Absence of structural homology between *sup*1 and *sup*2 genes of yeast *Saccharomyces cerevisiae* and identification of their transcripts

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The results of Southern blotting demonstrate that *sup*2 is a unique gene in *Saccharomyces cerevisiae* that does not possess homologous sequences in the yeast genome. The direct hybridization of DNA fragments, containing cloned *sup*1 and *sup*2 genes, did not reveal any structural homology between these two genes. By Northern blotting analysis the sizes of the transcripts were determined to be 1.6 kb for *sup*1 gene and 2.5 and 1.4kb for *sup*2 gene. Experiments with RNA isolated from yeast mutant with impaired splicing demonstrated that *sup*1 and *sup*2 genes do not contain introns.

Southern blotting *Northern blotting* (Saccharomyces cerevisiae) *Gene homology*

1. INTRODUCTION

In the yeast *Saccharomyces cerevisiae* in addition to dominant suppressors encoding tRNA, recessive suppressors *sup*1 and *sup*2 have been described [1–3]. Mutations in these two genes are allele-specific but gene non-specific, acting towards all three types of nonsense mutations located in different genes [2]. The study of pleiotropic effects of the suppressor mutations as well as the data on a high level of ribosomal ambiguity in the suppressor strains indicate that *sup*1 and *sup*2 genes encode for ribosome-associated proteins participating in the decoding of genetic information [4]. Recently both genes were cloned in shuttle vectors p3030 [5] and YEp13 [6]. Here, the transcripts for *sup*1 and *sup*2 genes are identified. Moreover, the data on the absence of structural homology between these two genes are presented.

2. MATERIALS AND METHODS

DNA was extracted from *S. cerevisiae*, strain 6V-D307 (a *ade* 1-14 *his*3 *leu*2 *lys*2 *sup*2) as described in [7], treated by restriction endonucleases, electrophoresed through agarose gel and transferred to nitrocellulose membrane [8].

Southern blot hybridization was carried out in 6 × SSC, at 68°C [9].

To analyse the homology between *sup*1 and *sup*2 genes hybridization was performed in the same buffer [9] at 60°C.

RNA for Northern analysis was extracted [10] from yeast strain carrying the *rna*2-1 mutation [11], preincubated under permissive (25°C) or nonpermissive (37°C) conditions. 30 μg total RNA were electrophoresed on an agarose-urea gel [12] and transferred to DBM paper [13]. Hybridization was performed at 42°C in 50% formamide [9]. As ^{32}P hybridization probes nick-translated plasmids containing *sup*1 gene (pYS1 and pPBM16), *sup*2 gene (pSTR7 and pSTR16) or their restriction fragments were used [5,6]. Plasmids pYS1 and pPBM16 contained *sup*1 gene in vectors p3030 and pACYC177, respectively [5]. Plasmid pSTR7 contained *sup*2 gene in yeast-*E. coli* shuttle vector YEp13 [6]. Plasmid pSTR16 contained the *Hin*dIII-*Sal*I fragment of the *sup*2 gene cleaved

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from pSTR7 and inserted in vector pBR322. This plasmid did not contain yeast chromosomal DNA unrelated to the *sup*2 gene sequence. Both pACYC177 and pBR322 were not homologous to yeast chromosomal DNA. Plasmids pYS1 and pSTR7 have homology in their vector sequences [5,6].

3. RESULTS AND DISCUSSION

3.1. *Identification of transcripts for* sup*1 and* sup*2 genes*

Fig.1 shows the results of Northern blotting analysis of RNA isolated from yeast *rna*2-1 mutant incubated under permissive (25°C) or restrictive (37°C) conditions.

Plasmid pPBM16, containing the *sup*1 gene, hybridizes with a unique 1.6 kb RNA species. Since no alterations in electrophoretic mobility were noticed when RNA was isolated from the yeast cells with impaired splicing (*rna*2-1 mutant incubated at 37°C) (fig.1a) we drew a conclusion about the absence of introns in *sup*1 gene. This is in agreement with the finding of a single RNA band of 1.5–1.6 kb for the *sup*45 gene of *S. cerevisiae*, presumably similar or identical to *sup*1 [14] and the size of the open reading frame for the *sup*1 gene [15].

