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Title: Maf1, a general negative regulator of RNA polymerase III in yeast

Article Type: SI: Transcription by Odd Pols

Keywords: Transcription regulation, RNA polymerase III, tRNA synthesis, Maf1 repressor

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Abstract: tRNA synthesis by yeast RNA polymerase III (Pol III) is down-regulated under growth-limiting conditions. This control is mediated by Maf1, a global negative regulator of Pol III transcription. Conserved from yeast to man, Maf1 was originally discovered in Saccharomyces cerevisiae by a genetic approach. Details regarding the molecular basis of Pol III repression by Maf1 are now emerging from the recently reported structural and biochemical data on Pol III and Maf1. The phosphorylation status of Maf1 determines its nuclear localization and interaction with the Pol III complex and several Maf1 kinases have been identified to be involved in Pol III control. Moreover, Maf1 indirectly affects tRNA maturation and decay. Here I discuss the current understanding of the mechanisms that oversee the Maf1-mediated regulation of Pol III activity and the role of Maf1 in the control of tRNA biosynthesis in yeast.

Response to Reviewers: Response to Reviewer #1:
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However, I do not agree with the opinion that the model of Pol III regulation presented in Fig. 2 is speculative - it is consistent with the results of several groups working on Maf1. Maf1 binds to Pol III machinery after the imitation step as concluded by Patric Cramer on the basis of a biochemical and kinetic study (Vannini et al, Cell, 2010). The essential role of CK2 in Pol III regulation has been known for a long time. Although the role of Maf1 phosphorylation by CK2 in Pol III reactivation could be redundant(what I emphasized in the revised manuscript), there are other CK2 substrates in Pol III machinery beyond Maf1 (like TFIIIB) which mediate the CK2 signal. All studies of other groups which contribute to the model are now cited in the legend to Fig. 2
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Cover Letter

Warsaw, October 1, 2012

Dear Dr. Bhargava,

Thanks for the reviews of my article " **Maf1, a general negative regulator of RNA polymerase III in yeast**" submitted to BBA-GRM Special issue. While the first referee made several specific comments which are very appropriate and useful, the second review is only general and reflects more personal feelings. I tried my best to address the concerns of both referees and to improve the indicated points on the coverage of the literature, especially on Maf1 phosphorylation. All changes in text are marked in blue. I hope the revised version of the manuscript is more balanced and comprehensive.

Best regards,
Magdalena Boguta

***Response to Reviewers**

Reviewer #1: : The author has provided a thorough review of Maf1 literature which is very useful, especially the listing of different phenotypes of maf1.

Suggestions:
Throughout the review, in several places, citations are needed to support the statements (this is a literatire review), e.g., latter part of first para of p. 8, and 3rd para on p. 11 are just two examples.

It would be strengthen the review to elaborate on the crystal structure of Maf1 from point of view of highly conserved regions and mutations that abolish function.

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Highlights

- Phenotypes of yeast *maf1* mutants
- Family of eukaryotic Maf1 proteins
- Regulation of the activity of yeast Maf1
- Model of Pol III regulation by Maf1 and CK2 kinase
- Indirect effect of Maf1 on tRNA processing and decay

Maf1, a general negative regulator of RNA polymerase III in yeast

A brief, informative title: Regulation of Pol III by Maf1

Magdalena Boguta

[1]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02 106 Warsaw, Poland; [2]Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, Poland

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Abstract

tRNA synthesis by yeast RNA polymerase III (Pol III) is down-regulated under growth-limiting conditions. This control is mediated by Maf1, a global negative regulator of Pol III transcription. Conserved from yeast to man, Maf1 was originally discovered in *Saccharomyces cerevisiae* by a genetic approach. Details regarding the molecular basis of Pol III repression by Maf1 are now emerging from the recently reported structural and biochemical data on Pol III and Maf1. The phosphorylation status of Maf1 determines its nuclear localization and interaction with the Pol III complex and several Maf1 kinases have been identified to be involved in Pol III control. Moreover, Maf1 indirectly affects tRNA maturation and decay. Here I discuss the current understanding of the mechanisms that oversee the Maf1-mediated regulation of Pol III activity and the role of Maf1 in the control of tRNA biosynthesis in yeast.

Introduction

The existence of three RNA polymerases (Pol) is well documented for all investigated eukaryotes. Pol I synthesizes ribosomal RNA (rRNA), Pol II produces mainly mRNAs, and Pol III generates tRNAs, 5S rRNA and other small noncoding RNAs. Recent studies reveal substantial conservation between the transcription initiation machineries of the three polymerases (Pol I, II and III) [1]**.** Each core transcription initiation complex consists of a promoter DNA, a polymerase, TBP (TATA-binding protein), a TFIIB-like factor, a TFIIF-like protein, and TFIIE or proteins with TFIIE-related domains. Despite the high similarity between the general factors and the conserved basal structure of the polymerase complexes, the mechanisms of their regulation are different. Possibly the mechanisms that are specific for given categories of RNA provide a selective advantage by separately controlling mRNA, rRNA and tRNA production. Pol II uniquely employs the so-called mediator complex and carries an extra C-terminal domain (CTD) on the largest subunit, Rpb1. CTD undergoes dynamic phosphorylation during the progression from initiation through elongation to termination [2,3]. The specific phosphorylation patterns that predominate at each stage of transcription recruit appropriate sets of mRNA-processing and histone-modifying factors [3]. These dynamic interactions provide a means for coupling and coordinating specific stages of transcription with other events determining levels of individual mRNAs.

In contrast to the highly diverse population of mRNAs, the levels of Pol I and Pol III transcripts are predominantly controlled in a global manner. These two polymerases, sometimes collectively called “Odd Pols”, specialize in high-level synthesis of fundamental non-coding RNAs, together accounting for well over 90% of all cellular RNA by mass. In the yeast *Saccharomyces cerevisiae* Pol I synthesizes 35S precursor of 28S and 18S rRNA whereas the Pol III transcriptome includes 5S rRNA, tRNAs, U6 splicesomal RNA, the RNA subunit of the signal recognition particle (encoded by *SCR1*), RNase P RNA, and Snr52 snoRNA [4,5]. Critically for cellular economy, Pol I and Pol III need to be

repressed when growth is inhibited. Although controlled in a coordinated manner, these two polymerases use different regulatory proteins.

The activity of the yeast Pol I enzyme is specifically modulated by Rrn3, which renders Pol I competent for transcription initiation. The interaction with the Pol I complex is dependent on Rrn3 phosphorylation and is regulated by growth factor signaling pathways that adjust rRNA production to nutrient availability [6]. Pol III transcription in turn is uniquely controlled by a general repressor Maf1 which is regulated by phosphorylation and relays diverse negative signals to the Pol III machinery. The molecular basis of the Pol III transcription repression by Maf1 is now emerging due to the structural and biochemical data on Pol III and Maf1 recently reported. These results have highlighted the multiple levels of regulation of Maf1 activity and uncovered previously unanticipated secondary effects of Maf1. Some of these findings will be discussed in this review.

Genetics of Maf1

Maf1, the key player in the repression of RNA Pol III transcription, was originally discovered in *S. cerevisiae* by a genetic approach. The purpose of the screen which led to the isolation of the *maf1-1* mutant was the identification of *trans*-acting factors affecting the sub-cellular distribution of the tRNA-isopentenyltransferase Mod5 [7]. The parental strain used for that genetic screen contained a nonsense suppressor *SUP11*; *maf1-1* was selected among colonies that showed decreased suppression following random mutagenesis. Since the *SUP11* activity requires the cytoplasmic pool of Mod5, selection for a loss of suppression assumed a lower amount of Mod5 in the cytoplasm. Indeed, according to an original observation, Mod5 was mislocalized to the nucleus in the *maf1-1* mutant [7]. Despite nearly two decades elapsing since the original discovery, the mechanism by which Maf1 affects Mod5 localization is not understood. A decreased level of A37 isopentenylation in tRNAs, although never addressed experimentally, could be one reason of lower tRNA-mediated suppression in *maf1-1*.

In addition to decreasing the *SUP11* efficiency, *maf1*-1 was also temperature-sensitive when grown on nonfermentable carbon sources. Both phenotypes were fully complemented by *MAF1,* a gene of unknown function when cloned [8]. An intriguing genetic interaction between *MAF1* and Pol III was revealed by a search for multicopy suppressors of *maf1-1*; diverse variants of the *RPC160* gene with deletions in the 3' part of its open reading frame suppressed the *maf1-1* phenotypes when over-expressed [8]. Direct examination by Northern blotting revealed increased tRNA levels in *maf1-1* cells indicating the function of Maf1 as a negative Pol III regulator [9]. Full inactivation of *MAF1* resulted in the same phenotypes as those caused by the initially selected *maf1-1* allele.

Thus, although Maf1 is, apparently, the unique global regulator of Pol III transcription in yeast, the *maf1-Δ* mutant is viable and under standard conditions its growth is not compromised. One as yet unsolved question is why the mutant cells cannot tolerate an excess of tRNA and other Pol III transcripts under unfavorable growth conditions in media with a nonfermentable carbon source. The growth defect of *maf1-Δ* on medium with glycerol pointed to a deficiency in mitochondrial function. In yeast *S. cerevisiae* two Pol III synthesized tRNAs were reported as mitochondrially targeted, namely $tRNA^{Lys}$ and $tRNA^{Gln}$. Mitochondrial functions of these tRNAs are not completely clear although there is indirect evidence for their role in mitochondrial translation [10]. Since Pol III transcription is elevated in the absence of Maf1, one can assume increased amounts of some cytosolic tRNAs in mitochondria exerting a negative effect on mitochondrial translation in *maf1-Δ* mutant. Defects in mitochondrial translation most common cause of the degradation of mtDNA resulting in the formation of *petite* colonies known as *rho*$^-$. However, no increased *rho*$^-$ accumulation was observed in *maf1-Δ* strains arguing against this assumption [11].

Lastly, Maf1 could exert a specific effect on gene(s) required for growth on non-fermentable carbon sources and located close to tRNA gene(s) in the genome. In this mechanism called tRNA-gene-mediated silencing (tgm), actively transcribed tRNA genes negatively regulate adjacent Pol II-dependent genes. Indeed a role of Maf1 in tgm silencing has been reported by Moir et al. 2006 [12].

They studied the silencing of the *HIS3* gene by upstream adjacent *SUP4* tRNA gene, both expressed from a plasmid in a *his3* yeast strain. When *SUP4* was actively transcribed, the strain remained His- due to the tgm silencing, and preventing *SUP4* transcription by mutation in tRNA gene promoter resulted in the His+ colonies [13]. Unexpectedly, also the *maf1-Δ* mutation resulted in the His+ phenotype, although one should assume an opposite effect – a upregulation of *SUP4* transcription due to the lack of Maf1 and a consequent strong silencing of *HIS3* gene. That, as yet unexplained result sheds new light on Maf1 functioning which may be dual since tgm silencing effect of Maf1 may be unrelated to the effect of Maf1p on transcription by Pol III.

Similarly, the effect of Maf1 on the efficiency of tRNA-mediated suppression is opposite to that expected. Although one would assume that increased cellular tRNA levels should improve the efficiency of tRNA-mediated nonsense suppression, data show quite the opposite. Importantly the overall fidelity of translation is increased in the *maf1-Δ* mutant. As has been shown using a dual-gene reporter system, *maf1-Δ* cells have two-fold lower levels of UAA and UAG stop codon read-through [14]. This counterintuitive result could be due to the accumulation of unprocessed tRNA precursors which could limit the role of mature tRNAs in translation (indirect effect of Maf1 on tRNA processing is described later in this review).

