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Cloning and Regulation of *Schizosaccharomyces pombe* Gene Encoding Ribosomal Protein S20

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A cDNA clone encoding the ribosomal protein S20 has been isolated from the *Schizosaccharomyces pombe* cDNA library by colony hybridization. The insert contained in the original plasmid pYJ10 was transferred into shuttle vector pRS316 to generate plasmid pYJ11. The cDNA insert of plasmid pYJ11 contains 484 nucleotides and encodes a protein of 118 amino acids with a calculated mass of 13,544 daltons. The deduced amino acid sequence of *S. pombe* ribosomal protein S20 is very homologous with fruit fly, rat, and budding yeast counterparts. It is also homologous with *Xenopus* S22 ribosomal protein. *S. pombe* ribosomal protein S20 appears to be relatively hydrophobic except the C-terminal region. The 728 bp upstream region of the S20 gene was amplified from chromosomal DNA and transferred into the *Bam*HI/*Eco*RI site of the promoterless β-galactosidase gene of the vector YEp357R, which resulted in fusion plasmid pYS20. The synthesis of β-galactosidase from the fusion plasmid appeared to be the highest in the mid-exponential phase. The *S. pombe* cells with the fusion plasmid grown at 35°C gave lower β-galactosidase activity than the cells grown at 30°C. Computer analysis showed the consensus sequence CAGTCACA in the upstream regions of various ribosomal protein genes in *S. pombe*, which would be involved in the coordinated expression of small ribosomal proteins.

***Key words*:** cDNA, fission yeast, ribosomal protein S20, regulation, *Schizosaccharomyces pombe*

Ribosomes are complex ribonucleoproteins catalyzing peptide bond formation in all organisms. Eukaryotic ribosomes are composed of two subunits, 60S and 40S. The 40S subunit consists of about 30 ribosomal proteins and one rRNA molecule (9). The biogenesis of the eukaryotic ribosome is a complex process involving the coordinated expression of four ribosomal RNAs and approximately 75 ribosomal proteins. Ribosomal protein S20 is one of the proteins constituting the 40S subunit. The genes encoding ribosomal protein S20 were isolated from fruit flies (2), rats (3), *Saccharomyces cerevisiae* (7), *Xenopus laevis* (10), and rice (14). Ribosomal protein S20 participates in post-translational inhibition of the polyamine biosynthetic enzymes ornithine and arginine decarboxylase (15). Interestingly, the expression of the S20 mRNA was down-regulated early during the induction of apoptosis in human leukaemic cells, prior to the onset of DNA fragmentation and other morphological changes associated with cell death, suggesting some degree of involvement of the S20 gene in the biochemical events that occur during the onset of cell death (4).

In this communication we report the cDNA cloning and regulation of ribosomal protein S20 from the fission yeast *Schizosaccharomyces pombe* (8, 11, 12, 19), which displays different physiology from the budding yeast *S. cerevisiae* and is probably more closely related to higher eukaryotes.

Materials and Methods

Chemicals

Ampicillin, X-Gal, IPTG, glucose, EDTA, ONPG, and SDS were purchased from Sigma Chemical Co. (St. Louis, USA). Restriction enzymes (*Eco*RI, *Xho*I, *Bam*HI), T4 DNA ligase, proteinase K, DIG high prime labeling and Detection Starter Kit I, and RNase A were obtained from Roche Molecular Biochemicals (Mannheim, Germany). Seakem LE agarose was from BioProducts (Maine, USA). Agar, tryptone, and yeast extract were from Amer-

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sham Pharmacia Biotech Inc. (Piscataway, USA) *S. pombe* cDNA library was purchased from Clontech Laboratories, Inc. (Palo Alto, USA). The 5'-DIG labeled DNA probe and the two PCR primers (primer 1, 5'-GCCCA-ACTTGGTGACGGGATCCCATTCCTT-3'; primer 2, 5'-GAATGCGGTGAACAGTAGAGGAATTCTGTTGTTCC-3') were purchased from TaKaRa Shuzo Co. Ltd. (Shiga, Japan).

