RNA polymerase III under control: repression and de-repression

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Abstract

The synthesis of tRNA by yeast RNA polymerase III (Pol III) is regulated in response to changing environmental conditions . This control is mediated by Maf1, the global negative regulator of Pol III transcription conserved from yeast to man. Details regarding the molecular basis of Pol III repression by Maf1 are now emerging from recently reported structural and biochemical data on Pol III and Maf1. Efficient Pol III transcription, following the shift of cells from a non-fermentable carbon source to glucose, requires phosphorylation of Maf1. One of the newly identified Maf1 kinases is the chromatin-bound casein kinase II (CK2). Current studies allowed us to propose an innovative mechanism of Pol III regulation. We suggest that CK2-mediated phosphorylation of Maf1, occurring directly on tDNA chromatin, controls Pol III recycling.

Control of RNA polymerase III activity

In yeast RNA synthesis by RNA polymerases I and III (Pol I and Pol III) represents more than 80% of all nuclear transcription [1]. Pol I is dedicated to the synthesis of rRNA whereas tRNAs, 5S rRNA and several other small noncoding RNAs are synthesized by Pol III

(Box 1). Under favourable growth conditions during each cell cycle, 3–6 million tRNA molecules are synthesized in a yeast cell at a rate of 2–4 transcripts/gene/s [2]. This high rate of tRNA gene transcription is achieved through many rounds of re-initiation by Pol III on a stably DNA-bound initiation factor TFIIIB. The rate of subsequent rounds increases at least 5-fold through a process known as facilitated recycling, which couples the termination of transcription with reinitiation in a manner that is not yet precisely understood [3, 4]. Because of the high energy cost of transcription, exact coordination of Pol III activity with environmental growth conditions provides a powerful selective advantage. Relatively little is known about how the persistence of activated or repressed Pol III states is controlled directly on tRNA genes, despite tremendous advances in deciphering the Pol III structure, recruitment of its auxiliary factors and modes of their regulation.

When stress conditions occur or nutrients turn out to be limiting, tRNA transcription is rapidly repressed by Maf1, a negative regulator of Pol III. Since the level of the basal transcription of class III genes is very high, we suggest that repression is the major form of Pol III regulation. Below we propose that association of Maf1 with tDNA chromatin is controlled at every Pol III re-initiation step and, depending on environmental conditions , results in transcription repression or de-repression.

Maf1: central actor of Pol III regulation in yeast

Highly conserved from yeast to man, Maf1 was identified as a general and direct repressor of Pol III transcription [5]. In addition, at least human and yeast Maf1 are phosphoproteins [6-9]. In yeast, Maf1 is the only Pol III negative regulator that acts as an effector of several signalling pathways [10, 11]. In addition to downregulation that normally occurs in stationary phase, Maf1 is also required for Pol III repression accompanying starvation, respiratory growth, as well oxidative and replication stress [12-14]. Signalling

pathways activated by growth-limiting conditions lead to dephosphorylation of Maf1 by the PP2A phosphatase and import of Maf1 into the nucleus (Figure 1) driven by two nuclear localization sequences (NLS) [6, 15]. In the nucleus, hypophosphorylated Maf1 binds to the Pol III complex. The increased association of Maf1 with class III chromatin under repressing conditions is correlated with dissociation of Pol III from tRNA gene [6, 8]. Prolonged repression results in dissociation of TFIIIB as well, but during acute repression TFIIIB remains associated with the promoter [8].

In favourable growth conditions, Maf1 is inactivated by phosphorylation which operates at several levels to counteract Pol III repression (Figure 1). Apart from decreasing direct binding of Maf1 to Pol III, phosphorylation also acts to both facilitate Maf1 export from the nucleus and to preclude import of cytoplasmic Maf1 to the nucleus. Different kinases perform these functions by phosphorylating Maf1. PKA kinase inactivates NLS signals and thereby prevents nuclear localization of Maf1 [15]. Sch9-mediated phosphorylation of nuclear Maf1 presumably enables its interaction with Msn5 exportin and transport out of the nucleus. Besides affecting its nuclear transport, Maf1 phosphorylation by Sch9 has an impact on its interaction with Pol III since inactivation of all potential Sch9 phosphorylation sites results in increased Maf1-Pol III association [16-18].

