compromise Maf1 exit from the nucleus. Immunofluorescence microscopy analysis showed that the Maf1-specific signal was uniformly distributed throughout the cell in YPD medium and concentrated in the nuclear compartment in YPGly, which was accompanied by Maf1 dephosphorylation (Fig. S4, compare lanes 1 and 2). Upon transfer of the cells back to YPD, supplemented with DMSO for control, Maf1 was re-phosphorylated and relocated to the cytoplasm (lane 3) but remained in the dephosphorylated state in the nucleus when TBBt was added (lane 4). The effect of TBBt was reversible since Maf1 was re-phosphorylated and relocated out of the nucleus following TBBt removal (lane 6). We thus concluded that phosphorylation by CK2 mediates Maf1 export to the cytoplasm following transfer of yeast from a medium with a nonfermentable carbon source to one with glucose.

CK2 controls Maf1 association with tRNA genes. Previous work implicated Maf1 association with tRNA genes in a nutrient-dependent manner (8, 21). Since tRNA transcription is also regulated by carbon metabolism (11), we monitored carbon source-dependent Maf1 occupancy on class III genes and its dependence on CK2. Chromatin immunoprecipitation (ChIP) analyzed by real-time quantitative PCR showed moderate but significant enrichment of Maf1 on tRNA genes when glucose-grown cells were harvested in exponential growth phase (Fig. 6). In glycerol medium, however, the Maf1 occupancy on tRNA genes increased significantly. Strikingly, we were unable to detect Maf1 on 5S rDNA chromatin in either condition. Shifting the cells back to glucose medium led to the dissociation of Maf1 from tRNA genes and, significantly, supplementation of the medium with the TBBt inhibitor impaired this dissociation dramatically. Altogether, these data suggest that CK2 mediates dissociation of Maf1 from tRNA genes following transfer of yeast from nonfermentable (glycerol) to fermentable (glucose) growth conditions.

To investigate the possible effect of CK2 activity on Maf1 interaction with Pol III we used yeast cells expressing HA-tagged Rpc160, the largest Pol III subunit. Pol III was immunopurified from crude extracts and the immunoprecipitate was analyzed by Western blot (Fig. 6B). Equal amounts of Rpc160 were precipitated from yeast grown in glucose and glycerol media, whereas the levels of co-immunopurified Maf1 varied depending on growth conditions. In glucose medium, little Maf1 is bound to Pol III (Fig. 6B, lane 1), while in the glycerol medium the amount is over nine fold higher (Fig. 6B lane 2 and Fig 6C). Maf1 dissociated from the Pol III complex when cells were shifted back to the glucose medium supplemented with DMSO (lane 3), but this dissociation was over five fold less pronounced when the medium was supplemented with TBBt (Fig. 6B lane 4

and Fig 6C). We concluded that CK2 activity is required to release Maf1 from the Pol III complex and tRNA genes upon exit from nonfermentable to fermentable growth conditions.

Maf1 mediates CK2 signal transduction to Pol III. Knowing that CK2 regulates Maf1 interaction with Pol III and its occupancy at tRNA genes we asked how Maf1 phosphorylation by CK2 influences Pol III transcription activity *in vivo*. We took advantage of the fact that yeast lacking Maf1 are viable. Total RNA was isolated from wt and *maf1-Δ* cells grown in glucose medium, transferred to glycerol medium and transferred back to glucose medium supplemented or not with TBBt. To estimate the amounts of newly synthesized tRNA, we used an intron probe complementary to pre-tRNA^{Leu} (Fig. 7). Quantification of the intron-containing tRNA precursor revealed repression of Pol III activity in the wild-type strain but not in *maf1-Δ* upon transition of exponentially growing cells from glucose to glycerol, consistent with our previously published data (11, 15). After transfer back to the glucose medium, the pre-tRNA transcript levels in wild-type and *maf1-Δ* returned nearly to those before shifting cells to the glycerol medium (compare lanes 3, 7 and 1, 5). In contrast, transition to the glucose medium supplemented with TBBt precluded pre-tRNA derepresion in the wild-type strain, whereas in the *maf1-Δ* strain the pre-tRNA level was similar in the medium without TBBt (compare lanes 3, 7 and 4, 8). Altogether these data suggest that CK2 activates Pol III transcription via Maf1 phosphorylation.