Strains and growth conditions

The cDNA library was constructed in *Escherichia coli* strain DH10B, and was amplified by growing in LB medium containing 50 μl/ml ampicillin. LB medium contained 10 g tryptone, 5 g yeast extract, and 10 g NaCl per 1 L. The wild-type *S. pombe* KP1 (*h*$^+$ *leu1-32 ura4-294*) was used for transformation. *E. coli* strain MV1184 was used for subcloning. The *S. pombe* cells were grown in minimal medium which contained KH phthalate (3 g), Na_2HPO_4 (1.8 g), NH_4Cl (5 g), glucose (20 g), 1000X vitamin mixture (1 ml), 1000X minerals (0.1 ml), 50X salts (20 ml), adenine (250 mg), leucine (250 mg), and uracil (250 mg) per 1L.

Plasmids

The *E. coli*-yeast shuttle vector pRS316 (18), which contains T7 and T3 promoters, was used for the cloning of the ribosomal protein S20 cDNA fom *S. pombe*. The fusion vector YEp357R (13) was used for subcloning the upstream region of ribosomal protein S20 gene from *S. pombe*.

Colony hybridization

To screen the cDNA encoding ribosomal protein S20 from the *S. pombe* cDNA library (Clontech Laboratories, Inc., Palo Alto, USA), colony hybridization was performed according to the procedure described in 'The DIG System User's Guide for Filter Hybridization' prepared by Roche Molecular Biochemicals (Mannheim, Germany). The summarized procedure is as follows. Colonies on agar plates were pre-cooled for approximately 30 min at 4°C, and a membrane disc was carefully placed onto the surface. The transferred DNA was then cross-linked by baking the dry membrane for 1 h at 80°C. It was next placed on a clean piece of aluminum foil and treated with proteinase K. The membrane disc was pre-hybridized for 1 h in pre-hybridization solution. After the pre-hybridization solution was discarded, the disc was placed in a hybridization solution containing 5'-DIG labeled DNA probe. The hybridization reaction was conducted at 65°C for about 15 h. After hybridization and washing, the disc was subjected to colorimetric detection with NBT and BCIP.

DNA Techniques

PCR was performed as described in the users guide offered by Roche Molecular Biochemicals. The PCR conditions used in this study were 94°C (1 min), 60°C (1 min) and 72°C (2 min) for 35 cycles. The nucleotide sequences were determined with an automatic DNA sequencer from Bionex, Inc. (Seoul, Korea). The determined nucleotide sequence reported in this study has been submitted to the GenBank database under accession number AF282863. The chromosomal DNA was isolated from *S. pombe* cells according to the procedure as previously described (6). The other recombinant DNA techniques used in this study were performed according to 'Molecular Cloning: A Laboratory Manual' (17).

β-Galactosidase assay

β-Galactosidase activity in the extracts was measured by the spectrophotometric method (5) using ONPG as a substrate. Protein contents in extracts were measured by the Bradford method (1) using BSA as a standard.

Results and Discussion

cDNA cloning

A *S. pombe* cDNA library, which is commercially available, was constructed in plasmid vector pGAD GH (15). The *Eco*RI/*Xho*I site of vector pGAD GH was used for constructing the library of *S. pombe*, which is evolutionarily distant from *S. cerevisiae*. A nucleotide sequence homologous to that of the ribosomal protein S20 genes was identified on the genomic DNA sequence of *S. pombe* in the GenBank database. It didn't contain an intron. Based on the plausible nucleotide sequence of the coding region, a 30 bp 5'-DIG labeled DNA probe was prepared, and used in the screening of the ribosomal protein S20 cDNA clone from the *S. pombe* cDNA library (Clontech Laboratories, Inc., Palo Alto, USA). Plasmid DNA was isolated from the positive clones and named pYJ10. Plasmid pYJ10 was found to harbor an insert of about 500 bp estimated from the digestion with *Eco*RI and *Xho*I. The cDNA insert contained in plasmid pYJ10 was transferred into the *Eco*RI -*Xho*I site of yeast-*E. coli* shuttle vector pRS316, and the resultant plasmid was named pYJ11, which was subsequently sequenced from both directions.