Defects in tRNA biosynthesis, both in tRNA maturation and in nuclear export, induce translational derepression of the ubiquitous transcription factor Gcn4, a response conserved from yeast to mammals [15,16]. In yeast Gcn4 responds to amino acid deprivation due to accumulation of uncharged tRNAs that interact with Gcn2, the protein kinase that phosphorylates translation initiation factor eIF2. Phosphorylation of eIF2 by Gcn2 results in decreased levels of general translation, but increased translation of the transcription regulator Gcn4, which in turn results in transcription of numerous genes involved in amino acid and nucleotide biosynthesis (for review, see [17]. Remarkably, deletion of *MAF1* led to activation of Gcn4 as well [18], confirming the existence of a system communicating tRNA quality control in the nucleus to the translation machinery. However,

the component elements of this system require further investigation since accumulation of immature tRNAs induced Gcn4 independently of eIF2 phosphorylation [15,18].

Another impact of Maf1 on the yeast physiology is its role in sporulation. While competent for growth on media with a nonfermentable carbon source at the regular temperature, the *maf1-Δ / maf1-Δ* homozygous diploid is sporulation deficient [19] . Molecular basis of this phenotype needs to be addressed experimentally. Moreover, *maf1-Δ* is lethal when combined with a number of other deletions as revealed by a synthetic genetic array analysis [20]. Besides genes from the functional categories linked to the role of Maf1 as a regulator of Pol III transcription, that screen identified as synthetically lethal with *maf1-Δ* also genes involved in ribosome biogenesis, RNA pol II transcription, tRNA modification and ubiquitin-dependent proteolysis.

While the current studies on Maf1 tend to focus on its role in Pol III control, the often puzzling genetic phenotypes observed for yeast *maf1* mutants may serve as an inspiration for a broader investigations.

Family of Maf1 proteins

Maf1 protein is found in human, animals, plants and lower eukaryotes. It contains three highly conserved regions called A, B and C, which are shared among species [9,21,22] but do not correspond to structural modules or defined surface patches in Maf1 protein [23]. Neither potential prokaryotic orthologs nor substantial homology with protein domains of known function have been identified resulting in a striking lack of information on the functional significance of those regions and included signature sequences PDYDFS and WSfnYFFYNkklKR. Point mutagenesis of yeast *MAF1* has only highlighted the importance of several serine residues (mostly non-conserved ones) and two nuclear localization sequences [12,24,25].

A comparison of the arrangement of representative Maf1 sequences is presented in Figure 1. The distance between the B and C segments of approximately 10 aa is constant in evolution, with the

exception of *Aspergilus nidulans* (insertion of 15 aa). In contrast, the distance between regions A and B largely varies between species. The A and B domains are fused in *Encephalitozoon cuniculi* whereas in the yeast *S. cerevisiae* and *Candida glabrata* they are separated by a long linker of 182 aa and 174 aa, respectively. The Maf1 sequences of *C. elegans* and *H. sapiens* have relatively shorter linkers.

Limited proteolysis of recombinant human Maf1 resulted in two stable fragments (aa 1-45 and aa 75-234) corresponding to the evolutionarily conserved regions. When two open reading frames corresponding to these two fragments were co-expressed in bacteria, they co-purified and co-eluted during size exclusion chromatography as soluble complex. However, when expressed individually, neither fragment formed a soluble, protease-resistant module. These biochemical data suggest that the N- and C- terminal regions of Maf1 form modules that do not fold independently but rather need to be co-expressed to form a stable and soluble entity [26]. In line with the biochemical data reported for human Maf1, a physical interaction of the N- and C-terminal regions of yeast Maf1 has been shown using the yeast two-hybrid system and a genetic suppressor approach [26]. More importantly, X-ray crystallography of Maf1 at 1.55 A ° resolution revealed that Maf1 forms a globular structure and conserved sequence regions do not correspond to structural modules or defined surface patches [23] . Crystallized variant of human Maf1 lacked two regions which appear solvent-exposed and unstructured: the linker connecting A and B conserved sequences and the C-terminal acidic tail. These two unstructured regions are not required for Pol III binding since both a full-length recombinant yeast Maf1 and a shorter variant that lacked the linker and the C-terminal tail formed a complex with Pol III that could be purified by size-exclusion chromatography [23]. Moreover, partial deletion of linker region is dispensable for Maf1 function, as shown recently by molecular analysis [27]. Nevertheless, unstructured regions could be involved in fine-tuning regulation of Maf1 activity by phosphorylation, as described in the next section.

Regulation of Maf1 activity

Function of Maf1 as a negative effector of Pol III synthesis was presumed on the basis of molecular and analysis of *maf1-1* mutant [8,9]. First, Northern analysis indicated that tRNA levels are elevated in *maf1* mutant cells . Second, mutations in the *RPC160* gene encoding the largest subunit of Pol III which reduce tRNA levels were identified as suppressors of the *maf1-1* growth defect. Third, physical interaction between Maf1 and Pol III suggested a direct role for Maf1 in Pol III down-regulation [9]. Importanly, based on Northern blot analysis of selected precursor tRNAs, Maf1 was found to mediate several signaling pathways by repressing Pol III transcription in response to diverse stresses [28].

Maf1 activity is regulated via its phosphorylation state-dependent cellular localization [12,24,25,29,30,31,32,33]. Pol III repression requires Maf1 in dephosphorylated state [24,29,33,34]. Differentially phosphorylated forms of Maf1 can be resolved by SDS-PAGE and it is the least phosphorylated form that binds Pol III [22,25,29]. The increased association of Maf1 with Pol III under repressing conditions is correlated with dissociation of Pol III from tRNA gene [24,29,33].

In favourable growth conditions, Maf1 is located predominantly in the cytoplasm, although is never fully excluded from the nucleus [12,35]. The Maf1's cytoplasmic location is mediated by two mechanisms: a phosphorylation-dependent inactivation of the Maf1 nuclear location signals (NLSs) [12], and nuclear export of phosphorylated Maf1 by the exportin Msn5 [35]. Although the changing distribution of Maf1 between the nucleus and the cytoplasm could, in principle, account for the regulation of Poll III transcription by Maf1, two lines of evidence suggest that the phosphorylation is the main mechanism by which Maf1 activity is controlled and the nuclear–cytoplasmic dynamics of Maf1 may instead be a mechanism to fine-tune regulation. First, Maf1 is still able to regulate Pol III transcription in cells lacking Msn5, that is required to export phosphorylated Maf1 from the nucleus [35]. Second, although Maf1 is constitutively located in the nucleus in the common W303 yeast strain background, the regulation of Pol III is nevertheless responsive to environmental signaling [32].

The predicted phosphorylation sites are mostly found in the unstructured linker located between conserved A and B fragments of Maf1 sequence. The migration of yeast Maf1 in SDS gels is especially sensitive to phosphorylation at particular serines (S177 and S178) in the linker region [31]. Phosphorylation within the long unstructured linker likely affects the interaction between the conserved A and BC regions of the yeast Maf1 necessary for the full repression of Pol III activity [26]. Biochemical studies regarding the significance of the unstructured regions for the regulation of Maf1 activity by phosphorylation are, however, limited since their presence in the recombinant yeast Maf1 greatly reduces solubility and promotes protein aggregation.

Maf1 is also phosphorylated in mammalian cells. Human Maf1 becomes largely dephosphorylated after stress, and it is the dephosphorylated form of Maf1 that associates with Pol III [22,36,37].

Maf1 as mediator of signaling pathways

In yeast, Maf1 is the only Pol III negative regulator that acts as an effector of several signalling pathways [28]. In addition to the down-regulation that normally occurs in the stationary phase, Maf1 is also required for Pol III repression accompanying rapamycin treatment, starvation, secretion defects, as well as oxidative and replication stress [24,25,28,29,30,38]. Cells depleted of Maf1 remain alive under stress conditions, but, surprisingly, Maf1-mediated regulation is essential during the transition from fermentative to glycerol-based respiratory growth [11]. This observation underscored the critical function of Maf1 in the coupling of Pol III transcription to metabolic processes and/or energy production dependent on the carbon source [11,12].

The molecular mechanisms that trigger Maf1 activity in response to different signaling pathways are only partially understood. The main Maf1 phosphatase is protein phosphatase 4 (PP4) [34]. The PP4 action is most likely direct, as a portion of cellular PP4 co-precipitates with Maf1. By dephosphorylating Maf1 PP4 mediates rapid Pol III repression in response to diverse stresses [34].

In response to favourable growth conditions yeast Maf1 is phosphorylated at different sites by several kinases. So far four of them, PKA, Sch9, TORC1 and CK2 have been described [12,25,31,32,33], but other Maf1 kinases are likely to be identified in the future . The impact of each of those kinases on the control of Maf1 interaction with the Pol III complex is, however, difficult to estimate using available experimental tools.

Sch9 certainly effects the Maf1 interaction with Pol III since inactivation of all potential Sch9 phosphorylation sites (which are the same as PKA sites) in a Maf1-7A mutant promoted its nuclear localization and increased the Maf1-Pol III association [25,31,39]. Importantly in the Maf1-7A mutant cells Pol III was not constitutively repressed and responded robustly to rapamycin [25]. Moreover, the Pol III activity was still sensitive to rapamycin in Maf1-7E "phosphomimetic" mutant. Those results suggested that regulation by Sch9/PKA is insufficient for confer Pol III control and some additional step is required. Either these kinases must target factor(s) in addition to Maf1, or Maf1-7E has to be phosphorylated in additional positions (and, conversely, Maf1-7A additionally dephosphorylated) [25].

A further opportunity for the control of Maf1 activity is provided by chromatin-bound kinases TOR and CK2. Although both yeast and human TOR phosphorylate Maf1 [32,37,40,41], control by TOR seems not to be conserved in evolution. The mTOR kinase localizes to mammalian tRNA and 5S rRNA genes by interaction with TFIIIC, a DNA binding factor that recognizes the promoters of those genes. By this association, mTOR-mediated phosphorylation of Maf1 functionally contributes to the regulation of the repressive activity of Maf1 at mammalian tRNA genes [40]. In contrast, the yeast TORC1 kinase binds to rDNA chromatin, 35S as well as 5S genes, and is not detected on tRNA genes [32]. TORC1 has been postulated to interact with and phosphorylate Maf1 at the rDNA loci, in this way regulating its translocation from the nucleolus to the nucleoplasm [32]. However, phosphorylation by TORC1 was detected for recombinant, but not native, yeast Maf1 and the phosphorylation site has not been identified. Moreover, Maf1 association with 5S rDNA genes is

controversial [33]. Remarkably, TORC1 phosphorylates Sch9 at multiple sites and this phosphorylation is required for the catalytic activity of Sch9, another kinase of yeast Maf1 [25]. Thus, TORC1 could control yeast Maf1 indirectly, via Sch9 in the nucleoplasm.

Maf1 is also a target of CK2 kinase [27,33] which is enriched on promoters of Pol III genes [33]. Maf1 also binds to tRNA genes, presumably indirectly by association with Pol III, and likely is located in close proximity to CK2. Indeed, their direct interaction was confirmed by co-immunoprecipitation [33]. The phosphorylation of Maf1 by CK2 is correlated with important events required for Pol III activation: release of Maf1 from chromatin and dissociation of Maf1 from the Pol III complex [33]. However, it is difficult to estimate impact of CK2-mediated Maf1 phosphorylation on Pol III activation. The experimental model used to study the role of Maf1 phosphorylation by CK2 in Pol III control included transferring yeast between repressive conditions in a medium with a non-fermentable carbon source to favourable conditions in glucose medium [27,33]. Although mutation of all potential CK2 sites in Maf1 resulted in less efficient in Pol III transcription in glucose medium [33], almost no effect on Pol III reactivation upon transfer from glycerol to glucose has been observed [27]. These data do not support the unconditional requirement for CK2 phosphorylation of Maf1 during derepression of Pol III transcription. Nevertheless, CK2 is a promiscuous kinase and certainly Maf1 is not its only substrate associated with Pol III activity since the components of Pol III machinery in yeast and humans (namely TFIIIB subunits and SNAP190 factor) are phosphorylated or controlled by CK2 [42,43,44,45]. Concerning other CK2 targets, phosphorylation of Maf1 is probably one of several other trials triggering Pol III activation upon transfer of yeast from repressive respiratory growth conditions to glucose.

