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Short communication

Cloning and Regulation of *Schizosaccharomyces pombe* Gene Encoding Ribosomal Protein L11

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The cDNA encoding ribosomal protein was identified from a cDNA library of *Schizosaccharomyces pombe*. The nucleotide sequence of the 548 bp cDNA clone reveals an open reading frame, which encodes a putative protein of 166 amino acids with a molecular mass of 18.3 kDa. The amino acid sequence of the *S. pombe* L11 protein is highly homologous with those of rat and fruit, while it is clearly less similar to those of prokaryotic counterparts. The 1,044 bp upstream sequence, and the region encoding N-terminal 7 amino acids of the genomic DNA were fused into the promoterless β-galactosidase gene of the shuttle vector YEp357 in order to generate the fusion plasmid pHY L11. Synthesis of β-galactosidase from the fusion plasmid varied according to the growth curve. It decreased significantly in the growth-arrested yeast cells that were treated with aluminum chloride and mercuric chloride. However, it was enhanced by treatments with cadmium chloride (2.5 μM), zinc chloride (2.5 μM), and hydrogen peroxide (0.5 mM). This indicates that the expression of the L11 gene could be induced by oxidative stress.

Keywords: cDNA, Fission yeast, β-Galactosidase fusion, L11, Regulation, Ribosomal protein, *Schizosaccharomyces pombe*

Introduction

The biogenesis of the eukaryotic ribosome is a complex process that involves the coordinated expression of four ribosomal RNAs and approximately 75 ribosomal proteins. In eukaryotic cells, ribosomal proteins are coded by monocistronic mRNAs from widely separated genes. The ribosomal protein L11 is found in the large subunit of eukaryotic ribosomes. The gene encoding the ribosomal protein L11 was cloned and sequenced from various prokaryotic organisms, such as *Escherichia coli* (Post *et al.*, 1979), *Proteus vulgaris* and *Serratia marcescens* (Sor and Nomura, 1987), *Haloarcula marismortui* (Arndt and Wiegel, 1990), *Halobacterium cutirubrum* (Shimmin and Dennis, 1989), and *Thermus thermophilus* (Triantafillidou *et al.*, 1998). However, the eukaryotic cDNA, encoding the cytoplasmic ribosomal protein L11, was isolated and sequenced from only two organisms, such as rats (Chan *et al.*, 1992) and *Drosophila melanogaster* (Larochelle and Suter, 1995). The L11 ribosomal protein from *Thermus thermophilus* was overproduced and purified to homogeneity using a two-step purification protocol (Triantafillidou *et al.*, 1999).

Ribosome formation is proportional to the growth rate, depending on growth conditions or developmental stages (Nischt *et al.*, 1987). Upon starvation of *Dictyostelium discoideum* cells, the ribosomal protein L11 mRNA were lost from polysomes (Agarwal *et al.*, 1999). In the crystal structure, part of the role of L11 was found to stabilize an unusual RNA fold within the ribosome (Conn *et al.*, 1999). The RNA binding domain of ribosomal protein L11 is strikingly similar to the homeodomain class of eukaryotic DNA binding proteins (Xing *et al.*, 1997). The C-terminal domain of ribosomal protein L11 was reported to bind in the distorted minor groove of a helix within a 58 nucleotide domain of 23S rRNA (Guha Thakurta and Draper, 2000). The fission yeast *Schizosaccharomyces pombe* is morphologically and physiologically distinct from the budding yeast *Saccharomyces cerevisiae*, although both belong to the same taxonomic family, Ascomycetes. *S. pombe* is more closely related to higher eukaryotes than *S. cerevisiae*. Here we report the identification and regulation of the *S. pombe* gene that encodes the ribosomal protein L11.

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Materials and Methods

Chemicals Ampicillin, X-Gal, IPTG, glucose, O-nitrophenyl β-D-galactopyranose (ONPG), EDTA, and SDS were purchased from the Sigma Chemical Co (St. Louis, USA). Restriction enzymes (*Bam*HI, *Hin*dIII), *Pyrobest*® DNA polymerase, T4 DNA ligase were from the TaKaRa Shuzo Co. Ltd., Japan. Proteinase K and RNase A were obtained from Roche Molecular Biochemicals (Mannheim, Germany). Seakem LE agarose was from Bioproducts (Rockland, USA). Agar, tryptone, and yeast extract were from United States Biochemicals (Cleveland, USA). The two PCR primers (primer 1, 5'-TCTAGTTGGTCAAGTTTTGGGATCCAA GGGCAAC-3'; primer 2, 5'-CCCAAAGAAATGTTCAAGACAA GCTTGGAGATACG-3') were ordered from the TaKaRa Shuzo Co. Ltd., Japan.