Inhibition of Pol III transcription by mutations in catalytic subunits of CK2 is less effective in *maf1-Δ* strain. Earlier data showed that the Pol III transcription activity was very low in *cka1-Δ cka2^{ts}* mutant cells even at permissive growth temperature (27, 30). To extend these studies to Maf1 we constructed *cka1-Δ cka2^{ts} maf1-Δ* triple mutant and examined the amounts of newly synthesized tRNA using the intron probe complementary to pre-tRNA^{Leu}. Total RNA was isolated from the *cka1-Δ cka2^{ts}* and *cka1-Δ cka2^{ts} maf1-Δ* mutant cells grown in glucose medium and transferred to the same medium pre-warmed to 37°C (non-permissive temperature) (Fig. 8). Quantification of Northern blot revealed that deletion of the *MAF1* gene in the *cka1-Δ cka2^{ts}* double mutant led to increased pre-tRNA^{Leu} synthesis both at the permissive and non-permissive temperatures. We posit that *cka1-Δ cka2^{ts}* double mutation leads to the constitutive presence of repressive hypophosphorylated forms of Maf1 (as shown in Fig. 1) that inhibit Pol III transcription both at 30°C and 37°C.

CK2 associates with tRNA genes in Maf1-sensitive manner. The previously reported CK2 co-purification with TFIIIB (25) prompted us to investigate the presence of CK2 at class III genes. We

used a control wild-type strain and an isogenic *maf1-Δ* mutant, both expressing Myc-tagged α catalytic subunit of CK2 (Cka1-Myc) to perform ChIP analysis followed by real-time quantitative PCR. Slight but significant association of CK2 with $tRNA^{Leu}$ and $tRNA^{Trp}$ genes (2-4 fold above background) was observed for the wild type strain. The occupancy of CK2 at tRNA genes was, however, significantly increased in the *maf1-Δ* strain, especially when cells were grown under repressive conditions in glycerol medium (7-8 fold above background). To our surprise, little or no association of Cka1-Myc was found with other class III genes tested - *RDN5* (5S rRNA gene), *SCR1* and *SNR6* - in the wild type and *maf1-Δ* strains, regardless of the growth conditions (Fig. 9). These data suggest that CK2 is present at some Pol III-transcribed genes but the level of this occupancy is modulated by Maf1.

Discussion

In this work we present evidence that Maf1 is phosphorylated by CK2 kinase. We show that Maf1 phosphorylation by CK2 contributes directly to activation of Pol III transcription by stimulating Maf1 dissociation from the Pol III complex and from tRNA genes.

We found that yeast Maf1 is phosphorylated by CK2 on S388 and on at least two residues within serine tract S159-S162. Significantly, these sites are located in two highly flexible regions of Maf1. S388 is located in the C-terminal tail whereas S159-S162 lie in a mobile linker separating two conserved, highly structured A and BC fragments. Notably, sites known to be phosphorylated in Maf1 by PKA/Sch9 are S177-180 (17, 19) are located in the second serine tract located between the A and BC boxes (Fig S5). Human Maf1 has the same organization of two structured A and BC regions separated by a linker, which, however, is shorter and not homologous to the yeast one. Human Maf1 is phosphorylated by mTOR, and, significantly, the phosphorylation sites are within the linker region (32-34). Thus, the presence of phosphosites in the region separating the A and BC fragments has been conserved from yeast to humans, even though the specific amino acid sequence has not. The phosphorylation state of the linker therefore appears to be a key factor that changes the distance between the A and BC regions modulating their interaction and Maf1 association with Pol III, supporting the hypothesis presented by Gajda et al (35). Here we propose that CK2 phosphorylation promotes Maf1 dissociation from Pol III in yeast.

