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Molecular Basis of RNA Polymerase III Transcription Repression by Maf1

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SUMMARY

RNA polymerase III (Pol III) transcribes short RNAs required for cell growth. Under stress conditions, the conserved protein Maf1 rapidly represses Pol III transcription. We report the crystal structure of Maf1 and cryo-electron microscopic structures of Pol III, an active Pol III-DNA-RNA complex, and a repressive Pol III-Maf1 complex. Binding of DNA and RNA causes ordering of the Pol III-specific subcomplex C82/34/31 that is required for transcription initiation. Maf1 binds the Pol III clamp and rearranges C82/34/31 at the rim of the active center cleft. This impairs recruitment of Pol III to a complex of promoter DNA with the initiation factors Brf1 and TBP and thus prevents closed complex formation. Maf1 does however not impair binding of a DNA-RNA scaffold and RNA synthesis. These results explain how Maf1 specifically represses transcription initiation from Pol III promoters and indicate that Maf1 also prevents reinitiation by binding Pol III during transcription elongation.

INTRODUCTION

The eukaryotic genome is transcribed by the multisubunit enzymes Pol I, II, and III, which catalyze DNA-dependent RNA synthesis. Pol III transcribes genes encoding short, untranslated RNAs, including transfer RNAs, 5S ribosomal RNA (rRNA), the spliceosomal U6 small nuclear RNA (snRNA), and the signal recognition particle 7SL RNA. Pol III genes are essential and involved in fundamental processes such as ribosome and protein biogenesis, RNA processing, and protein transport. Pol III transcription is coregulated with Pol I activity, accounting together for up to 80% of nuclear gene transcription in growing cells (Paule and White, 2000; Grummt, 2003; Willis et al., 2004). Pol III activity is a critical determinant of cell growth.

Pol III is the most complex of the nuclear RNA polymerases. It has a total molecular weight of around 700 kDa and comprises 17 subunits (Schramm and Hernandez, 2002). Five of its subunits, Rpb5, 6, 8, 10, and 12, are common to Pol I, II, and III. Subunits AC40 and AC19 are common to Pol I and III and are homologous to Pol II subunits Rpb3 and Rpb11, respectively. The two largest Pol III subunits C160 and C128 are homologous to Pol II subunits Rpb1 and Rpb2, respectively, and form the active center of the enzyme. Subunits C17 and C25 form a subcomplex with homology to the Pol II subcomplex Rpb4/7 (Ferri et al., 2000; Jasiak et al., 2006; Sadhale and Woychik, 1994), whereas subunit C11 shares homology with Pol II subunit Rpb9. The Pol III-specific subunits C82, C53, C37, C34, and C31 form two subcomplexes. The C53/37 subcomplex shows weak homology to the Pol II initiation factor TFIIF and is involved in promoter opening, elongation, termination, and reinitiation (Cramer et al., 2008; Carter and Drouin, 2009; Kassavetis et al., 2010; Landrieux et al., 2006), whereas the C82/34/31 subcomplex is involved in promoter recognition and initiation. C34 interacts with TFIIIB, which recruits Pol III to promoters (Thuillier et al., 1995; Wang and Roeder, 1997; Werner et al., 1993) and is involved in open complex formation (Brun et al., 1997). To date, structural information on Pol III is limited to a cryo-electron microscopic (cryo-EM) map that revealed the approximate location of the two Pol III-specific subcomplexes (Fernández-Tornero et al., 2007), a homology model for the 10-subunit core enzyme, and the crystal structure of C25/17 (Jasiak et al., 2006).

Rapid repression of Pol III transcription ensures cell survival during stress (Warner, 1999). Pol III repression is mediated by Maf1, a protein that is conserved from yeast to human (Pluta et al., 2001; Upadhya et al., 2002). Maf1 represses Pol III in response to DNA damage, oxidative stress, growth to stationary phase, treatment with rapamycin or chlorpromazine, and blocking of the secretory pathway (Upadhya et al., 2002; Willis et al., 2004). In growing yeast, Maf1 is phosphorylated and localized in the cytoplasm. Stress conditions lead to Maf1 dephosphorylation and nuclear import (Oficjalska-Pham et al., 2006; Roberts et al., 2006), which is directed by two nuclear localization signal (NLS) sequences (Lee et al., 2009; Moir et al., 2006). In the nucleus, Maf1 binds Pol III to prevent its interaction with TFIIIB and promoters (Desai et al., 2005; Moir et al., 2006; Roberts et al., 2006). Maf1 also binds Brf1, a subunit of TFIIIB that resembles the Pol II initiation factor