Strain and growth condition The *E. coli* strain MV1184 (Vieira and Messing, 1987), the genotype of which is *ara* Δ (*lac-proAB*) *rpsL thi* (Φ*80 lacZ* Δ *M15*) Δ (*srl-recA*) *306*::*Tn10* (*tet*^{r}), was used for the subcloning. It was grown in a LB medium that contained 50 μg/ml ampicillin. The LB medium contained 10 g tryptone, 5 g yeast extract, and 10 g NaCl per 1 L. The *S. pombe* KP1 (h$^+$ *leu1-32 ura4-294*) (Cho *et al.*, 2001; Kang *et al.*, 2001) was used for transformation. 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), 1,000× vitamin mixture (1 ml), 10,000× minerals (0.1 ml), 50× salts (20 ml), and leucine (250 mg) per 1 L.

Plasmid The *E. coli*-yeast shuttle vector plasmid pRS316 (Sikorski and Hieter, 1988), which contains T3 and T7 promoters, was used for the cloning of the ribosomal protein L11 cDNA from *S. pombe*. The *E. coli*-yeast shuttle vector YEp357 was used for construction of the fusion plasmid.

PCR PCR was performed as described in the users sheet offered by the TaKaRa Shuzo Co. Ltd., Japan. The PCR conditions used in this study were 94°C (1 min), 59°C (1 min), 72°C (2 min) for 32 cycles.

Nucleotide sequencing The nucleotide sequences were performed with an automatic DNA sequencer in Bionex, Inc. (Seoul, Korea). The determined nucleotide sequence in this article was submitted to the GenBank database under the accession number AF201080.

Isolation of chromosomal DNA from *S. pombe* The chromosomal DNA was isolated from *S. pombe* cells, according to the procedure previously described (Hoffman, 1987).

β-Galactosidase assay β-Galactosidase activity in the extracts was measured by the spectrophotometric method (Guarente, 1983) using ONPG as a substrate. Protein contents in the extracts were measured by the Bradford method (Bradford, 1976) using BSA as a standard.

General techniques The other recombinant DNA techniques used in this study were performed according to Sambrook *et al.* (1989).

Results and Discussion

cDNA cloning A *S. pombe* cDNA library, which is commercially available, was constructed in the plasmid vector pGAD GH (van Aelst *et al.*, 1993). The *Eco*RI/*Xho*I site of the vector pGAD GH was used for the construction of the *S. pombe* library. From this library, the cDNA encoding *S. pombe* thioltransferase (glutaredoxin) was previously screened and characterized (Kim *et al.*, 1999a,b,c; Cho *et al.*, 1999; Cho *et al.*, 2000a,b,c). In the course of the cloning and analysis of the *S. pombe* thioltransferase (glutaredoxin) cDNA, it was noticed that the original clone, the recombinant plasmid pKG10 (Kim *et al.*, 1999), had the 1.11kb insert, which was too large for only the thioltransferase (glutaredoxin) cDNA. Further characterization indicated that the plasmid pKG10 contained the approximately 570 bp *Eco*RI DNA fragment, in addition to the *Eco*RI/*Xho*I DNA fragment that harbors the thioltransferase (glutaredoxin) cDNA. It was estimated that the insert, contained in the plasmid pKG10, was produced by random ligation during the library construction. Partial sequencing of the plasmid pKG10 revealed that the *Eco*RI DNA fragment might contain the homologous sequence to the ribosomal protein L11, which was estimated from the sequence comparison with the database stored in GenBank. Then, in the present study, the *Eco*RI DNA fragment was isolated from the plasmid pKG10. The isolated fragment was ligated into the *Eco*RI site of the shuttle vector pRS316 in order to generate the recombinant plasmid pJYL1. It was confirmed by determination of the insert size after the white colonies were chosen on the X-gal/IPTG plate. The plasmid pJYL1 contains the cDNA that

```
1    tcaaaaccccatgaaggagcttcgtatctccaagcttgtcttgaacatttctttgggaga
1              M  K  E  L  R  I  S  K  L  V  L  N  I  S  L  G  E
61   atctggtgatcgtctcacccgtgctgccaaggttttggagcaactttcgggtcaaacccc
18     S  G  D  R  L  T  R  A  A  K  V  L  E  Q  L  S  G  Q  T  P
121  cgtttttttctaaggctcgttataccattcgtcgttttggtatccgccgtaatgagaagat
38     V  F  S  K  A  R  Y  T  I  R  R  F  G  I  R  R  N  E  K  I
181  tgcttgccatgttactgttcgtggtcccaaggccgaggagattttagagcgtggccttaa
58     A  C  H  V  T  V  R  G  P  K  A  E  E  I  L  E  R  G  L  K
241  ggtcaaggaatacgagttgaaaaagcgtaacttttctgccaccggtaactttggttttgg
78     V  K  E  Y  E  L  K  K  R  N  F  S  A  T  G  N  F  G  F  G
301  tatccaggagcacattgacttgggcatcaagtacgatccttccattggtatttatggtat
98     I  Q  E  H  I  D  L  G  I  K  Y  D  P  S  I  G  I  Y  G  M
361  ggatttctacgttgtcatggaccgccctggtatgcgtgtagctcgtcgtaaggcccaacg
118    D  F  Y  V  V  M  D  R  P  G  M  R  V  A  R  R  K  A  Q  R
421  tggccgtgttggttacactcacaaaatcaatgctgaggacaccatcaactggttcaagca
138    G  R  V  G  Y  T  H  K  I  N  A  E  D  T  I  N  W  F  K  Q
481  aaagtatgatgccgtcgttttaggaaagtaaacttcatttggtactactgtgcttacatt
158    K  Y  D  A  V  V  L  G  K  -
541  caattgaa
```

