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Structural rearrangements of the RNA polymerase III machinery during tRNA transcription initiation☆

Ewan Phillip Ramsay, Alessandro Vannini*

The Institute of Cancer Research, London SW7 3RP, UK

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ABSTRACT

RNA polymerase III catalyses the synthesis of tRNAs in eukaryotic organisms. Through combined biochemical and structural characterisation, multiple auxiliary factors have been identified alongside RNA Polymerase III as critical in both facilitating and regulating transcription. Together, this machinery forms dynamic multi-protein complexes at tRNA genes which are required for polymerase recruitment, DNA opening and initiation and elongation of the tRNA transcripts. Central to the function of these complexes is their ability to undergo multiple conformational changes and rearrangements that regulate each step. Here, we discuss the available biochemical and structural data on the structural plasticity of multi-protein complexes involved in RNA Polymerase III transcriptional initiation and facilitated re-initiation during tRNA synthesis. Increasingly, structural information is becoming available for RNA polymerase III and its functional complexes, allowing for a deeper understanding of tRNA transcriptional initiation. This article is part of a Special Issue entitled: SI: Regulation of tRNA synthesis and modification in physiological conditions and disease edited by Dr. Boguta Magdalena.

1. Introduction

In the eukaryotic nucleus, transcription of genomic DNA is carried out using three distinct multi-subunit complexes, namely RNA Polymerase (Pol) I, II and III. Identified more than forty years ago by chromatographic separation from sea urchin preparations [1], Pols have since been characterised as highly functionally distinct enzymes, transcribing different classes of RNAs. The Pol III enzyme is devoted to the transcription of the entire pool of transfer RNAs (tRNAs) and other non-coding RNA transcripts such as 5S rRNA, U6 small nuclear RNA (snRNA), micro RNAs (miRNA), RNase P RNA and the 7SL RNA, amongst others [2,3]. The high efficiency of the specialised Pol III machinery allows for high levels of transcription, with as many as 3–6 million tRNA molecules produced per population doubling in *S. cerevisae* [4].

Recently, significant progress has been made in the understanding of the structure and mechanism of initiation of the Pol III enzyme. In this review, we summarise the different promoter architectures and their associated auxiliary factors that are essential for tRNA transcription, with a particular emphasis on the best-characterised model organism *S. cerevisiae*. We describe the structural rearrangements involved during the process of polymerase recruitment, DNA promoter opening and transcriptional initiation. Finally, we discuss the models of facilitated recycling which may be behind the extraordinary ability of Pol III to produce high levels of tRNAs.

2. A diverse set of promoters are recognised by TFIIIC to initiate tRNA transcription

tRNAs represent the most populated class of genes, known as class II, transcribed by the Pol III enzyme, with all members in *S. cerevisae* sharing a consensus gene structure [2,5]. Early studies suggested an intragenic architecture of tRNA promoters, with no observable sequence conservation in the 5′ flanking regions, and deletions leaving as little as 6 base pairs 5′ of the transcribed gene still supporting transcription [6–9]. Deletion or mutation of internal gene sequences was found to severely disrupt Pol III transcription, with sequences located both within the 5′ and 3′ regions of the tRNA coding region required for efficient transcription [10,11]. Subsequent deletion and sequence analysis in *Xenopus laevis* identified two highly conserved consensus sequences in the 5′ and 3′ regions of tRNA genes, named the ‘A-box’ and ‘B-box’ respectively [12]. These regions, encoding the D and T loops of the tRNA, were found to be essential for transcription of many tRNAs, suggesting a common mechanism of regulation [13,14].

The A and B boxes of the tRNA gene promote transcription through recruitment of the transcription factor TFIIIC in the first stage of tRNA

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* Corresponding author.
E-mail address: alessandro.vannini@icr.ac.uk (A. Vannini).

