Characterization of yeast U14 snoRNA interactions required for rRNA processing, and development of a novel in vivo rDNA system for dissecting ribosome biogenesis

Wen-Qing Liang, *University of Massachusetts Amherst*

Abstract

U14 small nucleolar RNA (snoRNA) is required for processing of 18S ribosomal RNA. It was hypothesized that U14 might base pair with 18S RNA through two highly conserved U14 sequence elements known as domains A and B. Using Saccharomyces cerevisiae as the experimental system, I showed that: (1) the domain A and B elements are functionally interdependent, and (2) single-point mutations in domain A combined with complete substitution of domain B causes lethality while either mutation alone does not. Direct interaction of U14 with 18S RNA was shown by demonstrating that a lethal mutation in U14 domain A can be suppressed with a mutation which restores complementarity in the corresponding region of 18S RNA.^ Y-domain in yeast U14 was postulated to serve as a recognition element for vital intermolecular or intramolecular interactions. Consistent with this assumption, mutations in several conserved nucleotides of the loop cause growth defects. In contrast, alterations to the stem have little or no effect. Using a lethal mutation in the loop, three different intragenic suppressor mutations were mapped to three positions adjacent to the primary mutation, and are predicted to influence the structure of the loop.^ An extragenic suppressors (UF1) able to rescue a cold-sensitive mutation in the loop encodes an essential putative ATP-dependent RNA helicase. Loss of UF1 gene expression caused a reduction in 18S rRNA production, without affecting accumulation of 25S rRNA or U14 snoRNA. Pulse-chase analysis showed that depletion of UF1 protein impaired pre-18S rRNA processing.^ Finally, an effort was made to define minimum pre-rRNA substrates that can be used to produce functional 18S and 25S rRNAs in vivo. The rDNA operon was split either between the 18S RNA and 5.8S/25S coding units, or between the 18S/5.8S RNA and 25S RNA coding units. The test fragments were expressed from GAL7 promoters. The results showed that functional rRNAs could be produced in trans, but only when the operon was divided between the 18S RNA and 5.8S/25S RNA coding sequence. ^

Subject Area

Molecular biology|Cellular biology

Recommended Citation