Interaction of yeast Maf1 with Pol III apparatus and mechanism of Pol III repression

The Pol III apparatus consists of three complexes: the Pol III enzyme and the general factors TFIIIB and TFIIIC required for transcription initiation and promoter recognition, respectively. An

additional factor, TFIIIA, is required only for 5S rRNA gene transcription. *In vitro*, the primary step in the transcription of a tRNA gene is the binding of TFIIIC to the intragenic promoter elements known as the A and B boxes. A promoter-bound TFIIIC recruits the TFIIIB complex upstream of the transcription initiation site. TFIIIB is composed of the TBP (TATA-binding protein), Brf1 and Bdp1 subunits. Brf1 participates in TFIIIB-DNA complex formation by creating an extended connection between the opposite sides of the bent DNA, while Bdp1 generates an additional bend between the transcription start site and upstream of the TBP-interacting region, extending the TFIIIB-DNA contacts upstream of the TATA-box. The TFIIIB-DNA complex is sufficient to recruit the Pol III complex for multiple transcription cycles (reviewed in [46,47]). Efficiency and selectivity of Pol III transcription also depends on ist ability to recognize precisely positioned termination signals [48].

The Pol III complex (0.7 MDa) comprises 17 subunits. Five subunits are common to the three Pols, two are common with Pol I and are paralogs of Pol II subunits, five are paralogs of Pol I and Pol II subunits, and five are unique to Pol III. The structural core of Pol III is formed by nine subunits, C160, C128, AC40, AC19, ABC27, ABC23, ABC14.5, ABC10β and ABC10α. On the periphery of the core enzyme are eight additional subunits which form three distinct subcomplexes: C53-C37, C82-C34-C31 and C17-C25 [23,49]. C53-C37 is involved in promoter opening, elongation and termination, but re-initiation requires activity of C37-associated subunit C11 which additionally assures intrinsic cleavage activity of Pol III [50,51,52,53,54]. The heterotrimer C82-C34-C31, required for promoter-dependent transcription initiation, is partially related to the Pol II initiation factor TFIIE at least for C82 and C34 subunits that, similarly to TFIIE components, contain winged helix (WH) domains [1,55]. The structure of C82-C34-C31 located at the Pol III clamp over the active center cleft is rearranged by Maf1 which binds to the Pol III clamp at the rim of the cleft [23]. Most of the density assigned to the C34 WH domains in the Pol III-DNA-RNA complex was absent in the Pol III-Maf1 complex, indicating a Maf1-dependent displacement of these domains. The relocation of a specific WH domain of the C34 subunit is thought to weaken its interaction with the Brf1 subunit of the TFIIIB initiation factor,

suggesting that Maf1 impairs Pol III recruitment to promoters. Indeed, although free Pol III stably binds the Brf1–TATA-binding protein (TBP)–DNA complex, the Pol III–Maf1 complex does not [23]. This is consistent with earlier evidence that recruitment of Maf1 to class III chromatin correlates with the displacement of Pol III and TFIIIB [21]. Importantly, Maf1 does not inhibit the catalytic activity of Pol III, allowing DNA binding and RNA synthesis [23,56].

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 [23,56] and the interactions of Pol III with Maf1 and a Brf1-TBT-DNA are mutually exclusive [23]. It is consistent with earlier evidence that Maf1 prevents Pol III promoter interaction [21,24]. Significantly, Maf1 does not impair binding of a DNA-RNA scaffold nor Pol III elongation to the end of the template [23].

The observation that Pol III in complex with nucleic acids in its active center can simultaneously bind Maf1 suggested Maf1 binding at the elongation step [23]. This recruitment could occur by an undefined yet event causing Pol III repositioning during elongation or termination. During transcriptional elongation, the Pol III conformation is flexible [49]. Earlier hypothesis assumed a competition between the two Pol III catalytic states – fast stepping elongation state and slow stepping cleavage state - to influence the duration of pausing and the termination efficiency [50,57]. It was shown that the duration of this pause and termination is controlled by heterodimer C37–C53 [51]. Additionally, pausing and termination is linked Pol III cleavage activity mediated by C11 subunit [50,51,53,54]. Interestingly C11 has dual activity since, independently on a role in cleavage, it is also responsible for Pol III reinitiation, possibly by affecting the transition from the cleavage state to the elongationstate of Pol III [51,54]. The demonstration that C53/C37 lies near the pol III active site and participates inpromoter opening [52], is consistent with this hypothesis. With a model of the two Pol III catalytic states in mind, one could imagine that one catalytic state favors Maf1 binding during Pol III elongation or Maf1 interfering with one catalytic state may favor the other. Presumably Maf1 binding is related to or promotes “slow steping” cleavage state taking account the increased

occupancy of Maf1 at Pol III genes under repressive conditions [24,29,33]. If Maf1 interferes with C11 activity is currently unknown.

The rate of Pol III transcription increases at least fivefold through a process known as facilitated recycling, which couples the termination of transcription with reinitiation in a manner that is not yet precisely understood [58]. Facilitated recycling of both yeast and human Pol III was studied in the in vitro mechanism [56,59]. In course of studying effect of Maf1 on Pol III recycling it was shown that Pol III bound to preinitiation complexes or in elongation complexes is protected from repression by Maf1 and can undergo several rounds of initiation [56]. These results indicated that recombinant Maf1 is unable to inhibit facilitated recycling in the in vitro system [56]. Situation in the living cell is, however, different because, both Pol III machinery and Maf1, are under dynamic regulation by environmental conditions and additionally Pol III complex is rearranged during transcription cycle. In vivo mechanism of Maf1-mediated repression was proposed that allows immediate adjusting of Pol III activity to changing environmental conditions [60]. This model assumes Maf1 binding to the Pol III elongation complex at each cycle and its dissociation prior to initiation of the next cycle. Experimental data show unequivocally that a small amount of dephosphorylated, Pol III-associated Maf1 is present even in glucose-grown yeast [29,35]. CK2 kinase, which is present directly on the Pol III complex, ensures a high rate of transcription via phosphorylation of both Maf1 ,TFIIIB and potentially other Pol III components [33,42,43,45]. (Figure 2a). Conversely, when cells encounter unfavourable growth conditions, the CK2 catalytic subunit dissociates from the Pol III complex and is no longer able to stimulate transcription [33,42]. Moreover, dephosphorylated Maf1 is imported from the cytoplasm increasing its concentration in the nucleus [12]. This is the time when Maf1 takes over control and inhibits transcription (Figure 2b). This mechanism promises constant monitoring of the environment and a transcription shut-down immediately after the conditions become adverse.

In light of the high costs of tRNA synthesis, such a redundant system preventing a wasteful use of limited resources seems justified.

Indirect effect of Maf1 on tRNA processing

Individual stages of transcription are often coordinated with posttranscriptional events necessary for proper gene expression. Pol II transcription is directly coupled with mRNA maturation via CTD which serves as a binding platform for mRNA processing factors [2]. Co-transcriptional processing occurs also during rRNA synthesis [61]. Although it is unknown whether early tRNA maturation occurs co-transcriptionally, Pol III transcription in yeast is synchronized with the processing of tRNA precursors and Maf1, as the master Pol III regulator plays a major role in this synchronization [18].

tRNA processing involves multiple steps that occur in yeast at different subcellular locations [for review see [62]. Initial transcripts are extended at both the 5' and 3' termini and ten tRNA gene families in yeast contain introns. The processing of the 5' leader by RNaseP and the trimming of 3' trailer involving La protein, Rex1 exonuclease and RNaseZ occur in the nucleus. The end-processed tRNAs are then transported to the cytoplasm by the exportin Los1 and introns are removed by cytoplasmic splicing machinery located at the outer surface of mitochondria. tRNA molecules are modified in many ways and these modifications are added throughout the processing, both in the nucleus and in the cytoplasm.

Earlier reported relationship between tRNA transcription and processing in yeast focused on a dual role of Bdp1, essential subunit of TFIIIB factor [63]. Deletion of a small internal fragment of Bdp1 resulted in aberrant maturation of tRNA and temperature-sensitive phenotype, which could be overcome by overexpression of *RPR1* gene encoding subunit of RNaseP. Moreover, Bdp1, separately or in conjunction with TFIIIB, interacts physically with RNaseP complex suggesting its role in coupling of tRNA transcription and processing [63].

Direct role of tRNA processing factors in Pol III transcription was shown in mammalian cells. RNAseP is required for synthesis of tRNA in HeLa cells and acts as a catalytic ribonucleic protein in Pol III transcription excreting its role through interaction with Pol III complex and chromatin of active tRNA and 5S rRNA genes [64]. Mammalian La protein mediates transcript release and Pol III termination [65]..

An initial experimental observation to suggest a role of Maf1 in the posttranscriptional steps of tRNA biosynthesis in yeast came from analyses employing Pol III-specific microarrays [11]. A comparison of Pol III-transcribed RNAs from *maf1-Δ* and wild type cells shifted to non-permissive conditions showed that the levels of individual tRNA species were increased in the mutant from less than two-fold to over ten-fold. Significantly, most of the tRNAs encoded by intron-containing genes were elevated more than were the tRNAs encoded by intron-lacking genes. For example, the intron-containing $tRNA^{Phe}$ (GAA) was increased in *maf1-Δ* over 11-fold, $tRNA^{Trp}$ (CCA) – over 10-fold, and $tRNA^{Leu}$ (CAA) – nearly 9-fold [11]. A further study of tRNAs *maf1-Δ* cells by Northern hybridization highlighted an imbalance between the rate of tRNA synthesis and efficiency of its maturation. Both the initial transcripts and end-processed, intron-containing tRNA precursors accumulated in the absence of Maf1. This pre-tRNA accumulation could be overcome by transcription inhibition, arguing against a direct role of Maf1 in tRNA maturation and suggesting saturation of the processing machinery by the increased amounts of primary transcripts. Indeed saturation of the tRNA exportin Los1 was shown as one reason why end-matured intron-containing pre-tRNAs accumulated in cells lacking Maf1 [18]. It is likely that beside the Los1-mediated export other processes can be limiting for efficient pre-tRNA processing in *maf1*-Δ cells, especially under unfavorable growth conditions. However, the systematic regulation of tRNA processing by environmental conditions has not been addressed experimentally so far.

Maf1-mediated repression of RNA polymerase III activity inhibits tRNA degradation via RTD pathway

RNA levels are determined by two opposing distinct processes; transcription and decay. Transcription occurs in the nucleus whereas the major RNA decay pathways operate in the cytoplasm. Despite their location in different compartments, the two processes are tightly coordinated by an unknown yet mechanism. Several recent papers report a mutual feedback between mRNA synthesis and degradation [66,67]. In general, impairing mRNA transcription increases its stability. A similar relation has recently been reported for tRNA: Maf1-mediated repression of Pol III transcription has been shown to inhibit tRNA degradation [68] (Figure 3).

