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Regulation of tRNA synthesis by the general transcription factors of RNA polymerase III - TFIIIB and TFIIIC, and by the MAF1 protein

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ABSTRACT

The synthesis of transfer RNA (tRNA) is directed by RNA polymerase III (Pol III) specialized in high-level transcription of short DNA templates. Pol III recruitment to tRNA genes is controlled by two general initiation factors, TFIIIB and TFIIIC. They are multi-protein complexes regulated at the level of expression of individual subunits, as well as through phosphorylation and interaction with partner proteins. Here, we describe particular aspects of TFIIIB and TFIIIC control in yeast and human cells. Under stress conditions, tRNA synthesis is negatively regulated by the MAF1 protein, which interacts directly with Pol III. Sequence and function of MAF1 are conserved among eukaryotic organisms from yeast to humans. MAF1 is a phosphoprotein which mediates diverse regulatory signals to Pol III. Interestingly, there is a subset of housekeeping tRNA genes, both in the yeast and human genome, which are less sensitive to MAF1-dependent repression. The possible mechanisms responsible for this differential regulation of tRNA synthesis by MAF1 are discussed.

1. Introduction

Transfer RNAs (tRNAs) are adaptor molecules that play a role in translation by linking messenger RNA (mRNA) with amino acids during protein synthesis. tRNAs are transcribed by RNA polymerase (Pol) III, a highly evolutionarily conserved complex that consists of 17 subunits [1]. Apart from tRNAs, Pol III synthesizes several other essential components of the protein biosynthetic machinery, including 5S rRNA, 7SL RNA (component of the signal recognition particle ribonucleoprotein complex; SRP) and a subset of small non-coding RNAs required for maturation of other RNA molecules and for translation control. The rate of Pol III transcription is tightly regulated in response to different conditions.

For unicellular eukaryotes, such as yeast (*Saccharomyces cerevisiae*), coordination of tRNA levels and ribosome function is important for optimal utilization of nutrients and for survival. Consequently, transcription of tRNA genes by Pol III is down-regulated in the stationary phase [2], by nutrient starvation [3] or by a non-fermentable carbon source [4], secretion defects, DNA damage, and chemical treatments [5,6].

In mammals, Pol III is subjected too much broader regulatory influences than in yeast. Its activity is decreased in response to differentiation and stress signals, such as starvation, DNA damage, and hypoxia [7–9], and increased in response to growth factors, hormones, nutrients, and inflammatory signals [10–12]. As there is a spectrum of factors that modulate Pol III activity both in yeast and mammalian cells, appropriate mechanisms must be engaged to control this crucial enzyme.

In eukaryotes, Pol III recruitment to tRNA genes is mediated by two general transcription factors, TFIIIB and TFIIIC, and the contribution of a yet unknown gene-specific regulatory protein(s) cannot be fully excluded. Pol III transcription is also subjected to global down-regulation. In yeast cells, signals that repress Pol III converge on a central negative regulator, the MAF1 protein [6]. The function of MAF1 as a Pol III repressor is conserved among eukaryotes, including mammals; however, mammalian cells contain an expanded repertoire of regulatory circuits and elements that modulate Pol III activity. Several other important regulators, including p53, Myc, and Rb, are also engaged in direct control of Pol III.

Here, we briefly describe the mechanisms underlying the expression, posttranslational modifications and assembly of Pol III initiation factors, TFIIIB and TFIIIC. We also review the modes of regulation of tRNA gene transcription, both in yeast and mammalian cells, with particular focus on MAF1, TFIIIB and TFIIIC.

2. Transcription factors - TFIIIB and TFIIIC, and tRNA genes

tRNA genes contain two internal promoters, box A and box B, which are specifically recognized and bound by a large multi-subunit protein complex, TFIIIC. Both yeast and human TFIIIC consist of six

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Table 1
Yeast and human subunits of TFIIIB and TFIIIC.

Yeast	Human
TFIIIB	
TBP (TATA-binding protein)	TBP (TATA-binding protein)
Brf1	Brf1/Brf2
Bdp1	Bdp1
TFIIIC	
τA	
Tfc1 (τ95)	TFIIIC63
Tfc4 (τ131)	TFIIIC102
Tfc7 (τ55)	TFIIIC35
τB	
Tfc3 (τ138)	TFIIIC220
Tfc6 (τ91)	TFIIIC110
Tfc8 (τ60)	TFIIIC90

polypeptides organized into two globular domains, τA and τB (Table 1). τB binds with high affinity to the B box and favors A box binding by τA (reviewed in [13,14]). Contrary to mammals, in yeast cells all tRNA genes are bound by TFIIIC [15–18]. The correlation between TFIIIC binding and *in vivo* tDNA transcription remains unknown.

Both in yeast and mammalian cells, TFIIIB consists of three subunits: TFIIB-related Brf1, TATA-box binding protein (TBP), common also for the other two RNA polymerases, and Pol III-specific subunit, Bdp1 (Table 1). In vertebrates, a variant form of TFIIIB was found, in which Brf1 is substituted with Brf2. This form of TFIIIB is required for transcription of type III genes with external promoters, including the U6 spliceosomal RNA and selenocysteine tRNA genes [14]. TFIIIB plays an important role in recruiting Pol III to its target genes; however, recruitment of TFIIIB itself is one of the most important steps in the regulation of Pol III transcription. Recently, a possible molecular mechanism of TFIIIB recruitment to its target genes was described [19]. TFIIIB is also required for and sufficient to sustain multiple rounds of transcription [20], which makes it a good target for regulation. Accordingly, in mammalian cells, several proteins that regulate tRNA gene transcription extensively exploit TFIIIB modulation (see below for details).

a. The role of TFIIIC in TFIIIB recruitment in yeast cells

Yeast TFIIIB assembles upstream of the transcription start site through interaction between Brf1 and Bdp1 with Tfc4, a subunit of the TFIIIC τA module. Based on yeast two-hybrid studies and genetic analyses, recruitment of both Brf1 and Bdp1 was shown to be directed by the tetratricopeptide repeat domains (TPR) of Tfc4 [21–23] (for a more detailed description see review by E. Ramsay and A. Vannini, in this issue).

