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The yeast *Yarrowia lipolytica* has two, functional, signal recognition particle 7S RNA genes

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Summary. Cells containing a deletion of either the *SCR1* or *SCR2* genes, which code for the 7SL RNA component of the signal recognition particle (SRP) homologue, were found to be viable. Two independent approaches demonstrated that cells containing deletions of both genes were inviable. Therefore, *Yarrowia lipolytica* contains two (and only two) functional 7SL RNA genes.

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

Introduction

The signal recognition particle (SRP) is a ribonucleoprotein composed of a single RNA species (7SL RNA) and six polypeptides (Walter and Blobel 1980, 1982). SRP is required for the translocation of secretory proteins into the endoplasmic reticulum (ER) (Walter and Blobel 1980, 1981). From the study of in vitro systems a model in which SRP functions as an adapter between the translation machinery in the cytoplasm and the translocation machinery in the ER membrane has been proposed (Walter and Lingappa 1986). The functions of individual SRP proteins have been identified by the in vitro study of perturbed SRPs reconstituted with modified SRP proteins or with a subset of the SRP proteins (Siegel and Walter 1985, 1988). However, classical genetic studies of the in vivo functions of SRP and its components have not been feasible.

The isolation of SRP homologues in two genetically tractable organisms, the yeasts *Yarrowia lipolytica* (He et al. 1989; Poritz et al. 1988) and *Schizosaccharomyces pombe* (Brennwald et al. 1988; Poritz et al. 1988; Ribes et al. 1988), marks the first step in identifying the in vitro functions of SRP. The genes coding for the 7SL RNA components of these SRP homologues have been cloned and sequenced, and they resemble 7SL RNAs of higher eukaryotes with regards to size, transcriptional start signals, 5′ end structure, potential secondary structure, and binding under stringent conditions to mammalian SRP proteins (Brennwald et al. 1988; He et al. 1989; Poritz et al. 1988; Ribes et al. 1988).

S. pombe contains a single essential 7SL RNA gene (Brennwald et al. 1988; Ribes et al. 1988) and a conserved tetranucleotide loop, which is important for function, has been identified (Liao et al. 1989). Originally, based on Southern blotting results it was reported that *Y. lipolytica* also contained only one 7SL RNA gene (*SCR1*; Poritz et al. 1988). However, recently He et al. (1989) cloned a second 7SL RNA gene (*SCR2*) which has 94% sequence homology with *SCR1* in coding region but a quite different sequence in the flanking regions. The major goal of this study was to determine if both 7SL RNA genes were functional. The basic strategy was to construct gene deletions and to determine their effects on viability.

Materials and methods

Media, genetic procedures. Complete medium was YPD, and minimal medium was YNB-glucose as described by Sherman et al. (1986). Mating, tetrad and random spore analyses, and scoring of segregants were carried out as described previously (Ogrydziak et al. 1978; Beckerich et al. 1984). Diploids were sporulated either on V-8 medium (Gaillardin et al. 1973) or on CSM medium (Barth and Weber 1985).

Transformation, gene disruption. The protocol of Davidow et al. (1985), as modified by Xuan et al. (1988), was used for transformation of *Y. lipolytica*. One-step gene disruptions were done as described by Rothstein (1983).

Hybridization. Southern blotting and DNA hybridizations were as described by Xuan et al. (1988) or by using the alkaline transfer method (Reed et al. 1985) with Zeta probe nylon membrane (Bio-Rad). Probes were prepared by nick-translation (Xuan et al. 1988) or by random primed DNA labelling (Boehringer Mannheim). Total RNA, prepared as described by Davidow et al. (1987), was

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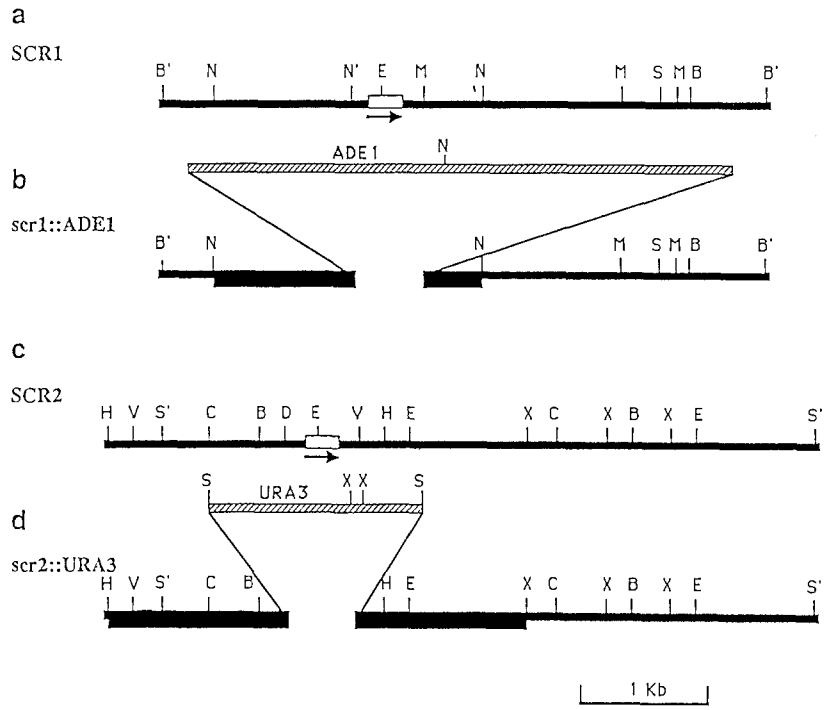