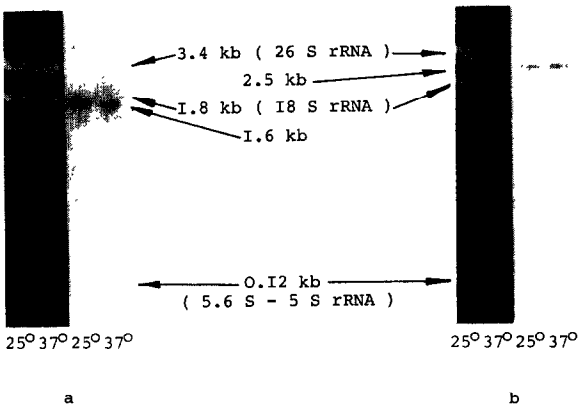

Fig.1. Northern blotting hybridization between total yeast RNA and ^{32}P DNA probes. Total RNA was isolated from the cells of *rna*2-1 mutant preincubated at permissive (25°C) or restrictive (37°C) temperature. (a) Hybridization with ^{32}P plasmid pPBM16 (*sup*1), (b) hybridization with ^{32}P plasmid pSTR16 (*sup*2). RNA staining by ethidium bromide is shown on the left and radioautographs to the right of both panels.

When nick-translated plasmid pSTR16, containing *sup*2 gene, was hybridized with total RNA a unique 2.5 kb band was found (fig.1b). No changes in electrophoretic mobility of this RNA band were observed when RNA was isolated from *rna*2-1 mutant incubated under conditions of impaired splicing indicating the absence of introns in the *sup*2 gene. However, when plasmid pSTR16 was used for hybridization with poly(A^+) RNA, another minor RNA band with a length of 1.4 kb was found (fig.2). No hybridization was found with poly(A^-) RNA (material non-adsorbed on oligodeoxy(T)-cellulose) suggesting that both 1.4 and 2.5 kb RNAs are polyadenylated. Since plasmid pSTR16 contains as an insert an internal part of the *sup*2 gene only [6], hybridization with two RNAs with different length may point to the existence of two stable transcripts for *sup*2 gene in yeast cells. Their presence is presumably due to the initiation or termination of transcription at two different sites.

3.2. *Hybridization of* sup*2 gene-containing probes with yeast chromosomal DNA*

Plasmid pSTR16, as well as a fragment of pSTR7 plasmid, both containing *sup*2 gene, hybridize with a unique fragment of yeast chromosomal DNA obtained after cleavage by different restriction endonucleases. The length of *Xho*I-*Eco*RI and *Xho*I-*Bam*HI fragments hybridized with pSTR16 coincides with the length

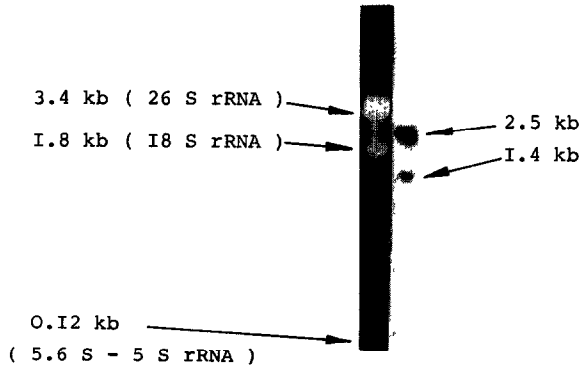

Fig.2. Northern blotting hybridization between yeast poly(A^+) RNA and ^{32}P nick-translated plasmid pSTR16 (*sup*2 gene). Electrophoresis of poly(A^+) RNA stained by ethidium bromide (left); hybridization with ^{32}P plasmid pSTR16 (*sup*2) (right).

of the corresponding restriction fragments of plasmid pSTR7 carrying the *sup*2 gene (see fig.3, tracks 7,8; fig.4). Hybridization with other fragments of chromosomal DNA was not observed indicating that *sup*2 gene is unique in yeast genome.