Fig. 1. The nucleotide sequence and deduced amino acid sequence of *S. pombe* ribosomal protein L11 cDNA. The nucleotides are numbered from 5' to 3' in the upper row, whereas the putative amino acid sequence is numbered from N-terminal to C-terminal end in the lower row. A short bar indicates the stop codon.

Table 1. Amino acid composition of the ribosomal protein L11 predicted from the nucleotide sequence of *S. pombe* cDNA.

Amino Acid		Number
Nonpolar, aliphatic R groups		
Glycine	G	16
Alanine	A	10
Valine	V	12
Leucine	L	12
Isoleucine	I	13
Proline	P	4
Aromatic R groups		
Phenylalanine	F	7
Tyrosine	Y	7
Tryptophan	W	1
Polar, uncharged R groups		
Serine	S	7
Threonine	T	7
Cysteine	C	1
Methionine	M	4
Asparagine	N	6
Glutamine	Q	5
Negatively charged R groups		
Aspartate	D	7
Glutamate	E	11
Positively charged R groups		
Lysine	K	16
Arginine	R	17
Histidine	H	3
Total amino acids		166

encodes the homologous ribosomal protein L11 from *S. pombe* as an insert.

Nucleotide sequence analysis The recombinant plasmid clone, harboring the *S. pombe* ribosomal proteins L11 cDNA, was subjected to automatic DNA sequencing. Since the shuttle vector pRS316 contains T7 and T3 promoter sequences at the both sides of the multiple cloning site, the two strands of the insert DNA were sequenced from the recombinant plasmid pJYL1. The complete sequence was verified by the overlapping of the two DNA strand sequences and confirmed (Fig. 1). The cDNA clone contained a 548 bp insert, excluding the synthetic *Eco*RI adaptors (Fig. 1). The insert was found to encode 166 amino acid residues, starting from methionine. The putative protein has the molecular mass of 18.3 kDa. The *S. pombe* L11 cDNA sequence does not contain a ploy adenylation signal. The amino acid composition of the putative L11 protein is shown in Table 1. It contains unique cysteine and tryptophan. It is rich in charged amino acids and nonpolar amino acids. The hydropathic profile is shown in Fig. 2. It appears to be hydrophobic, except for the N-terminal 20 amino acid residues. It contains at least 3 hydrophobic domains. The hydropathic profiles of the ribosomal protein L11 from *S. pombe,* fruit flies, and rats appear to be very similar, with the exception of the N-terminal amino acid regions (Fig. 2). The difference in the N-terminal regions may be attributed to relatively less homology in the

