Z3, a novel antisense snoRNA from *Saccharomyces cerevisiae*

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Abstract Small nucleolar RNA (snoRNA) is one of the most important elements participating in eukaryotic ribosomal biogenesis. The present report describes the results of the identification of a novel snoRNA, Z3, from yeast *S. cerevisiae*. Z3 snoRNA is 106 nts in length. It contains box C, D elements and a 13 nt complementarity to 25S rRNA. This antisense segment, together with the downstream box D, guides a 2'-O-ribose methylation of cytidine acid at position 2956th in yeast 25S rRNA. Z3 snoRNA, encoded by an independent transcribed gene on the chromosome III of yeast, possesses the same functional elements responsible for the rRNA methylation site as that of the intron-encoded U35 snoRNA of vertebrate.

Keywords: Z3, snoRNA, yeast, rRNA methylation.

Most of events of eukaryotic ribosomal biogenesis are believed to take place in nucleolus. The maturation of pre-rRNA is one of the most important events and requires the participation of a number of small nucle-
ular RNAs (snoRNAs). Although many of the details remain to be defined, recent studies\cite{1, 2} have shown that the snoRNA plays a key role in rRNA processing, specific-site methylation of rRNA and pre-rRNA folding which is regarded as an important progress of molecular biology. Yeast S. cerevisiae is a suitable genetic system for eukaryotic gene structure and function study. Among the 80 species of snoRNAs reported by far, more than 40 are from yeast\cite{3}. S. cerevisiae shows many characteristics different from higher eukaryotes in snoRNA gene organization and expression. For example, most of the mammalian snoRNA genes are nested in introns of various protein genes, whereas many independent transcribed snoRNA genes have been reported constantly in yeast, suggesting existence of different biogenesis pathways and processing patterns between the two kinds of snoRNAs.

In this note, we report the characterization of a novel snoRNA, Z3, from S. cerevisiae. The functional significance of Z3 snoRNA and its possible homologous gene in mammal are also discussed.

1 Materials and methods

EMBL and Genbank DNA sequence database were screened using Blast and Fasta program. Sequence comparison and analysis were performed by PCgene 6.0 package.

The yeast strain JG1017 used for total and nucleolar RNA isolations was grown on YEPD (1% yeast extract, 2% bacto-tryptone, 2% glucose) liquid medium at 30˚C, shaking in 140 r/min until OD600 = 1.5. After the cells were ground into powder with liquid N2, total核酸 acid was first prepared by KAc method, total RNA was then purified from total nucleic acid by guanidinium-thiocyanate method\cite{2}. The preparation of nucleolar RNA was described in reference [5].

Z3 snoRNA sequence-specific oligonucleotide Pz3 (5'GCTCAGTACCACGCCCTGT 3') was 5'-end labeled using [γ-32P] ATP (5 000 Ci/mmol) as described by Sambrook et al.\cite{6} and used as probe for Northern analysis or as primer for reverse transcription. Fifteen micrograms of total RNA was separated on 8% denaturing polyacrylamide gels, then was electrotransferred to Nylon membrane (Ammersham). After cross-linked under the UV irradiation in 253 nm wavelength for 5 min, the membrane was hybridized with 5'-labeled Pz3 as described in reference [7].

Reverse transcription was carried out in 20 μL reaction mixture containing 15 μg of total RNA, 20 ng of 5'-labeled Pz3 and 250 μmol/L of dNTP. After denatured at 65˚C for 5 min and cooled to 42˚C, 10 units AMV reverse transcriptase (Promega) was added and extended at 42˚C for 30 min. The cDNA was then separated on 8% denaturing polyacrylamide gel and extracted from the slice which contained the Z3 cDNA. "G" was added to purified Z3 cDNA at 3' end by using terminal transferase (Promega) and PCR amplified with primer pair polyC16 (GGAAATTCCGATCCCCCCCCCCCCC) and Pz3 according to the previous method\cite{4}. PCR product was purified and subcloned into pGEM T-vector (Promega) and then sequenced using the Sequenase Sequencing Kit (Life Science).

2 Results and discussion

We have searched the DNA database in EMBL and Genbank for the sequences exhibiting the common features of snoRNA gene structure as described previously\cite{8}, a number of snoRNA gene candidates have been discovered (Qu Lianghu et al., unpublished). One of them, named Z3 DNA, found in cosmid 9711 (EMBL number is 49 211) was selected for further analysis. Z3 DNA was defined as 134 bp in length (fig. 1), possessing one box C(TGATGA), one box D(CTGA) motifs and a 13 nt long segment complementary to a phylogenetically conserved segment of 25 S rRNA. There are two 6 nt long inverted repetitions located at 5' and 3' termini, which are liable to form stable terminal stem structure similar to most of snoRNA genes. Those features show that Z3 DNA sequence may encode a novel species of snoRNA, named Z3 snoRNA, of S. cerevisiae. To identify Z3 snoRNA, Northern hybridization and reverse transcription were carried out using Z3 snoRNA sequence-specific oligonucleotide Pz3 as probe and primer. The results of Northern analysis confirmed that Z3 is a 106 nt long, metabolically stable snoRNA (fig. 2). In addition, the Northern analysis of the RNA from different fractions of yeast cell showed that Z3 snoRNA was enriched, as expected, in nucleolus (data not shown). Reverse transcription gave rise to only one, 104 nt long cDNA product (fig. 3) using 5'-labeled Pz3 as primer, indicating that 5'-end of Z3 snoRNA is homogeneous and able to be positioned at the sixth nucleotide upstream from the box C. The cDNA was amplified by PCR, cloned and sequenced. The cDNA sequence determined from PCR product