The folding and stability of tRNA is affected by specific modifications of individual nucleotides in tRNA of nucleotides, with each type of tRNA carrying an unique modification pattern [69,70]. $tRNA^{Val(AAC)}$, lacking the m^7G_{46} and m^5C_{49} modifications, is a substrate for the rapid tRNA decay (RTD) pathway. In the absence of the respective tRNA-metlyltransferases in a *trm4Δtrm8Δ* mutant, $tRNA^{Val(AAC)}$ is subject to rapid tRNA decay and is degraded at 37°C by 5'→3' exonucleases, Xrn1 and Rat1, leading to a temperature-sensitive growth [71]. The phenotype of the *trm4Δtrm8Δ* mutant can be suppressed by overexpression of the Maf1-encoding gene or, more efficiently, expression of the unphosphorylable Maf1-7A mutant that constitutively binds the Pol III complex and reduces tRNA transcription [68]. The suppression by overexpressed *MAF1* is accompanied by a two-fold stabilization of hypomodified $tRNA^{Val(AAC)}$, while the Maf1-7A mutant gives an even stronger effect. Additionally a 5'-terminal part of the *RPC160* gene has been also cloned as an autonomous suppressor of the *trm4Δtrm8Δ* phenotype. Indeed, similar suppressor actions of an overdose of Maf1 and the N-terminal part of Rpc160 on the *trm4Δtrm8Δ* growth defect were expected basing on earlier data. Overexpression of a 5'-terminal fragment as well as point mutations in the *RPC160* gene

were identified previously as suppressors of the *maf1Δ* growth phenotype. That suppression was accompanied by reduction of tRNA levels in *maf1Δ* cells [8,9].

Along the same lines, inhibition of tRNA synthesis in a Maf1-independent manner, either by a point mutation in the RNA polymerase III subunit Rpc128 or a decreased expression of the Rpc17 subunit, also suppressed the degradation of hypomodified $tRNA^{Val(AAC)}$. Similar suppression was achieved by overexpression of *TEF1* and *VAS1*, encoding, respectively, elongation factor eEF1A and valyl-tRNA synthetase, which likely protect hypomodified $tRNA^{Val(AAC)}$ by direct interactions [68]. The evidence that elongation factor eEF1A competes with the rapid decay pathway for substrate tRNAs was also presented by independent study of Eric Phizicky group [72]. According to the presented model inhibition of tRNA transcription leads to stabilization of hypomodified $tRNA^{Val(AAC)}$ due to an altered protein : RNA ratio and consequent more efficient protection by tRNA-interacting proteins.

Concluding remarks

The role of Maf1 as a general repressor of Pol III transcription appears to be conserved in eukaryotes. Human Maf1 also negatively regulates Pol III transcription [22,73,74] and *Drosophila* Maf1 represses tRNA synthesis in vivo in larvae [75,76]. That conserved function suggests also a conserved mechanism of Pol III regulation by Maf1.

As an unicellular organism, easily amenable to genetic manipulations, yeast are a convenient model for studying a basic regulatory mechanism of a tight Pol III control. However, relatively little is known about how the persistence of activated or repressed Pol III states is controlled directly on tRNA genes, despite the tremendous advances in deciphering the Pol III structure, recruitment of its auxiliary factors and modes of their regulation. There are still numerous questions that remain open and require further investigation. It is of great interest to know when exactly Maf1 binds the Pol III complex during the cycling of Pol III and what the mode of Pol III function in the absence of Maf1 is.

Maf1 interacts physically with Pol III complex, therefore one may assume that the alterations in Pol III structure/activity upon changed environmental conditions would be affected by the absence of Maf1. What is the exact role of CK2 in the regulation of Pol III machinery and by which mechanism does Maf1 affects the CK2 association with Pol III chromatin? What is the role of other Maf1 kinases? And finally how many more kinases phosphorylates Maf1? These questions should be addressed in further research.

Legends to Figures

Figure 1

Alignment of Maf1 sequences. Schematic representation of Maf1 protein sequences from different species: *E.c.*; *Encephalitozoon cuniculi* (gi|19069247|, size: 161 aa), *C.e*; *Caenorhabditis elegans* (gi|17506011|, size: 245 aa), *A.t.*; *Arabidopsis thaliana* (gi|22326767|, size: 224 aa), *D.d.*; *Dictyostelium discoideum* (gi|66816633|, size: 278 aa), *C.i.*; *Ciona intestinalis* gi|198415188|, size: 233 aa), *D.m.*;*Drosophila melanogaster* (gi|46409204|, size: 226 aa), *D.r.* ;*Danio rerio* (gi|47087413|, size: 247 aa), *H.s.*; *Homo sapiens* (gi|49065352|, size: 256 aa), *A.n.*; *Aspergillus nidulans* (gi|67901388|, size: 314 aa), *S.p.*; *Schizosaccharomyces pombe* (gi|254745531|, size: 238 aa), *C.g.*; *Candida glabrata* (gi|49529111|, size: 391 aa) and *S.c.* ; *Saccharomyces cerevisiae* (gi|1170854|, size: 395 aa). Protein sequences have been aligned with MUSCLE multiple alignment software and the figure created with Geneious Pro 4.5.4 software. Conserved regions A, B and C are boxed and localization of signature sequences is indicated. Localization of point mutation of *S. cerevisiae* Maf1 essential for Pol III repression R232H, K331A R332A; L242A D248A, P247D D248A, P247A R332A [24]; D248/D250A, D40/R41A [12]; K35E [26] was marked with asterisks. For the alignment of yeast and human Maf1 proteins including structural features reader is referred to Fig. 3A in ref. [23].

Figure 2.

Model of Pol III regulation by Maf1. Maf1 is recruited to Pol III, with a probability dependent on the growth conditions, at each transcription cycle. Maf1 binds to Pol III machinery after imitation step as concluded on the basis of biochemical and kinetic study [23] Following termination, Maf1 and CK2 confront each other in the Pol III initiation complex. Under favourable growth conditions (Figure 2a), Maf1 is released from Pol III [24,25,29] due to CK2 phosphorylation of Maf1 and/or activation of other components of Pol III machinery [42, 43,45]. Maf1 release allows reinitiation and efficient Pol III transcription. Subsequent export to the cytoplasm decreases the Maf1 concentration in the nucleus [12,35] and lowers the probability of its re-recruitment to the Pol III complex. Under adverse conditions (Figure 2b), the catalytic subunits of CK2 dissociate from TBP and CK2 becomes inactive [42]. Maf1, associated with the elongation complex, cannot be re-phosphorylated and remains bound to Pol III and precludes re-initiation. Additionally, dephosphorylated Maf1 is imported from the cytoplasm increasing its concentration in the nucleus [12,35]. In a short time estimated in minutes , all Pol III becomes bound by dephosphorylated Maf1 and is released from tRNA genes. Since Pol III genes are short and elongation is rapid, this stops all Pol III transcription almost instantaneously.

Figure 3.

Relationship between transcription and posttranscriptional steps of tRNA biosynthesis in yeast. Primary Pol III-synthesized transcripts are end-processed in the nucleus and exported to the cytoplasm by Los1. Introns are spliced out at the other membrane of mitochondria by tRNA endonuclease (Sen complex). CCA-addition at 3' terminus is followed by tRNA charging with aminoacids, binding to elongation factor (eEF1-α) and delivery to ribosomes. Turnover of mature tRNAs is controlled in the cytoplasm by rapid decay (RTD) pathway. Initial processing of tRNA in the nucleus is affected by Bdp1 subunit of TFIIIB [64]. Control of Los1-mediated export of tRNA from the nucleus is coordinated with regulation of Pol III transcription by Maf1 [18]. tRNA rapid decay pathway interacts with both, Pol III transcription and translation machinery [68,72].

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Maf1, a general negative regulator of RNA polymerase III in yeast

A brief, informative title: Regulation of Pol III by Maf1

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Abstract

tRNA synthesis by yeast RNA polymerase III (Pol III) is down-regulated under growth-limiting conditions. This control is mediated by Maf1, a global negative regulator of Pol III transcription. Conserved from yeast to man, Maf1 was originally discovered in *Saccharomyces cerevisiae* by a genetic approach. Details regarding the molecular basis of Pol III repression by Maf1 are now emerging from the recently reported structural and biochemical data on Pol III and Maf1. The phosphorylation status of Maf1 determines its nuclear localization and interaction with the Pol III complex and several Maf1 kinases have been identified to be involved in Pol III control. Moreover, Maf1 indirectly affects tRNA maturation and decay. Here I discuss the current understanding of the mechanisms that oversee the Maf1-mediated regulation of Pol III activity and the role of Maf1 in the control of tRNA biosynthesis in yeast.

Introduction

The existence of three RNA polymerases (Pol) is well documented for all investigated eukaryotes. Pol I synthesizes ribosomal RNA (rRNA), Pol II produces mainly mRNAs, and Pol III generates tRNAs, 5S rRNA and other small noncoding RNAs. Recent studies reveal substantial conservation between the transcription initiation machineries of the three polymerases (Pol I, II and III) [1]**.** Each core transcription initiation complex consists of a promoter DNA, a polymerase, TBP (TATA-binding protein), a TFIIB-like factor, a TFIIF-like protein, and TFIIE or proteins with TFIIE-related domains. Despite the high similarity between the general factors and the conserved basal structure of the polymerase complexes, the mechanisms of their regulation are different. Possibly the mechanisms that are specific for given categories of RNA provide a selective advantage by separately controlling mRNA, rRNA and tRNA production. Pol II uniquely employs the so-called mediator complex and carries an extra C-terminal domain (CTD) on the largest subunit, Rpb1. CTD undergoes dynamic phosphorylation during the progression from initiation through elongation to termination [2,3]. The specific phosphorylation patterns that predominate at each stage of transcription recruit appropriate sets of mRNA-processing and histone-modifying factors [3]. These dynamic interactions provide a means for coupling and coordinating specific stages of transcription with other events determining levels of individual mRNAs.

In contrast to the highly diverse population of mRNAs, the levels of Pol I and Pol III transcripts are predominantly controlled in a global manner. These two polymerases, sometimes collectively called “Odd Pols”, specialize in high-level synthesis of fundamental non-coding RNAs, together accounting for well over 90% of all cellular RNA by mass. In the yeast *Saccharomyces cerevisiae* Pol I synthesizes 35S precursor of 28S and 18S rRNA whereas the Pol III transcriptome includes 5S rRNA, tRNAs, U6 splicesomal RNA, the RNA subunit of the signal recognition particle (encoded by *SCR1*), RNase P RNA, and Snr52 snoRNA [4,5]. Critically for cellular economy, Pol I and Pol III need to be

repressed when growth is inhibited. Although controlled in a coordinated manner, these two polymerases use different regulatory proteins.

The activity of the yeast Pol I enzyme is specifically modulated by Rrn3, which renders Pol I competent for transcription initiation. The interaction with the Pol I complex is dependent on Rrn3 phosphorylation and is regulated by growth factor signaling pathways that adjust rRNA production to nutrient availability [6]. Pol III transcription in turn is uniquely controlled by a general repressor Maf1 which is regulated by phosphorylation and relays diverse negative signals to the Pol III machinery. The molecular basis of the Pol III transcription repression by Maf1 is now emerging due to the structural and biochemical data on Pol III and Maf1 recently reported. These results have highlighted the multiple levels of regulation of Maf1 activity and uncovered previously unanticipated secondary effects of Maf1. Some of these findings will be discussed in this review.