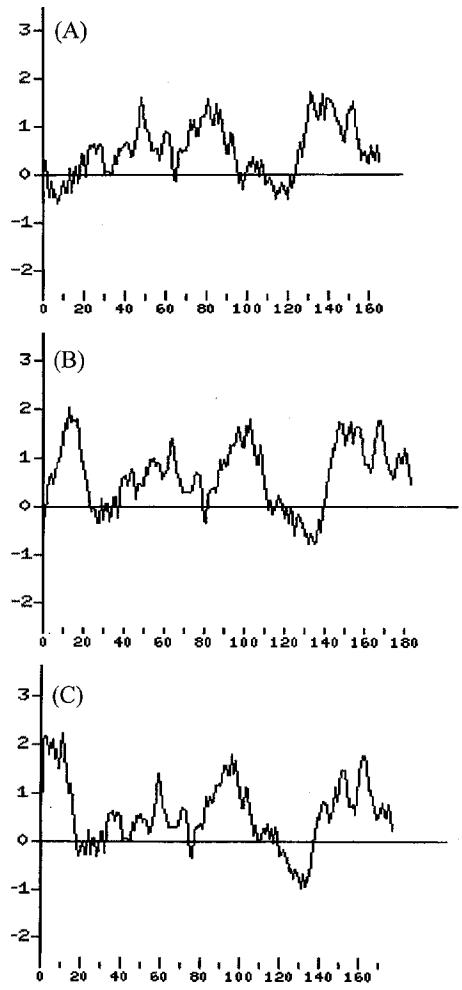

Fig. 2. Hydropathic profile of the ribosomal protein from *S. pombe* (A), fruit fly (B), and rat (C).

amino acid sequences of the N-terminal regions (Fig. 3). Its secondary structure was found to contain lots of helix structures that were estimated from the sequence analysis (data not shown). The sequence alignment of the putative L11 protein with other known eukaryotic L11 proteins is shown in Fig. 3. As shown in Fig. 3, high homology was observed among all three L11 proteins. The homology is more significant in the N-terminal and middle regions. However, they were found to be relatively less homologous at the C-terminal regions. The DNA-deduced protein sequence for L11 from *S. pombe* exhibits a 75.3%, and 70.5% identity with the equivalent eukaryotic proteins from rats and fruit flies, respectively (data not shown). However, it shares 12.3%, 9.6%, and 11.4% identity with the equivalent prokaryotic proteins from *Thermus thermophilus*, *Streptomyces* sp., and *Dictyostelium discoideum,* respectively. These results clearly indicate that the *S. pombe* ribosomal protein L11 is more homologous with the L11 proteins of the higher eukaryotes. Based on the nucleotide sequence, the *S. pombe* ribosomal protein L11 shares a 66.8% and 67.3% identity with the cDNA from rats and fruit flies, respectively (data not shown).

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R. rattus          -MAQDQGEKENPMR----ELRIRKLCLNICVGESGDRLTRAAKVLEQLTGQTPVFSKARY 55
D. melanogaster    MAAVTKKIKRDPAQEPDEDLHIRKLCLNICVGESGDRLTRAAKVLEQLTGQQPVPSKARY 60
S. pombe           -------MK---------ELRISKLVLNISLGESGDRLTRAAKVLEQLSGQTPVFSKARY 44
                          *          * * ** ***  ****************** ** ** *****

R. rattus          TVRSFGIRRNEKIAVHCTVRGAKAEEILEKGLKVREYELRKNNFSDTGNFGFGIQEHIDL 115
D. melanogaster    TVRSFGIRRNEKIAVHCTVRGAKAEEILERGLKVREYELRRENFSSTGNFGFGIQEMIDL 120
S. pombe           TIRRFGIRRNEKIACHVTVRGPKAEEILERGLKVKEYELKKRNFSATGNFGFGIQEHIDL 104
                   * * ********** * **** ******** **** ****   *** *** ****** ***

R. rattus          GIKYDPSIGIYGLDFYVVLGRPGFSIADKKRRTGCIGAKHRISKEEAMRWFQQKYDGIIL 175
D. melanogaster    GIKYDPSIGIYGLDFYVVLGRPGYNVNHRKRKSGTVGFQHRLTKEDAMKWFQQKYDGIIL 180
S. pombe           GIKYDPSIGIYGMDFYVVMDRPGMRVARRKAQRGRVGYTHKINAEDTINWFKQKYDAVVL 164
                   ************ *****  ***      *  *  *  *    *    ** ****   *

R. rattus          PGK- 178
D. melanogaster    NTKK 184
S. pombe           GK-- 166
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Fig. 3. Alignment of primary sequences of the ribosomal protein L11 from *S. pombe* cDNA (AF201080, this study), rat (*Rattus rattus*) (Chan *et al.*, 1992), and fruit fly (*Drosophila melanogaster*) (Larochelle and Suter, 1995). The conserved amino acid residues were indicated by asterisks below the sequences.