Because CK2 activity is required for cell cycle progression at G1-S and G2/M (30), we asked whether the observed defect of Maf1 phosphorylation in CK2-deficient cells is an indirect effect of cell cycle blockage or is a direct result of the lowered CK2 activity. The relationship between checkpoint signaling and regulation of tRNA synthesis was analyzed extensively by a recent study showing that neither normal progression trough S phase nor alpha-factor-mediated G1 arrest or G2/M arrest by nocodazole are associated with alterations in tRNA transcription (13). These results

indicate that the Maf1-mediated effect on tRNA transcription correlated with CK2 depletion cannot be attributed to cell-cycle defects.

Yeast cells synthesize 3–6 million molecules of tRNA every cell cycle at a rate of 2–4 transcripts/gene/s (6). This high rate of transcription is achieved through many rounds of Pol III reinitiation on stable DNA-bound complexes of the initiation factor TFIIIB. Studies in yeast have shown that the CK2 phosphorylation of TBP is required for efficient TFIIIB recruitment to Pol III promoters in unstressed cells (25). The rate of re-initiation is increased by facilitated recycling, a process of repeated reloading of the polymerase on a given transcription unit. However, when nutrients become limiting, stress conditions are encountered or yeast are transferred from glucose to a medium with non-fermentable carbon source, Pol III transcription is rapidly repressed through the action of Maf1 protein which, in the unphosphorylated state, binds to Pol III and obstructs transcription. Importantly, the Pol III repression by Maf1, at least that in response to DNA damage, is correlated with dissociation of CK2 catalytic subunits from the TFIIIB complex (25).

Exactly how Maf1 is recruited to Pol III during ongoing transcription is unknown. Maf1 does not bind to a preassembled Pol III-Brf1-TBP-DNA initiation complex (6, 9). Moreover, a biochemical study clearly shows that Maf1 is not a Pol III inhibitor, as it prevents neither nucleic acid binding in the active center nor RNA synthesis (9). The Maf1 recruitment to Pol III could be brought about by an unknown event causing Pol III reposition and occurring after initiation step, during elongation or termination. According to a model presented by Riva, Carles and co-workers, a conformational change occurs in Pol III during a transitory pause at the elongation step prior termination and duration of this pause is controlled by the C37-C53 heterodimer (36). One interesting hypothesis could be that this pausing facilitates Maf1 binding.

Here we examine the relationship between the Maf1-mediated repression and Pol III control by CK2. We demonstrate that phosphorylation of Maf1 by CK2 is critical for the release of Maf1 from tRNA genes and for the high rate of Pol III transcription after a shift from repressing (glycerol) to optimal growth conditions (glucose). In repressing conditions, Maf1 is physically associated with the Pol III complex and with tDNA chromatin (8). Inhibition of CK2 hampers the dissociation of Maf1 from the Pol III complex and its export out of the nucleus as normally observed upon the shift from respiration to fermentation. Moreover, the dissociation of CK2 catalytic subunit from Pol III chromatin is less efficient in the deletion strain *maf1*-Δ. These results suggest that Maf1 assists the arrangement of CK2 complex thus preventing CK2-mediated phosphorylation of TBP during repression.

