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Two genes encode 7SL RNAs in the yeast *Yarrowia lipolytica*

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Summary. We have identified an abundant cytoplasmic 7S RNA in crude extracts of the yeast *Yarrowia lipolytica*. A cDNA probe was prepared from this RNA and used to screen a genomic library. The DNA sequence of a positive clone was determined and the end positions of the 7S RNA gene established by comparison with the sequence of the extremities of 7S RNA. This gene, designated *SCR2*, encodes a 270-nucleotide RNA that can be folded into a secondary structure similar to that of 7SL RNAs. This RNA is 94.4% homologous to a previously identified 7S RNA from this yeast, but is encoded by a separate gene with highly divergent flanking sequences.

Key words: Yeast – 7SL RNA – *Yarrowia lipolytica*

Introduction

7SL RNA has been shown to be one of the components of the Signal Recognition Particle (SRP) (Walter and Blobel 1980; Walter and Blobel 1982). In in vitro systems, SRP plays a crucial role in protein translocation from the cytoplasm across the endoplasmic reticulum membrane. Six proteins are associated with 7SL RNA to constitute the SRP and the particle must interact with components of the ribosomes and of the endoplasmic reticulum.

In this paper, we present the cloning and sequencing of the gene, *SCR2*, which encodes a 7SL-like RNA in the yeast *Yarrowia lipolytica*. The cloning of *SCR1*, which also encodes a 7SL RNA in this yeast, has already been reported (Poritz et al. 1988). We present evidence for the presence of two 7SL RNA genes in *Y. lipolytica*.

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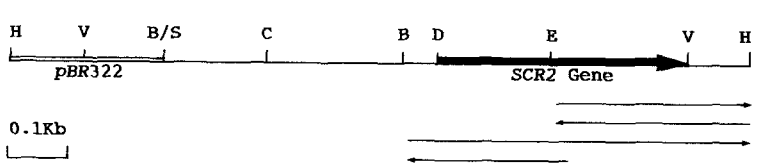
Materials and methods

Yarrowia lipolytica strain W29 is the wild type reference strain used in our laboratory. Strain CX161-1B was a gift of D. Ogrydziak (University of California at Davis). We have previously reported the preparation of a genomic DNA library from strain W29 (Xuan et al. 1988). *Y. lipolytica* strain W29 was grown in YEPD liquid complete medium (1% Difco yeast extract, 1% Difco Bactopeptone, 2% Glucose in 50 mM citrate buffer pH 5.0) to OD_{600} 1.0, harvested and washed with distilled water. The isolation procedure of the 7S RNA was adapted from Ribes et al. (1988).

Results

Identification of a 7SL RNA candidate from Yarrowia lipolytica

Based on the properties of mammalian and *Schizosaccharomyces pombe* SRP (Ribes et al. 1988), an enrichment scheme for the purification of the SRP from *Y. lipolytica* was adopted. A cytoplasmic fraction was obtained from a low-salt lysate of spheroplasts of *Yarrowia lipolytica* by centrifugation at low speed to discard the nuclei and cell debris. The supernatant was centrifuged at high-speed to pellet the microsomes and ribosomes, which were then resuspended in low-salt buffer and again pelleted. The pellet was resuspended in high-salt buffer to release SRP and again centrifuged. RNA was prepared from an equivalent fraction of the supernatant at each stage and separated on polyacrylamide-urea gel. A single abundant cytoplasmic RNA species was observed in the post-ribosomal supernatant which was close in size to 7S. Unexpectedly, this 7S-sized RNA was mainly in the supernatants of the first two high-speed centrifugations. Thus, no stable association between this 7S-sized RNA and ribosomes was detected, in contrast to mammalian 7SL RNA and *S. pombe* 7SL RNA.



B

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          -230       -220       -210       -200       -190       -180       -170       -160       -150
     atctgcttccaaatatagaatcataacaagggttagggtgtgattatataatattggtcttaattgatgtgctagggctttaaaagttggttaaaa

-140       -130       -120       -110       -100       -90        -80        -70        -60        -50
taacgctctaatgccttttttaatatattgtctttttcaaaatctcaaatcggacacttcttcgtgtatgagactccatttttttggctccgtcacgtgat
                                                                                                 :::::::
           atatggttttacccaatcaaccgatttatattcttgaaaattgcgtcgaaaggtggttaaaatcgcacttgaagttgggcaacacgtgac

 -40        -30        -20        -10          1         10         20         30         40         50
atgtattatcagctatagtggtgtaaacaaagtttttta--ct-AGCTGTAATGGCATTTTGTCGGAGTGGTAAATCGCCTTCTTGTTGTGCGTTCGAG
 :: :::  : :   : :::  ::   ::: : :::::::  :: :::::::::::::::::::::::::::::::::::: :::::::::::::::::::
ttg-attggctggg-t-gtgtagtcc-caacgctttttaaaCTTAGCTGTAATGGCATTTTGTCGGAGTGGTAAATCGTCTTCTTGTTGTGCGTTCGAG