Regulation The regulation of ribosomal protein genes may play an important role in its physiological functioning. To independently monitor the expression of the *S. pombe* L11 gene, its upstream sequence was fused to the promoterless β-galactosidase gene of the *E. coli*-yeast shuttle vector YEp357. Based on the sequence deposited in the GenBank, the upstream region of the *S. pombe* L11 gene was amplified from the chromosomal DNA by PCR using primers 1 and 2, which contain the *Bam*HI and *Hin*dIII sites, respectively. Primer 2 was designed to adjust the reading of the L11 gene into the coding region of the β-galactosidase gene. The amplified DNA fragment was electro-eluted from 0.8% agarose gel, and digested with *Bam*HI and *Hin*dIII. The digested DNA fragment ligated the *Bam*HI/*Hin*dIII site of the vector YEp357. After transformation into the *E. coli* strain MV1184, the designed subclone was confirmed by restriction mapping and sequencing. It was named pHY L11. The upstream sequence, contained in the recombinant plasmid pHY L11, is shown in Fig. 4. Plasmid pHY L11 contains a 1,044 bp upstream sequence and the region encoding the N-terminal 7 amino acids of the *S. pombe* L11 protein. The upstream sequence contains the putative binding sites for AP-1, HSF, and Mata1p (Fig. 4). Plasmid pHY L11 was introduced into *S. pombe* cells. The *S. pombe* cells, harboring the fusion plasmid, were grown in minimal medium at 30°C. According to the growth, their extracts were prepared for the curve, the same number of cells were harvested and a β-galactosidase assay was performed. The results are shown in Fig. 5. The synthesis of β-galactosidase from the fusion plasmid was found to vary according to the growth phases. It was elevated at the exponential phase, whereas, it was decreased in the cells at the early stationary phase. This finding corresponds to the need of ribosomal protein in dividing cells. However, when the growth of the yeast cells was arrested by aluminum chloride (Fig. 6), the synthesis of β-galactosidase from the fusion plasmid drastically dropped to almost zero. This

```
1    TTGAAAATAG CCGTGCCTGG TGGTTTGATT GGTGTTGGTA CAACAGTCGA TCCTACTCTT
61   TGCCGTGCCG ATCGTCTAGT TGGTCAAGTT TTGGGTTCCA AGGGCAACCT TCCAGAAGTT
                                                          ----> HSF
121  TATACCGAGC TCGAAATTAA CTACTTTTTG CTCCGTCGTC TTTTGGGTGT AAAGAGTGGT
181  GATAAAAACA CCACTAAAGT TCAAAAACTT GCCAAAAACG AAGTGTTGAT GGTCAACATC
241  GGTTCCACTT CCACTGGTGG CCGTGTTATG ATGGTTAAAG CAGATATGGC AAAGATCCTT
301  CTTACCGCCC CAGCATGTAC CGAAATTGGT GAAAAGGTGG CTCTCAGCAG ACGTATTGAA
361  AAACACTGGC GGCTTATCGG TTGGGCTAAG GTGGTTGAGG GTAAGACTCT AAAAGTTTAG
421  ATTTTATCGT TGTAAACATT GTATAAGATG AATTCTCATT TTTTAACTTT TAAAAAATAA
481  ACAATGGTAA TGTCTACGGT TAAACTTAAC GATGTCTCGT ACACGGTGTG TGATTTTAGT
541  ACAGTTGATG AATCATGTAG TTGATACACA GTGCTAATAG ATGCGTATCT ACATCCCTAT
          ----------> AP-1
                ----------> MATa1p
601  TTTATACATT CGGATCTCTG TGCAGTATTT AATGTTTCCA TTATTAATTA GTTAGACTAT
661  TTTCAACATT TACTCTACAG CGAATTTATA CCTATTTAGT ACACCTGATG GTACTAGTTC
721  CTTATAGTAA AGCAATAAGT AAATAGAAGA GTCATAAATC TATATTTACT ATTATTTGAT
                           -----> HSF
781  TCTAATCAAT TATGCTTATT TACTTGACTC TTTCGTTTTG TGGTATTTCA TTCATAGTAT
841  GTATCTGTAA AATATGCTTT CACTTCCCAG ACTTTTGGAA GCCTACAAAA AAAAATATGA