We believe that CK2 controls the dynamic association of Maf1 with chromatin by assessing Pol III during re-initiation. Regardless of the growth conditions, Maf1 could be recruited to the Pol III complex with each transcription cycle, during elongation or termination. Our previous study

shows that a small amount of dephosphosphorylated Maf1 is Pol III-bound even in cells growing exponentially on glucose (8). We propose that this dephosphorylated nuclear Maf1 is associated with the re-initiating complex. Following termination, before the new transcription cycle of re-initiating Pol III, Maf1 encounters CK2 bound to TFIIIB in promoter region. Under favorable growth conditions, CK2 phosphorylates Maf1, thus releasing it from Pol III and enabling re-initiation. At the same time CK2 activates Pol III transcription by binding and phosphorylating TBP. Subsequent export of the phosphorylated Maf1 to the cytoplasm decreases its concentration in the nucleus lowering the probability of association with Pol III complex. Upon stress, the catalytic subunits of CK2 dissociate from TBP and CK2 becomes inactive. The Pol III-associated Maf1 cannot be re-phosphorylated, remains with Pol III and re-initiation is not possible. Simultaneously TBP cannot be phosphorylated by CK2. Moreover, dephosphorylated Maf1 is imported from the cytoplasm to the nucleus to strengthen the Pol III repression. Altogether, the presented idea fits with, and even strengthens the model of Pol III regulation by CK2 proposed previously by Ghavidel and Schultz (25).

Maf1 is regulated by a variety of pathways to adjust Pol III transcriptional rate to changing conditions. Besides CK2, three other kinases, PKA, Sch9 and TORC1, contribute to Maf1 phosphorylation in yeast. A question arises about the sequence of their action and priority in Maf1-dependent Pol III transcription regulation. We suggest that CK2 plays a major role by directly controlling Maf1-Pol III association whereas phosphorylation by other kinases mainly contributes to cellular localization of Maf1. According to our model presented above, the nuclear abundance of Maf1 supports the control of Pol III activity by CK2. Maf1 phosphorylation by PKA and Sch9 contributes to excluding Maf1 from the nucleus, although inactivation of all potential Sch9 phosphorylation sites resulted in constitutive Maf1 association with Pol III (19). Phosphorylation of Maf1 by TORC1 is thought to take place on chromatin in nucleoli and to contribute to Maf1 regulation by excluding it from chromatin (21).

Interestingly, TORC1 in yeast has been detected on 5S rRNA rather than on tRNA genes, in contrast to CK2 preferentially present on tRNA genes. The results presented here indicate that Maf1 has a higher affinity for tRNA genes than for 5S rRNA genes. Initiation complexes assembled on tRNA genes compositionally different from complexes assembled on 5S rRNA genes, which could be the reason of the different Maf1 occupancy. The absence of Maf1 on 5S genes correlates with the lack an *in vivo* effect of Maf1 on 5S rRNA experession (7). Additionally *MAF1* deletion failed to rescue the decreased 5S rRNA synthesis resulting from Sch9 inhibition in contrast to the effect on decreased tRNA synthesis (19). Interestingly, tRNA and 5S rRNA levels were also differentially affected by mutations in genes encoding the Pol III subunits C34, C31, C82, C53 and C160, which decreased of tRNA synthesis but did not alter the transcription of 5S rRNA (37-40).

In cells lacking Maf1, CK2 still binds to tRNA genes and this association is even increased upon the shift to repressive (glycerol) conditions. We propose that dissociation of the CK2 catalytic subunits from the TBP subunit of TFIIIB is precluded in the absence of Maf1. Therefore, one hypothesis is that Maf1 is required to block TFIIIB activation by CK2 thereby effecting Pol III repression. This raises the question of whether Maf1 may influence the CK2 holoenzyme at the heart of Pol III preinitiation complex.

One unanswered but fundamental question is the mechanism of CK2 regulation. The presence of many discrete CK2 subpopulations is crucial in resolving the current conflicting views on whether CK2 is constitutively active or is modulated in response to specific stimuli is. Each local CK2 population may be regulated in a distinct manner to carry out its precise function(s). Most likely the CK2 activity is controlled through interactions of its regulatory subunits with other proteins. Apart from the transient formation of the holoenzyme with catalytic subunits of CK2 in humans, regulatory subunits can interact with at least 30 different proteins but is rapidly degraded when unassembled [for review see (22)]. In humans, CK2-mediated phosphorylation of p53 protein is regulated by the FACT complex (hSpt16 and SSRP1) (41), and Pin-1 protein inhibits phosphorylation of topoisomerase IIα by CK2 (42). We propose here a model in which Maf1 regulates Pol III directly and also modulates CK2 activity towards TFIIIB. To confirm this conjecture, further studies are needed on how Maf1 influences the association of CK2 subunits with the TBP component of TFIIIB.

