Z25, a novel intronic snoRNA encoded in mammalian nucleolin gene*

ZHOU Hui (周 惠) and QU Lianghu (屈良鹄)**

(Biotechnology Research Center, Zhongshan University, Guangzhou 510275, China)

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Abstract A novel intronic small nucleolar RNA (snoRNA), termed Z25, was identified from mammals by computer analysis and experimental sequence methods. Z25 is a 69 nucleotides long RNA containing typical boxC/D motifs, terminal stem and an 11-nucleotide sequence complementary to 18S rRNA. In theory, Z25 functions as an RNA guide for the 2′-O-ribose methylation of adenine at position 1678 (human 18S rRNA coordinate) in 18S rRNA. Z25 snoRNA gene was found to be located in the fifth intron of nucleolin gene of human, mouse and rat, demonstrating that the mammalian nucleoline gene is a host gene encoding multiple snoRNAs.

Keywords: Z25, snoRNA, nucleolin, mammal.

Small nucleolar RNAs (snoRNAs) are a kind of novel RNA regulators, which play key roles in ribosome biogenesis of eukaryote. So far, the researches have shown that at least six snoRNAs are involved directly in the eleavage of rRNA precursor; they are the essential components in the complex of rRNA processing[1]. Recent discoveries have demonstrated that snoRNAs serves as RNA guides for the chemical modifications of rRNA, such as guiding the 2′-O-ribose methylation and pseudouridylation[2—4]. Many snoRNAs are crucial to the folding of rRNA precursor[5]. The clarification of the molecular mechanism of the chemically modified rRNA revealed the relationship between the number of certain modified nucleotides and the number of snoRNA genes, which sheds light on the finding of new snoRNAs. For example, the finding of 55 2′-O-ribose methylated nucleotides in *S. cerevisiae* 18S and 25s rRNA has led to the identification of numerous antisence snoRNAs responsible for guiding the 51 methylation nucleotides[6]. At present, 105 2′-O-ribose methylated nucleotides have been found in mammal rRNA, but only 57 antisence snoRNAs have been characterized. Therefore, it is of interest to identify and characterize new snoRNAs from mammals.

In the past, finding new snorRNAs was mainly through experimental method. With the achievements of human genome project, a great number of DNA sequences have been documented in the international molecular biology databases. Previously we found a dozen of novel snoRNAs by computer analysis of intronic sequences[2,7,8] and based on these studies, a new method was developed[9], which is capable of screening the international databases directly. Also we found several peculiar gene organizations in yeast and rice, and some new host genes from mammals[10,11]. Here, we report a novel antisense snoRNA gene, termed Z25, nested in the fifth intron of nucleolin

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** Corresponding author.

gene of human, mouse and rat, and demonstrate that mammalian nucleolin gene is a host to multiple snoRNAs.

1 Materials and methods

1.1 Computer analysis

The computer searches on Genbank and EMBL databases were performed according to the antisense snoRNA finding program[9]. The sequences exhibiting snoRNA gene features were selected and further analyzed by a PC gene 6.0 package.

1.2 Northern hybridization and cDNA analysis of Z25 snoRNA

The oligonucleotide PZ25 (5′ KTTCAGTATTAAGTCCCTTTGT 3′) was used as a primer for reverse transcription and as a probe for Northern hybridization of Z25 snoRNA. It was labeled at the 5′ end with γ-^{32}P ATP as described[12]. The total RNA was isolated from human cell lines and mouse liver by guanidinium thiocyanate method[13]. A total of 20 μg RNA was separated by electrophoresis on a 8% acrylamide gel containing 8 mol/L urea, then transferred to Hybond$^+$ nylon membrane (Ammersham) with the Pharmacia multiphore II electrophoresis system. After a 5 min UV light (253 nm) irradiation, the nylon membrane was hybridized with the 5′end-labeled probe Pz25 as described in ref. [7]. The reverse transcriptions were carried out in 20 μl reaction mixtures containing 20 μg of total RNA and 20 ng of 5′end-labeled oligonucleotide in the presence of 250 μmol/L dNTPs. The mixture was pre-incubated for 5 min at 65℃, cooled to 42℃ before the addition of 200 units of MMLV reverse transcriptase (Promega) and incubated for 30 min at 42℃. The product was analyzed by electrophoresis on the same gel and autoradiography was carried out. Plasmid pBR322 was used as the molecular weight marker after digestion by restriction endonuclease *Taq* Ⅰ and *Hae* Ⅲ and radiolabeled at 5′ end with γ-^{32}P. The cDNA cloning and sequencing were performed as described previously[7]. The plasmid sequence was directly determined using a sequenase sequencing kit (Life Science).