901  AAATTTAAAG AAAGTGAACG ATTCAATTCT TTGAATACAA AAATTCTATC AAATAAGCGA
          -----> HSF
                -----> HSF
961  TTTTGATCTT TTCCCAATTA GCAAACTCTA AGGCAACGAC ATTATTCAGA GCCCTAACCT
1021 AAACAAGTGA CTGTTAATTT CGAATGCAAA CAACACTAGG AGACGCAAAA CGAGCTACCT
1081 ACATCAAACA CCATGGCAGA GAAGGCTCAA AACCCCATG
                           -----> HSF
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Fig. 4. The nucleotide sequence of the upstream region of the *S. pombe* ribosomal protein L11 genomic DNA contained in the fusion plasmid pHY L11. The initiation codon is underlined. Putative binding sites for AP-1, HSF, and Mata1p are represented.

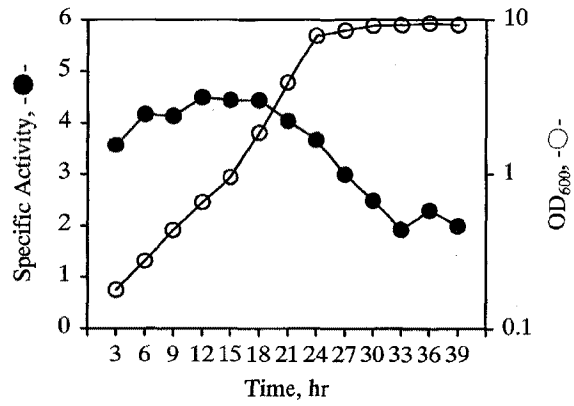

Fig. 5. Growth-dependent synthesis of β-galactosidase from the fusion plasmid pHY L11. The growth of the fission yeast cells (-○-) was monitored by the absorbance at the wavelength of 600 nm. β-Galactosidase activity (-●-) was assayed as described in 'Materials and Methods'. The specific activity of β-galactosidase was represented by μmol/min/mg protein.

suggests that the expression of the L11 gene is shut down in the condition of restrained growth. This could be due to the lower need of protein synthesis in the growth-arrested cells. Interestingly, the synthesis of β-galactosidase from the fusion plasmid was enhanced in the presence of cadmium chloride (2.5 μM) and zinc chloride (2.5 μM) (Fig. 6). Although the enhancement is not very high, it could have some physiological meanings. The enhancement may relate to the synthesizing proteins that are involved in stress response. Hydrogen peroxide (0.5 mM) also enhanced the synthesis of

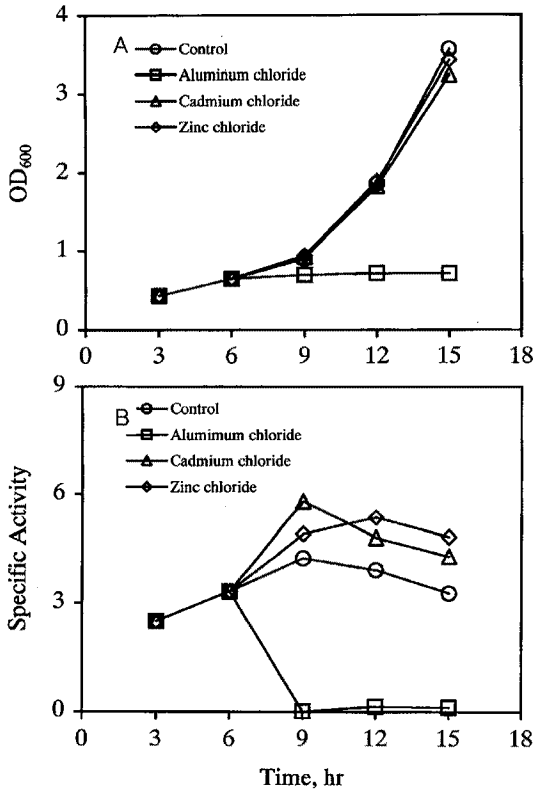

Fig. 6. Effects of aluminum chloride, cadmium chloride, and zinc chloride on the β-galactosidase synthesis from the fusion plasmid pHY L11 in *S. pombe* cells. The *S. pombe* cells, harboring the fusion plasmid pHY L11, were grown in minimal medium, and split at the early exponential phase. Aluminum chloride (2.5 μM, - □ -), cadmium chloride (2.5 μM, -△-), and zinc chloride (2.5 μM, -◇-) were added into the separate culture flasks, and the culture flask were continually shaken. (A) Growth curve. (B) β-Galactosidase activity. The β-galactosidase activity was determined at 25°C by the spectrophotometric assay using ONPG as a substrate. Its specific activity was expressed in μmol/min/mg protein.

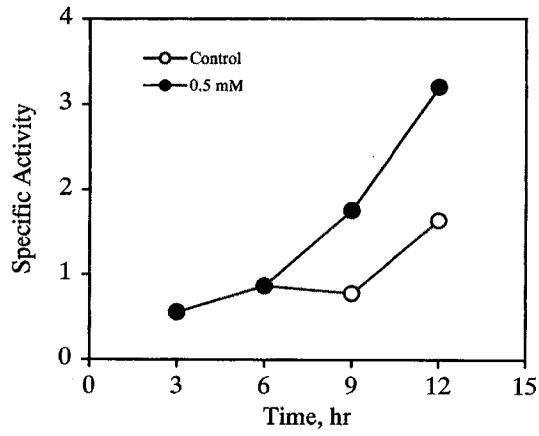

Fig. 7. Effect of hydrogen peroxide on the β-galactosidase synthesis from the fusion plasmid pHY L11 in *S. pombe* cells. The yeast cells, containing the fusion plasmid pHY L11, were grown in minimal medium, and split at the early exponential phase. Hydrogen peroxide (0.5 mM, -●-) was added into one culture flask, and the culture flasks were continually shaken. Open circles (-○-) indicate the untreated cells. The β-galactosidase activity was determined at 25°C by the spectrophotometric assay using ONPG as a substrate. Its specific activity was expressed in μmol/min/mg protein.

β-galactosidase from the fusion plasmid (Fig. 7). The physiological meaning in the enhancement by hydrogen peroxide remains elusive. However, it could reflect the urgent need of various stress response proteins. The induction of the *S. pombe* L11 gene could be interpreted by the existence of the AP-1 site in the upstream sequence (Fig. 4). However, its detailed mechanism remains unsolved. Moreover, the induction response of the L11 gene in the condition of oxidative stress raises an other interesting problem.

