Yeast ribosomal proteins: XII. YS11 of *Saccharomyces cerevisiae* is a homologue to *E.coli* S4 according to the gene analysis

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ABSTRACT

We isolated and sequenced a gene, *YS11A*, encoding ribosomal protein YS11 of *Saccharomyces cerevisiae*. *YS11A* is one of two functional copies of the YS11 gene, located on chromosome XVI and transcribed in a lower amount than the other copy which is located on chromosome II. The disruption of *YS11A* has no effect on the growth of yeast. The 5'-flanking region contains a similar sequence to consensus UAS_{rpg} and the T-rich region. The open reading frame is interrupted with an intron located near the 5'-end. The predicted amino acid sequence reveals that yeast YS11 is a homologue to *E. coli* S4, one of the *ram* proteins, three chloroplast S4s and others out of the ribosomal protein sequences currently available.

INTRODUCTION

Ribosomes and their components have been regarded as appropriate molecular materials for evolutionary studies (see 1−3). The protein part containing many protein molecules provides an opportunity to examine both the evolution of respective protein species and the co-evolution of related proteins (4−7). To thoroughly understand the evolutionary messages from them, we need to accept further varied data concerning the ribosomal proteins from a comprehensive range of organisms. Most of all the data on lower eukaryotes are necessary to be accumulated because less is known about them. Under this situation, we have hitherto reported complete or partial sequences of yeast ribosomal proteins (8−13). Consecutively, we present the primary structure of the gene for ribosomal protein YS11 from *S. cerevisiae*.

It was suggested that YS11 plays an important role in translational fidelity since *SUP46* mutant had protein YS11 electrophoretically altered (14). The dominant suppressor, *SUP46*, acts on a wide range of mutations(15). Ribosomes derived from a *SUP46* strain produce abnormally high rates of error in a cell-free translation system (16). These properties resemble those of *ram* (ribosomal ambiguity) suppressors of *E. coli* (17). In this study, we will reveal that ribosomal protein YS11 of yeast is equivalent to *E. coli* S4, one of the *ram* proteins, according to the entire amino acid sequence predicted after the gene analysis of YS11. The analyzed gene for YS11 is in a complementary pair together with the gene cloned by Vincent and Liebman, which complements *SUP46* mutation (15th International Conference on Yeast Genetics and Molecular Biology, July 21−26, 1990, The Hague).

MATERIALS AND METHODS

Strains and plasmids

S. cerevisiae strain A364A (ATCC22244) was used for the preparation of poly(A)$^+$RNA and chromosomal DNA, strain MT127-31A (*MATα* rna2 met14 pet ade gal1) for the preparation of poly(A)$^+$RNA, and strain YNN281 (*MATa lys2-801 ura3-52 ade2-1 trp1-Δ* his3-Δ200 gal) for the disruption of the *YS11A* gene. *E. coli* strain JM109 was used for DNA library propagation (18). Plasmids pLS10 (19; constructed by Dr. L. Symington), pJS7 (20) , and pSM13 (constructed by Dr. D. Schild) containing the *rad52* gene cloned into the *Bam*HI site of pBR322 were kindly provided by Drs. S. W. Liebman and A. Vincent.

Transformation

Yeast was transformed by the lithium acetate transformation method (21) and *E. coli* by the method of Hanahan (22).

Isolation of nucleic acids and genomic DNA library formation

Chromosomal DNA of *S. cerevisiae* was isolated as described by Cryer *et al.* (23) with slight modification. Genomic DNA digested with *Bam*HI and *Bgl*II was applied to an agarose gel electrophoresis. Out of the separated DNA fragments, the appropriate fraction, deduced from a Southern blot analysis, was extracted and ligated with *Bam*HI-digested vector pUC118. The resultant genomic DNA library was propagated in *E. coli*. Plasmid DNA was prepared by the alkaline method (24). Total yeast RNAs were isolated according to Jensen *et al.* (25) and poly(A)$^+$RNA was purified with oligo(dT)-cellulose (Pharmacia LKB Biotechnology, Inc.) as recommended by the supplier.