Recently, a model of TFIIIC architecture was proposed, based on the structural information on individual subunits and cross-linking data [19]. The model allowed mapping and analyzing the previously described TFIIIC mutations. Additionally, it served to design polypeptides for biochemical pull-down experiments aimed at studying Tfc4 interaction with the Tfc3 subunit of τB and Bdp1, the subunit of TFIIIB. Based on the model, TFIIIC-directed assembly of TFIIIB is initiated by Brf1 binding to the N-terminal domain of Tfc4, followed by TBP recruitment *via* Brf1 and Tfc8 subunit of τB, and, finally, binding of Bdp1 to Tfc4. Competition between Bdp1 and Tfc3 τB subunits in binding to the same domain of Tfc4 suggests that recruitment of Bdp1 induces a conformational change, leading to displacement of the τB module and, consequently, to dissociation of TFIIIC from the gene [19].

Earlier co-immunoprecipitation studies, performed in cross-linked chromatin, have revealed increased association between TFIIIC and TFIIIB under repressive conditions [3]. The established TFIIIC-TFIIIB interaction may be quickly reconstructed through transfer of the TFIIIB to the tRNA gene and that transcription will be restored under favorable growth condition. Furthermore, dissociation of TFIIIC (the whole complex or the τB module only) from tRNA is questionable. *In vitro* studies have shown that TFIIIC is required for TFIIIB and Pol III assembly, but is displaced from DNA in the post-initiation step [24,25]. In addition, *in vitro* transcription of human tRNA genes containing TATA boxes does not require TFIIIC [26,27]. *In vivo* data show, however, that TFIIIC is present at all transcriptionally-active Pol III genes, although its absolute binding efficiency is relatively low compared to TFIIIB and Pol III [28,29].

Interestingly, binding of TFIIIC to Pol III-transcribed genes greatly increases during acute repression [3,28], suggesting that it may impair Pol III progression during elongation. This proposal is further supported by a recent genome-wide analysis of nascent transcripts attached to Pol III, in which a remarkably uneven polymerase distribution along the transcription units was observed, suggesting local slow-down of elongation or transient pausing of the polymerase [30]. Inspection of individual tRNA genes showed a predominant pattern with a high density of nascent transcripts over the 5′ end and a weaker peak before the 3′ end of the gene. In addition, the 5′ and 3′ peaks of transcribing Pol III coincided with the beginning of the A box and the B box of the internal promoter, respectively, suggesting that TFIIIC bound to these sites could slow the Pol III elongation rate leading to transient pausing. Similar observations were made in biochemical studies, in which TFIIIC-dependent slow-down of Pol III upstream of the B box was detected [24]. However, the exact role of TFIIIC during Pol III transcription is not clear and requires further investigations.

In contrast to the increased occupancy of TFIIIC under stress conditions, the binding of TFIIIB to tRNA genes under the same conditions is reduced (Fig. 1). Analysis of the occupancy of tRNA genes by the Pol III machinery revealed a decrease in recruitment of TFIIIB and Pol III during the stationary growth phase, as well as upon rapamycin and hydroxyurea treatment. It is important to note, however, that decrease of tRNA genes transcription and concomitant dissociation of Pol III, precede TFIIIB dissociation [2,18,31]. Occupancy of Pol III factors on tRNA genes is controlled by phosphorylation of the subunits, regulation of their expression levels and interaction with regulatory proteins (see details below).

b. Regulation of TFIIIB recruitment to mammalian tRNA genes

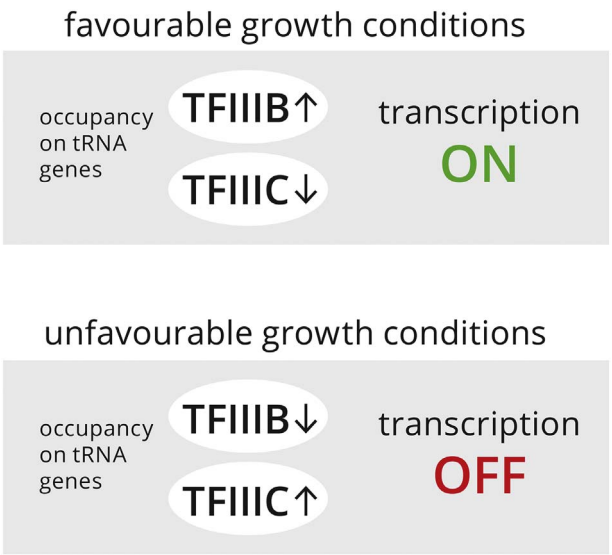

Fig. 1. Occupancies of tRNA genes by TFIIIB and TFIIIC are inversely correlated. In favorable growth conditions, TFIIIB shows strong binding to tRNA genes, TFIIIC is only weakly associated with these genes, and Pol III has high transcriptional activity [3]. Conversely, in unfavorable growth conditions, TFIIIB dissociates from tRNA genes, TFIIIC strongly binds to these genes, and Pol III has low transcriptional activity [2].