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The ribosomal protein L32-2 (RPL32-2) of *S. pombe* exhibits a novel extraribosomal function by acting as a potential transcriptional regulator

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Abstract **Ribosomal proteins play important roles in stabilizing the rRNA structure to facilitate protein synthesis in ribosome. In the present study, we analyzed the potential extraribosomal function of the ribosomal protein L32-2 (RPL32-2), which was expressed by a gene clone isolated from a cDNA library of *Schizosaccharomyces pombe* (*S. pombe*). RPL32-2 fused with the GAL4 DNA-bind domain or the GAL4 transcriptional activating domain could, respectively, activate transcriptions of reporter genes in yeast strain AH109. The RPL32-2 mutants with truncation of either the N- or the C-terminal domain resulted in abolishment of this regulatory effect. The DNA binding site for RPL32-2 of *S. pombe* was identified by using a random oligonucleotide selection strategy and gel motility shift assay and Western blotting confirmed its binding specificity. Moreover, we found RPL32-2 was also able to interact with a to-be-identified AT sequence binding protein. These data suggest that RPL32-2 of *S. pombe*, besides its ribosomal function, may also act as a potential transcriptional regulator in nucleus.**

Keywords: Ribosomal protein L32; Transcription; Regulation; *Schizosaccharomyces pombe*

1. Introduction

The ribosome, consisting of ribosomal proteins and RNAs, is an important molecular machine responsible for protein synthesis by executing two main functions: decoding the genetic message and the formation of peptide bonds [1]. The ribosome proteins, which are highly conserved among diverse organisms, mainly function as stabilizers of the rRNA structure [2]. However, some ribosomal proteins may also possess extraribosomal functions, e.g, DNA repair, regulation of transcription, cell growth and apoptosis [3–8].

Yeast ribosomes contain 78 ribosomal proteins [9]. Balance must be maintained in synthesis of the ribosomal proteins so that equimolar amounts of the proteins are supplied [8–12]. However, some ribosomal proteins, e.g., L32 (RPL32), are over-expressed in some type cells, e.g., human prostate cancer cells [10] and CD4+ cells of the young mice [12]. Therefore, it is interesting to investigate whether these ribosomal proteins may play a role in regulation of transcription.

In this study, we isolated a gene clone from a cDNA library of *Schizosaccharomyces pombe* (*S. pombe*), which encodes the protein RPL32-2, a homologue of the RPL32 of *Saccharomyces cerevisiae*. Our data indicate that the fusion of RPL32-2 with GAL4 DNA-bind domain or the RPL32-2 and GAL4 transcriptional activating domain can, respectively activate transcription of reporter gene in the GAL4-base hybrid system. Furthermore, we found this protein could interact with an AT-binding protein, suggesting that RPL32-2 is a potential transcriptional regulator.

2. Materials and methods

2.1. Plasmid constructions

The plasmid pGADT7-RPL32-2 was constructed by inserting an *Eco*RI/*Xho*I RPL32-2 from cDNA library of *S. pombe* into the *Eco*RI/*Xho*I site in pGADT7 for expressing the fusion protein of GAL4 activation domain (GAD) and RPL32-2 (GAD-RPL32-2) under the control of the ADH1 promoter. Transcription and translation of HA-RPL32 fusion protein can be controlled under T7 promoter for using in vitro rabbit reticulocyte lysate system. The plasmid pGBKT7-RPL32-2 was constructed by cloning the same *Eco*RI/*Xho*I fragment of RPL32-2 into the *Eco*RI/*Bam*HI site of pGBKT7 for expression of fusion protein of GAL4 DNA-binding domain (GBD) and RPL32-2 (GBD-RPL32-2). To express GAD-RPL32-2ΔN (aa 1–79) and GAD-RPL32-2ΔC (aa 81–127) fusion proteins, the corresponding *Sac*I/*Xho*I and *Eco*RI/*Sac*I fragments were inserted into pGADT7 vector. To express GBD-RPL32-2ΔN (aa 1–79) and GBD-RPL32-2ΔC (aa 81–127) fusion proteins, the corresponding plasmids were constructed by inserting the *Eco*RI/*Sac*I and *Sac*I/*Pst*I fragments, respectively, into the pGBKT7 vector. The resultant plasmids were verified by PCR with the primer from DNA BD and AD insert screen amplification set.

