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Review

Yeast RNA polymerase III transcription factors and effectors ☆

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ABSTRACT

Recent data indicate that the well-defined transcription machinery of RNA polymerase III (Pol III) is probably more complex than commonly thought. In this review, we describe the yeast basal transcription factors of Pol III and their involvements in the transcription cycle. We also present a list of proteins detected on genes transcribed by Pol III (class III genes) that might participate in the transcription process. Surprisingly, several of these proteins are involved in RNA polymerase II transcription. Defining the role of these potential new effectors in Pol III transcription in vivo will be the challenge of the next few years. This article is part of a Special Issue entitled: Transcription by Odd Pols.

1. Introduction

The RNA polymerase III (Pol III) enzyme was discovered at the beginning of the 70s [1] and the factors required for in vitro transcription were identified 10 years later [2,3]. For more than 20 years the molecular mechanisms involving these transcription factors were explored, largely through in vitro studies, to obtain a simple but robust working model. Recent in vivo data have dramatically complicated our view of Pol III transcription and regulation. More specifically, the use of chromatin immunoprecipitation (ChIP) techniques combined with genome wide approaches has led to the discovery of unexpected proteins at Pol III-transcribed genes. The role of these proteins in Pol III transcription remains largely unexplored. This review will focus on our knowledge of the proteins known to play a role in Pol III transcription or its regulation in *Saccharomyces cerevisiae*.

2. The discovery of the basal Pol III transcription machinery

The presence of conserved sequences within the promoters of class III genes (genes transcribed by Pol III, Fig. 1A) suggests that transcription factors common to all class III genes might interact with these sequences. In the 80s, fractionation experiments of cell extracts from several organisms revealed that the specific in vitro transcription of class III genes requires three transcription factors in addition to Pol III: two general transcription factors, TFIIIB and TFIIIC, and a specific transcription factor, TFIIIA, required only for the synthesis of 5S rRNA [2,4–7]. The presence of DNA binding proteins in these fractions was rapidly highlighted in DNase I footprinting, electrophoretic mobility shift assays or template commitment studies. These experiments have also helped define the order of assembly of the transcription complexes. For the 5S rRNA genes, TFIIIA binds to DNA first, followed by TFIIIC and then TFIIIB. For tRNA genes, TFIIIC is the first to associate with DNA and helps in assembling TFIIIB. Finally, Pol III binds these initiation transcription complexes (see Fig. 1B). The DNA binding properties of factors TFIIIA and TFIIIC have facilitated their purification and the study of their functions. In contrast, the identification of TFIIIB components was more difficult due to an absence of intrinsic DNA binding activity and to the spontaneous dissociation of TFIIIB activity into separate components.

2.1. TFIIIA

TFIIIA is required for the transcription of 5S rRNA genes. TFIIIA is a ~40 kDa protein that is extremely abundant in amphibian oocytes which led to the *Xenopus laevis* protein being the first eukaryotic transcription factor to be purified to homogeneity [3]. TFIIIA was shown to interact with the Internal Control Region (ICR) of 5S rRNA genes [8,9]. In *S. cerevisiae*, the ICR is composed of a C box of 14 nucleotides (Fig. 1A) [10] and TFIIIA occupies a 35 bp region encompassing part of the C box [11]. TFIIIA is the first member of the C_2H_2 zinc finger family of DNA-binding proteins. TFIIIA proteins from different organisms are poorly conserved and their similarities are mainly limited to the C_2H_2 zinc finger domain. Of the 9 zinc fingers in the *S. cerevisiae* TFIIIA protein [12,13], DNA binding minimally requires the first three zinc fingers [14] and zinc fingers 1 and 7 are essential for the transcription machinery assembly on 5S rRNA genes. Residues within the first zinc finger were shown to be involved in TFIIIC recruitment, but not in DNA binding [15]. *S. cerevisiae* TFIIIA contains a domain of 81 amino acids between fingers 8 and 9 [12,13] that is not required for TFIIIA binding to DNA but whose presence is essential for its transcriptional activity in vitro [14]. A short leucine-rich segment NGLNLLLN included in this domain

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contributes to the ability of TFIIIA to promote transcription perhaps by mediating an interaction with TFIIIC [16]. In *S. cerevisiae*, the only essential function of TFIIIA is its role in the transcription of 5S rRNA genes. Cells are able to survive in the absence of TFIIIA when 5S rRNA is expressed from a *RPR1* promoter [17]. In contrast, TFIIIA is not essential in ascomycete *Yarrowia lipolytica* where TFIIIA-independent transcription of 5S rRNA can occur via dicistronic units of tRNA-5S rRNA genes [18].

To our knowledge, no chromatin immunoprecipitation experiments have been reported using TFIIIA as bait. It is thus possible that TFIIIA binds additional DNA loci in vivo. However, these potential binding sites are unlikely to be essential according to the well-defined role of TFIIIA.

2.2. TFIIIC

In *S. cerevisiae*, TFIIIC is organized in two large subassemblies called τA and τB that were identified by limited proteolysis and electron microscopy [19,20]. Remarkably, TFIIIC is a flexible factor that is able to bind both the A and B box of tRNA genes even though these boxes are separated by variable distance [19]. The reconstitution of an active recombinant TFIIIC defined yeast TFIIIC as a large protein complex composed of 6 subunits with an apparent migration rate of 138-, 131-, 95-, 91-, 60- and 50-kDa on SDS-PAGE [21–26].