Oligonucleotide probes

Residue positions 11 to 20 of the known N-terminal amino acid sequence of ribosomal protein YS11(8), YSTPKRPYQS, were chosen to obtain a mixed probe containing oligonucleotides whose sequences had been designed according to the codon usage preferred by yeasts (13,26). The mixture of the four differently

designed DNAs, each 29 nucleotides in length, was synthesized on an Applied Biosystems model 381A DNA Synthesizer. Three oligonucleotides, α, β and γ (see the legend of Fig. 4),were synthesized on an Applied Biosystems model 391 PCR-MATE EP DNA Synthesizer.

Labeling of DNAs, Southern and Northern blots and DNA sequencing

Labeling of DNAs and Southern hybridization were performed as previously described (13). Northern blot analysis described previously (13) was modified to use 1.5% agarose gel and 0.16−1.77kb RNA ladder (Bethesda Research Laboratories) as a size marker. Using the gel containing chromosomes of *S. cerevisiae* separated by a pulsed field electrophoresis, purchased from Clontech Laboratories, Inc., Southern hybridization, in a search for chromosomal locations, was carried out as recommended by the supplier. DNA sequencing was performed according to Suzuki *et al.* (13).

Gene disruption

YS11A was disrupted by the one-step gene replacement procedure (27). Briefly mentioned, pUC118 digested with *Eco*RI was filled in with the large fragment of *E. coli* DNA polymerase I and self-ligated. The resultant vector was treated with *Pst* I, and a 2.0kb *Pst* I fragment derived from pYS11A was inserted. The plasmid was digested with *Eco*RI and ligated to a 1.2kb *Eco*RI fragment containing *URA3* gene, obtained from plasmid pLS10. The 3.2kb *Sac*I-*Sph*I fragment by the restriction sites within pUC118 was transformed in haploid cell YNN281. Transformants were selected using a medium without uracil.

Sequence similarity analysis

Computer programs RELATE and ALIGN (28) were used to assess possible evolutionary relationships between YS11 and other ribosomal proteins. The scoring matrix was prepared by MDM'78 (28). Further sequence similarities were examined using a computer program that quantitatively evaluates the extent of similarities as correlation coefficients (29). Details of the method have been described previously (7,30).

RESULTS

Cloning of ribosomal protein YS11 gene

In cloning a gene encoding protein YS11 from a genomic library of *S. cerevisiae* using a synthetic oligonucleotide mixture as a probe, two positive colonies out of 2,400 transformants were obtained. The physical maps revealed that the plasmids contained a common insert of 3.5kb in length, one of which was termed pYS11A. A restriction map of its insert is shown in Fig. 1.

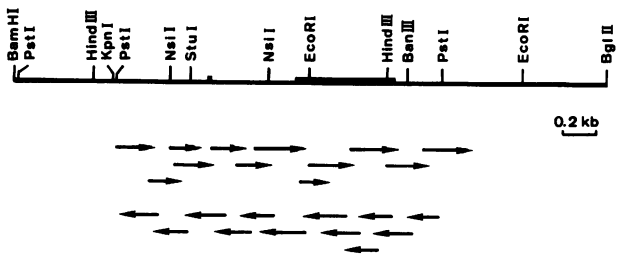

Fig. 1. Restriction map and sequencing strategy of the pYS11A. The arrows show the extent of nucleotide sequencing and the solid bar represents the coding region.

Sequence analysis of *YS11A*

The partial nucleotide sequence of the insert of pYS11A was determined from both strands according to the strategy presented in the lower part of Fig. 1. The open reading frame encoding a protein predicted to consist of 197 amino acids is interrupted with an intron of 501bp (Fig. 2). The 5'-flanking sequence contains an UAS_{rpg}-like sequence at nucleotide positions −244 to −231 and the T-rich region from −219 to −172. At 148 bases downstream of the stop codon, a polyadenylation signal AATAA is found.