2.2. Cell survival test and β-galactosidase assay

DNA of the plasmids was introduced into AH109 strain by LiAc-mediated yeast transformation. The cells transformed with the plasmids pGADT7-RPL32-2, pGADT7-RPL32-2ΔN and pGADT7-RPL32-2ΔC were incubated in the synthetic dropout (SD)/-Leu medium for 4 days at 30 °C and then transferred into SD/-Leu/-His/-Ade medium for culture at 30 °C for additional 4 days. Similarly, the cells transformed with the plasmids pGBKT7-RPL32-2, pGBKT7-RPL32-2ΔN and pGBKT7-RPL32-2ΔC were incubated on synthetic dropout (SD)/-Trp medium for 4 days at 30 °C, followed by culturing the cells in SD/-Trp/-His/-Ade medium for 4 days at 30 °C.

The β-galactosidase activity was determined by colony-lift filter assay as previously described [27]. Briefly, the fresh colonies were grown in a plate at 30 °C for 4 days to 2–3 mm in diameter. A sterile Whatman #5 filter was placed over the surface of the plate of colonies and gently rubbed the filter with the side of the forceps until the filter was evenly wetted. The filter was carefully lift off the agar plate with

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forceps and transferred (colonies facing up) to a pool of liquid nitrogen. After the filter had frozen completely (about 10 s), it was removed and allowed to thaw at room temperature. The filter was then carefully placed, with the colony side up, on another filter that was pre-soaked in a clean 90-mm plate containing 3 ml of Z buffer X-gal solution. The filters were incubated at 30 °C and checked periodically for the appearance of blue colonies.

2.3. In vitro translation and nuclear extract preparation

In vitro the HA-tagged RPL32-2 was transcripted and translated by TNT T7 coupled reticulocyte lysate system (Promega) [12]. Yeast nuclear extracts were prepared using the method described by Symington [13]. The yeast strain (WT, AKU4220) was obtained from China General Microbiological Culture Collection Center (Beijing, China).

2.4. Oligonucleotide selection

Oligonucleotide selection was performed with a DNA fragment (N24) that contained a 24-bp random sequence flanked on either side by 18 bases of non-random sequence [5′-GAATTCGGATCCTCTAGA (N24)CTGCAGAAGCTTCTCGAG-3′]. The single-stranded oligonucleotide containing the random sequence was PCR-amplified to a double-stranded oligonucleotide with the primers as outlined. The products of PCR were purified by phenol/chloroform extraction. Binding reactions were performed in a final volume of 25 μl at room temperature for 30 min. The mixture of reaction contained 5 μl (0.5 ng/μl) of N24 double-stranded DNA fragment, 3 μl (0.5 μg/μl) poly (dI–dC) and 10 μl (1μg/μl) of the protein of HA-RPL32-2 of *S. pombe* from cell-free translation. The binding buffer contained 10 mM Tris–HCl (pH7.5), 50 mM NaCl, 1 mM DTT, 5% (vol/vol) glycerol, 0.1% NP-40, and 0.5 μg/μl of bovine serum albumin (BSA). The binding complexes were isolated by immunoprecipitation used BD Matchmarker Co-IP kit (BD biosciences). Briefly, 10 μl (1 μg/μl) of HA-Tag polyclonal antibody was added to the mixture of binding reaction. After incubation at room temperature for 1 h, 3 μl of protein A beads was added and incubated at room temperature for 1 h. The beads were washed for 5 times with the Wash Buffer 1 and Wash Buffer 2 from the BD Co-IP kit, resuspended in 100 μl of H_2O and boiled for 5 min. Following phenol extraction, the DNA was recovered by ethanol precipitation. The recovered DNA was amplified by PCR (94 °C, 1 min, 52 °C, 40 s, and 72 °C, 1 min for 30 cycles). The amplified DNA was extracted by phenol/chloroform and recovered by ethanol precipitation. The selection and enrichment procedure was repeated for six rounds. After the last round of PCR amplification, the gel-purified DNA fragments were cloned into PMD-18 T vector (TA cloning) (TaKaRa Biotechnology Co., Dalian, China). The clones containing single insets were identified and constructs were verified by DNA sequencing. The binding potential of selected sites at the site of N24 DNA fragments was analyzed with the method of combination of computer-generated and visual alignment.