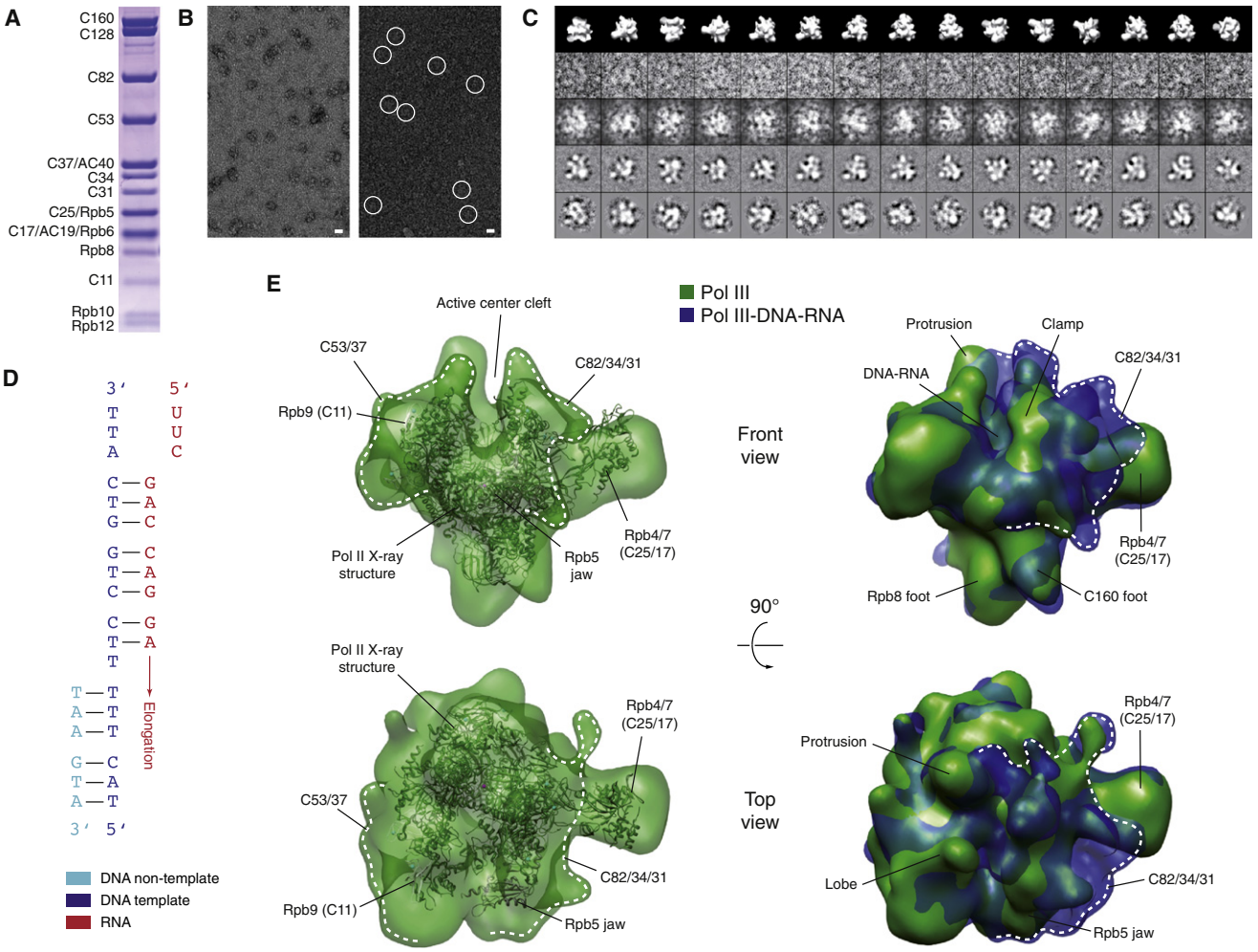

Figure 1. Cryo-EM Structures of Pol III and Pol III-DNA-RNA Complex

(A) SDS-PAGE of pure yeast Pol III. The identity of the 17 subunits was confirmed by mass spectrometry.

(B) EM micrographs of Pol III in negative stain (left) and vitrified ice (right). Scale bars represent 10 nm.

(C) Views of the Pol III reconstruction (first row) with corresponding raw single-particle images (second row), low pass-filtered single-particle images (third row), class averages (forth row), and reference-free averages (fifth row).

(D) DNA-RNA scaffold used in complex formation.

(E) Cryo-EM reconstruction of Pol III (green) and Pol III-DNA-RNA complex (blue). The Pol II X-ray structure (Armache et al., 2005) was fitted to the Pol III map and is shown as a ribbon model. White dashed lines indicate additional densities between the lobe and Rpb9 (C11), attributed to the C53/37 subcomplex, and between the clamp and Rpb5, attributed to the C82/34/31 subcomplex, that gets ordered in the DNA-RNA complex.

See also Figures S1 and S4 and Movie S1.

TFIIB (Desai et al., 2005). Maf1-mediated repression is associated with reduced Brf1 and Pol III occupancy at Pol III genes (Oficjalska-Pham et al., 2006; Roberts et al., 2006). Similar results have been obtained with human cells, establishing Maf1 as a conserved global repressor of Pol III transcription (Reina et al., 2006).

Here, we report cryo-EM structures of Pol III in its free form and in complex with a DNA-RNA scaffold, assign the locations of Pol III subunits, present the Maf1 crystal structure, and combine the resulting information with a cryo-EM structure of a Pol III-Maf1 complex. Together with functional studies, these results establish the mechanism for Pol III transcription repression by Maf1.

RESULTS AND DISCUSSION

Pol III EM Structure Reveals C82/34/31 Mobility

We established a protocol for large-scale purification of Pol III from the yeast *Saccharomyces cerevisiae* (Experimental Procedures). Pure Pol III samples comprised all 17 subunits (Figure 1A), were monodisperse, and appeared homogeneous in EM with negative stain (Figure 1B). We collected high-quality cryo-EM data after vitrification under native conditions. A reconstruction of Pol III from 20,480 single particles led to a map at 21 Å resolution (Figure 1E; Figure S1 available online; Experimental Procedures) that generally agrees with the previously published map (Fernández-Tornero et al., 2007).