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Regulation of tRNA gene transcription by the chromatin structure and nucleosome dynamics☆

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ABSTRACT

The short, non-coding genes transcribed by the RNA polymerase (pol) III, necessary for survival of a cell, need to be repressed under the stress conditions *in vivo*. The pol III-transcribed genes have adopted several novel chromatin-based regulatory mechanisms to their advantage. In the budding yeast, the sub-nucleosomal size tRNA genes are found in the nucleosome-free regions, flanked by positioned nucleosomes at both the ends. With their chromosomes-wide distribution, all tRNA genes have a different chromatin context. A single nucleosome dynamics controls the accessibility of the genes for transcription. This dynamics operates under the influence of several chromatin modifiers in a gene-specific manner, giving the scope for differential regulation of even the isogenes within a tRNA gene family. The chromatin structure around the pol III-transcribed genes provides a context conducive for steady-state transcription as well as gene-specific transcriptional regulation upon signaling from the environmental cues.

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1. Introduction

Transcription lies at the heart of the gene expression, which plays a vital role in major transitions of life and life forms in course of evolution. Three eukaryotic RNA polymerases are multi-subunit enzymes, each specialized and dedicated for synthesizing the transcript from a defined, non-overlapping set of genes. This division of labor is further specified by a set of general transcription factors of each, which help them faithfully transcribe the target genes. RNA polymerase (pol) I is reserved for the transcription of the large ribosomal RNA genes and contributes to the major transcriptional activity of the cell. Pol III transcribes the genes coding for the short and stable non-coding RNAs like tRNAs, while pol II is responsible for the synthesis of the protein-coding mRNAs plus some other small, non-coding and regulatory RNAs. The genes transcribed by pol III fall in the category of "housekeeping" genes, which play important roles in the basic cellular activities like translation, RNA processing and ribogenesis. Apart from these classical roles, in the recent past several striking revelations about the diverse roles of tRNAs in cellular physiology and disease manifestation have brought renewed focus on the tRNA biology [1–4].

Regulatory mechanisms, which involve chromatin and may not be explained by the classical genetic approaches, are termed epigenetic. Much of the understanding of these mechanisms has evolved out of the studies performed with pol II-transcribed genes using budding yeast as the reference system. In the last few years, efforts to understand the pol III transcription in greater depth have revealed novel regulatory mechanisms, uniquely associated with the pol III-transcribed genes. The chromatin based epigenetic mechanisms are one of them [5]. Recent research has witnessed a surge of studies exploring involvement of epigenetic mechanisms in pol III transcription, which were considered irrelevant for pol III transcription at one time. The scope of this review is kept on those mechanisms, which establish the chromatin context of pol III transcription *in vivo*, focusing mostly on the studies in the budding yeast and including other studies only wherever available and relevant. A lot of exciting and interesting research in the past several years has brought into focus a variety of 'extra-transcriptional' [6], roles of the chromatin configuration at the pol III-transcribed genes and a number of excellent reviews on the topic have been written [6–9]. Several other new and fascinating aspects of pol III transcription regulation have been reviewed recently elsewhere [10–12].

2. Pol III transcription system

Pol III requires two general transcription factors, TFIIIB and TFIIIC

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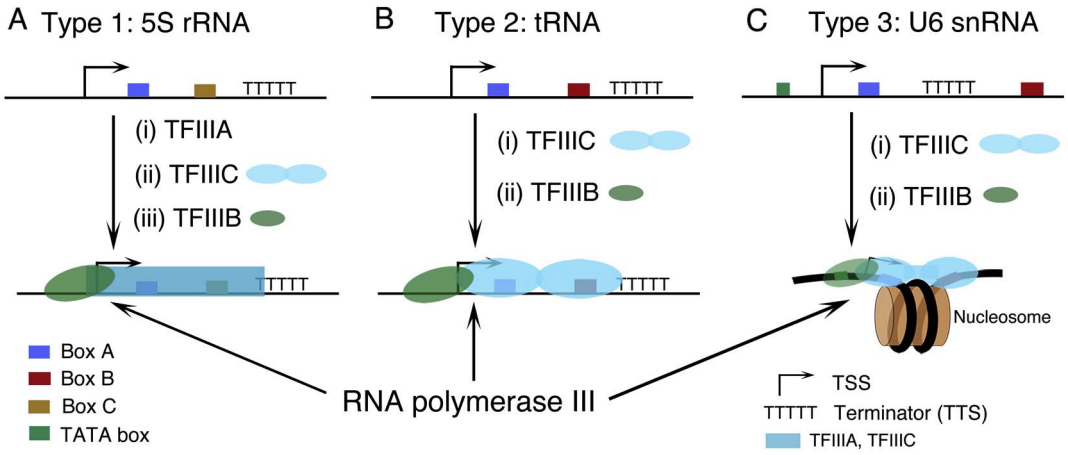

Fig. 1. Schematic representation of the yeast pol III TC assembly on the three types of genes. The transcription complex components join the genes in the order shown. TFIIIA, which binds to the type 1, 5S rRNA gene is not shown for the sake of clarity. On the type 3, U6 snRNA gene, TFIIIC binding results in the positioning of a nucleosome between the boxes A and B [79]. TFIIIB binds to the upstream TATA box (green box) subsequently. Pol III binds to the gene-TFIIIC-TFIIIB (plus TFIIIA in case of Type 1 gene) complex at the last in each case.

