中山大学学报 (自然科学版)
第37卷 第2期 ACTA SCIENTIARUM NATURALIUM Vol. 37 No. 2
1998年 3月 UNIVERSITATIS SUNYATSENI Mar. 1998

Identification of A Novel Antisense Small Nucleolar RNA from Yeast*

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Abstract A novel small nucleolar RNA, Z6, was identified and characterized from yeast *Saccharomyces cerevisiae*. Z6 snoRNA is 109 nt. in length, encoded by an independent transcribed gene which probably belongs to a snoRNA gene cluster on the chromosome XIII of yeast. It contains box C, box D motifs and a 11 nt. long segment complimentary to a conserved sequence of 25S rRNA. The antisense element, together with the downstream box D', guides a 2'-O-ribose methylation of cytidine acid at position 2195 in yeast 25S rRNA.

Keywords Z6, snoRNA, yeast, rRNA methylation
Classification number Q 74

In the recent years, the small nucleolar RNAs (snoRNAs) have been the interesting subject when this kind of RNAs, which enrich in nucleolus of eukaryotic cells and function as ribonucleoprotein, are found to play key roles in ribosomal biogenesis[1]. Most of the known snoRNA can be classified into two groups which are structurally and functionally distinct. One group of snoRNA, the boxC/D group containing boxC (UGAUGA) and boxD (CUGA) motifs, has been proved function as guides for the 2'-O-ribose methylation of rRNA by base pairing[2,3]. With the similar pattern, the other group which characterized by box H (ANANNA), ACA consensus at 3' end and "hairpin-hinge-hairpin-tail" structure, is involved in site-specific pseudouridylation of rRNA[4]. Although much work has been done, the process of ribosomal biogenesis is still considered to be a complicated event, especially the rRNA maturation. Many evidences have demonstrated that the rRNA maturation need the participating of a myriad of moleculars. Despite the functions as guides for methylated and pseudouridylated modification, several snoRNAs are essential for site-specific cleavage in pre-rRNA processing[1], and others are proposed to play other roles such as rRNA chaperon[5]. For further understanding the mechanism of rRNA maturation and assembly of rRNA and ribosomal protein, more snoRNA species and other associated functional elements remain to be identified and characterized. In this report, a new snoRNA is identified from yeast *S. cerevisiae*. Its structural features and functional significance are also discussed.

1 Materials and methods

1.1 Computer screening of sequence data bases

Genbank and EMBL DNA sequence data bases were screened by using Blast and Fasta

* 国家自然科学基金重点项目 (39730300) 资助项目
收稿日期: 1997-11-17 陆勇军, 男, 32岁, 讲师

programm. Sequences exhibiting snoRNA gene features were selected and further analyzed by using PC gene 6.0 package.

1.2 Oligonucleotide probe and primers

The sequence of oligonucleotide Pz6 used as primer for reverse transcription and as probe for Northern analysis is 5′-GATCAGTTGCGCGGGTTTTC-3′. PolyC16 [5′ GGAATTCGGAT (C) 16] is used as reverse primer in PCR amplification of cDNA. Pz6 was radiolabeled at 5′-end with [γ-^{32}P] ATP as described in reference[6].

1.3 Preparation of yeast total RNA

The yeast strain JG1017 was grown on YEPD (yeast extracts 1%, Bacto-tryptone 2%, glucose 2%) liquid medium at 30℃ with shaking at 140 r/min. until the A_{600}= 1.5. The cell was centrifuged and the pellet was ground into powder with liquid N_2. Total RNA was extracted and purified according to guanidinium-thiocyante method[6].