2.5. Binding probes

The DNA fragment (WT) was prepared by PCR amplification of plasmids containing the selected binding site with oligonucleotide primer (NA and NB, as above). The PCR product was digested by BamH I and was radiolabeled at 3′-terminal with [α-^{32}P]dGTP by DNA polymerase I large (Klenow) fragment for the probe of RPL32-2 binding DNA assays. For preparing the competitor DNA probes (mut 1–6), the single-base of GTTGGT of RPL32-2 binding DNA sequence in WT was substituted by A/C/C/A/A/C in order, respectively. The DNA probe (ATWT) was prepared by PCR amplification of WT fragment with the sense primer (NA) and the antisense primer (ATNB) containing an AT sequence (5′-CGATATAAAACTCGA GAAGCTT-3′). The radiolabeled probe (ATNB) was prepared in the same way as described above. The probes were purified by electrophoresis through a 12% non-denaturing polyacrylamide gel, eluted and quantitated.

2.6. DNA binding assay

The HA-RPL32-2 fusion protein was generated by TNT T7 lysate coupled translation system and immunoprecipitated by BD Matchmarker Co-IP Kit, as described above. The protein was eluted with 0.1 M glycine–HCl (pH 2.5) and neutralized by 1 M Tris–HCl (pH 8.0) immediately. To determine the binding of the eluted protein to the DNA fragment, the fraction containing the eluted protein was added to the buffer containing 10 μg WT fragment, 0.3 μg of poly-(dI–dC), 10 mM Tris–HCl (pH 7.5), 50 mM NaCl, 1 mM DTT, 5% (vol/vol) glycerol, 0.1% Nonidet P-40, and 0.5 μg/μl BSA. The mixture was incubated for 30 min at room temperature to allow the formation of complexes. For competition analysis, the unlabelled oligonucleotides (mut 1–6) at a concentration of 50-fold higher than the radiolabeled WT DNA fragment were included in the binding reaction buffer. For detecting the interaction between the RPL32-2-DNA and AT binding protein, 10 μg nuclear extract mixed with 10 μl in vitro transcription and translation the HA-RPL32-2 was incubated at room temperature for 1 h. The complex was immunoprecipitated and eluted as described above. The same protocol was used for DNA binding and competition reaction. A mobility shift assay was performed by loading the complexes onto an 8% native polyacrylamide gel in order to verify RPL32-2 with DNA or containing AT sequence DNA and its binding protein interaction.

2.7. Western blot assay

The proteins complexes were eluted by 0.1 M glycine–HCl (pH 2.5) and the pH was neutralized by addition of 1 M Tris–HCl (pH 8.0) immediately. The elutes were analyzed by non-denaturing polyacrylamide gel electrophoresis and transferred to a nitrocellulose filter without using methanol in the transfer buffer. The filter was incubated in 3% BSA in Tris–buffer saline (TBS) for 1 h at room temperature. After wash with the TTBS (0.1% Tween-20 TBS), the filter was incubated with rabbit polyclonal antibodies against HA-Tag (1:50 dilution in TBS containing 0.3% BSA) at 37 °C for 1 h with shaking slowly. After washing, it was incubated sequentially with biotinylated sheep anti-rabbit IgG (second antibody) at room temperature for 30 min and with aivdin–biotin–peroxidase complex at room temperature for 30 min. After 4 washes by TTBS, the filter was developed in diammobenzidine tetrahydrochloride buffer. The eluate WT fragments bound to RPL32 protein were shown on the filter.

3. Results

3.1. Sequence analysis of RPL32-2

We isolated ribosomal protein RPL32-2 gene (NP_594182.1) from cDNA library of *S. pombe*. The gene is located on chromosome I and is 97.8% identity with RPL32 on chromosome II. The amino acid residues Ile4, Val7, Leu21, and Ser95 in RPL32 are substituted with Val4, Ile7, Arg21, Gly95 in RPL32-2, respectively. Though the RPL32 of *S. pombe* contains no cysteine residues, its amino acid sequence resembles highly with the N-terminal fragment of the protein of *Ustilago maydis 521* (gi|46096876|gb|EAK82109.1|) and at the C-terminal region of the protein contain TAFII55 (TAF7) protein conserved region [Identities = 86/122 (70%), Positives = 104/122 (85%). TAFII55 is a TBP-associated factor in transcription factor TFIID and play a role in the regulation of gene transcription by RNA polymerase II [14]. A computer blast of amino acid sequences represented in all non-redundant GenBank CDS indicated that RPL32-2 of *S. pombe* has low similarity with the zinc finger (C3HC4-type RING finger) family protein (gi|15234116|ref|NP192036 .1|) [*Arabidopsis thaliana*] [Identities = 18/56 (32%), Positives = 29/56 (51%) in the region of aa 55–110] and Zinc finger protein on ecdysone puffs (gi|730297|sp|P41073|PEP_DROME) [*Drosophila melanogaster*] [Identities = 33/106 (31%), Positives = 47/106 (44%) in the region of aa 5–102].