Although Maf1 shuttles between the nucleus and cytoplasm, phosphorylation of Maf1 but not its export is essential for Pol III activation following a shift from adverse to favourable growth conditions [16, 19, 20]. Several lines of evidence indicate that yeast can regulate Maf1 activity without excluding it from the nucleus [15, 19]. The subnuclear localization of Maf1 is also relevant since Pol III transcription is localized to the nucleolus [20]. Importantly, although under favourable growth conditions Maf1 is mostly cytoplasmic, it is never fully excluded from the nucleus [6].

The latest findings allow us to propose how phosphorylation of Maf1 is coordinated with other events occurring on chromatin that establish Pol III control. First, structural and biochemical data on Pol III and Maf1 have been reported recently [21, 22]. Second, phosphorylation of Maf1 by two central cellular kinases, CK2 and TORC1, occurring directly on chromatin, has been documented [9, 20, 23-25].

Maf1 structure and mode of interaction with Pol III

Maf1 structure was determined recently by X-ray crystallography at 1.55Å resolution, and the structure of the Maf1-Pol III co-complex was reconstructed from cryo-electron microscopy data [21]. The location of Maf1 in the co-complex confirmed previous genetic and biochemical data indicating that Maf1 binds to the N-terminal fragment of the largest Pol III subunit C160, and located it to the clamp domain of Pol III [5, 6, 21, 26]. Maf1 binding rearranges the complex of subunits C82/34/31 within Pol III that are required for transcription initiation. The relocation of a specific domain of 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 [21]. Indeed, whereas free Pol III stably bound the Brf1-TBP-DNA complex, the Pol III-Maf1 complex did not [21]. This is consistent with previous evidence that recruitment of Maf1 to class III chromatin correlated with the displacement of Pol III and TFIIIB [11]. Importantly, Maf1 does not inhibit the catalytic activity of the Pol III, allowing nucleic acid binding in the active site and RNA synthesis [2, 21].

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, [2, 21], but this does not exclude a possibility that Maf1 might be recruited during sequential binding of TBP, Brf1 and Bdp1 giving rise to unproductive preinitiation complex. More likely Maf1 is

recruited after initiation step. Maf1 recruitment could occur by an unknown event causing Pol III repositioning and after the initiation step, during elongation or termination. During transcriptional elongation, the Pol III conformation is flexible [22]. A conformational change appears to occur in Pol III during a transitory pause in the elongation step prior to termination, and the duration of this pause is controlled by the C37-C53 heterodimer [27]. One interesting hypothesis is that this pausing facilitates Maf1 binding.

Phosphorylation of chromatin-associated Maf1

Phosphorylation, occurring in favourable growth conditions directly on tRNA genes, is the main way of inactivating yeast Maf1 by preventing its interaction with Pol III [6-8]. One opportunity for such direct control is provided by chromatin-bound kinases [28, 29]. TOR ,is the main metabolic kinase operating on Pol III (and Pol I) chromatin [18] . Although both yeast and human TOR phosphorylate Maf1 [9, 20, 24, 30] Pol III 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 [9].

In contrast, the yeast TORC1 kinase binds to rDNA chromatin, 35S as well as 5S genes, and is not detected on tRNA genes [20]. It has been postulated that TORC1 interacts with and phosphorylates Maf1 at the rDNA loci, in this way regulating its translocation from the nucleolus to the nucleoplasm [20]. However, phosphorylation by TORC1 was detected only for recombinant yeast Maf1 and the phosphorylation site has not been identified [20]. Moreover, Maf1 association with 5S rDNA genes is controversial [23]. TORC1 phosphorylates Sch9 at multiple sites and this phosphorylation is required for catalytic

activity of Sch9, another kinase of yeast Maf1 [16]. Thus, TORC1 might control yeast Maf1 indirectly, via Sch9 in the nucleoplasm (Figure 1).