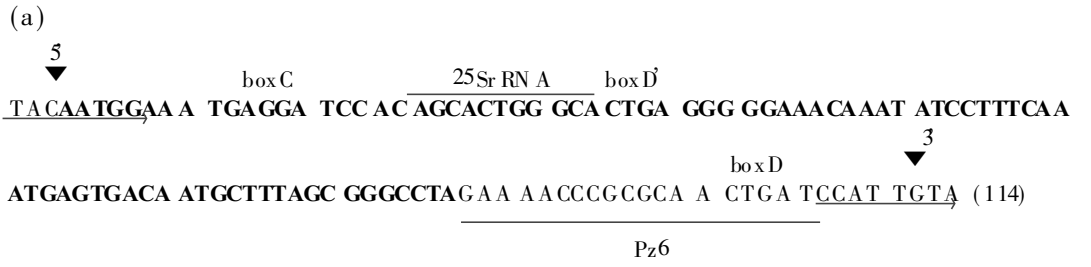
1.4 Northern hybridization, cDNA cloning and sequencing

Procedures for Northern hybridization analysis, reverse transcription and cDNA purification were as previously described[7]. The purified cDNA was tailed with an oligo-(dG) tract and PCR amplified by using Pz6 and polyC(16) as primers. PCR products were purified, cloned into the SmaI site of pTZ19 plasmid. Sequencing was performed using the Sequenase sequencing kit (Life Science).

2 Results and discussion

In the past two years, a computer screening method employed to analyze on EMBL and Genbank database has been proved to be considerable effective in finding of new snoRNA genes[7,8]. With the same method, a yeast snoRNA gene candidate, termed Z6 DNA, locating on a non protein encoding sequence between two protein genes of yeast 13th chromosome, was screened from database. Z6 DNA is defined as 114 nt in length, contains boxC, boxD motifs and possesses a 11 nt long segment potential to form perfect duplexes with the conserved sequence of 25S rRNA. There are two repetitions of 9 nt at 5′ and 3′ termini (Fig. 1-a, noted by arrows) which make the precusor transcript to form stable terminal stem structure similar to most of snoRNA genes. Except C and D boxes, Z6 DNA did not share significant sequence similarity to any known snoRNA genes, suggesting that it may encode a novel snoRNA, that is, Z6 snoRNA.

To confirm the above inference, a sequence-specific oligonucleotide, Pz6, was synthesized and 5′-end radiolabeled. Northern hybridization was performed by using labeled Pz6 as probe and gave rise to an unique band of 109 nt under stringent conditions (Fig. 2-a). Reverse transcription experiment using Pz6 as primer, on the other hand, gave single band cDNA product of 103 nt (Fig. 2-b). The cDNA product was purified, PCR amplified and cloned. The positive clones were then subjected to sequence analyses. The results showed identical sequence between the cDNA and Z6 DNA (Fig. 1-a, bold letters), further confirming that Z6 snoRNA was the transcript of Z6 DNA. According to the results of reverse transcription and cDNA sequence, the 5′ end of this newly defined snoRNA can be positioned at the 8th nucleotide upstream from box C. Referring to the result of Northern analysis, the 3′ end of Z6 snoRNA is situated at the 8th nucleotide downstream from boxD (Fig. 1-a, indicated by arrowheads).



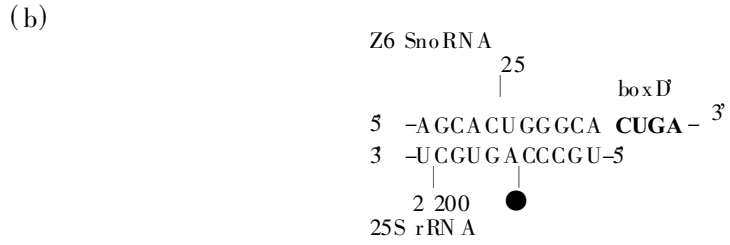


Fig. 1　Structural features of Z6 DNA and snoRNA
(a) the sequence and conserved elements of Z6 DNA; (b) Duplex between Z6 snoRNA and 25S rRNA