The molecular weight calculated from the predicted amino acid sequence, 22,443, is close to that of the purified YS11 protein, previously estimated by a SDS-polyacrylamide gel electrophoresis (31). The predicted sequence at amino acid positions 2 to 24 is identical, except for two residues, to the known N-terminal sequence (8) including the part used for designing the synthetic probe. The amino acid composition is also in good agreement with the previous one (Table 1). The biased codon usage corresponds to those of highly expressed genes in yeasts (13, 26; data not shown).

Functional two copies of YS11 gene and their chromosomal locations

To determine the copy number of YS11 gene, a Southern hybridization analysis of genomic DNA was performed as follows: when the *Eco*RI-*Hin*dIII fragment from the coding region

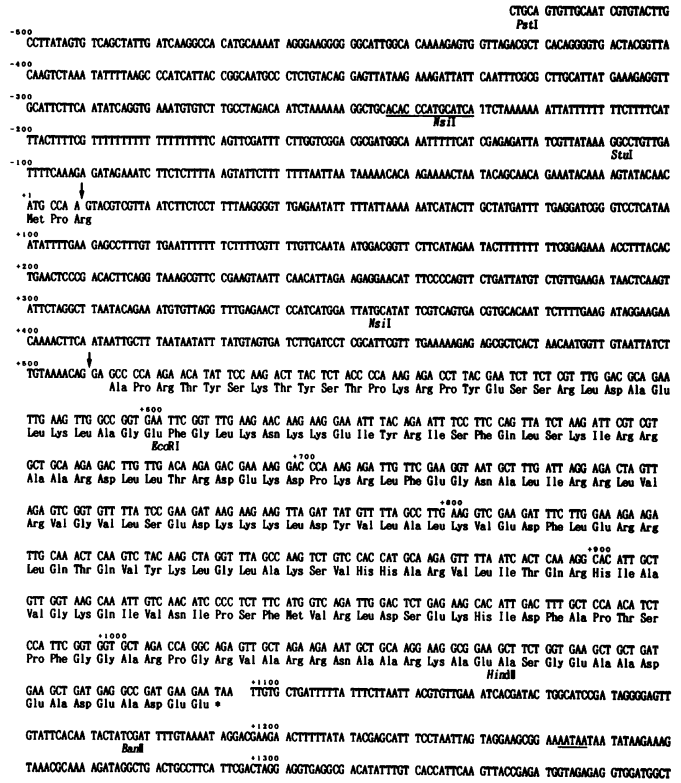

Fig. 2. Nucleotide sequence of the *YS11A* gene and predicted amino acid sequence of yeast ribosomal protein YS11. The nucleotides are numbered with respect to the translation initiation codon, designated +1. A candidate for a potential UAS_{rpg} and a consensus polyadenylation signal are underlined. Arrows indicate the deduced splice sites. Several restriction sites are shown for a reference to the restriction map presented in Fig. 1.

was used as a probe, two hybridizing bands were found indicating the existence of two copies (Fig. 3A). The one more copy besides *YS11A* exactly corresponds to the gene cloned by Vincent and Liebman (see INTRODUCTION). On the other hand, only one hybridizing band appeared when a fragment mainly containing the 3'-flanking region of *YS11A* was used (Fig. 3B). The one band means that the probe specifically reacted with *YS11A* since the nucleotide sequences of the two copies at the 3'-flanking region extremely differ from each other, compared our data with the above report by Vincent and Liebman. To examine whether the two copies of YS11 gene are functional, various DNA fragments were provided as probes of a Northern blot analysis. As shown in Fig. 4A, the *Hin*dIII-*Ban*III fragment, which contains both the 3'-flanking and the coding regions of *YS11A* to be expected to hybridize more preferably with *YS11A*, brought two bands of nearly similar intensity (lane 1). It may mean the unbalanced expression of the two genes whose transcripts differ in length from each other. The *Eco*RI-*Hin*dIII fragment from the *YS11A* coding region resulted in an intense, broad band which clearly follows a faint one, corresponding to the range of the two bands in lane 1. Additional narrow band above them is speculated to be one of their precursors because of the position for size (lane 2). Thus, it is suggested that the upper band of a pair by the intense and the faint ones resulted from *YS11A* which is transcribed in a low amount, and the lower one did from the other gene which is expressed in a large amount. To make a clear distinction between the transcripts of the two gene copies to address the respective bands, three DNA probes, α, β and γ, were synthesized as mentioned in the legend of Fig. 4. As probes α (lane 3) and β (lane 4) were used, only the upper or lower band was detected, respectively. Probe γ (lane 5) presented a