Fig. 1. Deletion and disruption of *SCR1* and *SCR2*. **a** restriction map of the wild-type *SCR1* locus. **b** the disrupted allele of *SCR1*, created by deletion of the coding region and insertion of the *Y. lipolytica ADE1* gene. **c** restriction map of the wild-type *SCR2* locus. **d** the disrupted allele of *SCR2*, created by the deletion of the coding region and insertion of the *Y. lipolytica URA3* gene. The *Hind*III-*Xho*I fragment was cloned into a plasmid polylinker site, and a *Bam*HI-*Apa*I fragment containing pieces of the polylinker at the ends was used for the one-step gene disruption. *Open boxes* are the coding regions of *SCR1* and *SCR2*. *Thick lines* indicate the fragments used for one-step gene disruptions. *SCR1* was not checked for digestion by *Cla*I, *Dra*I, *Eco*RV, *Sau*3A and *Xho*I. *SCR2* was not checked for digestion by *Nde*I, *Mlu*I and *Nco*I. *B* = *Bgl*II, *B'* = *Bam*HI, *C* = *Cla*I, *D* = *Dra*I, *E* = *Eco*RI, *H* = *Hin*dIII, *M* = *Mlu*I, *N* = *Nco*I, *N'* = *Nde*I, *S* = *Sal*I, *S'* = *Sau*3A, *V* = *Eco*RV, *X* = *Xho*I

electrophoresed on an agarose gel containing formaldehyde as described by Maniatis et al. (1982), and the RNA was transferred to Zeta probe nylon membrane according to the manufacturer's instructions. Labelled nucleotides were purchased from Amersham.

Results

Deletion of SCR1 is not lethal

For the one-step gene deletion of *SCR1*, a 560 base pair *Nde*I-*Mlu*I fragment which includes the entire coding region was deleted, and a 4.3 kb fragment containing the *Y. lipolytica ADE1* gene was inserted. A partial *Nco*I fragment carrying the *scr1::ADE1* allele (Fig. 1) was transformed into the diploid DX547 (*ade1/ade1 leu2/leu2 pro1/pro1*). Ade$^+$ strains heterozygous for the *SCR1* disruption were identified by Southern analysis, sporulated on V-8 medium and segregants from tetrad dissection were scored. As expected for an essential gene, the spore viability decreased significantly, but surprisingly Ade$^+$ spores were viable. The presence of the *scr1::ADE1* allele in the Ade$^+$ segregants was confirmed by Southern hybridization, and a 7SL RNA species was detected on Northern blots of total RNA from these segregants. Therefore, *SCR1* is not essential and there are at least two 7SL RNA genes in *Y. lipolytica*.

Deletion of SCR2 is not lethal

For the one-step gene deletion of *SCR2*, a 570 base pair *Dra*I-*Eco*RV fragment which includes the entire coding region was deleted, and a 1.7 kb fragment containing the *Y. lipolytica URA3* gene was inserted. The *Bam*HI-*Apa*I linear fragment (cut in the plasmid polylinker site) carrying the *scr2::URA3* allele (Fig. 1) was transformed into diploid 15/45 (*ura3/ura3 LEU2/leu2 ade1/ADE1; Xuan et al. 1988*). *Ura*$^+$ strains heterozygous for the *SCR2* deletion were identified by Southern hybridization and sporulated on CSM medium. Following random spore analysis, Ura$^+$Leu$^-$Ade$^-$ spores were selected, and the deletion of the *SCR2* gene was verified by Southern hybridization (Fig. 2). Total DNA from the parental and deletion strains was cut with *Cla*I and probed with a mixture of labelled *SCR1 Mlu*I-*Nde*I and *SCR2 Dra*I-*Eco*RV fragments. The 9.0 kb band corresponds to *SCR1*. In a Ura$^+$Leu$^-$ Ade$^-$segregant (lane 2), the 2.5 kb band corresponding to *SCR2* has disappeared. Therefore, the *SCR2* deletion is not lethal to the cell.

Deletion of both SCR1 and SCR2 is lethal

To confirm that there are only two 7SL RNA genes and that a functional 7SL RNA gene is required for growth, two different approaches were taken. First, a Ura$^+$Leu$^-$Ade$^-$ haploid strain with the *scr2::URA3* allele was transformed with autonomously replicating plasmid pINA441 carrying the *LEU2* gene, the *ARS18* fragment (P. Fournier, pers. comm.) and the 1.2 kb *Cla*I-*Hind*III *SCR2* fragment. The deletion of the *SCR1* gene was performed by transforming this Leu$^+$Ura$^+$Ade$^-$ strain with a partial *Nco*I fragment containing the *scr1::ADE1* allele (Fig. 1). The Ade$^+$Ura$^+$Leu$^+$ transformants carrying the *scr1::ADE1* disruption were identified by Southern hybridization (Fig. 2). In the *SCR1-SCR2* double deletion strain, only the 1.2 kb *SCR2* fragment on the plasmid was detected (Fig. 2,lane 4). No loss of the Leu$^+$ replicative plasmid was observed among more than 5,000 clones after 20 generations of growth on nonselective medium thus