3.2. RPL32-2 of S. pombe activates the reporter genes in a GAL4-base hybrid system

The clone of RPL32-2 of *S. pombe* was used to constructed plasmids encoding the fusion proteins of RPL32-2 and GAL4-

binding DNA domain (pGADT7-RPL32-2) or the fusion proteins of RPL32-2 and GAL4-transcription-activation domain pGBKT7-RPL32-2. After the plasmid pGADT7-RPL32-2 was transformed into the yeast AH109 cells which could grow in SD/-Leu/-His/-Ade medium, it activated the LacZ reporter gene to express β-galactosidase as shown in the colony-lifter assay (Fig. 1A1 and A2). Similar result was obtained when pGBKT7-RPL32-2 was transformed into the yeast AH109 cells, i.e., the fusion protein of RPL32-2 and GAL4-binding DNA domain could promote expression of the His, Ade and LacZ reporter genes (Fig. 1B1 and B2). These date suggest that RPL32-2 has the ability of binding DNA and transcriptional activation in Gal4-base hybrid system.

To determine whether the RPL32-2 contains the independent DNA binding domain and transcription-activation domain, the protein was truncated by deletion of its N- or C-terminal region. After amplification by PCR, the bands of various truncated cDNA were revealed (Fig. 2A). Unlike the cells transformed with both wide-type plasmids pGADGH-RPL32-2 and pGADT7-RPL32-2, neither pGADT7-RPL32-2ΔN nor pGADT7-RPL32-2ΔC-transformed AH109 cells could grow in SD/-Leu/-His/-Ade medium (Fig. 2B). Similarly, the cells transformed with pGBKT7-RPL32-2ΔN and pGBKT7-RPL32-2ΔC could not grow in SD/-Trp/-His/-Ade medium either (Fig. 2C). These results indicate that the both the N- and C-terminal regions of RPL32-2 are important for its ability of DNA binding and transcriptional activation in GAL4-hybrid system and the domains responsible for these two functions may be associated.

3.3. The DNA binding specificity of RPL32-2

If the RPL32-2 of *S. pombe* is a transcriptional regulator, it could exhibit an ability to bind DNA. The random oligonucleotide selection procedure has been successfully to identify optimal DNA binding sites for a variety of transcriptional regulatory proteins [14,22]. We used this method to investigate the DNA binding specificities of the RPL32-2 protein. Binding selection was performed with a double-stranded DNA fragment that contains a 24-bp random sequence. Those DNA fragments interacted with the RPL32-2 protein were separated by immunoprecipitation of the bound complexes and PCR amplification repeatedly. After the sixth round, the DNA sites selected with HA-RPL32-2 were cloned and sequenced. After alignment of 64 different sequences (with a combination of computer-generated alignment and visual alignment), the site, GTTGGT, was identified. To determine the binding specificity, six unlabeled mutational site probes (mut 1–6) at 50-fold higher concentration were added to reaction mixtures containing the WT DNA probe and the bands were visualized by gel shift analysis and Western blotting. As shown in Fig. 3A, the DNA fragments GCTGGT (lane 3) and GTTAGT (lane 5) could not substitute WT fragment. Binding of the WT DNA fragment to the fusion protein of HA-RPL32-2 resulted in significant reduction of mobility, as shown by Western blotting assay (Fig. 3B).