Table 1. Amino acid composition[a] of yeast ribosomal protein YS11. I: deduced from the *YS11A* gene; II: inferred from the isolated protein (31).

Amino acids	I	II
Ala	10.6	10.2
Arg	11.7	11.3
Asp and Asn	8.1	8.2
Cys	0.0	n.d.[b]
Glu and Gln	10.6	11.6
Gly	5.1	5.6
His	2.0	2.0
Ile	4.6	4.5
Leu	10.2	10.4
Lys	9.1	9.5
Met	1.0	0.3
Phe	3.6	3.7
Pro	4.6	5.2
Ser	6.1	5.0
Thr	3.6	3.5
Trp	0.0	n.d.[b]
Tyr	3.0	2.9
Val	6.1	6.3

[a] The values are in mol percent
[b] not determined

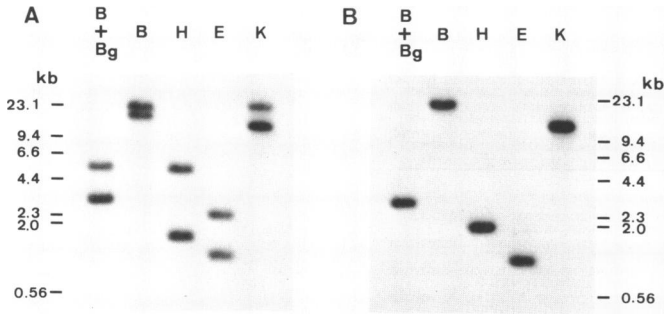

Fig. 3. Southern blot analysis of chromosomal DNA from *S. cerevisiae* using the DNA fragments derived from the *YS11A* gene as probes. Chromosomal DNA (3μg) was digested with the restriction endonuclease(s) indicated, subjected to an agarose gel electrophoresis and transferred onto a nylon membrane as described in MATERIALS AND METHODS. One hybridization probe (A) was 0.45kb *Eco*RI-*Hin*dIII fragment of positions +599 to +1,046, and the other (B) was 0.8kb *Hin*dIII-*Eco*RI fragment downstream of *Hin*dIII-site at position +1,047. *Hin*dIII-digested phage λ-DNA was used as a size marker. B: *Bam*HI, Bg:*Bgl*II, H: *Hin*dIII, E: *Eco*RI, K: *Kpn*I.