This study represents the cloning and regulation of the gene that encodes the ribosomal protein L11 from *S. pombe*. This is the first L11 cDNA that was isolated from eukaryotic microorganisms, whereas it is the third known L11 cDNA among the eukaryotes. The putative amino acid sequence of the *S. pombe* ribosomal protein L11 is highly homologous with the L11 proteins of higher eukaryotes, such as rats and fruit flies, which are estimated from the evolutionary aspect. The expression of the *S. pombe* L11 gene varied according to the growth curve. It was completely shut down in the growth arrested yeast cells. These findings indicate the relationship between cell growth and the expression of the L11 gene. Another interesting result was that the expression of the *S. pombe* L11 gene was enhanced by oxidative stress. Further research would give information about the regulatory mechanism of the *S. pombe* L11 gene, and its physiological role in the biogenesis of eukaryotic ribosomes.

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References

Agarwal, A. K., Parrish, S. N. and Blumberg, D. D. (1999) Ribosomal protein gene expression is cell type specific during development in *Dictyostelium discoideum*. *Differentiation* **65**, 73-88.

Arndt, E. and Wiegel, C. (1990) Nucleotide sequence of the genes encoding the L11, L1, L10 and L12 equivalent ribosomal proteins from the archaebacterium *Halobacterium marismortui*. *Nucleic Acids Res.* **18**, 1285.

Bradford, M. M. (1976) A rapid and sensitive method for the quantition of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254.

Chan, Y.-L., Olvera, J., Paz, V. and Wool, I. G. (1992) The primary structure of rat ribosomal protein L11. *Biochem. Biophys. Res. Comm.* **185**, 356-362.

Cho, Y.-W., Park, E.-H. and Lim, C.-J. (1999) A second

thioltransferase from Chinese cabbage: purification and characterization. *J. Biochem. Mol. Biol.* **32**, 133-139.

Cho, Y.-W., Park, E.-H. and Lim, C.-J. (2000a) Glutathione S-transferase activities of S-type and L-type thioltransferase from *Arabidopsis thaliana*. *J. Biochem. Mol. Biol.* **33**, 179-183.

Cho, Y.-W., Park, E.-H. and Lim, C.-J. (2000b) Catalase, glutathione S-transferase and thioltransferase respond differently to oxidative stress in *Schizosaccharomyces pombe*. *J. Biochem. Mol. Biol.* **33**, 344-348.

Cho, Y.-W., Park, E.-H. and Lim, C.-J. (2000c) Regulation of thioltransferase activity from *Schizosaccharomyces pombe*. *J. Biochem. Mol. Biol.* **33**, 422-425.

Cho, Y.-W., Park, E.-H., Ahn, K.-S., Kim, D.M. and Lim, C.-J. (2001) Growth-dependent variations in antioxidant and redox enzyme activity of *Schizosaccharomyces pombe*. *J. Biochem. Mol. Biol.* **34** (in press).

Conn, G. L., Draper, D. E., Lattman, E. E. and Gittis, A. G. (1999) Crystal structure of a conserved ribosomal protein-RNA complex. *Science* **284**, 1171-1174.

Guarente, L. (1983) Yeast promoters and *lacZ* fusion designed to study expression of cloned genes in yeast. *Methods Enzymol.* **101**, 181-191.