Nucleotide sequence analysis

Since shuttle vector pRS316 contains T7 and T3 promoter sequences on both sides of the multiple cloning site, two strands of the insert DNA were sequenced from recombinant plasmid pYJ11. The complete sequence was verified and confirmed as shown in Fig. 1. The cDNA clone harbors a 484 bp insert excluding the synthetic adaptors (Fig. 1). The insert contains a unique open-reading frame encoding a protein of 118 amino acids with a calculated mass of 13,544 daltons. It also contains 27 nucleotides of

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   1                                          ccgctgtctacaaaatgagtcaagtcgcc
   1                                                    M  S  Q  V  A

  43  aaagatcaaaaggaacaacaaattccctctactgttcaccgcattcgcattacccttact
   6   K  D  Q  K  E  Q  Q  I  P  S  T  V  H  R  I  R  I  T  L  T

 103  tctcgtaacgttcgtaacttggagaaggtttgctctgacttggtcaaccgtgccaaggac
  26   S  R  N  V  R  N  L  E  K  V  C  S  D  L  V  N  R  A  K  D

 163  aagcaattgcgtgttaagggtcctgttcgtcttcccaccaagatcttgaaaatcactacc
  46   K  Q  L  R  V  K  G  P  V  R  L  P  T  K  I  L  K  I  T  T

 223  cgtaagactcctaacggtgaaggttccaagacctgggaaacctatgagatgcgtatccac
  66   R  K  T  P  N  G  E  G  S  K  T  W  E  T  Y  E  M  R  I  H

 283  aagcgtctcatcgacctccactctccttccgagattgtcaagcaaattacttccatccac
  86   K  R  L  I  D  L  H  S  P  S  E  I  V  K  Q  I  T  S  I  H

 343  atcgagcctggtgttgaggttgaggttaccattgcccagtaaggtgcttgttccatttgg
 106   I  E  P  G  V  E  V  E  V  T  I  A  Q  -