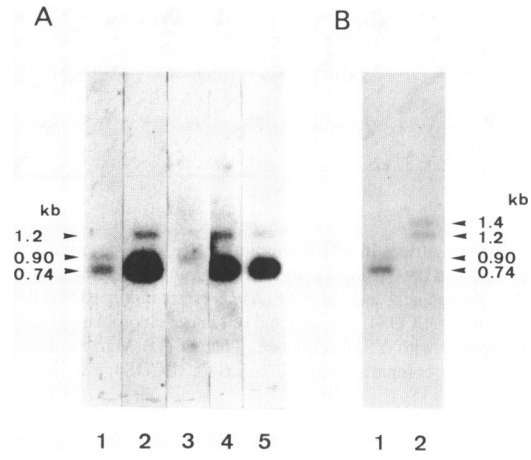

Fig. 4. Northern blot hybridization of poly(A)$^+$ RNA from *S. cerevisiae*. (A) poly(A)+ RNA (0.42μg) from strain A364A was analyzed using various ^{32}P-labeled DNA fragments. lane 1: 0.13kb *Hin*dIII-*Ban*III fragment of positions +1,047 to +1,176; lane 2: 0.45kb *Eco*RI-*Hin*dIII fragment of positions +599 to +1,046; lane 3: synthetic oligonucleotide α specific to *YS11A*, 5'-CTAGT-CTCCTAATCAAAGC-3' corresponding to positions +724 to +742; lane 4: synthetic oligonucleotide β specific to the other YS11 gene copy, whose sequence was kindly provided by Drs. A. Vincent and S.W. Liebman before publication, 5'-ACCAATCTTCTGATCAAGG-3'; lane 5: synthetic oligonucleotide γ common in the two genes, 5'-CAATTCTGCGTCCAAACGAG-3' corresponding to positions +566 to +585. (B) poly(A)$^+$ RNAs (0.46μg, respectively) from *rna2* mutant grown at permissive (lane 1) and restrictive (lane 2) temperatures were hybridized with the ^{32}P-labeled 0.45kb *Eco*RI-*Hin*dIII fragment from the coding region. The numbers indicate the size of poly(A)$^+$ RNA, deduced from size marker RNA ladder (see MATERIALS AND METHODS).

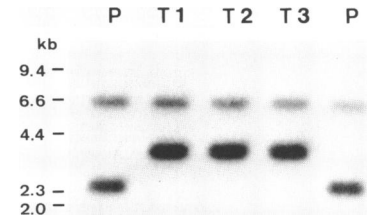

Fig. 5. Southern blot analysis of chromosomal DNA from the *YS11A* gene-desrupted strain. DNA samples (2μg) were digested with *Kpn*I and *Bgl*II, subjected to an agarose gel electrophoresis and transferred onto a nylon membrane. The membrane was used for hybridization with the ^{32}P-labeled 0.45kb *Eco*RI-*Hin*dIII fragment from the coding region. P: the parental haploid strain YNN281, T1-3: the transformed haploid strains.

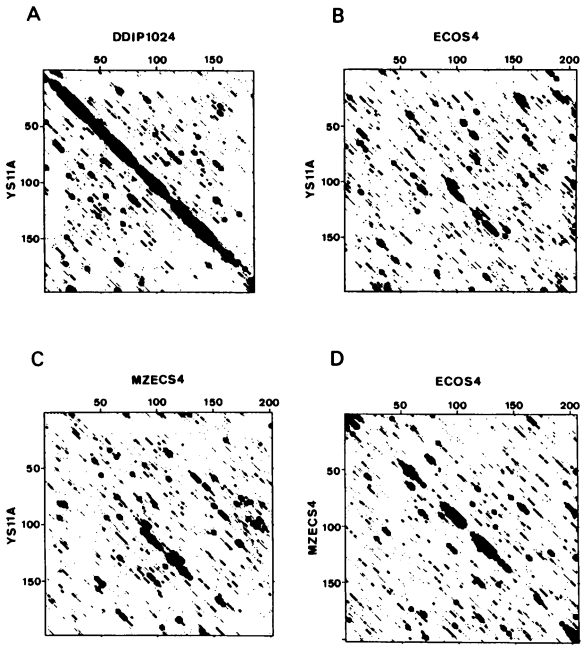

Fig. 6. Comparison matrices in which are plotted C(i, j) values greater than 0.3 with gradually enlarged dots, using a computer program that searches for sequence similarities (29). Amino acid sequences of (A) *Dictyostelium* rp1024 (DDIP1024, ref. 38), (B) *E. coli* S4 (ECOS4, ref. 33), and (C) maize chloroplast S4 (MZECS4, ref 36) are compared with YS11 sequence. That of (D) Maize chloroplast S4 (MZECS4, ref 36) is compared with *E. coli* S4's (ECOS4, ref. 33).

