Structural basis of co-transcriptional rRNA folding and processing

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Biosynthesis of ribosomal (r) RNA is a prime example for co-transcriptional RNA folding and processing and it is coordinated with co-transcriptional RNP assembly. During the transcription of primary transcripts containing 16S, 23S, 5S rRNA and intervening tRNAs, RNA polymerase (RNAP) is modified by transcription factors NusA, NusG, NusB, NusE and SuhB to form a transcription anti-termination complex (*rrn*TAC) and a multi-factor RNA chaperone at the RNA exit channel of RNAP that supports the folding of the nascent RNA [1, 2]. Co-transcriptional rRNA folding is a prerequisite for subsequent processing of the primary transcript by several nucleases to yield mature 16S, 23S, 5S rRNA and tRNA [3]. RNase III is a double strand-specific endoribonuclease and the nuclease that acts first during rRNA maturation [4, 5]. Following completion of 16S rRNA transcription, a long, double-stranded RNA stem is formed (16S stem) that harbors the recognition motif for RNase III cleavage to generate pre-16S rRNA. While the role of RNase III during ribosome biogenesis has been well characterized [3-5], the molecular details of RNA recognition and processing during active transcription and co-transcriptional RNA folding still remain elusive.

By using single-particle cryogenic electron microscopy (cryo-EM), we have determined several structures of *rrn*TACs that contain variants of the 16S stem, harboring the RNase III cleavage motif and resembling the pre-processed transcript of pre-16S RNA (Fig. 1A, B). The structures reveal further insights into the chaperoning activity of the modifying RNP. By fixing the 5' end of the 16S stem close to the RNA exit channel, NusA and the SuhB dimer provide a platform for the formation of dsRNA in a locally defined region. We also elucidated the structure of an *rrn*TAC associated with an inactive variant of RNase III bound to the 16S stem (Fig. 1C). The dimeric RNase III engages the 16S stem on the opposite side of the RNAP-associated RNA chaperone and forms direct contacts not only to the dsRNA, but also to NusA and to one of the SuhB subunits. Structure-informed functional analyses revealed that SuhB residues forming direct contacts to nascent RNA are important for efficient rRNA folding and/or processing as mutating these residues reduced RNase III cleavage efficiency.

Our results underline the importance of co-transcriptional RNA folding to obtain native and functional RNAs or targets for further maturation. We uncovered the structural basis of co-transcriptional, long-range RNA secondary structure formation by keeping the 5' part of the nascent RNA fixed to a modifying RNP and close to the emerging 3' part to support the formation of dsRNA.

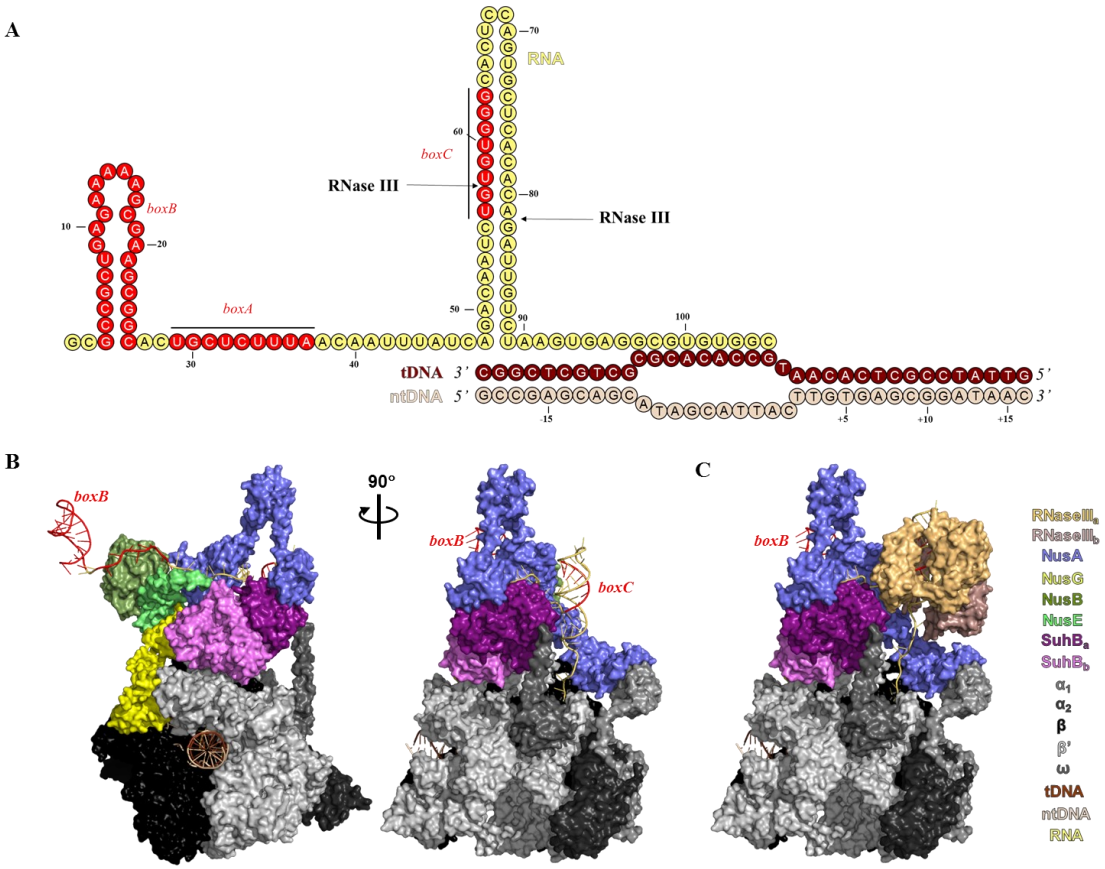

Figure 1. Cryo-EM structure of *rrn*TAC in the absence and presence of RNase III inactivated variant.

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