 403  tcggggttcgggatgtcatgtttgcatcgacagtcccctccagtgaaataaaattttact

 463  tttaaaaaaaaaaaaaaaaaac
```

Fig. 1. The nucleotide sequence of the *S. pombe* cDNA encoding ribosomal protein S20 and its deduced amino acid sequence. The N-terminal and C-terminal of the deduced polypeptide are indicated by start and stop codons. The short bar indicates the termination codon. One putative polyadenylation signal is underlined.

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                  1          15 16          30 31          45
S. pombe          --MSQVAKDQKEQQI PSTVHRIRITLTSRN VRNLEKVCSDLVNRA  43
S. cerevisiae     MSDFQKEKVEEQEQQ QQQIIKIRITLTSTK VKQLENVSSNIVKNA  45
D. melanogaster   MAAAPKDIEKPHVGD SASVHRIRITLTSRN VRSLENVCRDLINGA  45
R. norvegicus     --MAFKDTGKTPVEP EVAIHRIRITLTSRN VKSLEKVCADLIRGA  43
X. laevis         --MAFKDPGKAPVDQ EVAIHRIRITLTSRN VKSLEKVCADLIRGA  43
A. thaliana       -----MKPGKAGLEEP LEQIHKIRITLSSKN VKNLEKVCTDLVRGA  41
O. sativa         -----MKSGKIGFESS QEVQHRIRITLSSKS VKNLEKVCGDLVKGA  41
                                  ***** *  *  ** *       *

                  46          60 61          75 76          90
S. pombe          KDKQLRVKGPVRLPT KILKITTRKTPNGEG SKTWETYEMRIHKRL  88
S. cerevisiae     EQHNLVKKGPVRLPT KVLKISTRKTPNGEG SKTWETYEMRIHKRY  90
D. melanogaster   KNQNLRVKGPVRMPT KTLRITTRKTPCGEG SKTWDRFQMRIHKRI  90
R. norvegicus     KEKNLKVKGPVRMPT KTLRITTRKTPCGEG SKTWDRFQMRIHKRL  88
X. laevis         KEKNLKVKGPVRMPT KTLRITTRKTPCGEG SKTWDRFQMRIHKRL  88
A. thaliana       KDKRLRVKGPVRMPT KVLKITTRKAPCGEG TNTWDRFELRVHKRV  86
O. sativa         KDKSLKVKGPVRMPT KVLHITTRKSPCGEG TNTWDRFEMRVHKRV  86
                     *  ***** ** * * * *** * ***   **     * ***

                  91          105 106         120 121
S. pombe          IDLHSPSEIVKQITS IHIEPGVEVEVTIAQ -    118
S. cerevisiae     IDLEAPVQIVKRITQ ITIEPGVDVEVVVAS N    121
D. melanogaster   IDLHSPSEIVKKITS INIEPGVEVEVTIAN -    120
R. norvegicus     IDLHSPSEIVKQITS ISIEPGVEVEVTIAD A    119
X. laevis         IDLHSPSEIVKQITS ISIEPGVEVEVTIAD A    119
A. thaliana       IDLFSSPDVVKQITS ITIEPGVEXEVTIAD S    117
O. sativa         IDLVSSADVVKQITS ITIEPGVEVEVTISD Q    117
                  ***      ** **  * *****  **
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Fig. 2. Amino acid sequence alignment of *S. pombe* ribosomal protein S20 with the most similar sequences identified in the GenBank database. Protein sequences deduced from the cloned *S. pombe* S20 cDNA, the budding yeast *Saccharomyces cerevisiae* (7), *Drosophila melanogaster* (2), *Rattus norvegicus* (3), *Xenopus laevis* (10), *Arabidopsis thaliana* (Accession No. AL353992.1), and *Oryza sativa* (14). The amino acid residues are indicated by standard single letter notation. The asterisks indicate identical amino acid residues.

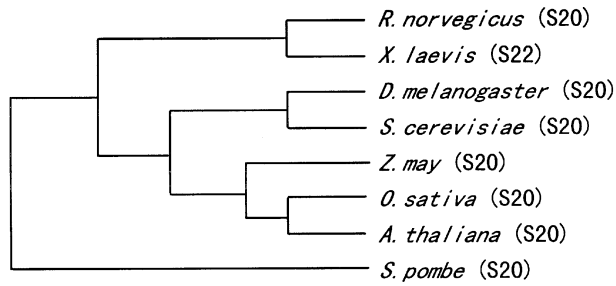

Fig. 3. A phylogenetic tree based on the amino acid sequence of ribosomal protein S20. The phylogenetic tree was constructed by PROTPARS.

the 5' noncoding region and 100 nucleotides of the 3' untranslated region. The putative S20 protein is rich in basic amino acids such as lysine and arginine. One potential polyadenylation signal is 64 bp downstream of the termination codon. The hydropathic profile of the S20 protein exhibits a relatively hydrophobic character except the C-terminal region (data not shown). The predicted amino acid sequence of *S. pombe* ribosomal protein S20 shows 47% homology with fruit fly (2), 47% homology with rat (3), and 45% homology with *S. cerevisiae* (7) counterparts (Fig. 2). It also shows 44% homology with *Xenopus laevis* (10) S22 protein (Fig. 2). It appears to be also homologous with those of some plants such as *Arabidopsis thaliana* (Accession No. AL353992.1) and rice (14). Homology is more conspicuous in the central region, whereas the N-terminal regions are relatively dissimilar among the known S20 proteins of eukaryotes. A phylogenetic tree was constructed based on the amino acid sequences of ribosomal protein S20 using PROTPARS (Fig. 3). The *S. pombe* S20 protein appeared to be distant from the *S. cerevisiae* S20 protein.

Regulation

The regulation of ribosomal protein genes may play an important role in its physiological function. The expression of the S20 mRNA was downregulated early during the induction of apoptosis in the human leukaemic cells (4). To directly monitor the expression of the *S. pombe* S20 gene, the upstream region of the S20 gene was fused into the promoterless β-galactosidase gene of vector YEp357R. The upstream sequence of the *S. pombe* S20 gene was amplified from the chromosomal DNA by PCR using primer 1 and primer 2, which contain *Bam*HI and *Eco*RI sites, respectively. Primer 2 was designed to adjust the reading frame of the S20 gene into the coding region of β-galactosidase gene. The amplified DNA was cloned to the vector YEp357R and it was named pYS20. The upstream sequence contained in recombinant plasmid pYS20 is shown in Fig. 4, which was found to be identical with the sequence stored in the GenBank database. Plasmid pYS20 contains a 728 bp upstream sequence and