Genetics of Maf1

Maf1, the key player in the repression of RNA Pol III transcription, was originally discovered in *S. cerevisiae* by a genetic approach. The purpose of the screen which led to the isolation of the *maf1-1* mutant was the identification of *trans*-acting factors affecting the sub-cellular distribution of the tRNA-isopentenyltransferase Mod5 [7]. The parental strain used for that genetic screen contained a nonsense suppressor *SUP11*; *maf1-1* was selected among colonies that showed decreased suppression following random mutagenesis. Since the *SUP11* activity requires the cytoplasmic pool of Mod5, selection for a loss of suppression assumed a lower amount of Mod5 in the cytoplasm. Indeed, according to an original observation, Mod5 was mislocalized to the nucleus in the *maf1-1* mutant [7]. Despite nearly two decades elapsing since the original discovery, the mechanism by which Maf1 affects Mod5 localization is not understood. A decreased level of A37 isopentenylation in tRNAs, although never addressed experimentally, could be one reason of lower tRNA-mediated suppression in *maf1-1*.

In addition to decreasing the *SUP11* efficiency, *maf1*-1 was also temperature-sensitive when grown on nonfermentable carbon sources. Both phenotypes were fully complemented by *MAF1,* a gene of unknown function when cloned [8]. An intriguing genetic interaction between *MAF1* and Pol III was revealed by a search for multicopy suppressors of *maf1-1*; diverse variants of the *RPC160* gene with deletions in the 3' part of its open reading frame suppressed the *maf1-1* phenotypes when over-expressed [8]. Direct examination by Northern blotting revealed increased tRNA levels in *maf1-1* cells indicating the function of Maf1 as a negative Pol III regulator [9]. Full inactivation of *MAF1* resulted in the same phenotypes as those caused by the initially selected *maf1-1* allele.

Thus, although Maf1 is, apparently, the unique global regulator of Pol III transcription in yeast, the *maf1-Δ* mutant is viable and under standard conditions its growth is not compromised. One as yet unsolved question is why the mutant cells cannot tolerate an excess of tRNA and other Pol III transcripts under unfavorable growth conditions in media with a nonfermentable carbon source. The growth defect of *maf1-Δ* on medium with glycerol pointed to a deficiency in mitochondrial function. In yeast *S. cerevisiae* two Pol III synthesized tRNAs were reported as mitochondrially targeted, namely $tRNA^{Lys}$ and $tRNA^{Gln}$. Mitochondrial functions of these tRNAs are not completely clear although there is indirect evidence for their role in mitochondrial translation [10]. Since Pol III transcription is elevated in the absence of Maf1, one can assume increased amounts of some cytosolic tRNAs in mitochondria exerting a negative effect on mitochondrial translation in *maf1-Δ* mutant. Defects in mitochondrial translation most common cause of the degradation of mtDNA resulting in the formation of *petite* colonies known as *rho*$^-$. However, no increased *rho*$^-$ accumulation was observed in *maf1-Δ* strains arguing against this assumption [11].

Lastly, Maf1 could exert a specific effect on gene(s) required for growth on non-fermentable carbon sources and located close to tRNA gene(s) in the genome. In this mechanism called tRNA-gene-mediated silencing (tgm), actively transcribed tRNA genes negatively regulate adjacent Pol II-dependent genes. Indeed a role of Maf1 in tgm silencing has been reported by Moir et al. 2006 [12].

They studied the silencing of the *HIS3* gene by upstream adjacent *SUP4* tRNA gene, both expressed from a plasmid in a *his3* yeast strain. When *SUP4* was actively transcribed, the strain remained His- due to the tgm silencing, and preventing *SUP4* transcription by mutation in tRNA gene promoter resulted in the His+ colonies [13]. Unexpectedly, also the *maf1-Δ* mutation resulted in the His+ phenotype, although one should assume an opposite effect – a upregulation of *SUP4* transcription due to the lack of Maf1 and a consequent strong silencing of *HIS3* gene. That, as yet unexplained result sheds new light on Maf1 functioning which may be dual since tgm silencing effect of Maf1 may be unrelated to the effect of Maf1p on transcription by Pol III.

Similarly, the effect of Maf1 on the efficiency of tRNA-mediated suppression is opposite to that expected. Although one would assume that increased cellular tRNA levels should improve the efficiency of tRNA-mediated nonsense suppression, data show quite the opposite. Importantly the overall fidelity of translation is increased in the *maf1-Δ* mutant. As has been shown using a dual-gene reporter system, *maf1-Δ* cells have two-fold lower levels of UAA and UAG stop codon read-through [14]. This counterintuitive result could be due to the accumulation of unprocessed tRNA precursors which could limit the role of mature tRNAs in translation (indirect effect of Maf1 on tRNA processing is described later in this review).

Defects in tRNA biosynthesis, both in tRNA maturation and in nuclear export, induce translational derepression of the ubiquitous transcription factor Gcn4, a response conserved from yeast to mammals [15,16]. In yeast Gcn4 responds to amino acid deprivation due to accumulation of uncharged tRNAs that interact with Gcn2, the protein kinase that phosphorylates translation initiation factor eIF2. Phosphorylation of eIF2 by Gcn2 results in decreased levels of general translation, but increased translation of the transcription regulator Gcn4, which in turn results in transcription of numerous genes involved in amino acid and nucleotide biosynthesis (for review, see [17]. Remarkably, deletion of *MAF1* led to activation of Gcn4 as well [18], confirming the existence of a system communicating tRNA quality control in the nucleus to the translation machinery. However,

the component elements of this system require further investigation since accumulation of immature tRNAs induced Gcn4 independently of eIF2 phosphorylation [15,18].

Another impact of Maf1 on the yeast physiology is its role in sporulation. While competent for growth on media with a nonfermentable carbon source at the regular temperature, the *maf1-Δ / maf1-Δ* homozygous diploid is sporulation deficient [19] . Molecular basis of this phenotype needs to be addressed experimentally. Moreover, *maf1-Δ* is lethal when combined with a number of other deletions as revealed by a synthetic genetic array analysis [20]. Besides genes from the functional categories linked to the role of Maf1 as a regulator of Pol III transcription, that screen identified as synthetically lethal with *maf1-Δ* also genes involved in ribosome biogenesis, RNA pol II transcription, tRNA modification and ubiquitin-dependent proteolysis.

While the current studies on Maf1 tend to focus on its role in Pol III control, the often puzzling genetic phenotypes observed for yeast *maf1* mutants may serve as an inspiration for a broader investigations.

Family of Maf1 proteins

Maf1 protein is found in human, animals, plants and lower eukaryotes. It contains three highly conserved regions called A, B and C, which are shared among species [9,21,22] but do not correspond to structural modules or defined surface patches in Maf1 protein [23]. Neither potential prokaryotic orthologs nor substantial homology with protein domains of known function have been identified resulting in a striking lack of information on the functional significance of those regions and included signature sequences PDYDFS and WSfnYFFYNkklKR. Point mutagenesis of yeast *MAF1* has only highlighted the importance of several serine residues (mostly non-conserved ones) and two nuclear localization sequences [12,24,25].

A comparison of the arrangement of representative Maf1 sequences is presented in Figure 1. The distance between the B and C segments of approximately 10 aa is constant in evolution, with the

exception of *Aspergilus nidulans* (insertion of 15 aa). In contrast, the distance between regions A and B largely varies between species. The A and B domains are fused in *Encephalitozoon cuniculi* whereas in the yeast *S. cerevisiae* and *Candida glabrata* they are separated by a long linker of 182 aa and 174 aa, respectively. The Maf1 sequences of *C. elegans* and *H. sapiens* have relatively shorter linkers.

Limited proteolysis of recombinant human Maf1 resulted in two stable fragments (aa 1-45 and aa 75-234) corresponding to the evolutionarily conserved regions. When two open reading frames corresponding to these two fragments were co-expressed in bacteria, they co-purified and co-eluted during size exclusion chromatography as soluble complex. However, when expressed individually, neither fragment formed a soluble, protease-resistant module. These biochemical data suggest that the N- and C- terminal regions of Maf1 form modules that do not fold independently but rather need to be co-expressed to form a stable and soluble entity [26]. In line with the biochemical data reported for human Maf1, a physical interaction of the N- and C-terminal regions of yeast Maf1 has been shown using the yeast two-hybrid system and a genetic suppressor approach [26]. More importantly, X-ray crystallography of Maf1 at 1.55 A ° resolution revealed that Maf1 forms a globular structure and conserved sequence regions do not correspond to structural modules or defined surface patches [23] . Crystallized variant of human Maf1 lacked two regions which appear solvent-exposed and unstructured: the linker connecting A and B conserved sequences and the C-terminal acidic tail. These two unstructured regions are not required for Pol III binding since both a full-length recombinant yeast Maf1 and a shorter variant that lacked the linker and the C-terminal tail formed a complex with Pol III that could be purified by size-exclusion chromatography [23]. Moreover, partial deletion of linker region is dispensable for Maf1 function, as shown recently by molecular analysis [27]. Nevertheless, unstructured regions could be involved in fine-tuning regulation of Maf1 activity by phosphorylation, as described in the next section.

Regulation of Maf1 activity

Function of Maf1 as a negative effector of Pol III synthesis was presumed on the basis of molecular and analysis of *maf1-1* mutant [8,9]. First, Northern analysis indicated that tRNA levels are elevated in *maf1* mutant cells . Second, mutations in the *RPC160* gene encoding the largest subunit of Pol III which reduce tRNA levels were identified as suppressors of the *maf1-1* growth defect. Third, physical interaction between Maf1 and Pol III suggested a direct role for Maf1 in Pol III down-regulation [9]. Importanly, based on Northern blot analysis of selected precursor tRNAs, Maf1 was found to mediate several signaling pathways by repressing Pol III transcription in response to diverse stresses [28].

Maf1 activity is regulated via its phosphorylation state-dependent cellular localization [12,24,25,29,30,31,32,33]. Pol III repression requires Maf1 in dephosphorylated state [24,29,33,34]. Differentially phosphorylated forms of Maf1 can be resolved by SDS-PAGE and it is the least phosphorylated form that binds Pol III [22,25,29]. The increased association of Maf1 with Pol III under repressing conditions is correlated with dissociation of Pol III from tRNA gene [24,29,33].

In favourable growth conditions, Maf1 is located predominantly in the cytoplasm, although is never fully excluded from the nucleus [12,35]. The Maf1's cytoplasmic location is mediated by two mechanisms: a phosphorylation-dependent inactivation of the Maf1 nuclear location signals (NLSs) [12], and nuclear export of phosphorylated Maf1 by the exportin Msn5 [35]. Although the changing distribution of Maf1 between the nucleus and the cytoplasm could, in principle, account for the regulation of Poll III transcription by Maf1, two lines of evidence suggest that the phosphorylation is the main mechanism by which Maf1 activity is controlled and the nuclear–cytoplasmic dynamics of Maf1 may instead be a mechanism to fine-tune regulation. First, Maf1 is still able to regulate Pol III transcription in cells lacking Msn5, that is required to export phosphorylated Maf1 from the nucleus [35]. Second, although Maf1 is constitutively located in the nucleus in the common W303 yeast strain background, the regulation of Pol III is nevertheless responsive to environmental signaling [32].

The predicted phosphorylation sites are mostly found in the unstructured linker located between conserved A and B fragments of Maf1 sequence. The migration of yeast Maf1 in SDS gels is especially sensitive to phosphorylation at particular serines (S177 and S178) in the linker region [31]. Phosphorylation within the long unstructured linker likely affects the interaction between the conserved A and BC regions of the yeast Maf1 necessary for the full repression of Pol III activity [26]. Biochemical studies regarding the significance of the unstructured regions for the regulation of Maf1 activity by phosphorylation are, however, limited since their presence in the recombinant yeast Maf1 greatly reduces solubility and promotes protein aggregation.