to transcribe the target genes, which generally consist of intra-genic promoter elements [13]. Depending on the promoter architecture, these genes are majorly grouped into three types [14]. The type 1 is solely represented by the 5S rDNA, which requires binding of a specific transcription factor TFIIIA before TFIIIC and TFIIIB can bind and recruit pol III (Fig. 1). Type 3 genes produce a variety of small, non-coding RNAs including U6 snRNA. The major transcriptome of pol III is constituted by the type 2 genes coding for the tRNAs (275 genes in case of the budding yeast). In addition to these genes, pol III also transcribes *RPR1* (coding the RNase P RNA involved in tRNA processing), *SCR1* (coding the RNA component of the signal recognition particle), and, in most metazoan, the gene coding for the 7SK RNA, which binds and inhibits the pol II transcription elongation factor P-TEFb. Mammalian pol III has been shown to transcribe miRNAs as well, expanding the repertoire of pol III transcripts [15]. For type 2 and type 3 genes of yeast, TFIIIC is the first to bind the intra-genic boxes A and B which helps in recruiting TFIIIB at −30 bp position, upstream of the transcription start site (TSS). As depicted in the Fig. 1, pol III is the last to join the initiation complex on all the genes [16,17].

On the pol III-transcribed tRNA genes, TFIIIC binds the intra-genic promoter elements, the boxes A and B. However, the upstream recruitment of TFIIIB by TFIIIC is influenced by the upstream sequences within −50 bp position to the TSS of the tRNA genes [13]. It has been known since long that the 5′ flanking sequences have positive influence on the transcription of yeast tRNA genes [18–20]. These sequences constitute a conserved pattern including a TATA box or TATA-like element, which may conceivably influence the binding of the TBP-containing TFIIIB at the −30 bp position [21]. This dependence of TFIIIB binding on the TATA box and its variant sequences varies in different species [22,23].

2.1. Transcription by pol III is regulated

Transcription of pol III-transcribed genes is presumably constitutive as their expression is required in all cell types and most environmental conditions for the cell survival. Therefore, pol III transcription is primarily tied to the regulation of cell growth and the cell cycle, changing according to the growth conditions and external stimuli [24–26]. Out of the three RNA polymerases, pol III consists of the largest number of subunits but the simplest transcription complex, especially when compared to pol II. The house-keeping functions of the pol III transcripts warrant their constant and high level cellular supply. Although greatly outnumbered by pol II-transcribed mRNA-encoding genes; pol III targets are transcribed at very high frequencies *in vivo* and contribute to 10–15% of the total cellular transcription [27]. However, under stress conditions, when cell reduces all its physiological activities, it is necessary to regulate the expression of these genes in order to save and preserve the cellular energy.

Pol III activity is repressed by the negative regulator Maf1 under stress conditions [28,29]. Maf1, p53 and RB are negative regulators while Sub1 shows a stimulating effect on pol III transcription [30–33]. RB and p53 are not found in the budding yeast and Maf1/Sub1 do not show sequence-specific DNA binding [34]. Interestingly, one unconventional prefoldin Bud27, which is necessary for cytoplasmic assembly of the functional pol III [35], was required for proper biogenesis of the two largest pol III subunits Rpc160 and Rpc128 [35]. Bud27 helps the assembly of all the three RNA polymerases and interacts with their common subunit Rpb5 [36]. Chromatin remodeler RSC, which influences pol III transcription (discussed in later sections), has also been reported to interact with all three RNA polymerases *via* their common subunit Rpb5 [37]. Bud27 deletion was found to reduce pol III transcription and interaction of Rpc160 with the chromatin remodeler RSC [35]. Both these effects could be related to the role of Bud27 in regulating levels of pol III itself. Thus, Bud 27 could be one of the positive effectors of pol III transcription.

Multiple examples in the literature show that pol II and its factors regulate pol III transcription and *vice-versa*. Using the genome-wide ChIP-chip studies in yeast, known regulators of pol II transcription such as Reb1, Fkh1, Yap6 and Hda1, have been found in the immediately upstream regions of the tRNA genes [38]. Besides these, a number of accessory factors including a histone acetylase p300, the La protein and NF1, Nhp6, TFIIS [39] have been proposed to influence pol III transcription without apparent gene specificity. Taking together the above-mentioned findings, it is evident that the two RNA polymerase activities may have common components, which may be targeted or utilized to impose common regulatory mechanisms.

2.2. Regulating a constitutive transcription system

Gene expression in eukaryotes is a highly regulated process. While classical activators and repressors are known to bind the cis DNA elements directly and influence the target gene transcription, other transcription factors influence the activity of the basal transcriptional machinery *via* protein-protein interactions. Unlike pol II-transcribed genes, pol III-transcribed genes are generally not known to have upstream regulatory elements, away from the site of the assembly of the transcription pre-initiation complex (PIC) [40]. This may be the reason why very few DNA-binding proteins have been found so far to regulate the pol III transcription.

Many proteins have been known to influence the expression of