CK2 is also present in the human Pol III complex and phosphorylates the TFIIIB subunits Brf1 (26) and Bdp1 (28). Our results indicate that human Maf1 can also be phosphorylated by CK2 *in vitro*, although we have not tested this *in vivo*. Such evolutionary conservation suggests that phosphorylation of Maf1 by CK2 acting as a master switch of Pol III transcription is of fundamental importance.

Supporting Information

SI includes result of mass spectrometry analysis (Table S1), supplementary figures, description of strains and plasmids (Tables S2 and S3) and Supplemental Experimental procedures,.

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Figure Legends

Fig. 1. ***cka2^{ts}* mutations affects Maf1 phosphorylation**. Control strains (wt and *cka1-Δ*) and isogenic thermosensitive double *cka1-Δ cka2^{ts}* mutant (constructed by D. Glover) were grown to exponential phase in rich glucose medium (YPD Exp) at 30°C, transferred to pre-warmed rich glucose medium (YPD), incubated at 37°C and harvested as indicated. Crude extracts were prepared from cells by TCA-precipitation and analyzed using SDS-PAGE with a modified acrylamide-bisacrylamide ratio followed by immunoblotting with polyclonal anti-Maf1 antibodies. Arrows indicate phosphorylated forms of Maf1.

Fig. 2. **Maf1 interacts with catalytic subunit of CK2.** YPH499 strain expressing HA-tagged Cka2 and WT control strain were grown in rich glucose medium to exponential phase (YPD), transferred to a nonfermentable glycerol medium (YPGly) and incubated for 3 h. Subsequently, cellular extracts were prepared and subjected to immunoprecipitation with magnetic beads coated with anti-HA antibodies, followed by elution of bound proteins. Total cellular extracts (TOT), flow through (FT) and immunopurified proteins were analysed by SDS-PAGE and Western blot using anty-HA and anty-Maf1 antibodies. Arrow indicates hyperphosphorylated Maf1 and asterisk indicates hypophosphorylated Maf1.

Fig. 3. **Recombinant yeast Maf1 is phosphorylated by yeast CK2 *in vitro*.** Recombinant *S. cerevisiae* Maf1 was expressed in *Escherichia coli* and purified by HIS-tag isolation. Autoradiogram shows [γ^{32}P]-ATP-labeled products of reactions containing: 100 ng yeast TAP-tag purified CK2 and: 1 μg casein (lane 0); increasing amounts of recombinant yeast Maf1 (0, 0.25, 0.5, 1, 2 μg – lanes 1-5); 2 μg recombinant yeast Maf1 and 4% (v/v) DMSO (lane 6); 2 μg recombinant yeast Maf1, 4% (v/v) DMSO and 10 μM TBBt (lane 7).

Fig. 4. **Mutations of CK2 sites prevent Maf1 phosphorylation.** Substitutions in *MAF1* cloned in pRS315 vector were made using mutagenic PCR. Mutants 1StA (S388A), 4StA (S159A, S160A, S161A, S162A), 5StA (S159A, S160A, S161A, S162A, S388A) and corresponding wt were grown overnight in SC –leu medium, then in glucose rich medium to exponential phase (YPD Exp), transferred to glycerol medium for 3 h (YPGly) and again transferred to YPD for 3h. TCA-precipitated proteins were examined by Western blot using anti-Maf1 antibody.