```
                                                ggcccaacttggtgacgggaacccattccttctcctcatcacgcttggctccacg
                                                          ----> HSF
acggccacgtccgcgaccacgaccaccacgtccacctcttccaaatcctctgggtgcgctttctgccatt

tctgctaattattccaagttcctaaaattgtgtaggtaacgaaggatgataaaaaatttggatgctcaaa

agcttctataatgagtgtgactgtttgggctcattagggtagggttctctctcgaatgagagttaagtaa
                                                                        --
gaatgacttaaaaaatttgttttgaaaacttataaggtttttaccttattttaatgattggattctttaa
--->HSF
---------->  GCN4
tggctccttgggaccaacttttgcttgtttttttttagttccctgtgaacgaattttcgttgttaccggc
                                                    ----> HSF
aatttgttacttgctcaatgttaggacttaaatccataaatttcttcgattcaattttcaactaattctc

tcaagcaacttccttttaattctcttattttaagttgcgatcaacatctgaacgcttctttgaccccgg
                                                          ----> HSF
tatgtacgattcgattgcctcgtctcatcaatgtaacaattagggtagggtatatgtttggaacagtcac
                                                                ----> HSF
tttttattagcaacacaaaatctacttcaacaacttcttctttcactaaaccccatcgtggtcaccgctg

tctacaaaatgagtcaagtcgccaaagatcaaaaggaacaacaaattccctctactgttcaccgcattcgc
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Fig. 4. Upstream nucleotide sequence of the gene encoding the ribosomal protein S20 from *S. pombe*. The indicated nucleotide sequence was amplified by PCR and fused into the promoterless β-galactosidase gene of the plamid YEp357R. Plausible transcription factor-binding elements are represented.

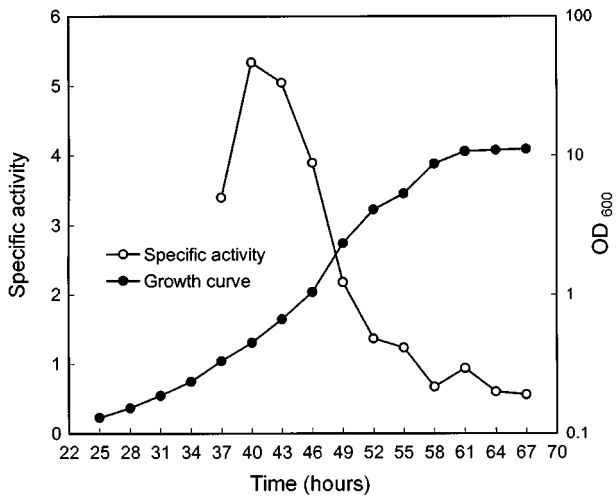

Fig. 5. The synthesis of β-galactosidase from the fusion plasmid pYS20 in *S. pombe* cells according to the growth curve. The yeast cells harboring the fusion plasmid were grown in minimal medium. The β-galactosidase activity was determined at 25°C by spectrophotometric assay using ONPG as a substrate, and its specific activity is expressed in ΔOD_{420}/min/mg protein.

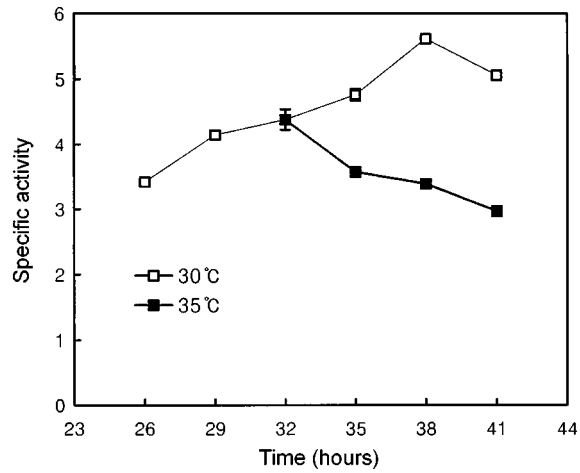

Fig. 6. Effect of incubation temperature on the synthesis of β-galactosidase from fusion plasmid pYS20 in *S. pombe* cells. The yeast cells harboring the fusion plasmid were grown in minimal medium at 30°C or 35°C. The β-galactosidase activity was determined at 25°C by the spectrophotometric assay using ONPG as a substrate and its specific activity is expressed in ΔOD_{420}/min/mg protein.