2 Results

With computer screening of DNA databases, a snoRNA gene candidate, termed Z25 DNA, was identified. This sequence is conserved in the fifth intron of nucleolin gene of human, mouse and rat (fig. 1), exhibiting the typical structural features of antisense snoRNA gene (fig. 2). It contains a TGATGA (boxC) sequence at its 5′ end and two CTGA (boxD′ and boxD) sequences in the middle and the 3′ end respectively. There are two repetitions (more than 6 bp long) at the two ends of the Z25 DNA encoding a stable terminal stem similar to the structure in most of snoRNA genes. It also possesses an 11 bp long tract complementary to 18S rRNA (broad lines in fig. 2) and a boxD′ is

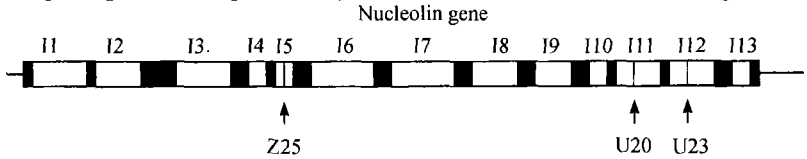

Fig. 1. Schematic diagram of the nucleolin gene of mammal. ■ Exon, □ intron.

located at its downstream. According to the theory of the structure and function of antisense snoRNA, this complementary sequence, together with the downstream boxD′, can guide a 2′-O-ribose methylation of adenine at the position of 1678 in 18S rRNA (human 18S rRNA coordinate). A1678 is a conserved nucleotide, which was identified as a 2′-O-ribose methylated nucleotide (i.e. Am1678) in human and amphibian *Xenopus lavies*[14]. So far, no snoRNA as a guide for this methylation site has been reported yet. So Z25 DNA may encode a novel snoRNA.

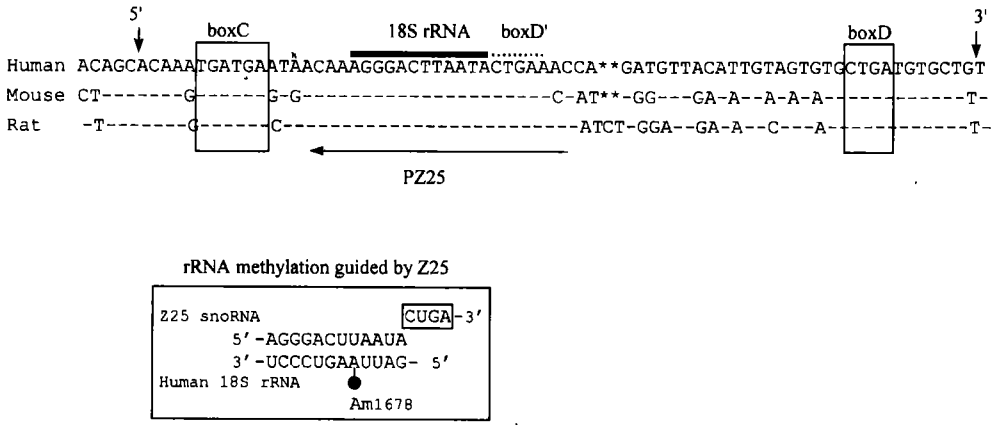

Fig. 2. Z25 snoRNA genes and its functional elements.