From in vitro experiments, it is clear that TFIIIC plays a key role in the transcription complex assembly process. TFIIIC binds the intragenic promoter elements of tRNA genes (the A and B boxes, Fig. 1A and B) and, once bound, promotes the binding of TFIIIB upstream of the transcription start site (TSS) [8,27,28]. Similarly, TFIIIC binds to and stabilizes preformed TFIIIA–5S rDNA complexes, before recruiting TFIIIB [11,29–31].

2.2.1. TFIIIC recognizes the promoter of class III genes

τB binds tightly to the B box of tRNA genes and other Pol III-transcribed genes (Fig. 1). Biochemical and genetic evidence indicates that τB likely comprises the three subunits of 138-, 91- and 60-kDa, Tfc3, Tfc6 and Tfc8, respectively [21,32–34]. The τA domain, visualized by electron microscopy [19], probably comprises the 131-, 95- and 55-kDa subunits, Tfc4, Tfc1 and Tfc7, respectively, which are thought to participate in A box binding [21,24,35]. The two subunits of 95- and 138-kDa are in close contact with DNA as shown by UV irradiation crosslink and are thought to play a central role in A- and B-box recognition, respectively [26]. Consistent with these data, the 95-kDa subunit was mapped over the A box by photocrosslinking experiments, while the138-kDa subunit was mapped to the vicinity of the B box [24]. On the 5S rRNA gene, despite differences in internal promoter elements compared to those of tRNA genes, the 95-kDa subunit occupies the same space in the absence of the A box sequence [35]. Fig. 2 provides a visual representation of the known functional domains of the TFIIIC subunits. The main functions and properties of the TFIIIC subunits are more exhaustively described in Table 1.

2.2.2. TFIIIC recruits TFIIIB to the DNA

In the TFIIIC–DNA complex, the 131-kDa subunit is positioned upstream of the TSS (Table 1) in a region occupied by TFIIIB [35,36]. The importance of this TFIIIC subunit for TFIIIB recruitment was confirmed by co-immunoprecipitation [37]. A two hybrid experiment was performed to map the domains of the 131-kDa subunit of TFIIIC that interact with two subunits of TFIIIB, Brf1 and Bdp1 [38,39]. TFIIIC is also involved in the recruitment of the TATA binding protein (TBP), the third component of TFIIIB. Genetic and co-immunoprecipitation data suggest that the 60-kDa subunit of TFIIIC interacts with TBP through its C-terminal domain

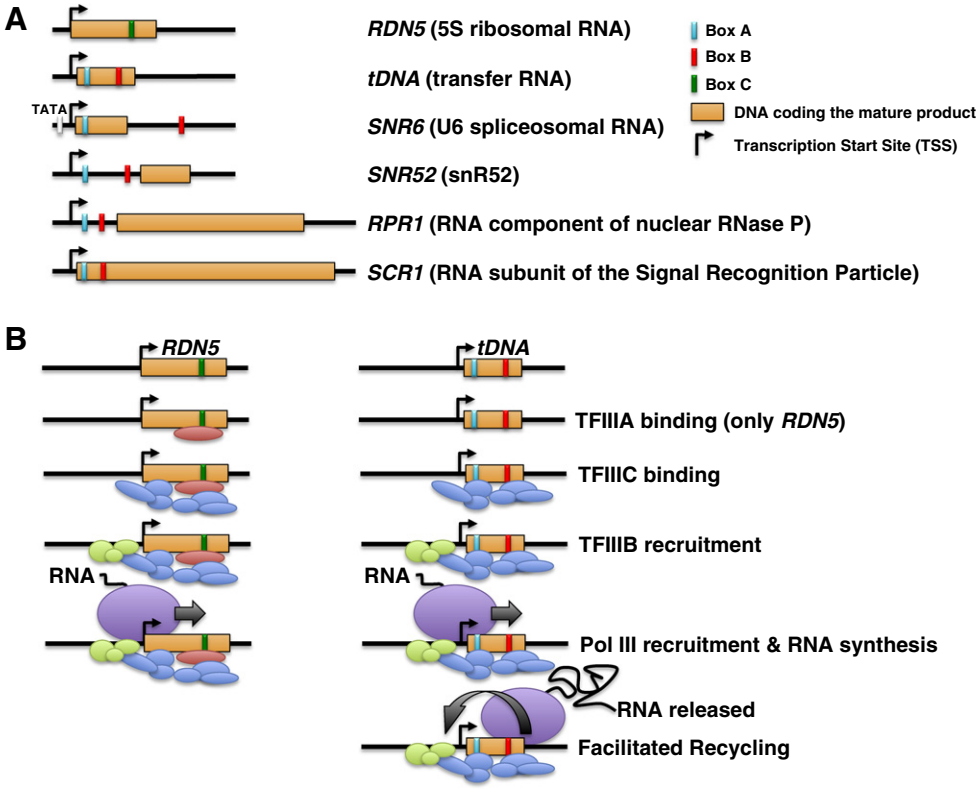

Fig. 1. Structure of RNA polymerase III-transcribed genes and in vitro assembly model of the transcription machinery. (A) Schematic representation of different Pol III-transcribed genes. The gene names are indicated on the right side with the encoded RNA between parentheses. The solid black bar represents upstream and downstream DNA, the open orange rectangle the mature product and the blue, red and green rectangles the A-, B- and C-boxes respectively. The horizontal arrow indicates the transcription start site (TSS). The *SNR6* TATA box is indicated with a white box. (B) Transcription complex assembly deduced from in vitro data on a yeast tRNA gene (*tDNA*, right) or 5S rRNA gene (*RDN5*, left). The components of TFIIIA, TFIIIB and TFIIIC are represented as red, green and blue ovals respectively. The 17-subunits of Pol III enzyme are represented as a unique purple oval.