Pol III control involves yet another player, CK2 kinase, known to be engaged in transcription-directed signalling and other critical cellular processes (Box 2). Previous work identified the role of CK2 in positive as well as negative Pol III regulation in yeast and humans [31-35]. It was shown a decade ago that CK2-mediated phosphorylation of the TBP subunit of yeast TFIIIB is required for efficient TFIIIB recruitment to Pol III promoters. Under favourable conditions CK2 physically interacts with TFIIIB, but upon genotoxic stress its catalytic subunits dissociate from the complex [32]. The mechanism of Pol III control by CK2 was, however, not fully understood and the participation of an additional factor was postulated [36]. Maf1 was unknown at that time and could not have been taken into account as such a factor. Recent work presents evidence that yeast CK2 is present on tRNA genes, but is not detected on 5S rRNA genes [23], in contrast to TORC1 being preferentially present on 5S rRNA genes [20]. Interestingly, Maf1 is also mainly detected on tRNA genes and is not found on 5S rRNA genes [23]. Moreover, Maf1 interacts with and is subject to CK2 phosphorylation, thus suggesting that Maf1 could be that additional factor involved in CK2-mediated Pol III regulation, the existence of which was previously suggested [23].

We postulate that Maf1 is a mediator of environmental conditions through which CK2 transduces the signal to Pol III. Our model posits that in favourable growth conditions, phosphorylation by CK2 at the same time deactivates Maf1 and activates TFIIIB to allow efficient Pol III transcription. This hypothesis is strongly supported by the fact that phosphorylation of Maf1 by CK2 appears to counteract repression directly at the level of the transcription unit, since both Maf1 and CK2 are associated with tRNA genes. Upon a shift to repressive conditions, the association of the CK2 catalytic subunit with tDNA chromatin is decreased [23]. This is in agreement with a previous report indicating dissociation of the

catalytic subunit of CK2 from the CK2-TFIIIB complex observed in response to stress [32]. In cells lacking Maf1, CK2 binds to tRNA genes as well and remains bound even in repressive conditions [23]. We propose that the dissociation of the CK2 catalytic subunits from the TBP subunit of TFIIIB is prevented in the absence of Maf1. Therefore, one hypothesis is that Maf1 is required for blocking the CK2–TFIIIB interaction, thereby triggering Pol III repression. This raises the question of whether Maf1 might influence the CK2 holoenzyme at the heart of the Pol III preinitiation complex, and if so, by what mechanism.

Pol III control by CK2 and Maf1: a model

Recent evidence has emphasized the importance of continued regulation of transcription throughout RNA synthesis. Several transcription factors controlling Pol II progression through the promoter-proximal region have been identified [37]. Since Maf1 does not inhibit the catalytic activity of Pol III, Maf1-mediated transcription arrest within a promoter-proximal region is considered to be unlikely.

Experimental data show unequivocally that a small amount of dephosphorylated, Pol III-associated Maf1 is present even in glucose-grown yeast [6]. According to our model (Figure 2), regardless of growth conditions, Maf1 can be recruited to the Pol III elongation complex at every transcription cycle. Following termination, Maf1 and CK2 confront each other in the heart of the Pol III initiation complex. Under favourable growth conditions, CK2 phosphorylates Maf1 thus releasing it from Pol III and enabling re-initiation. Subsequent export to the cytoplasm decreases Maf1 concentration in the nucleus and lowers the probability of its recruitment to the Pol III elongation complex. Despite the activity of CK2 on tRNA genes, Maf1 has some residual activity and restrains Pol III activity to a basal level. Indeed cells lacking Maf1 show higher pre-tRNA levels also in favourable conditions of