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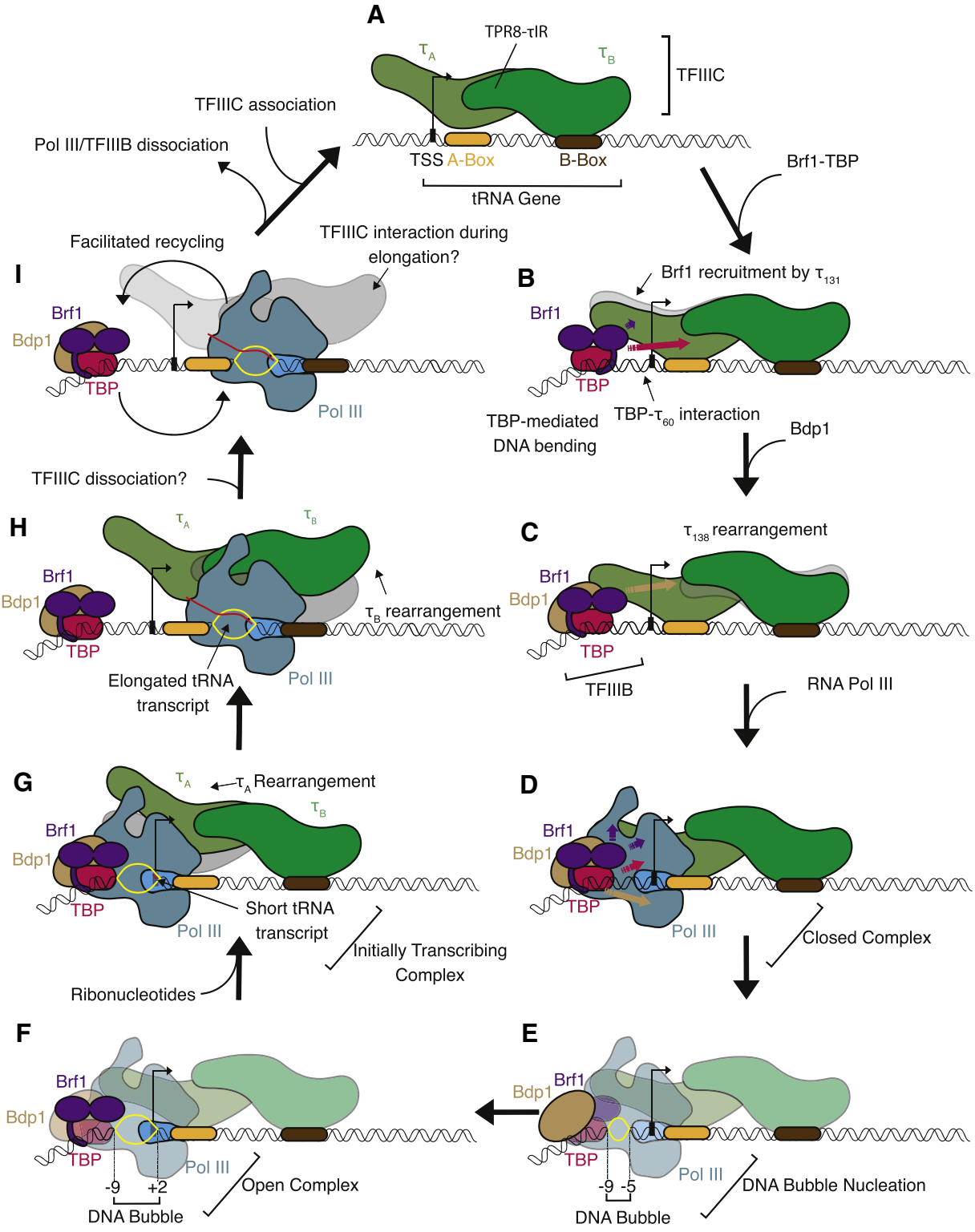

Fig. 1. Model of Pol III-mediated tRNA transcription. (A) TFIIIC recognises the A and B-box elements within the tRNA gene sequence via the τ_A and τ_B modules. (**B**) Brf1-TBP is recruited by the N-terminus of the τ_{131} subunit of TFIIIC (*purple arrow*). TBP association is facilitated via interactions with τ_{60} (*pink arrow*) and causes DNA bending, forming the B′-TFIIIC complex. (**C**) Bdp1 association competes for τ_{131} binding with τ_{138} (*gold arrow*), leading to structural rearrangement and formation of the stable TFIIIB-TFIIIC complex at the promoter. (**D**) The TFIIIB-TFIIIC assembly then recruits Pol III to the promoter via interactions between Brf1 and TBP and the C34/C17 Pol III subunits (*purple arrows*). Additional interactions between Bdp1 and C37 are also observed (*gold arrow*). The polymerase cleft closes around the DNA, forming a closed pre-initiation complex at the tRNA promoter. (**E**) The N-terminus of Bdp1 initiates promoter opening upstream of the transcriptional start site (TSS) between positions − 9 to − 5. (**F**) The DNA bubble is extended by the action of the N-terminal zinc ribbon domain of Brf1, forming the full DNA bubble, which extends to the TSS in the polymerase active site producing the open complex. (**G**) Initial synthesis of the complementary tRNA transcript on the template strand forms the initially transcribing complex. Subsequent promoter escape of this assembly is a rate-limiting step and likely requires τ_A rearrangement at the promoter. (**H**) Promoter escape leads to the formation of the elongating complex, which synthesises the tRNA transcript. The elongating polymerase assembly may rearrange the τ_B module, potentially removing TFIIIC from the active gene whilst leaving the stably bound TFIIIB upstream of the TSS. (**I**) TFIIIB directs multiple rounds of re-initiation on the same template through facilitated recycling, allowing for high yields of tRNA. Dissociation of these components requires TFIIIC re-association to reassemble the general transcription factor- polymerase complex and allow for further rounds of transcription.