For further confirmation of Z25 snoRNA, PZ25 was synthesized according to the Z25 DNA sequence (fig. 2). Northern hybridization was performed with the 5′ end-labeled PZ25 to the total RNA of human and mouse. A strong and unique signal corresponding to a 69 nucleotides long RNA, that is, Z25 snoRNA was detected under a stringent condition (fig. 3(a)). The length of the Z25 snoRNA was the same as expected. After a reverse transcription of human and mouse total RNA primed by Pz25, a single cDNA product, 36 nucleotides in length, was obtained (fig. 3(b)). The sequence determined from the cDNA was identical to Z25 DNA. Based on this result, the 5′ end of Z25 snoRNA could be mapped to the 5th nucleotide upstream from boxC (fig. 2(a)), shown by an

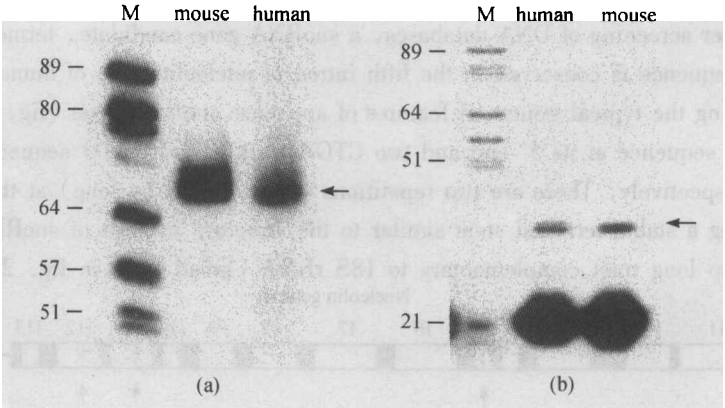

Fig. 3. Identification of Z25 snoRNA. (a) Northern blot; (b) reverse transcription.

arrow). Referring to the result of Northern hybridization, the 3′ end of Z25 snoRNA was mapped to the 7th nucleotide downstream from boxD.

3 Discussion

Nucleolin is a major protein of nucleolus and is highly conserved in a wide range of eukaryote. Nucleolin has already been found to be a multifunctional mulecule. It not only plays key roles in rDNA transcription, rRNA precursor processing and ribosome assembly, but also has many other functions such as maintaining chromosome structure and cellular signal transduction[15]. The mammalian nucleolin gene is 9 kb long and has 14 exons. But more than 70 percent of the gene is intronic constituent. Nicoloso et al. found U20, a methylation guide for 18S rRNA, in the 11th intron of mammalian nucleolin gene. It first demonstrated that mammalian nucleolin gene is a host gene[16]. Then we identified U23, a boxH/ACA snoRNA responsible for the pseudouridylation of uridine at position 97 (human 18S rRNA coordinate) in 18S rRNA, from the 12th intron of nucleolin gene of human, mouse, chicken, *X. laevis* and two fishes *Cyprinus carpio* and *Salmo gairdner* (Genbank accession Nos. Aj007015, Aj009729-Aj009731). The result provided the evidence for the functional significance of the introns of nucleolin gene. Now the discovery of Z25 gene in the 5th intron of nucleolin gene further demonstrates that mammalian nucleolin gene is a host gene encoding multiple snoRNAs.

Mammalian nucleolin gene has 13 introns, 3 introns have been found encoding snoRNA (ref. [16] and this work), the 4th intron is small in size (only 98—106 bp long), if there exists any RNA gene or functional element in the other 9 introns is unclear. It is worth to point out that the CIS1 (conserved intronic sequence 1) is highly conserved in the 1st intron of nucleolin gene of human, mouse and rat[17], but we did not find any structural features of snoRNA gene in it. Northern hybridization of human and mouse total RNA with the CIS1 specific probe has failed to detect any RNA molecule[1)]. However, the result can not exclude the possibility of the existence of some extremely rare or unstable RNA. The function of mammalian nucleolin gene introns and its origin need further studies.

The sequences of Z25 gene of human, mouse and rat have been deposited in the Genbank database under the accession numbers of Aj010666, Aj010667 and Aj010668 respectively.

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