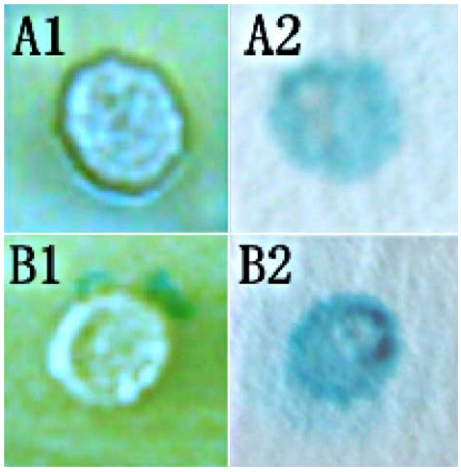

Fig. 1. Activation of the reporter genes in a GAL4-base hybrid system by the GADT7-RPL32-2 and GBKT7-RPL32-2 fusion proteins. (A1) The clone of cells transformed with pGADT7-RPL32 plasmid was spotted on SD/-Ade/-His/-Leu agar plate and incubated at 30 °C for 4 days. (A2) The blue colonies in A1 were lifted onto the filter and measured for β-galactosidase activity by colony-lift filter assay. (B1) The clone of cells transformed with pGBKT7-RPL32 plasmid was spotted on SD/-Ade/-His/-Trp agar plate and incubated at 30 °C for 4 days. (B2) The blue colonies in B1 was lifted onto the filter and measured for β-galactosidase activity by colony-lift filter assay.

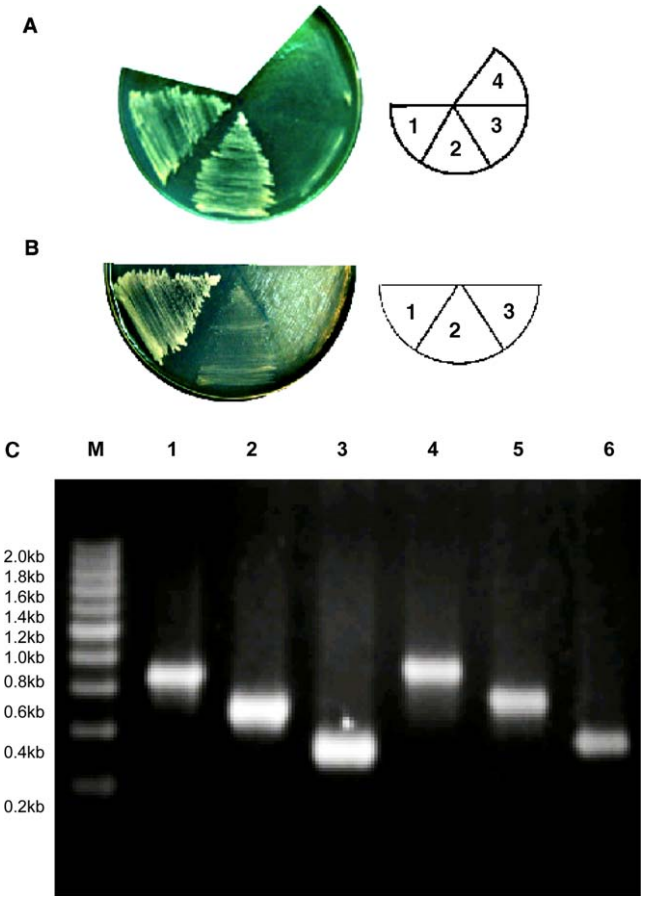

Fig. 2. Effects of the N- and C-terminal truncation of the RPL32-2 on activation of the reporter genes in a GAL4-base hybrid system. (A) The cells transformed with pGADGH-RPL32 (1) and pGADT7-RPL32 (2) could grow on SD/-Leu/-His/-Ade medium plate, but those with pGADGH-RPL32ΔN (3) and pGADT7-RPL32ΔC (4) could not grow. (B) The cells transformed with pGBKT7-RPL32 (1) could grow in SD/-Trp/-His/-Ade medium plate, but those with pGBKT7-RPL32ΔN (2) or pGBKT7-RPL32ΔC could not (3). (C) Constructed plasmids were verified by PCR. Lane 1: pGADT7-RPL32; lane 2: pGADT7-RPL32ΔN; lane 3: pGADT7-RPL32ΔC; lane 4: pGBKT7-RPL32; lane 5: pGBKT7-RPL32ΔN; lane 6: pGBKT7-RPL32ΔC: and M, DNA ladder marker.

