Ebp2p, the Yeast Homolog of Epstein-Barr Virus Nuclear Antigen 1-binding Protein 2, Interacts with Factors of Both the 60 S and the 40 S Ribosomal Subunit Assembly*

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Ebp2p, the yeast homolog of human Epstein-Barr virus nuclear antigen 1-binding protein 2, is essential for biogenesis of the 60 S ribosomal subunit. Two-hybrid screening exhibited that, in addition to factors necessary for assembly of the 60 S subunit, Ebp2p interacts with Rps16p, ribosomal protein S16, and the 40 S ribosomal subunit assembly factor, Utp11p, as well as Yil019w, the function of which was previously uncharacterized. Depletion of Yil019w resulted in reduction in levels of both 18 S rRNA and 40 S ribosomal subunit without affecting levels of 25 S rRNA and 60 S ribosomal subunits. 35 S pre-rRNA and aberrant 23 S RNA accumulated, indicating that pre-rRNA processing at sites A0–A2 is inhibited when Yil019w is depleted. Each combination from Yil019w, Utp11p, and Rps16p showed two-hybrid interaction.

Yeast ribosomes are composed of 4 rRNA and 78 ribosomal proteins. Three mature (25, 18, and 5.8 S) rRNAs are synthesized as a long precursor, 35 S pre-rRNA, in the nucleolus (1, 2). During transcription of 35 S pre-rRNA, a large nuclear U3 ribonucleoprotein (RNP) complex assembles at the 5′ end of the nascent pre-rRNA, forming terminal knobs (3). The 90 S pre-ribosome, initially identified by Udem and Warner (4), contains 35 S pre-rRNA ribosomal proteins, U3 snRNP, and other nonribosomal protein factors required for 40 S subunit assembly (5). Thirty-five protein factors were identified by proteome analysis has revealed that many factors are necessary for pre-rRNA processing and assembly of 60 and 40 S ribosomal subunits (5, 8–12). These analyses suggested that the 90 S particle is converted to precursors of the 40 and 60 S subunits by cleavage of the 35 S pre-rRNA at sites A0–A2 and assembly of other factors (for review, see Refs. 6 and 7). A combination of TAP purification and proteome analysis has revealed that many factors are required for pre-rRNA processing and assembly of 60 and 40 S subunits (5, 8–12). These analyses suggested that the 90 S particle contains many 40 S assembly factors but few 60 S factors. Association of many 60 S assembly factors occurs at or after processing of 35 S to 27 S A0, (13). However, it is unknown how these many factors and ribosomal proteins associate with pre-rRNA sequentially.

Here we screened genes with products that interact with Ebp2p, the yeast homolog of human Epstein-Barr virus nuclear antigen 1-binding protein 2. We previously demonstrated that Ebp2p is essential for maturation of 25 S rRNA and assembly of 60 S ribosomal subunits (14). Here we have shown that besides pre-rRNA processing and 60 S subunit assembly factors, Ebp2p interacts with Rps16p, ribosomal protein S16 (for nomenclature, see Ref. 15), and its associating factors, Utp11p and Yil019w. We also demonstrate that YIL019w, named FAF1 (40 S assembly factor), encodes a novel factor essential for pre-rRNA processing and 40 S subunit assembly.

MATERIALS AND METHODS

Plasmid Construction—pGEM-T-FAF1 was constructed as follows. A PCR fragment of FAF1, including its upstream and downstream regions, was produced from yeast genomic DNA using the primers 5′-CCGGATCCCAATCTTCTCAGGGTGTCAG-3′ and 5′-GGGTGAGTGTGTGACCGCTCATT-3′ and ligated into the pGEM-T vector. The FAF1 fragment in pGEM-T-FAF1 was cloned as a BamHI-SalI fragment into pRS316 (16) to generate pRS316-FAF1. pRS314-GAL-myc-BS, a pRS314-based (16) expression vector that contains the GAL1 promoter, the three-Myc epitope-encoding region just downstream of the initiation codon, a multicloning site, and the TDH3 terminator, was kindly provided by Dr. K. Tanaka. pRS314-GAL-myc-FAF1 was constructed by inserting the BamHI-SalI PCR fragment of the FAF1 open reading frame (from the first ATG to the stop codon), produced from yeast genomic DNA with the primers 5′-CCGGATCCGATTAATGGCTT-3′ and 5′-CCGGATCCGACCCCAACGAGGACGGAGGTTCTTCTTTGACC-3′ into the BamHI-SalI sites of pRS314-GAL1-myc-BS. A plasmid expressing Myc-tagged Faf1p by own promoter, pRS314-myc-FAF1, was constructed by replacing the Sacl-EcoRI region of the GAL1 promoter of pRS314-GAL-myc-FAF1 with the Sacl-EcoRI fragment of the upstream promoter region of GAL1 (1045 bp) produced from yeast genomic DNA with primers 5′-CCGGATCCGATTAATGGCTT-3′ and 5′-CCGGATCCGACCCCAACGAGGACGGAGGTTCTTCTTTGACC-3′. A plasmid expressing Nop1p-GFP in the YCP33 vector, YCP33-NOP1-GFP, was kindly provided by Dr. Y. Kikuchi.

Yeast Strains and Media—The yeast strains used were W303a, KM802 (W303a faf1::LEU2 pRS316-FAF1), KM804 (W303a faf1::LEU2 pRS314-GAL1-myc-FAF1), KM806 (W303a faf1::LEU2 pRS314-GAL1-myc-FAF1), and KM807 (KM806 YCP33-NOP1-GFP). Yeast cells were grown in YPD (yeast, peptone, dextrose) medium, YG (yeast, peptone, galactose) medium, synthetic complete medium containing 2% glucose (SC) or 2% galactose (SCGal), or SC dropout medium, depending on the plasmid markers (17). For plasmid shuffling, 0.67 mg/ml 5-fluoroorotic acid was added to SC medium. Yeast transformation was performed by a lithium acetate procedure (18).

Strain Construction—pGEM-T-FAF1::LEU2 was created by replacing the Aor51HI-EcoRI fragment of pGEM-T-FAF1 with the LEU2 gene. Diploid W303 was transformed with pRS316-FAF1 and subsequently with the DNA fragment containing the FAF1::LEU2 disruption. The disruptant was dissected to obtain KM802. KM804 and KM806 were constructed by plasmid shuffling.

Two-hybrid Screening—pBTM116-EBP2 (14) and the pACT (20)-
proteins were co-transformed into L40 strain cells. Leu plasmids in the cells were characterized. