

SFB 960-/BZR – Kolloquium

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H 53

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Connections between Pol I transcription and the early assembly and maturation of nascent pre-60S particles in yeast

The early steps of the production of the large ribosomal subunit are probably the least understood stages of eukaryotic ribosome biogenesis. We demonstrate that five assembly factors required for the production of the first pre-60S pre-ribosomal particle, Npa1p, Npa2p, Nop8p, Rsa3p and the helicase Dbp6p, can form a complex. Npa1p is a crucial component of this complex since it is required for its formation and stable integration within 90S pre-ribosomal particles. Mapping of the Npa1p-rRNA contacts by CRAC indicates that Npa1p binds adjacent to ribosomal protein Rpl3, structural protein Rrp5p and helicase Prp43p, all key players in early pre-60S particle assembly. Npa1p also crosslinks to snoRNAs involved in decoding center and peptidyl transferase center modifications and in the immediate vicinity of the binding sites of these snoRNAs on 25S rRNA. Our data strongly suggest that the Npa1p complex plays a key role in the compaction of the central core of 25S rRNA and the control of snoRNA-pre-rRNA interactions.

In an effort to determine how early maturation of the pre-60S particles is connected to Pol I transcription, we analyzed the consequences of pre-60S assembly factor depletion on Pol I transcription using run-on experiments. Depletion of components of the Npa1 complex or of the Noc1-Noc2-Rrp5 complex does not affect Pol I transcription. In contrast, Pol I transcription is strongly inhibited upon depletion the Rpf2-Rrs1 heterodimer, another early-associating pre-60S particle module involved in the incorporation of the 5S RNP into nascent pre-60S particles. This phenotype is correlated with strong perturbations of rDNA unit organization observed by Miller spreads. Our data suggest that Rpf2 and Rrs1 directly affect Pol I transcription through interactions with rDNA chromatin, with several rDNA-associated chromatin remodeling factors and with proteins affecting Pol I function. Our data support the hypothesis that the Rpf2-Rrs1 heterodimer is a major player in the functional coupling between rDNA transcription and pre-60S particle assembly and maturation.



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