the region encoding the N-terminal 11 amino acids of the *S. pombe* S20 protein. The *S. pombe* culture containing fusion plasmid pYS20 was grown in minimal medium at 30°C. According to the growth curve, the same number of cells was harvested and their extracts were prepared. The extracts were used for β-galactosidase assay. The results are shown in Fig. 5. β-Galactosidase synthesis appeared to be highest at the mid-exponential phase. This indicates that the *S. pombe* S20 protein is produced exclusively at the exponential phase. This might explain that the maximum production of the S20 protein could be required for exponential cell growth. In another experiment, the *S. pombe* cells harboring fusion plasmid pYS20 were grown in minimal medium and split at the early exponential phase. The addition of galactose into one culture flask stopped the growth of *S. pombe* cells. It indicated that the *S. pombe* cells were not successfully able to use extracellular galactose as an energy source. In the arrested cells, β-galactosidase activity was found to be lower than the control cells (data not shown). When the *S. pombe*

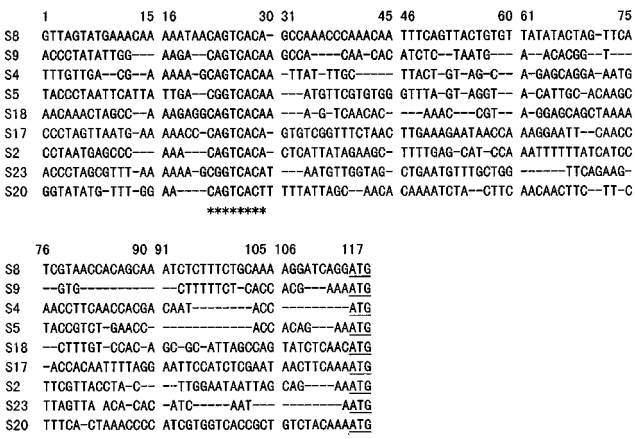

Fig. 7. Comparisons of the upstream nucleotide sequences of various genes encoding ribosomal proteins of small subunits from *S. pombe*. The nucleotide sequences were obtained from the GenBank database.

cells containing the fusion plasmid were grown at 30°C and 35°C, the *S. pombe* grown at 30°C gave much higher β-galactosidase activities than the cells grown at 35°C (Fig. 6). This indicates that the expression of the *S. pombe* S20 gene may be dependent on temperature. However, its physiological meaning remains elusive.

In this article, we report the cDNA cloning and regulation of ribosomal protein S20 from *S. pombe*. The regulatory mechanism of eukaryotic ribosomal proteins has not been clearly explained, although its coordinate production attracts interest. To investigate the regulation pattern of the *S. pombe* S20 gene, fusion into β-galactosidase was used, which makes expression level detection easier. Our results indicate that the expression of *S. pombe* S20 gene is regulated by growth phase and temperature. When the growth of *S. pombe* cells was arrested by the addition of galactose, the expression of the S20 gene was significantly decreased. This might be explained by the fact that the arrested cells don't require a large number of ribosomes. The expression of all ribosomal proteins would be coordinately regulated, since they are needed to assemble the ribosomal subunits together. Therefore the coordinate regulation of the ribosomal proteins is interesting. To obtain a preliminary idea on the coordinate regulation, the upstream regions of various small subunit protein genes of *S. pombe*, stored in the GenBank database, were compared (Fig. 7). The consensus sequence, CAGTCACA, was found about 85 bp upstream of translational initiation regions. It is a little similar to the cap signal (TCAGTCTT, 16). However, their similarity seems to be very slight. Considering the nucleotide sequence of the cDNA encoding the ribosomal protein S20, the consensus sequence should not be used simply as a cap signal. The existence of the consensus sequence would be involved in the coordinate regulation of the small subunit protein genes from *S. pombe*. This will be interpreted through further detailed investigations. Comparisons of upstream regions of the large subunit protein genes from *S. pombe* did not give any homologous sequences (data not shown). This indicates that the production of the large ribosomal subunit proteins may be regulated in a different manner.