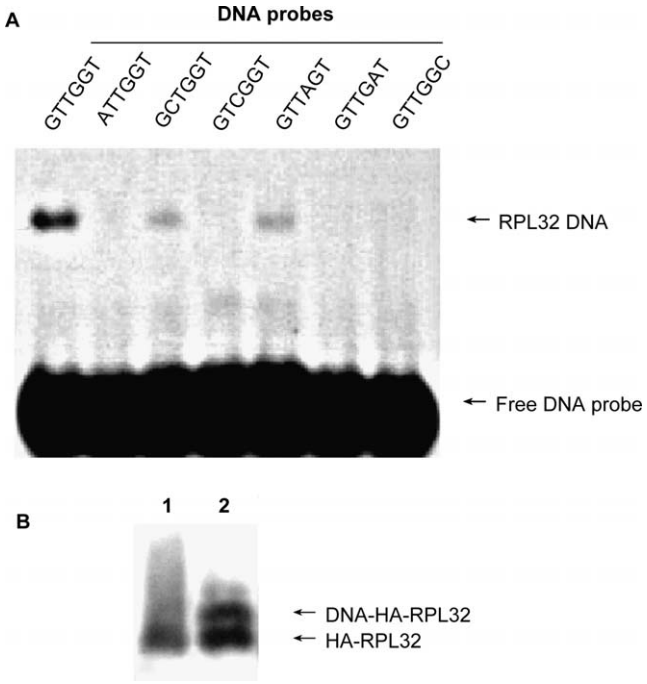

Fig. 3. Determination of the DNA binding specificity of RPL32-2. The double-stranded WT fragments were radiolabeled and combined with HA-RPL32-2 fusion protein to form complexes, which were purified by immunoprecipitation and analyzed by Western blotting assay. (A) Gel mobility shift assay indicated WT probe could interact with the protein of HA-RPL32-2. Lane 1: radiolabeled HA-RPL32 + WT fragment with GGTGTT sites; lanes 2–7: radiolabeled HA-RPL32 + WT fragment with GGTGTT sites plus 50-fold excess unlabeled DNA mutants (mut 1–6) as competitors. (B) Western blotting assay carried out the protein of RPL32-2 can bind the DNA fragment. Lane 1: the eluates of the HA-RPl32 without addition of the DNA probe (control); lane 2: the eluate of the HA-RPl32-2 with addition of the WT probe.

3.4. Cooperation of RPL32-2 and an AT binding protein

When oligonucleotide selection was performed with a DNA fragment (N24), the 24-bp random sequence flanked on either side by 18 bases of non-random sequence. If it contains an AT sequence in the 3′ non-random sequence, the HA-RPL32-2 protein binding oligonucleotide selection should lose order in random sequence region. Therefore, we prepared the radiolabeled probe (ATWT) containing a HA-RPL32-2 binding site and an AT sequence. After the blending of HA-RPL32-2 protein and nuclear extracts of *S. pombe* was immunoprecipitated and eluted, the eluate was mixed with the ATWT probe. As shown in Fig. 4, an extra band with reduced mobility was revealed in the lane where the mixture of nuclear extracts, RPL32-2 and ATWT probe was added, suggesting that HA-RPL32-2 may interact with a protein in the nuclear extracts and bind the ATWT probe simultaneously. This binding reaction could not be competed by the non-radiolabeled WT probe. These date indicate that the HA-RPL32-2 fusion protein can bind to an AT sequence binding protein.

4. Discussion

Ribosomal proteins are major components of ribosome responsible for stabilizing the rRNA structure in ribosome to guarantee the efficiency of protein synthesis [2]. However, there

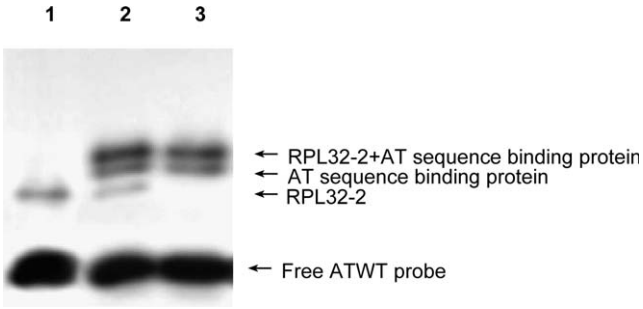

Fig. 4. Analysis of cooperation of RPL32-2 and an AT binding protein. Binding reactions were preformed using rabbit reticulocyte lysate of HA-RPL32-2 cDNA (plasmid T7ATG), nuclear extracts and radio-labeled ATWT probe. The complexes were isolated by immunoprecipitation and analyzed by gel mobility shift assay. Lane 1: mixture of ATWT probe and RPL32-2; lane 2: mixture of ATWT probe, RPL32-2 and the nuclear extracts; lane 3: mixture of ATWT probe, RPL32-2, the nuclear extracts and the non-radiolabeled ATWT probe at 50-fold higher concentration.

are several reports showing that a deficiency in some ribosomal proteins has been linked to developmental disorders in organisms as diverse as humans, fruitflies and plants [3,5,6,10]. Particularly the discovery of DNA-binding motifs and their relationship to transcription factors have suggested that a few ribosomal proteins may have extraribosomal functions in transcriptional regulation [3,5,15,16,18,19].