   60         70         80         90         100        110        120        130        140        150
TTCTGGACTCTGCACTGGGCTACTTTGTTTGTCCTTTCCGAATTCTGCGGTTGATGGGCGTCTCGGTCTGAGTAATCGGCTTTGAGATTTCCGTTCTAA
:  ::: ::::::::: ::: : :::: ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
TCTTGGGCTCTGCACTTGGCCA-TTTGGTTGTCCTTTCCGAATTCTGCGGTTGATGGGCGTCTCGGTCTGAGTAATCGGCTTTGAGATTTCCGTTCTAA

   160        170        180        190        200        210        220        230        240        250
GATTAACTGGGATACTTCAGTGGAGCAATCCAGCAGAGATCCAGTTGCCGTGGGTATGGCGGTGGGATAGCAACAAAGTGGTATATGTTATGGAAGGTA
:::::::::::: :::::::::::::::::::::::::::::::::::::::::::::::::::::::::: ::::: :::::::::::::::::::: ::
GATTAACTGGGAAACTTCAGTGGAGCAATCCAGCAGAGATCCAGTTGCCGTGGGTATGGCGGTGGGATCGCAACCAAGTGGTATATGTTATGGAAGATA

    260        270         +10        +20        +30        +40        +50        +60        +70        +80
TTTGCAATCACGACTCTCTttttgctaggtgatgttctatcttttgcttcatacgccaggataatgtaatttctcatttcaattggacctcgtgtcgaa
::: : ::::::: : : : :::
TTTACGATCACGATTttttgtttcgaacgaaggggcggatcatcagaccatttgtttttcagtcattctagcacgcaatctacaattacggtacttgtg

       +90        +100       +110       +120       +130       +140       +150       +160       +170
gtggatacttctagcgctacttttagtcgtcccatgatggtattcacttttcactcgatagctgatatcatttctcaactggtgatacccactttcctt

cgctcgtactcgctcgtactctcaccgtcaattctcacgcggttcacttgtcttaaagtttgctaaattaaaagtgacgcgt

       +190       +200       +210       +220       +230       +240       +250       +260
cgaaatgagtcaatgcagtcatgtccttttcatttatgttcatttttcatttatttcaatttctttcttaatatatttaatttcaagctt
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Fig. 1. A restriction map and sequencing strategy for *SCR2*. This gene, indicated by the *heavy arrow*, is located in the 0.8 Kb *Bgl*II-*Hind*III fragment. *B, Bgl*I; *C, Cla*I; *D, Dra*I; *E, Eco*RI; *H, Hind*III; *V, Eco*RV; *B/S, Bam*HI/*Sau*3A. The DNA sequencing strategy is indicated by *arrows* under the gene. **B** nucleotide sequence of the *SCR2* gene and comparison of the primary sequences of the *SCR1* and *SCR2* genes. DNA sequencing of *SCR2* was carried out by the dideoxynucleotide method. The boundaries of the gene were determined by direct RNA sequencing on 5′- and 3′-end-labelled 7SL RNA from *Y. lipolytica*. The upper line (in *bold letters*) shows the DNA sequence of *SCR2* gene. The lower line shows the DNA sequence of the *SCR1* gene published by Poritz et al. (1988). For both the *SCR1* and *SCR2* genes, the coding sequences are in *uppercase* and the flanking sequences in *lowercase*. The *underlined sequences* are consensus sequences defined for the A and B box elements of RNA polymerase III promoters (Sharp et al. 1981). The best fit alignments were determined by using the comparison method of Kanehisa (1984). Identical nucleotides are indicated (:)

Cloning of the 7S RNA gene

RNA prepared from the post-ribosomal supernatant was separated on a polyacrylamide-urea gel. The 7S RNA band was excised and eluted. A cDNA probe, made from the gel-purified 7S RNA, was used to screen a genomic library of *Y. lipolytica* (Xuan et al. 1988). Plasmids were extracted from positive clones and used to probe a Northern blot. One was found to hybridize to the 7S-sized RNA. This plasmid is designated pHF1 and was used for further study. Other plasmids hybridized to 5S and 5.8S rRNA.

The approximate location of the gene within the insert was determined by restriction mapping and Southern hybridization. The sequence of the 0.8 Kb *Bgl*II-*Hind*III fragment (Fig. 1) was determined by dideoxynucleotide sequencing (Sanger et al. 1977).

Primary and secondary structure of the 7S RNA

To determine the end points of the coding region of the 7S RNA gene, we sequenced the extremities of the 7S RNA. Direct sequencing of this RNA has shown that it possesses a unique 5′ initiation site and three adjacent termination sites which differ by single bases starting at position 270 (Fig. 1). The coding region contains 270/272 nucleotides.