Cloning and analysis of the nuclear gene MRP-S9 encoding mitochondrial ribosomal protein S9 of Saccharomyces cerevisiae

Abstract The Saccharomyces cerevisiae nuclear gene MRP-S9 was identified as part of the European effort in sequencing chromosome II. MRP-S9 encodes for a hydrophilic and basic protein of 278 amino acids with a molecular mass of 32 kDa. The C-terminal part (aa 153–278) of the MRP-S9 protein exhibits significant sequence similarity to members of the eubacterial and chloroplast S9 ribosomal-protein family. Cells disrupted in the chromosomal copy of MRP-S9 were unable to respire and displayed a characteristic phenotype of mutants with defects in mitochondrial protein synthesis as indicated by a loss of cytochrome c oxidase activity. Additionally, no activities of the gluconeogenetic enzymes, fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxykinase, could be observed under conditions of glucose de-repression. The respiration-deficient phenotype could not be restored by transformation of the disruption strain with a wild-type copy of MRP-S9, indicating that MRP-S9 disruption led to rho− or rho+ cells. Sequence similarities of MRP-S9 to other members of the ribosomal S9-protein family and the phenotype of disrupted cells are consistent with an essential role of MRP-S9 is assembly and/or function of the 30s subunit of yeast mitochondrial ribosomes.

Key words Ribosomal protein · Mitochondria · Yeast · Pet phenotype

Introduction

The two subunits of mitochondrial ribosomes of Saccharomyces cerevisiae consist of two RNA molecules (15s and 21s rRNA) and about 80 proteins. Of these, the two ribosomal RNAs and one protein (Var1) of the small ribosomal subunit are encoded by the mitochondrial genome (Dujon 1981). All other proteins of the mitochondrial ribosome are encoded by nuclear genes and must be transported into the mitochondrial matrix after synthesis in the cytoplasm (Tzagoloff and Myers 1986). In several laboratories more than 20 nuclear genes coding for mitochondrial ribosomal proteins (MRP) have been cloned and characterized. Seven of these genes, MRP2 (Myers et al. 1987), MRP7 (Fearon and Mason 1988), MRP-L8 (Kitakawa et al. 1990), YML33 (Kang et al. 1991), MRP20 (Fearon and Mason 1992), MRP4 (Davis et al. 1992) and MRP-L6 (Harrer et al. 1993), showed significant sequence similarities to eubacterial and/or chloroplast ribosomal-protein genes (Grohmann et al. 1991).

However, other identified mitochondrial ribosomal proteins, such as YMR26 and YMR31, showed no sequence similarity to any of the Escherichia coli ribosomal proteins (Kang et al. 1991). Furthermore, except for the YMR44 gene (Matsushita et al. 1989), the nuclear genes for mitochondrial ribosomal proteins only exist in single copy and have no introns, which contrasts with the intron-containing genes encoding cytoplasmic ribosomal proteins. Moreover, in contrast to their prokaryotic counterparts, the genes of mitochondrial ribosomal proteins are distributed over the entire yeast genome and are not clustered at a few loci. These features support the hypothesis that mitochondria have descended from prokaryotic endosymbionts (Schwartz and Dayhoff 1978). Considering this assumption, the genes for mitochondrial ribosomal proteins must have been transferred from the genome of the symbiont to the nucleus during mitochondrial evolution.

As part of the European BRIDGE program to sequence chromosome II of S. cerevisiae, we identified an open reading frame with strong homologies to members of the ribosomal S9-protein family of prokaryotic
ribosomes. These homologies, as well as the functional analysis of MRP-S9, lend further support to the conclusion that the MRP-S9 gene encodes the S9 protein of yeast mitochondrial ribosomes and that the nuclear MRP-S9 gene is of prokaryotic origin.