Fig. 5. **CK2 is necessary for regulation of Maf1 activity by carbon source.** Wild-type cells and isogenic mutants *cka1-Δ* , *cka2-Δ* and *cka1-Δ cka2^{ts}* were examined by Western blot using anti-Maf1 antibody. Cells were grown to exponential phase in rich glucose medium (YPD Exp) at 30^{o}C, transferred to glycerol medium (YPGly), incubated at 30^{o}C for 3 h, then transferred to (**A**). pre-warmed YPD, and incubated at 37^{o}C (**B**). pre-warmed rich glucose medium containing 4% v/v DMSO or 200 μM TBBt, and incubated at 30^{o}C. Cells were harvested as indicated.

Fig. 6. **Dissociation of Maf1 from tRNA genes following transfer of yeast from nonfermentable glycerol growth conditions to glucose is dependent on CK2 activity.** Relative occupancy of Maf1 at selected class III genes. Strain expressing Myc-tagged Maf1 was grown in rich glucose medium (YPD) to exponential phase and transferred to a nonfermentable glycerol medium (YPGly). Following 3 h incubation at 30^{o}C, the culture was split in half; one part was transferred to YPD with 2% (v/v) DMSO and the second - to YPD with 200 μM TBBt in DMSO. (A) Cross-linked chromatin was immunoprecipitated with antibodies against Myc epitope, followed by quantitative real time PCR. Occupancies of Maf1 at tRNA-Leu, tRNA-Trp and 5S rRNA genes were measured relative to occupancy at 35S rRNA. (B) Cellular extracts were incubated with magnetic beads coated with anti-HA monoclonal antibody followed by elution of bound proteins and Western analysis with anti-HA and anti-Maf1 antibodies. (C) Quantification of the amounts of Maf1 immunopurified with Rpc160. Experiment was performed in triplicate to estimate standard deviation.

Fig. 7. **Pol III transcription is activated via Maf1 phosphorylation by CK2.** (**A**) Northern blot analysis. Wild type and *maf1-Δ* strains were grown in rich glucose medium (YPD) to exponential phase and transferred to a nonfermentable glycerol medium (YPGly). Following 3 h incubation at 30^{o}C the culture was split in half; one part was transferred to YPD with 4% (v/v) DMSO and the second - to YPD with 200 μM TBBt in DMSO. RNA was isolated from cells and hybridized with tRNA^{Leu} intron and U3 probes. (**B**) Quantification of pre-tRNA^{Leu} transcript (upper band) normalized to U3 snoRNA transcript. Fold -change was calculated relative to expression in wild-type cells grown in glucose medium.

Fig. 8. **Deletion of *MAF1* gene restores Pol III transcription deficiency in *cka1-Δ cka2^{ts}* mutant** (**A**) Northern blot analysis. Isogenic mutants *cka1-Δ cka2^{t}* and *cka1-Δ cka2^{ts} maf1-Δ* were grown in glucose medium (YPD) at 30°C to exponential phase and transferred to YPD pre-warmed to 37°C for 1 h. RNA was isolated from cells and hybridized with tRNA^{Leu} intron and U3 probes. (**B**)

Quantification of pre-tRNA^{Leu} transcript (upper band) normalized to U3 snoRNA transcript. Fold - change was normalized to expression in *cka1-Δ cka2^{ts}* at 30°C.

Fig. 9. **CK2 binds to tRNA in carbon source- and Maf1-sensitive manner.** Relative occupancy of Cka1-Myc at selected class III genes. Strains wt and *maf1-Δ*, both expressing Myc-tagged α catalytic subunit of CK2 (Cka1-Myc) were grown in rich glucose medium (YPD) to exponential phase, transferred to a nonfermentable glycerol medium (YPGly) and incubated at 30^{o}C for 40 min. Cross-linked chromatin was immunoprecipitated with antibodies against Myc epitope, followed by real time quantitative PCR. All occupancies are expressed relative to *TEL15* region. The experiment was performed in triplicate to estimate S.D.