Guha Thakurta, D. and Draper, D. E. (2000) Contributions of basic residues to ribosomal protein L11 recognition of RNA. *J. Mol. Biol.* **295**, 569-580.

Hoftman, C. S. and Winston, F. (1987) A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. *Gene* **57**, 267-272.

Kang, S.-W., Cho, Y.-W., Park, E.-H., Ahn, K.-S. and Lim, C.-J. (2001) Cloning and expression of alkaline phosphatase gene from *Schizosaccharomyces pombe*. *J. Biochem. Mol. Biol.* **34**, 262-267.

Kim, H.-G., Cho, Y.-W., Park, E.-H., Park, S.-S., Ahn, K.-S. and Lim, C.-J. (1999a) Cloning, nucleotide sequence and expression of thioltransferase (glutaredoxin) cDNA from *Schizosaccharomyces pombe*. *Mol. Cells* **9**, 668-672.

Kim, H.-G., Park, E.-H. and Lim, C.-J. (1999b) A second thioltransferase of *Schizosaccharomyces pombe* contains glutathione S-transferase activity. *J. Biochem. Mol. Biol.* **32**, 535-540.

Kim, T.-S., Cho, Y.-W., Kim, J.-C., Jin, C.-D., Han, T.-J., Park, S.-S. and Lim, C.-J. (1999c) An L-type thioltransferase from *Arabidopsis thaliana* leaves. *J. Biochem. Mol. Biol.* **32**, 605-609.

Larochelle, S. and Suter, B. (1995) Molecular cloning of the *Drosophila* homologue of the rat ribosomal protein L11 gene. *Biochim. Biophys. Acta* **1261**, 147-150.

Nischt, R., Gross, T., Gatermann, K., Swida, U. and Käufer, N. F. (1987) Sequence and regulatory responses of a ribosomal protein gene from the fission yeast *Schizosaccharomyces pombe*. *Nucleic Acids Res.* **15**, 1477-1492.

Post, L. E., Strycharz, G. D., Nomura, M., Lewis, H. and Dennis, P. P. (1979) Nucleotide sequence of the ribosomal protein gene cluster adjacent to the gene for RNA polymerase subunit beta in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **76**, 1697-1701.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) *Molecular Cloning: a Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Schimmin, L. C. and Dennis, P. P. (1989) Characterization of the L11, L1, L10 and L12 equivalent ribosomal protein gene cluster of halophilic archaebacterium *Halobacterium cutirubrum*. *EMBO J.* **8**, 1225-1235.

Sikorski, R. S. and Hieter, P. (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122**, 19-27.

Sor, F. and Nomura, M. (1991) Cloning and DNA sequence determination of the L11 ribosomal protein operon of *Serratia marcescens* and *Proteus vulgaris*. *Mol. Gen. Genet.* **210**, 52-59.

Triantafillidou, D., Simitsopoulou, M., Franceschi, F. and Choli-Papadopoulou, T. (1998) *Thermus thermophilus* L11 ribosomal protein: cloning, overproduction, structural, and functional studies *in vivo* methylation of the overproduced protein. *J. Protein Chem.* **17**, 519-520.

Triantafillidou, D., Simitsopoulou, M., Franceschi, F. and Choli-Papadopoulou, T. (1999) Structural and functional studies on the overproduced L11 protein from *Thermus thermophilus*. *J. Protein Chem.* **18**, 215-223.

van Aelst, L., Barr, M., Marcus, S., Polverino, A. and Wigler, M. (1993) Complex formation between RAS and RAF and other protein kinases. *Proc. Natl. Acad. Sci. USA* **90**, 6213-6217.

Vieira, J. and Messing, J. (1987) Production of single-stranded plasmid DNA. *Methods Enzymol.* **153**, 3-11.

Xing, Y., Guha Thakurta, D. and Draper, D. E. (1997) The RNA binding domain of ribosomal protein L11 is structurally similar to homeodomains. *Nature Struct. Biol.* **4**, 24-27.