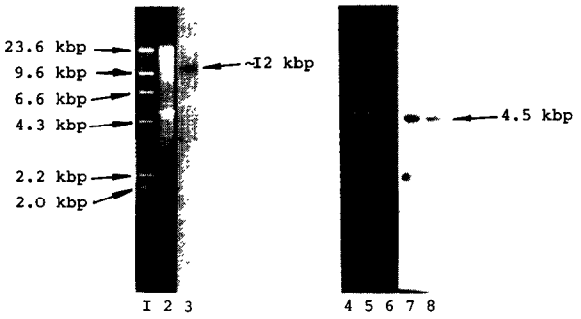

Fig.3. Southern blotting hybridization of yeast chromosomal DNA. ^{32}P nick-translated plasmid pSTR16 or *Xho*I-*Bam*HI fragment of pSTR7 was used as a probe. Lanes: 1,6, molecular mass standards (*Hin*dIII hydrolysate of λ phage); 2,3 *Xho*I hydrolysate of yeast DNA and corresponding blotting; 4,7, *Xho*I + *Bam*HI hydrolysate of yeast DNA and corresponding blotting; 5,8, *Xho*I + *Eco*RI hydrolysate and corresponding blotting.

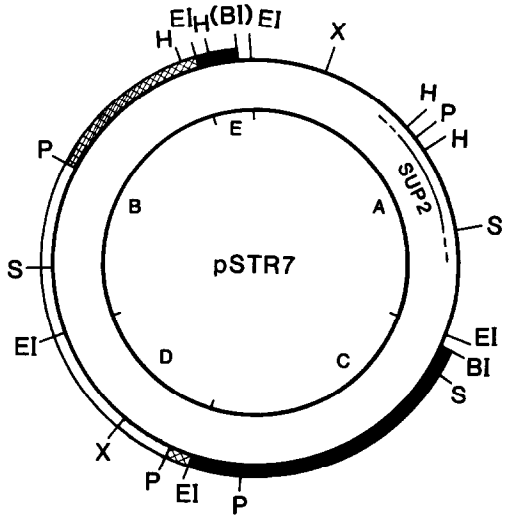

Fig.4. Restriction map of pSTR7 (*sup*2 gene containing plasmid). (▬) Sequence of pBR322; (▩) sequence of 2 μm DNA; (▭) sequence of *Leu*2 yeast gene; (——) sequence of yeast chromosomal DNA; (- - - -) sequence of *sup*2 gene. (A–E) Restriction fragments of pSTR7 plasmid after *Eco*RI treatment. BI, *Bam*HI; EI, *Eco*RI; P, *Pst*I; H, *Hin*dIII; S, *Sal*I; X, *Xho*I.

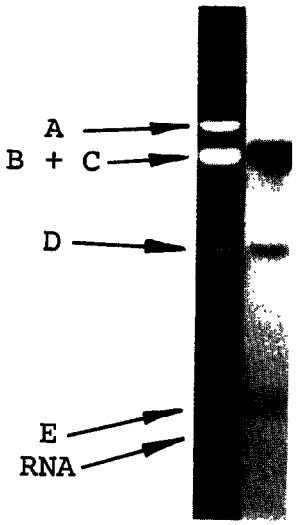

Fig.5. Southern blotting hybridization of the *Eco*RI-cleaved plasmid pSTR7 (*sup*2 gene) with ^{32}P nick-translated plasmid pYS1 (*sup*1 gene). Electrophoresis of *Eco*RI hydrolysate of pSTR7 (left) and corresponding blotting (right).

3.3. *Hybridization of plasmid pYS1 (gene* sup*1) with restriction fragments of plasmid pSTR7 (gene* sup*2)*

The results of hybridization of plasmid pYS1, containing *sup*1 gene, with pSTR7 (*sup*2 gene) restriction fragments obtained after *Eco*RI treatment are shown in fig.5. The *Eco*RIA fragment contains *sup*2 gene and does not contain the nucleotide sequence of YEp13 vector. All other restriction fragments of this plasmid contain nucleotide sequences of the YEp13 vector possessing vast homologous sequences with vector p3030, a constituent of plasmid pYS1.

No hybridization was found between plasmid pYS1 (*sup*1 gene) and *sup*2-containing fragment. These data demonstrate the absence of structural homology between *sup*1 and *sup*2 genes in the limits of the precision of the hybridization method used.

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