177 growth. Under stress conditions, the catalytic subunits of CK2 dissociate from TBP and CK2
178 becomes inactive. Maf1, associated with the elongation complex, cannot be re-
179 phosphorylated, remains bound to Pol III and re-initiation is not possible. Moreover,
180 dephosphorylated Maf1 is imported from the cytoplasm increasing its concentration in the
181 nucleus. In a short time estimated in minutes , all Pol III is bound by dephosphorylated Maf1.
182 Since Pol III genes are short and elongation is fast, this rapidly stops all Pol III transcription.
183
184 **Concluding remarks**
185 The highly active Pol III requires constant monitoring of the environment and a
186 transcription shut-down immediately after the conditions become adverse. The model
187 proposed here invokes an innovative mechanism of the control of Pol III recycling through
188 CK2-mediated phosphorylation of Maf1 occurring directly on tDNA chromatin. Maf1 binding
189 to the Pol III complex and subsequent Maf1 phosphorylation following dissociation during
190 every cycle of transcription constitute a 'probing' mechanism that allows immediate adjusting
191 of Pol III activity to changing environmental conditions. The constitutively active CK2
192 kinase, which is present directly on the Pol III complex, ensures a high rate of transcription
193 via both TBP and Maf1 phosphorylation. Conversely, when cells encounter unfavourable
194 growth conditions, the CK2 catalytic subunit dissociates from the Pol III complex and is no
195 longer able to stimulate transcription. This is the time when Maf1 takes over control and
196 inhibits transcription.
197 Although simple, the model presents an attractive mechanism of controlling Pol III
198 activity. It has never before been considered that Maf1 binds to the Pol III complex at every
199 cycle of transcription, only to be immediately phosphorylated and excluded from the
200 complex. At first glance this appears costly, but compared to the costs of tRNA synthesis it

might be beneficial and thus cost-saving, especially when the possibility of immediate Pol III shutdown is taken into account.

However, there are still some questions that remain open and require further investigation. It would be of great interest to know when exactly Maf1 binds the Pol III complex during the cycling of Pol III. By which mechanism does Maf1 influence CK2 association with TFIIIB? What factor(s) modulate the recurrent binding of the CK2 catalytic subunit to the Pol III complex? Why is CK2 present only on tRNA genes but not on 5S genes? What is the precise role of other kinases phosphorylating Maf1, is it only regulation of its cellular localization? These questions should be addressed in further research to support the model proposed in this article.

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Figure 1. **Maf1 is regulated at multiple levels.** The proportion of Maf1 in the nucleus and Maf1 interaction with Pol III is regulated by Maf1 phosphorylation mediated by nutrient and stress signalling. Rapamycin and other unfavourable growth conditions induce dephosphorylation of cytoplasmic Maf1 by PP2A or other phosphatases and Maf1 import to the nucleus. We speculate that in the nucleoplasm Maf1 is subjected to subsequent dephosphorylation allowing its localization to the nucleolus, where it binds to the Pol III complex and inhibits transcription. Upon switch from repression to favourable growth conditions (green), CK2-dependent phosphorylation of Maf1 induces its dissociation from the Pol III complex associated with tDNA. In parallel, TORC1-dependent phosphorylation of Maf1 induces its dissociation from the Pol III complex associated with 5S DNA allowing its transport from nucleolus to nucleoplasm. Downstream steps involve PKA-

and/or Sch9-dependent phosphorylation of Maf1 and Msn5-dependent export out of the nucleus thereby providing further separation between the Maf1 and the Pol III machinery. In the cytoplasm, newly synthesized Maf1 is also subject to phosphorylation by PKA and/or Sch9, thus preventing its import to the nucleus.

Figure 2. **Model of Maf1 action.** Under favourable growth conditions, the efficiency of Pol III transcription is increased by active recycling. CK2 phosphorylates Maf1 releasing it from the Pol III complex and enabling re-initiation (1). In every transcription cycle during elongation (2) or termination (3), Maf1 is recruited to the Pol III complex. Before re-initiation (4), Maf1 is confronted by the CK2 kinase, which phosphorylates and binds the TBP subunit of TFIIIB initiation factor in the promoter region. When unfavourable conditions are encountered, the catalytic subunits of CK2 dissociate from TBP and CK2 becomes inactive (5). Maf1 associated with Pol III cannot be re-phosphorylated and remains bound to the Pol III C160 subunit and Pol III C82/34/31 subcomplex (6). Maf1 prevents interaction of the C34 subunit with the Brf1 subunit of TFIIIB whereby re-initiation is not possible and Pol III is inhibited.