Maf1 is also phosphorylated in mammalian cells. Human Maf1 becomes largely dephosphorylated after stress, and it is the dephosphorylated form of Maf1 that associates with Pol III [22,36,37].

Maf1 as mediator of signaling pathways

In yeast, Maf1 is the only Pol III negative regulator that acts as an effector of several signalling pathways [28]. In addition to the down-regulation that normally occurs in the stationary phase, Maf1 is also required for Pol III repression accompanying rapamycin treatment, starvation, secretion defects, as well as oxidative and replication stress [24,25,28,29,30,38]. Cells depleted of Maf1 remain alive under stress conditions, but, surprisingly, Maf1-mediated regulation is essential during the transition from fermentative to glycerol-based respiratory growth [11]. This observation underscored the critical function of Maf1 in the coupling of Pol III transcription to metabolic processes and/or energy production dependent on the carbon source [11,12].

The molecular mechanisms that trigger Maf1 activity in response to different signaling pathways are only partially understood. The main Maf1 phosphatase is protein phosphatase 4 (PP4) [34]. The PP4 action is most likely direct, as a portion of cellular PP4 co-precipitates with Maf1. By dephosphorylating Maf1 PP4 mediates rapid Pol III repression in response to diverse stresses [34].

In response to favourable growth conditions yeast Maf1 is phosphorylated at different sites by several kinases. So far four of them, PKA, Sch9, TORC1 and CK2 have been described [12,25,31,32,33], but other Maf1 kinases are likely to be identified in the future . The impact of each of those kinases on the control of Maf1 interaction with the Pol III complex is, however, difficult to estimate using available experimental tools.

Sch9 certainly effects the Maf1 interaction with Pol III since inactivation of all potential Sch9 phosphorylation sites (which are the same as PKA sites) in a Maf1-7A mutant promoted its nuclear localization and increased the Maf1-Pol III association [25,31,39]. Importantly in the Maf1-7A mutant cells Pol III was not constitutively repressed and responded robustly to rapamycin [25]. Moreover, the Pol III activity was still sensitive to rapamycin in Maf1-7E "phosphomimetic" mutant. Those results suggested that regulation by Sch9/PKA is insufficient for confer Pol III control and some additional step is required. Either these kinases must target factor(s) in addition to Maf1, or Maf1-7E has to be phosphorylated in additional positions (and, conversely, Maf1-7A additionally dephosphorylated) [25].

A further opportunity for the control of Maf1 activity is provided by chromatin-bound kinases TOR and CK2. Although both yeast and human TOR phosphorylate Maf1 [32,37,40,41], control by TOR seems not to be conserved in evolution. The mTOR kinase localizes to mammalian tRNA and 5S rRNA genes by interaction with TFIIIC, a DNA binding factor that recognizes the promoters of those genes. By this association, mTOR-mediated phosphorylation of Maf1 functionally contributes to the regulation of the repressive activity of Maf1 at mammalian tRNA genes [40]. In contrast, the yeast TORC1 kinase binds to rDNA chromatin, 35S as well as 5S genes, and is not detected on tRNA genes [32]. TORC1 has been postulated to interact with and phosphorylate Maf1 at the rDNA loci, in this way regulating its translocation from the nucleolus to the nucleoplasm [32]. However, phosphorylation by TORC1 was detected for recombinant, but not native, yeast Maf1 and the phosphorylation site has not been identified. Moreover, Maf1 association with 5S rDNA genes is

controversial [33]. Remarkably, TORC1 phosphorylates Sch9 at multiple sites and this phosphorylation is required for the catalytic activity of Sch9, another kinase of yeast Maf1 [25]. Thus, TORC1 could control yeast Maf1 indirectly, via Sch9 in the nucleoplasm.

Maf1 is also a target of CK2 kinase [27,33] which is enriched on promoters of Pol III genes [33]. Maf1 also binds to tRNA genes, presumably indirectly by association with Pol III, and likely is located in close proximity to CK2. Indeed, their direct interaction was confirmed by co-immunoprecipitation [33]. The phosphorylation of Maf1 by CK2 is correlated with important events required for Pol III activation: release of Maf1 from chromatin and dissociation of Maf1 from the Pol III complex [33]. However, it is difficult to estimate impact of CK2-mediated Maf1 phosphorylation on Pol III activation. The experimental model used to study the role of Maf1 phosphorylation by CK2 in Pol III control included transferring yeast between repressive conditions in a medium with a non-fermentable carbon source to favourable conditions in glucose medium [27,33]. Although mutation of all potential CK2 sites in Maf1 resulted in less efficient in Pol III transcription in glucose medium [33], almost no effect on Pol III reactivation upon transfer from glycerol to glucose has been observed [27]. These data do not support the unconditional requirement for CK2 phosphorylation of Maf1 during derepression of Pol III transcription. Nevertheless, CK2 is a promiscuous kinase and certainly Maf1 is not its only substrate associated with Pol III activity since the components of Pol III machinery in yeast and humans (namely TFIIIB subunits and SNAP190 factor) are phosphorylated or controlled by CK2 [42,43,44,45]. Concerning other CK2 targets, phosphorylation of Maf1 is probably one of several other trials triggering Pol III activation upon transfer of yeast from repressive respiratory growth conditions to glucose.

Interaction of yeast Maf1 with Pol III apparatus and mechanism of Pol III repression

The Pol III apparatus consists of three complexes: the Pol III enzyme and the general factors TFIIIB and TFIIIC required for transcription initiation and promoter recognition, respectively. An

additional factor, TFIIIA, is required only for 5S rRNA gene transcription. *In vitro*, the primary step in the transcription of a tRNA gene is the binding of TFIIIC to the intragenic promoter elements known as the A and B boxes. A promoter-bound TFIIIC recruits the TFIIIB complex upstream of the transcription initiation site. TFIIIB is composed of the TBP (TATA-binding protein), Brf1 and Bdp1 subunits. Brf1 participates in TFIIIB-DNA complex formation by creating an extended connection between the opposite sides of the bent DNA, while Bdp1 generates an additional bend between the transcription start site and upstream of the TBP-interacting region, extending the TFIIIB-DNA contacts upstream of the TATA-box. The TFIIIB-DNA complex is sufficient to recruit the Pol III complex for multiple transcription cycles (reviewed in [46,47]). Efficiency and selectivity of Pol III transcription also depends on ist ability to recognize precisely positioned termination signals [48].

The Pol III complex (0.7 MDa) comprises 17 subunits. Five subunits are common to the three Pols, two are common with Pol I and are paralogs of Pol II subunits, five are paralogs of Pol I and Pol II subunits, and five are unique to Pol III. The structural core of Pol III is formed by nine subunits, C160, C128, AC40, AC19, ABC27, ABC23, ABC14.5, ABC10β and ABC10α. On the periphery of the core enzyme are eight additional subunits which form three distinct subcomplexes: C53-C37, C82-C34-C31 and C17-C25 [23,49]. C53-C37 is involved in promoter opening, elongation and termination, but re-initiation requires activity of C37-associated subunit C11 which additionally assures intrinsic cleavage activity of Pol III [50,51,52,53,54]. The heterotrimer C82-C34-C31, required for promoter-dependent transcription initiation, is partially related to the Pol II initiation factor TFIIE at least for C82 and C34 subunits that, similarly to TFIIE components, contain winged helix (WH) domains [1,55]. The structure of C82-C34-C31 located at the Pol III clamp over the active center cleft is rearranged by Maf1 which binds to the Pol III clamp at the rim of the cleft [23]. Most of the density assigned to the C34 WH domains in the Pol III-DNA-RNA complex was absent in the Pol III-Maf1 complex, indicating a Maf1-dependent displacement of these domains. The relocation of a specific WH domain of the C34 subunit is thought to weaken its interaction with the Brf1 subunit of the TFIIIB initiation factor,

suggesting that Maf1 impairs Pol III recruitment to promoters. Indeed, although free Pol III stably binds the Brf1–TATA-binding protein (TBP)–DNA complex, the Pol III–Maf1 complex does not [23]. This is consistent with earlier evidence that recruitment of Maf1 to class III chromatin correlates with the displacement of Pol III and TFIIIB [21]. Importantly, Maf1 does not inhibit the catalytic activity of Pol III, allowing DNA binding and RNA synthesis [23,56].

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 [23,56] and the interactions of Pol III with Maf1 and a Brf1-TBT-DNA are mutually exclusive [23]. It is consistent with earlier evidence that Maf1 prevents Pol III promoter interaction [21,24]. Significantly, Maf1 does not impair binding of a DNA-RNA scaffold nor Pol III elongation to the end of the template [23].

The observation that Pol III in complex with nucleic acids in its active center can simultaneously bind Maf1 suggested Maf1 binding at the elongation step [23]. This recruitment could occur by an undefined yet event causing Pol III repositioning during elongation or termination. During transcriptional elongation, the Pol III conformation is flexible [49]. Earlier hypothesis assumed a competition between the two Pol III catalytic states – fast stepping elongation state and slow stepping cleavage state - to influence the duration of pausing and the termination efficiency [50,57]. It was shown that the duration of this pause and termination is controlled by heterodimer C37–C53 [51]. Additionally, pausing and termination is linked Pol III cleavage activity mediated by C11 subunit [50,51,53,54]. Interestingly C11 has dual activity since, independently on a role in cleavage, it is also responsible for Pol III reinitiation, possibly by affecting the transition from the cleavage state to the elongationstate of Pol III [51,54]. The demonstration that C53/C37 lies near the pol III active site and participates inpromoter opening [52], is consistent with this hypothesis. With a model of the two Pol III catalytic states in mind, one could imagine that one catalytic state favors Maf1 binding during Pol III elongation or Maf1 interfering with one catalytic state may favor the other. Presumably Maf1 binding is related to or promotes “slow steping” cleavage state taking account the increased

occupancy of Maf1 at Pol III genes under repressive conditions [24,29,33]. If Maf1 interferes with C11 activity is currently unknown.

The rate of Pol III transcription increases at least fivefold through a process known as facilitated recycling, which couples the termination of transcription with reinitiation in a manner that is not yet precisely understood [58]. Facilitated recycling of both yeast and human Pol III was studied in the in vitro mechanism [56,59]. In course of studying effect of Maf1 on Pol III recycling it was shown that Pol III bound to preinitiation complexes or in elongation complexes is protected from repression by Maf1 and can undergo several rounds of initiation [56]. These results indicated that recombinant Maf1 is unable to inhibit facilitated recycling in the in vitro system [56]. Situation in the living cell is, however, different because, both Pol III machinery and Maf1, are under dynamic regulation by environmental conditions and additionally Pol III complex is rearranged during transcription cycle. In vivo mechanism of Maf1-mediated repression was proposed that allows immediate adjusting of Pol III activity to changing environmental conditions [60]. This model assumes Maf1 binding to the Pol III elongation complex at each cycle and its dissociation prior to initiation of the next cycle. Experimental data show unequivocally that a small amount of dephosphorylated, Pol III-associated Maf1 is present even in glucose-grown yeast [29,35]. CK2 kinase, which is present directly on the Pol III complex, ensures a high rate of transcription via phosphorylation of both Maf1 ,TFIIIB and potentially other Pol III components [33,42,43,45]. (Figure 2a). Conversely, when cells encounter unfavourable growth conditions, the CK2 catalytic subunit dissociates from the Pol III complex and is no longer able to stimulate transcription [33,42]. Moreover, dephosphorylated Maf1 is imported from the cytoplasm increasing its concentration in the nucleus [12]. This is the time when Maf1 takes over control and inhibits transcription (Figure 2b). This mechanism promises constant monitoring of the environment and a transcription shut-down immediately after the conditions become adverse.