similar pattern to that of lane 2, in which the *Eco*RI-*Hin*dIII fragment was used. These results strongly demonstrate that *YS11A* is transcribed in a considerably lower amount than the other copy, and its transcript is longer than the other's. The speculation for the additional band of 1.2kb in lane 2 of Fig. 4A was verified as follows (Fig. 4B): when poly $(A)^+$RNA from *rna2* mutant cells (32) grown at 23°C was hybridized with the *Eco*RI-*Hin*dIII fragment (lane 1), a similar pattern to lane 2 of Fig. 4A was presented. But strictly speaking, the bands of a pair from *YS11A* and the other gene were nearly similar in intensity. Moreover the additional band in question doubled appearing the two precursors (the resulting bands are not shown in the presented panel). Probably it indicates difference between strains. By the same hybridization analysis using the mutant cells shifted up to 36°C for 1hour after growing at 23°C (lane 2), two transcripts of 1.4kb and 1.2kb were accumulated showing that they are the precursors of *YS11A* and the other gene, respectively.

The chromosomal locations of the two gene copies were searched for. The gel carrying yeast chromosomes separated by a pulsed field electrophoresis was subjected to Southern hybridization with the 1.7kb *Bam*HI-*Eco*RI fragment which was expected to react with *YS11A* preferably. A strongly hybridizing band was then found on chromosome XVI, and a very faint band on chromosome II (data not shown), suggesting that gene *YS11A* is located on chromosome XVI and the other copy gene is on chromosome II. Since the electrophoretic position of chromosome XVI on the gel employed was close to that of chromosome XIII, the final judgment that the intense band is on chromosome XVI depended on the chromosome XIII marker, *rad52* gene, as well