Results and discussion

Cloning and sequence analysis of MRP-S9

As part of the European effort in sequencing chromosome II of *S. cerevisiae* we have determined the sequence of a 3-kb DNA fragment. This *XbaI/BamHII* fragment from the cosmid α2 kindly provided by H. Feldmann (University of Munich), and located on the right arm of chromosome II between the *SUP45* (Breinig and Piepersberg 1986) and *RPB5* (Woychik et al. 1990) genes, was subcloned into pBlueScriptII KS- (Stratagene, Heidelberg, Germany). A detailed restriction map of the *XbaI/BamHII* fragment is shown in Fig. 1. Two open reading frames were identified within this region. The first open reading frame encodes for a putative protein of 251 amino acids which shows significant homology to the known alcohol dehydrogenases, ADH1 (Bennetzen and Hall 1982), ADHII (Russel et al. 1983) and ADHIII (Young and Pilgrim 1985) of *S. cerevisiae*. The possible role of this additional alcohol dehydrogenase gene, called *ADH6*, in yeast metabolism is still not clear and is the subject of additional investigations (paper in preparation). The second open reading frame was designated MRP-S9 and was further analyzed. The 5' and 3' non-coding regions of the MRP-S9 gene showed typical motifs found normally in yeast genes (Fig. 2). Four potential TATA-sequences [consensus sequence: TA^{T}AA^{A}/AA; Bucher and Trifonov (1986)] were found at the region located 160 bp to 205 bp upstream of the AUG start codon (Fig. 2 underlined). Further motifs, such as a ribosomal recognition sequence [CACACAC; Kozak (1983)] or a CAAT-box (Grosvald et al. 1982) often found in yeast promoters, were not present in the MRP-S9 promoter. The 3'-end of the MRP-S9 sequence contained the TAG ... TAGT ... TTT sequence (Fig. 2) which has been described as a consensus sequence for transcriptional termination and polyadenylation in yeast (Zaret and Sherman 1982). Though common for

Materials and methods

Strains and media. *E. coli* strain DH5α was used for all subcloning and sequencing steps (Stratagene, Heidelberg, Germany). Gene replacements were carried out in the diploid *S. cerevisiae* strain ENY. MR17 (MATa/MATa his3-Δ1/his3-Δ1 leu2-3,112/leu2-3,112 ura3-52/ura3-52) Media for growth of *E. coli* and *S. cerevisiae* were prepared as described by Ausubel et al. (1989).

Transformation. Yeast transformation was performed as described by Dehmen et al. (1991). *E. coli* transformation was carried out according to the method of Hanahan (1985).

DNA manipulations and sequencing. Routine DNA manipulations, including plasmid preparation, subcloning, Southern blotting and agarose-gel electrophoresis, were all carried out as described in Maniatis et al. (1982).

Sequencing strategy. For sequencing, a 3-kb *BamHII/XbaI* DNA fragment from cosmid α2 was subcloned into pBlueScriptII KS- (Stratagene, Heidelberg, Germany), after which exonucleaseIII/mung bean nuclease (Boehringer Mannheim; Germany) deletions were constructed on both strands by the method of Hennikof (1984). When necessary, custom oligonucleotide primers were used to fill in gaps. The entire sequence was determined on both strands twice. Sequencing was performed on an ABI 373A DNA sequencer (Applied Biosystems Inc., Weiterstadt, Germany) using the *Tag Dye-Deoxy* terminator Cycle Sequencing Kit supplied by the manufacturer. The reaction products after PCR amplification were separated from non-incorporated dye-labelled ddNTP terminator nucleotides by CTAB precipitation according to the ABI protocol.

Computer-sequence analysis. *Dnasis/Prosis* software package (HITACHI) was used to assemble sequence data, to search for ORFs, and to analyse protein sequences. Database searches were done by the e-mail services offered by EMBL (FASTA; Pearson and Lipman 1988) and NCBI (BLAST; Altschul et al. 1990). Gene-deletion analysis. Plasmid pTM1, carrying the MRP-S9 gene on an *EcoRI/XbaI* fragment inserted into the vector pUC21 (Vieira and Messing 1991), was digested with *SalI* and ligated with a 1.3-kb *SalI/XhoI* fragment containing the complete *HIS3* gene (Struhl et al. 1979). Subsequently, the resulting plasmid pTM4, in which a part of the MRP-S9 gene was substituted by the *HIS3* gene, was used for homologous integration at the chromosomal locus by one-step gene replacement (Rothstein 1983).

Enzyme assays. Crude extracts were prepared as described by Ciriacy (1975). Protein was assayed with the micro-biuret method according to Zamenhoff (1957) using bovine serum albumin as a standard. The assay for phosphoenolpyruvate carboxykinase activity was performed according to Hansen et al. (1976). Fructose-1,6-bisphosphatase activity was measured as described by Gancedo and Gancedo (1971). Cytochrome c oxidase activity was determined by staining yeast colonies using the redox dye tetramethyl-p-phenylenediamine (McFwen et al. 1985).

Fig. 1. Restriction map of the 3-kb *XbaI/BamHII* fragment and gene disruption strategy for the MRP-S9 gene. The arrows indicate the coding region and the direction of transcription of the genes. The nucleotid sequence shown in Fig. 2 is bracketed