Box 1. RNA Polymerase III

RNA polymerase III (Pol III) is responsible for the synthesis of small non-coding RNAs involved in gene expression. Genome-wide identification of the Pol III transcriptome in the yeast *Saccharomyces cerevisiae* has revealed 5S rRNA, tRNAs, U6 splicesomal RNA, the RNA subunit of the signal recognition particle (encoded by *SCR1*), RNase P RNA, and Snr52 snoRNA to be synthesized by Pol III [38-40]. The Pol III apparatus consists of three complexes: the Pol III enzyme and the general factors TFIIIB and TFIIIC required for transcription initiation and for promoter recognition, respectively. An additional factor,

TFIIIA, is required only for 5S rRNA gene transcription. *In vitro,* the primary step in transcription of a tRNA gene is the binding of TFIIIC to intragenic promoter elements, known as the A and B boxes. TFIIIC binding to the promoter 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 suffices to recruit the Pol III complex for multiple transcription cycles [reviewed in [41]].

The Pol III complex (0.7 MDa) comprises 17 subunits. Five subunits are common to the three Pols, two are common to Pol I and are paralogs to Pol II subunits, five are paralogs to 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, Pol III contains eight additional subunits which form three distinct subcomplexes: C82-C34-C31, C17-C25, and C53-C37. The heterotrimer C82-C34-C31 is evolutionary related to the Pol II initiation factor TFIIE (at least for C82-C34) and is required for promoter-dependent transcription initiation [42]. This subcomplex bridges the initiation factors TFIIIC and TFIIIB. Moreover, C82-C34-C31 interacts directly with the C160 subunit. C17-C25 contributes to initiation complex assembly because C17 binds to Brf1, a subunit of TFIIIB, and to the C82-C34-C31 subcomplex. The C53-C37 heterodimer shows weak homology to the Pol II initiation factor TFIIF and is involved in Pol III termination (reviewed in [41]).

Box 2. The CK2 kinase

CK2 (casein kinase II) is a highly conserved eukaryotic protein kinase involved in transcription-directed signalling, gene control, and cell cycle regulation. CK2 has been implicated in critical cellular processes such as proliferation, apoptosis, differentiation, and transformation [43]. CK2 is mostly present as a tetramer composed of two catalytic and two regulatory subunits. The regulatory subunits stimulate the catalytic subunits, stabilize the CK2 heterotetramer, and act as a scaffold for specific kinase partners [44]. The enzyme is expressed constitutively at a modest level, and is thought to be constitutively active rather than being regulated by external stimuli [45]. How individual CK2-mediated phosphorylation events are regulated or how the access of CK2 to many of its substrates is controlled is not obvious. One idea is that there could be many discrete subpopulations of CK2 which exist within cells, and that each subpopulation is regulated in a unique manner. The repertoire of possible regulatory mechanisms include regulated expression and assembly of the CK2 holoenzyme, regulation through covalent modification (e.g. phosphorylation and ubiquitylation) and regulatory interactions with proteins or other cellular components [43, 46].

CK2 controls Pol III-mediated tRNA transcription in yeast and humans [23, 31-35]. Yeast CK2 interacts with and phosphorylates both the TBP subunit of TFIIIB initiation factor [32] and the Maf1 repressor [23], thereby dually stimulating tRNA gene transcription. In mammals CK2 is involved in Pol III regulation by the cell cycle. Pol III transcription is most active during the S and G2 phase [47] being positively regulated by CK2 [34]. During mitosis Pol III is repressed and CK2-mediated phosphorylation of the Bdp1 subunit of TFIIIB contributes to this negative regulation [33].

CK2 also exerts a positive effect on rDNA transcription by phosphorylation of different components of Pol I transcription machinery. In mammals CK2 also phosphorylates UBF, a Pol I initiation factor, and regulates the interaction between UBF and the TBP-containing

factor SL1 at the rDNA promoters in the nucleolus [48]. In mammals CK2 also phosphorylates the TIF-IA initiation factor promoting its dissociation from elongating Pol I [49]. Thus CK2 might provide a mechanism to co-regulate Pol I and Pol III transcription in the eukaryotic cell.

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