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References

1. Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248-254.
2. Chan, H.Y., Y. Zhang, and C.J. O'Kane. 1997. Identification and characterization of the gene for *Drosophila* S20 ribosomal protein. *Gene* 200, 85-89.
3. Chan, Y.L. and I.G. Wool. 1990. The primary structure of rat ribosomal protein S20. *Biochim. Biophys. Acta* 1049, 93-95.
4. Goldstone, S.D. and M.F. Lavin. 1993. Isolation of a cDNA clone, encoding the ribosomal protein S20, downregulated during the onset of apoptosis in a human leukaemic cell line. *Biochem. Biophys. Res. Commun.* 196, 619-623.
5. Guarente, L. 1983. Yeast promoters and *lac*Z fusion designed to study expression of cloned genes in yeast. *Methods Enzymol.* 101, 181-191.
6. Hoffman, C.S. and F. Winston. 1987. A ten-minute DNA preparation from yeast efficiently release autonomous plasmids for transformation of. *Escherichia coli*. *Gene* 57, 267-272.
7. Johnston, M., S. Andrews, R. Brinkman, J. Cooper, and H. Ding. 1994. Complete nucleotide sequence of *Saccharomyces cerevisiae* chromosome VIII. *Science* 265, 2077-2082.
8. Jun, J.H., Y.M. Kim, J.H. Lee, I.K. Chung, and D.M Kim. 1998. Factors affecting pheromone induction of *Schizosaccharomyces pombe* and isolation of pheromone induction mutants. *J. Microbiol.* 36, 196-202.
9. Kay, M.A. and M. Jacobs-Lorena. 1987. Developmental genetics of ribosome synthesis in *Drosophila. Trends Genet.* 3, 347-351.
10. Keiper, B.D. and W.M. Wormington. 1990. Nucleotide sequence and 40S subunit assembly of *Xenopus laevis* ribosomal protein S22. *J. Biol. Chem.* 265, 19397-19400.
11. Lee, J., I.W. Dawes, and J.-H. Roe. 1997. Isolation, expression, and regulation of the *pgr1*$^+$ gene encoding glutathione reductase absolutely required for the growth of *Schizosaccharomyces pombe*. *J. Biol. Chem*. 272, 23042-23049.
12. Lee, S.-I., J. Lee, and J.-H., Roe. 2000. Subcellular localization of catalase encoded by the *ctt1*$^+$ gene is *Schizosaccharomyces pombe*. *J. Microbiol.* 38, 156-159.
13. Myers, A.M., A. Tzagoloff, D.H. Kinney, and C.J. Lusty. 1986. Yeast shuttle and integrative vectors with multiple cloning sites suitable for construction of lacZ fusion. *Gene* 45, 299-310.
14. Nishi, R., H. Hashimoto, H. Uchimiya, and A. Kato. 1993. The primary structure of two proteins from the small ribosomal subunit of rice. *Biochim. Biophys. Acta* 1216, 113-114.
15. Panagiotidis, C.A., S.C. Huang, and E.S. Canellakis. 1995. Relationship of the expression of the S20 and L34 ribosomal

proteins to polyamine biosynthesis in *Escherichia coli. Int. J. Biochem. Cell Biol.* 27, 157-168.
16. Philipp, B. 1990. Weight matrix desciptions of four eukaryotic RNA polymerase II promoter elements derived from 502 unrelated promoter sequences. *J. Mol. Biol*. 212, 563-578.
17. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
18. Sikorski, R.S. and P. Hieter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* 122, 19-27.
19. Song, K.W., S.J Shin, and C.F. Albright. 1998. Isolation and caracterization of the new conditional-lethal mutation in *bry4* of *Schizosaccharomyces pombe* by *in vitro* mutagenesis. *J. Microbiol.* 36, 296-302.
20. van Aelst, L., M. Barr, S. Marcus, A. Polverino, and M. Wigler. 1993. Complex formation between RAS and RAF and other protein kinases. *Proc. Natl. Acad. Sci. USA* 90, 6213-6217.