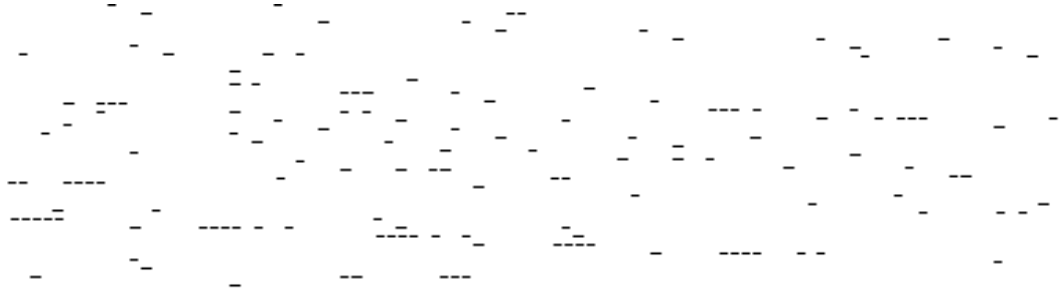


Fig. 2　(a) The result of Northern analyses using Pz6 as probe
(b) The result of reverse transcription of Z6 snoRNA
M, size marker; S1, S2 and R, 15μg total RNA of yeast (a) (b)

The known snoRNA genes have two distinct genomic organizations[1]. Many are nested within introns of protein genes where they are synthesized as part of pre-mRNA and then are released from the intron by processing. In the second type, the snoRNA coding sequences are flanked by promoters and hence independent transcribed. This dichotomy of gene organization referes different mechanism of expression and processing. Z6 snoRNA gene is located on a non-protein encoding sequence between two protein genes. A candidate TATA box has also been found at the 5'-end upstream from Z6 DNA. So we concluded that Z6 snoRNA is encoded by an independent-transcribed gene. Furthermore, two other snoRNA genes are being identified upstream of Z6 snoRNA gene, suggesting that Z6 snoRNA gene is probably a member of a snoRNA gene cluster (our unpublished results).

The C, D box containing snoRNAs always contain a segment perfect complimentarities to the universal core region of mature 18S or 25~28S rRNA[5]. The function of these so-called antisense snoRNA have been confirmed that the segment complimentary to rRNA, together with the downstream box D, provide the information necessary to select the target

nucleotide for 2′-O-ribose methylation of rRNA. Z6 snoRNA, the newly defined antisense snoRNA in this report, has also the function in guiding the formation of 2′-O-ribose methylation of cytidine acid at position 2 195 in yeast 25S rRNA (Fig. 1-b, depicted by black circle). It worth to note that this methylation site of rRNA is conserved between vertebrates and yeast, suggesting that a Z6 snoRNA homolog may exist in human and other higher organisms. The search for Z6 snoRNA gene homolog is now in progress in our laboratory.

Z6 snoRNA gene sequence has been deposited in EMBL Database under the accession number Z 69298.

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酿酒酵母中一种新的反义 snoRNA分子的鉴定

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摘 要 核仁小分子 RNA (snoRNA) 是一类在真核生物核糖体生物合成过程中起重要作用的小分子 RNA. 通过计算机直接分析国际分子生物学数据库及 RNA杂交分析、cDNA序列测定等方法, 在酿酒酵母 (*Saccharomyces cerevisiae*) 中发现和鉴定了 1个新的 snoRNA-Z6 snoRNA. 该 snoRNA长 109个核苷酸, 由位于酿酒酵母第 13号染色体上的 1个独立基因编码. Z6 snoRNA含有 boxC (UGAUGA)、boxD (CUGA) 等保守的结构元素, 属于反义 snoRNA家族, 即分子中有 1段 11个核苷酸的片段与 25S rRNA中 1段保守核心序列互补, 该 snoRNA片段连同其下游的 boxD共同指导与其互补的 rRNA序列第 2 195位胞苷酸的 2′-氧-核糖的甲基化.

关键词 Z6, snoRNA, 酿酒酵母, rRNA甲基化
分类号 Q 74

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