In the present study, we isolated a gene clone encoding the RPL32-2 of *S. pombe*. Like other ribosomal proteins, the RPL32-2 of *S. pombe* also shows a high degree of sequence conservation across diverse species at the protein level by BLAST searches (http://www.ncbi.nlm.nih.gov/BLAST/). To the best of our knowledge, we first demonstrated that the RPL32-2 of *S. pombe* possessed the intrinsic DNA-binding and transcriptional activation properties since the fusion protein of RPL32-2 of *S. pombe* and GAL4 DNA-binding domain can activate transcription of the reporter genes in GAL4-hybrid system.

The structural feature of RPL32-2 protein and its DNA make their interaction and binding possible. Up to now, there have been no reports indicating that RPL32 can interact with DNA. But some groups have shown that RPL32 of *S. cerevisiae* appears to interact with three distinct RNA molecules to influence different elements of RNA processing and function in three sites in the cell: (1) the processing of pre-rRNA in the nucleolus; (2) the splicing of the RPL32 transcript in the nucleolus; and (3) the translation of the spliced RPL32 mRNA in the cytoplasm [20,21].

Pollock et al. have designed a short random sequence oligonucleotide as a source of binding sites for amplification of the immunoprecipitated DNA by PCR [14]. Using this method, Rerngsamran et al. have discovered the binding sites for some transcription factor and the proteins present in crude nuclear extracts and in vitro translation reactions [22]. We used this method to select the RPL32-2 binding sites of and found that the authentic binding DNA site of RPL32-2 is GGTGTT.

Brent and Ptashne [23] have created a chimeric gene containing the coding regions for the transcription-activating domain of GAL4 and the DNA-binding domain of LexA and demonstrated the DNA-binding and transcription-activating domains of LexA are independent modules, i.e., the hybrid

protein containing the DNA-binding domain from one protein and the transcription-activating domain from another protein can still function as an activator [24]. This domain swap technique has been widely used to determine whether a domain possesses DNA binding or trans-activation capability [25]. Using the similar approach, we designed two chimeric genes encoding the GAL4AD-RPL32-2 and GAL4BD-RPL32-2 fusion proteins, respectively. Both fusion proteins exhibit the functions of DNA-binding and transcription-activating activities in GAL4-based two-hybrid system [26], suggesting that both RPL32-2 and GAL4 in the fusion proteins make their own contributions. These data indicate that RPL32-2, like GAL4, may also function as a potential particular transcriptional regulator.

Unlike typical activator, the DNA-binding and activation domains in RPL32-2 of *S. pombe* are not independent modules because truncation of either the N- or the C-terminal portion of the RPL32-2 resulted in complete loss of its DNA-binding and transcription-activating activity. The RPL32-2 of *S. pombe* may act in a similar way as a zinc finger protein since the zinc fingers function as independent modules.

It is unknown how transcription of four reporter genes in the fusion protein of RPL32-2 and GAL4 DNA-binding domain is activated in the GAL4-based two-hybrid system. Our data suggest that the transcriptional activating domain of RPL32-2 is different from that of general transcription factor. The RPL32-2 is a small protein consisting of only 127 amino acid residues and its DNA-binding or transcription-activating functions may not be mediated by two separated domains. Despite a domain swap could be used to show that the domain alone possesses DNA-binding or trans-activation capabilities [23], the studies have underscored the fact that activation and DNA-binding domain are quite complex [17]. In some cases, the two domains keep their own function by mutual dependence and control. [28,29]. Therefore, we inferred that activation and DNA-binding domain of the RPL32-2 of *S. pombe* is overlap or is controlled each other.

Our result also shown that the cooperative binding of RPL32-2 and the AT sequence binding protein relies on a direct protein–protein interaction. Thus, RPL32-2 may have a function in gene transcription regulation under given conditions.

In summary, the RPL32-2 of *S. pombe* exhibits the same function to activate reporter genes as the transcription activator GAL4 in GAL4-base hybrid system; but unlike GAL4, the RPL32-2 protein is not an independently DNA-binding domain or transcription-activating domain. Our results suggest that the RPL32-2 of *S. pombe* may act as a transcription activation associated protein beside its ribosomal function.

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