FUNCTIONAL ANALYSIS OF NIP7p, A CONSERVED PROTEIN INVOLVED IN PRE-rRNA PROCESSING IN S. CEREVISIAE

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Pre-rRNA processing occurs in the nucleolus, where a large number of factors associate transiently to the pre-rRNA leading to endo- and exonucleolytic cleavages and base modifications. Protein-rRNA interactions are critical during this process. S. cerevisiae Nip7p is required for 60S subunit biogenesis and was previously shown to interact with the nucleolar protein Nop8p and with the exosome subunit Rrp43p. In this work, we show that Nip7p interacts with Nop53p and Rrp15p, both involved in 60S subunit biogenesis, and with components of the box H/ACA pseudouridylation complex. Primer extension analysis showed that deficiency in Nip7p resulted in a moderate defect in pre-rRNA pseudouridylation whereas cleavage of the 27S prerRNA was severely affected. Consistent with these results, Nip7p can bind directly to the 5.8S, 5'ETS and ITS2 regions of the pre-rRNA. It is a conserved protein, presenting two alpha/beta domains in which the C-terminal region corresponds to the PUA domain. Two-hybrid and protein A-pull down assays revealed that the PUA domain is responsible for the interactions. These results indicate that Nip7p plays a central role in rRNA processing and that the PUA domain mediates its interaction with the complexes, possibly via binding to the pre-rRNA.

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