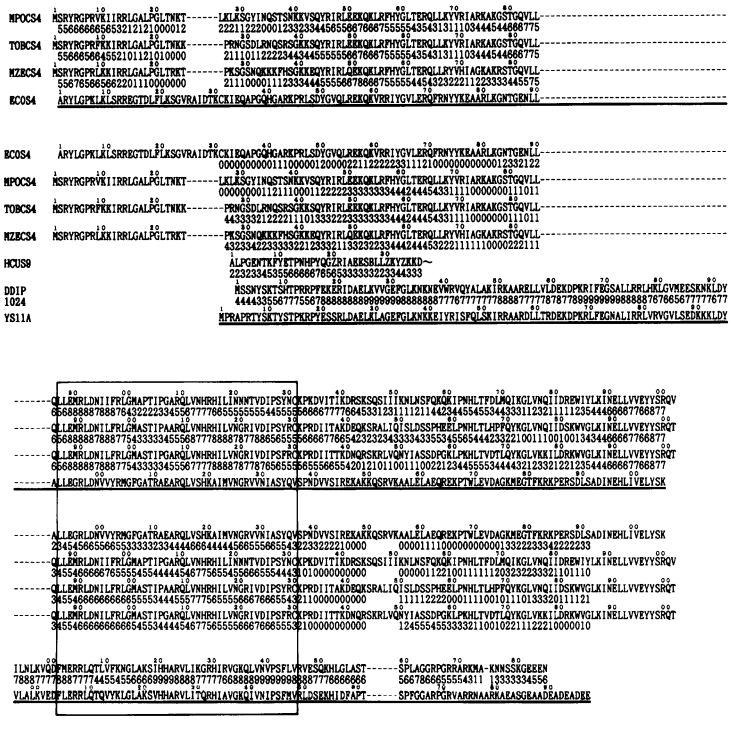

Fig. 7. Alignment of the seven YS11 family sequences by a single letter code: *E. coli* S4 (ECOS4, ref. 33), *Marchantia polymorpha* chloroplast S4 (MPOCS4, ref. 34), *Nicotiana tabacum* chloroplast S4 (TOBCS4, ref. 35), maize chloroplast S4 (MZECS4, ref 36), *Halobacterium cutirubrum* HS9 (HCUS9, ref 37) *Dictyostelium* rp1024 (DDIP1024, ref. 38) and yeast YS11. In the amino acid sequence of HCUS9, Asx and Glx are exchanged for Asp and Glu, respectively. Compared with *E. coli* or YS11 sequence (underlined), the similarity of each amino acid pair is indicated by the numerical value (×10) of the correlation coefficient. Small size numbers above the sequence indicate the position of the residues. A dashed line is a deleted site of one amino acid. Well-conserved regions throughout all sequences are emphasized with a frame.

as the chromosomes XVI and II markers, *TEF1* and *TEF2* genes, obtained from plasmids pSM13 and pJS7, respectively (see MATERIALS AND METHODS).

Disruption effect of *YS11A*

To examine the effect of *YS11A* gene disruption on the growth of yeast, the 1.2kb *Eco*RI fragment containing *URA3* was inserted into the *Eco*RI site within the coding region. A haploid strain carrying the *ys11a* mutant was obtained by directly transforming to haploid strain YNN281. Successful disruption of the *YS11A* was confirmed by a Southern blot analysis of the genomic DNA extracted from the haploid strain. In Fig. 5, a band of 2.6kb corresponds to *YS11A*. The replacement of *YS11A* with *ys11a::URA3* caused the disappearance of the 2.6kb band and the appearance of a band enlarged in the size of the *URA3* insertion. On the other hand, the upper band of 6.5kb, which corresponds to the other copy of YS11 gene, presented the same size in every lane in the analytical gel plate. All the haploid strains carrying *ys11a::URA3* grew as normally as their parent strain.

Protein YS11 is a homologue to *E. coli* S4 and others

The amino acid sequence of protein YS11, entirely predicted here, was compared with those of ribosomal proteins from various organisms in a data library (compiled by T.Tanaka, Ryukyu University) using the computer programs RELATE and additionally ALIGN (28). The sequences which were higher than 4.0 S.D. units in RELATE score were chosen for further detailed comparison. Out of the chosen ones, six are likely related to the YS11: *E. coli* S4 (33), *Marchantia polymorpha* chloroplast S4 (34), *Nicotiana tabacum* chloroplast S4 (35), maize chloroplast S4 (36), *Halobacterium cutirubrum* S9 (37) and *Dictyostelium* rp1024 (38). The comparison matrix prepared between YS11 and *Dictyostelium* rp1024 sequences depicts a single long well-matching segment with a minor gap of only one residue (Fig. 6-A). Even *H. cutirubrum* S9 sequenced up to only the N-terminal residue 36 shows a well matching segment to be related to YS11. Taking account of all the 21 matrices mutually compared among the 7 sequences, an overall alignment was constructed (Fig. 7). As boxed in the alignment, a common well-conserved region to the amount of 45 residues in length was recognized.