In light of the high costs of tRNA synthesis, such a redundant system preventing a wasteful use of limited resources seems justified.

Indirect effect of Maf1 on tRNA processing

Individual stages of transcription are often coordinated with posttranscriptional events necessary for proper gene expression. Pol II transcription is directly coupled with mRNA maturation via CTD which serves as a binding platform for mRNA processing factors [2]. Co-transcriptional processing occurs also during rRNA synthesis [61]. Although it is unknown whether early tRNA maturation occurs co-transcriptionally, Pol III transcription in yeast is synchronized with the processing of tRNA precursors and Maf1, as the master Pol III regulator plays a major role in this synchronization [18].

tRNA processing involves multiple steps that occur in yeast at different subcellular locations [for review see [62]. Initial transcripts are extended at both the 5' and 3' termini and ten tRNA gene families in yeast contain introns. The processing of the 5' leader by RNaseP and the trimming of 3' trailer involving La protein, Rex1 exonuclease and RNaseZ occur in the nucleus. The end-processed tRNAs are then transported to the cytoplasm by the exportin Los1 and introns are removed by cytoplasmic splicing machinery located at the outer surface of mitochondria. tRNA molecules are modified in many ways and these modifications are added throughout the processing, both in the nucleus and in the cytoplasm.

Earlier reported relationship between tRNA transcription and processing in yeast focused on a dual role of Bdp1, essential subunit of TFIIIB factor [63]. Deletion of a small internal fragment of Bdp1 resulted in aberrant maturation of tRNA and temperature-sensitive phenotype, which could be overcome by overexpression of *RPR1* gene encoding subunit of RNaseP. Moreover, Bdp1, separately or in conjunction with TFIIIB, interacts physically with RNaseP complex suggesting its role in coupling of tRNA transcription and processing [63].

Direct role of tRNA processing factors in Pol III transcription was shown in mammalian cells. RNAseP is required for synthesis of tRNA in HeLa cells and acts as a catalytic ribonucleic protein in Pol III transcription excreting its role through interaction with Pol III complex and chromatin of active tRNA and 5S rRNA genes [64]. Mammalian La protein mediates transcript release and Pol III termination [65]..

An initial experimental observation to suggest a role of Maf1 in the posttranscriptional steps of tRNA biosynthesis in yeast came from analyses employing Pol III-specific microarrays [11]. A comparison of Pol III-transcribed RNAs from *maf1-Δ* and wild type cells shifted to non-permissive conditions showed that the levels of individual tRNA species were increased in the mutant from less than two-fold to over ten-fold. Significantly, most of the tRNAs encoded by intron-containing genes were elevated more than were the tRNAs encoded by intron-lacking genes. For example, the intron-containing $tRNA^{Phe}$ (GAA) was increased in *maf1-Δ* over 11-fold, $tRNA^{Trp}$ (CCA) – over 10-fold, and $tRNA^{Leu}$ (CAA) – nearly 9-fold [11]. A further study of tRNAs *maf1-Δ* cells by Northern hybridization highlighted an imbalance between the rate of tRNA synthesis and efficiency of its maturation. Both the initial transcripts and end-processed, intron-containing tRNA precursors accumulated in the absence of Maf1. This pre-tRNA accumulation could be overcome by transcription inhibition, arguing against a direct role of Maf1 in tRNA maturation and suggesting saturation of the processing machinery by the increased amounts of primary transcripts. Indeed saturation of the tRNA exportin Los1 was shown as one reason why end-matured intron-containing pre-tRNAs accumulated in cells lacking Maf1 [18]. It is likely that beside the Los1-mediated export other processes can be limiting for efficient pre-tRNA processing in *maf1*-Δ cells, especially under unfavorable growth conditions. However, the systematic regulation of tRNA processing by environmental conditions has not been addressed experimentally so far.

Maf1-mediated repression of RNA polymerase III activity inhibits tRNA degradation via RTD pathway

RNA levels are determined by two opposing distinct processes; transcription and decay. Transcription occurs in the nucleus whereas the major RNA decay pathways operate in the cytoplasm. Despite their location in different compartments, the two processes are tightly coordinated by an unknown yet mechanism. Several recent papers report a mutual feedback between mRNA synthesis and degradation [66,67]. In general, impairing mRNA transcription increases its stability. A similar relation has recently been reported for tRNA: Maf1-mediated repression of Pol III transcription has been shown to inhibit tRNA degradation [68] (Figure 3).

The folding and stability of tRNA is affected by specific modifications of individual nucleotides in tRNA of nucleotides, with each type of tRNA carrying an unique modification pattern [69,70]. $tRNA^{Val(AAC)}$, lacking the m^7G_{46} and m^5C_{49} modifications, is a substrate for the rapid tRNA decay (RTD) pathway. In the absence of the respective tRNA-metlyltransferases in a *trm4Δtrm8Δ* mutant, $tRNA^{Val(AAC)}$ is subject to rapid tRNA decay and is degraded at 37^{o}C by 5'→3' exonucleases, Xrn1 and Rat1, leading to a temperature-sensitive growth [71]. The phenotype of the *trm4Δtrm8Δ* mutant can be suppressed by overexpression of the Maf1-encoding gene or, more efficiently, expression of the unphosphorylable Maf1-7A mutant that constitutively binds the Pol III complex and reduces tRNA transcription [68]. The suppression by overexpressed *MAF1* is accompanied by a two-fold stabilization of hypomodified $tRNA^{Val(AAC)}$, while the Maf1-7A mutant gives an even stronger effect. Additionally a 5'-terminal part of the *RPC160* gene has been also cloned as an autonomous suppressor of the *trm4Δtrm8Δ* phenotype. Indeed, similar suppressor actions of an overdose of Maf1 and the N-terminal part of Rpc160 on the *trm4Δtrm8Δ* growth defect were expected basing on earlier data. Overexpression of a 5'-terminal fragment as well as point mutations in the *RPC160* gene

were identified previously as suppressors of the *maf1Δ* growth phenotype. That suppression was accompanied by reduction of tRNA levels in *maf1Δ* cells [8,9].

Along the same lines, inhibition of tRNA synthesis in a Maf1-independent manner, either by a point mutation in the RNA polymerase III subunit Rpc128 or a decreased expression of the Rpc17 subunit, also suppressed the degradation of hypomodified $tRNA^{Val(AAC)}$. Similar suppression was achieved by overexpression of *TEF1* and *VAS1*, encoding, respectively, elongation factor eEF1A and valyl-tRNA synthetase, which likely protect hypomodified $tRNA^{Val(AAC)}$ by direct interactions [68]. The evidence that elongation factor eEF1A competes with the rapid decay pathway for substrate tRNAs was also presented by independent study of Eric Phizicky group [72]. According to the presented model inhibition of tRNA transcription leads to stabilization of hypomodified $tRNA^{Val(AAC)}$ due to an altered protein : RNA ratio and consequent more efficient protection by tRNA-interacting proteins.

Concluding remarks

The role of Maf1 as a general repressor of Pol III transcription appears to be conserved in eukaryotes. Human Maf1 also negatively regulates Pol III transcription [22,73,74] and *Drosophila* Maf1 represses tRNA synthesis in vivo in larvae [75,76]. That conserved function suggests also a conserved mechanism of Pol III regulation by Maf1.

As an unicellular organism, easily amenable to genetic manipulations, yeast are a convenient model for studying a basic regulatory mechanism of a tight Pol III control. However, relatively little is known about how the persistence of activated or repressed Pol III states is controlled directly on tRNA genes, despite the tremendous advances in deciphering the Pol III structure, recruitment of its auxiliary factors and modes of their regulation. There are still numerous questions that remain open and require further investigation. It is of great interest to know when exactly Maf1 binds the Pol III complex during the cycling of Pol III and what the mode of Pol III function in the absence of Maf1 is.

Maf1 interacts physically with Pol III complex, therefore one may assume that the alterations in Pol III structure/activity upon changed environmental conditions would be affected by the absence of Maf1. What is the exact role of CK2 in the regulation of Pol III machinery and by which mechanism does Maf1 affects the CK2 association with Pol III chromatin? What is the role of other Maf1 kinases? And finally how many more kinases phosphorylates Maf1? These questions should be addressed in further research.

Legends to Figures

Figure 1

Alignment of Maf1 sequences. Schematic representation of Maf1 protein sequences from different species: *E.c.*; *Encephalitozoon cuniculi* (gi|19069247|, size: 161 aa), *C.e*; *Caenorhabditis elegans* (gi|17506011|, size: 245 aa), *A.t.*; *Arabidopsis thaliana* (gi|22326767|, size: 224 aa), *D.d.*; *Dictyostelium discoideum* (gi|66816633|, size: 278 aa), *C.i.*; *Ciona intestinalis* gi|198415188|, size: 233 aa), *D.m.*;*Drosophila melanogaster* (gi|46409204|, size: 226 aa), *D.r.* ;*Danio rerio* (gi|47087413|, size: 247 aa), *H.s.*; *Homo sapiens* (gi|49065352|, size: 256 aa), *A.n.*; *Aspergillus nidulans* (gi|67901388|, size: 314 aa), *S.p.*; *Schizosaccharomyces pombe* (gi|254745531|, size: 238 aa), *C.g.*; *Candida glabrata* (gi|49529111|, size: 391 aa) and *S.c.* ; *Saccharomyces cerevisiae* (gi|1170854|, size: 395 aa). Protein sequences have been aligned with MUSCLE multiple alignment software and the figure created with Geneious Pro 4.5.4 software. Conserved regions A, B and C are boxed and localization of signature sequences is indicated. Localization of point mutation of *S. cerevisiae* Maf1 essential for Pol III repression R232H, K331A R332A; L242A D248A, P247D D248A, P247A R332A [24]; D248/D250A, D40/R41A [12]; K35E [26] was marked with asterisks. For the alignment of yeast and human Maf1 proteins including structural features reader is referred to Fig. 3A in ref. [23].

Figure 2.

Model of Pol III regulation by Maf1. Maf1 is recruited to Pol III, with a probability dependent on the growth conditions, at each transcription cycle. Maf1 binds to Pol III machinery after imitation step as concluded on the basis of biochemical and kinetic study [23] Following termination, Maf1 and CK2 confront each other in the Pol III initiation complex. Under favourable growth conditions (Figure 2a), Maf1 is released from Pol III [24,25,29] due to CK2 phosphorylation of Maf1 and/or activation of other components of Pol III machinery [42, 43,45]. Maf1 release allows reinitiation and efficient Pol III transcription. Subsequent export to the cytoplasm decreases the Maf1 concentration in the nucleus [12,35] and lowers the probability of its re-recruitment to the Pol III complex. Under adverse conditions (Figure 2b), the catalytic subunits of CK2 dissociate from TBP and CK2 becomes inactive [42]. Maf1, associated with the elongation complex, cannot be re-phosphorylated and remains bound to Pol III and precludes re-initiation. Additionally, dephosphorylated Maf1 is imported from the cytoplasm increasing its concentration in the nucleus [12,35]. In a short time estimated in minutes , all Pol III becomes bound by dephosphorylated Maf1 and is released from tRNA genes. Since Pol III genes are short and elongation is rapid, this stops all Pol III transcription almost instantaneously.

Figure 3.