DISCUSSION

As studied here, ribosomal protein YS11 is encoded by two gene copies. They are located on different chromosomes, chromosomes II and XVI. We have isolated the gene on chromosome XVI and termed it *YS11A*. According to the report by Vincent and Liebman concerning a gene which complements *SUP46* mutant (see INTRODUCTION), the cloned gene is the remainder copy of the YS11 gene. Ono *et al.* reported the *SUP46* locus to be on chromosome II (15). These reports support our data. Ribosomal protein genes in yeasts are duplicated in many cases (26). In those cases, both copies are functional as shown in ribosomal proteins RP51, S10, L16 and so on (39–41). Warner mentioned that the duplication is presumably a safety measure to ensure the production of ribosomal proteins (26). In this study, mutant *ys11a::URA3* in one of the two genes grows normally, as if *YS11A* gene were nonfunctional, whereas *YS11A* gene activity in the normal cell is certainly recognized. These properties are similar to those of L16, *e.g.*, even though the amount of mRNA in the deletion mutant was one-third less than the wild type level, it grew normally (41). It means that one copy can supply a deficiency of the other copy, supporting the above mention by Warner (26). On the other hand the very unbalanced expression between the two copies of YS11 gene (Fig. 4A) might result from the important role carried by the other gene. Concerning another yeast omnipotent supressor *SUP44*, protein YS5 gene is expressed as an essential single gene without any copy (42). The case of YS5 is just like one terminal stage of the unbalanced expression between duplicated genes. The expression in strain MT127-31A is the stage to be nearly balanced between the two copies (Fig. 4B). We would like to draw notice to the two distinct ways for the expression of the same genes. While the duplication is exactly advantageous for growing by way of a single cell, it remains unknown difference there may be between ribosomal protein species with and without a copy. Although the chromosomal locations of ribosomal protein genes are a little known, their wide knowledge may throw some light on the question concerning the protein species with and without a gene copy.

Being supported by the previous study on the purification and characterization of YS11 protein (31), we revealed an entire sequence predicted for the protein, and then an amino acid sequence similarity between two proteins, YS11 from yeast and S4 from *E. coli*, one of which give rise to the *ram* mutation. It was suggested that the omnipotent suppressors of yeast act similarly to the *ram* suppressors of *E. coli* (17). The first analysis of a ribosomal protein gene that affects translational ambiguity in a eukaryote, including yeast, was on yeast ribosomal protein YS5 concerning *SUP44* mentioned above and accordingly *E. coli* S5 *ram* protein (42). In there, the fact that they are equivalents together with others was discussed in terms of the structural and functional conservation between prokaryotes and eukaryotes. As a continuation, this study offers further protein species regarded as undergoing the *ram* mutation. The amino acid sequence at the C-terminal region of *E. coli* S4 is completely different among the wild type and the *ram* mutants since the changes are caused by a combination of a deletion and a frame shift (43). In the comparison matrix between YS11 and S4, there is no well-matching segment for the C-terminal regions. The function in yeast ribosomes might be expressed at different part(s) from the C-terminal region imagining the ribosomes as compound in nature. To resolve this problem, we hope for further experiments, including detailed follow-ups of the report of Vincent and Liebman mentioned above. As boxed in Fig. 7, six equivalents of the protein species show the existence of a common well-matching region to the amount of 45 residues in length indicating the important domain of this species in the ribosomal architecture through the eukaryotes, chloroplasts and *E. coli*. While within the eukaryotes or the prokaryotic group overall sequences match well each other (Figs. 6A, 6D and 7), between the two groups there is little notable region to be conserved through them except for the domain (Figs. 6B, 6C and 7). As drastic changes were requisite for the evolution, the eukaryotes lost the sequence corresponding to the prokaryotic N-terminal region of about 30 residues. On the other hand the prokaryotic group could not contain the sequence corresponding to the eukaryotic inner region to the amount of more than 40 residues, where the correlation coefficient values are extremely high between the eukaryotes. Recent reports relate that metabacterial (so-called archaebacterial) proteins resemble eukaryotics much more than eubacterials. Similarities of the metabacterials to the eubacterials are higher than those of the eukaryotics to the eubacterials (4, 44–46). It follows that metabacterial genes are organized like eubacterials,

situated just between the eukaryotes and eubacteria. The N-terminal sequence of *Halobacterium cutirubrum* S9 used here is also clearly close to the region of the eukaryotic ones. It is expected that its entire sequence contains the domain in common. Moreover the complete sequence from metabacterium *Halobacterium cutirubrum* located in the appropriate phylogenetic position is required for the further serial examination of the YS11 family proteins in term of the protein evolution, involved in the drastic sequence change between the eukaryotes and the prokaryotic group as mentioned above.

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