Relationship between transcription and posttranscriptional steps of tRNA biosynthesis in yeast. Primary Pol III-synthesized transcripts are end-processed in the nucleus and exported to the cytoplasm by Los1. Introns are spliced out at the other membrane of mitochondria by tRNA endonuclease (Sen complex). CCA-addition at 3' terminus is followed by tRNA charging with aminoacids, binding to elongation factor (eEF1-α) and delivery to ribosomes. Turnover of mature tRNAs is controlled in the cytoplasm by rapid decay (RTD) pathway. Initial processing of tRNA in the nucleus is affected by Bdp1 subunit of TFIIIB [64]. Control of Los1-mediated export of tRNA from the nucleus is coordinated with regulation of Pol III transcription by Maf1 [18]. tRNA rapid decay pathway interacts with both, Pol III transcription and translation machinery [68,72].

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Figure 1

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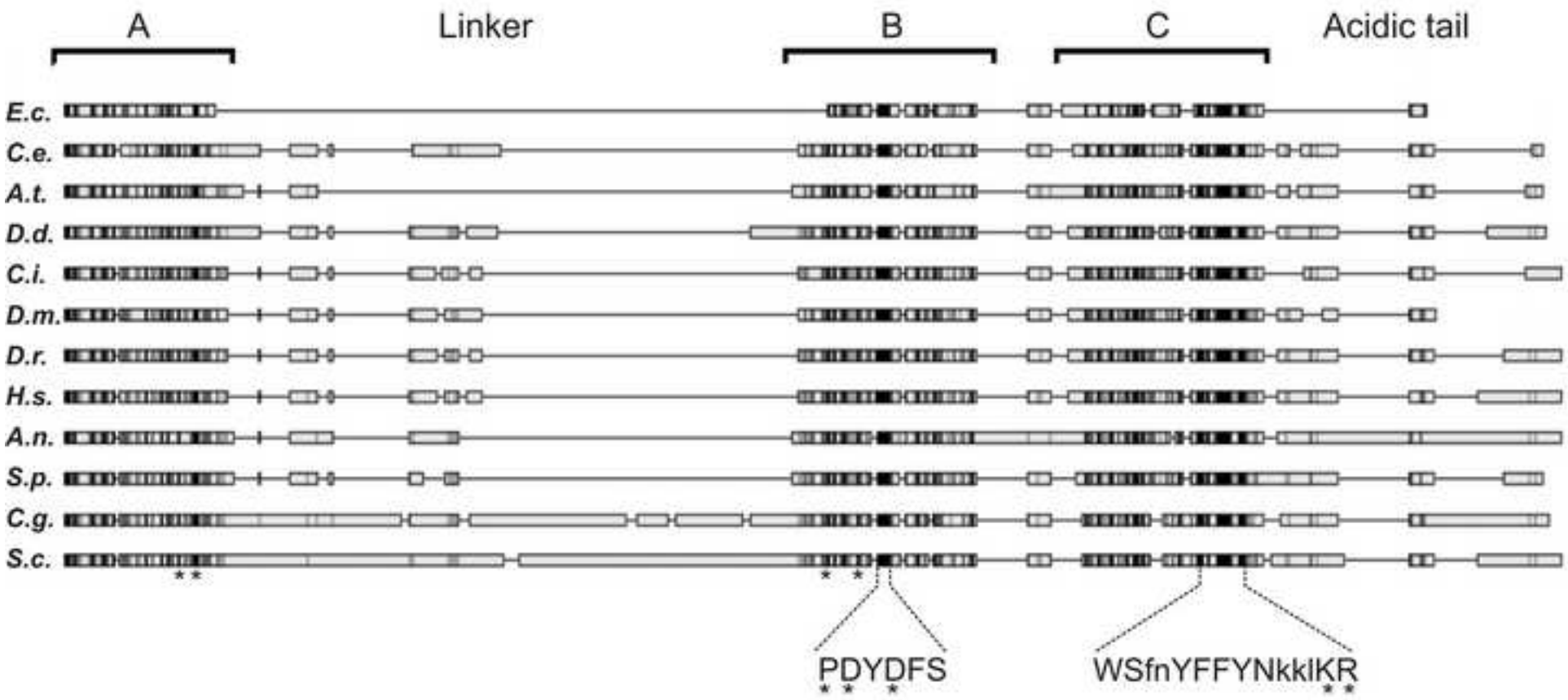

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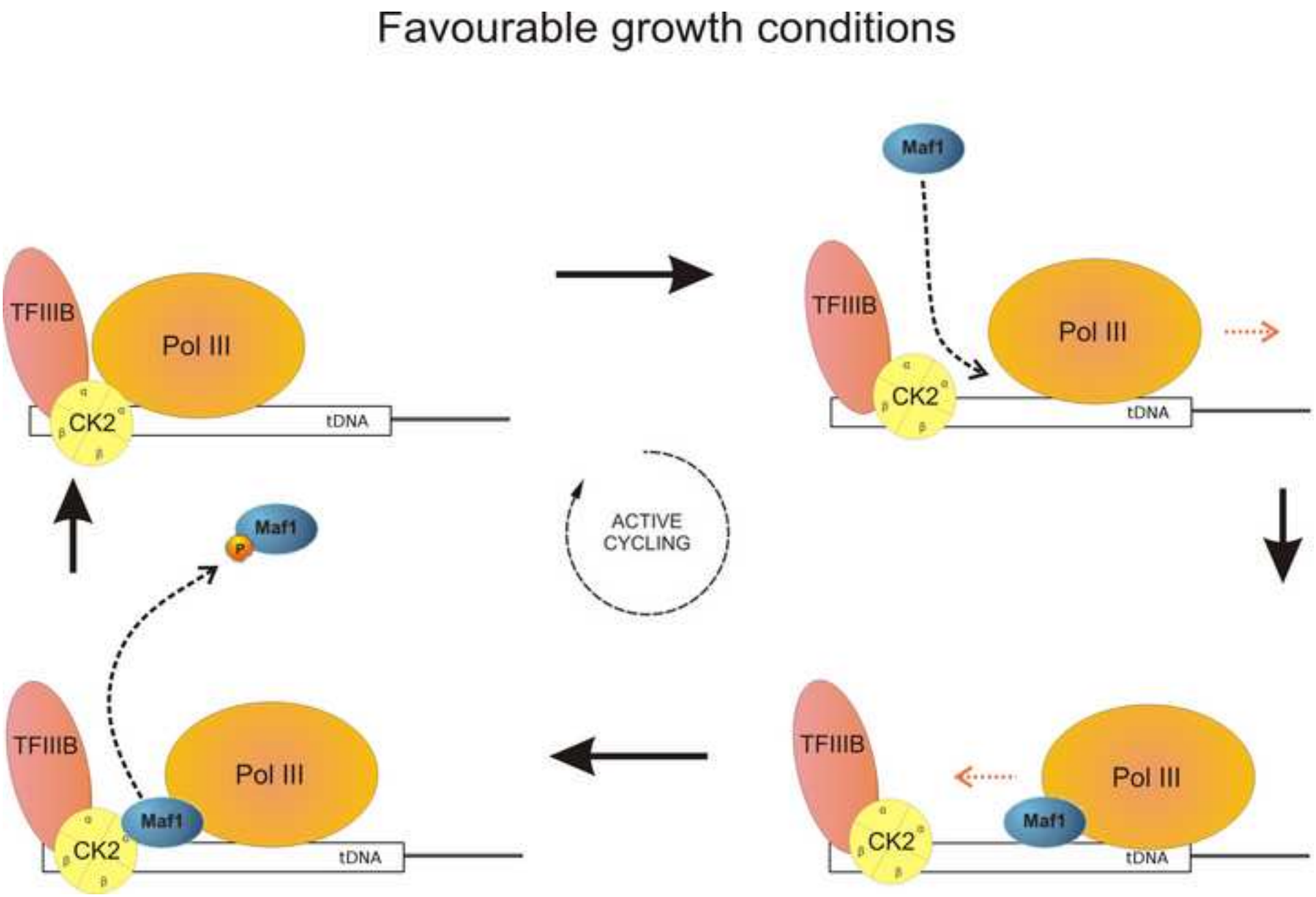

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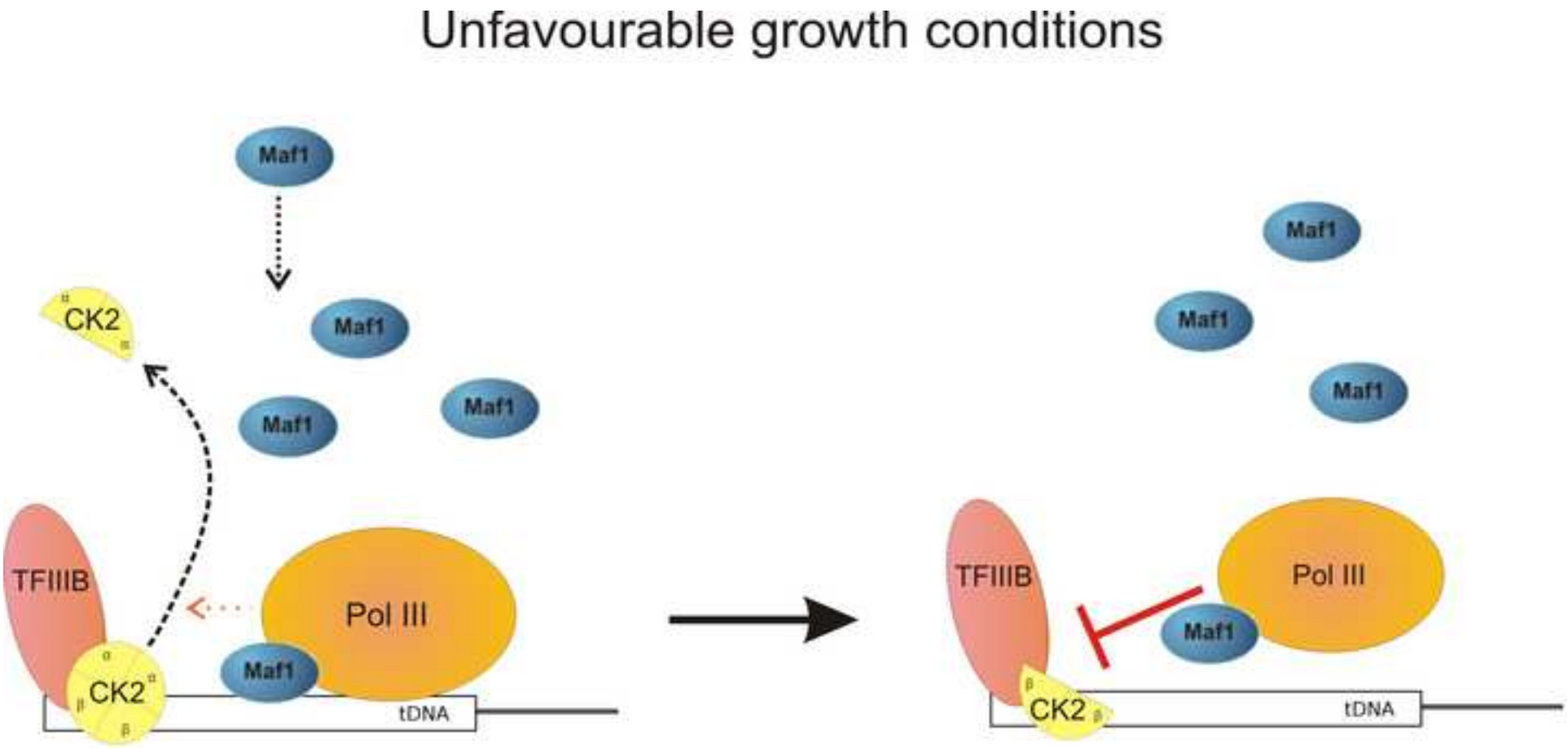

Figure 3

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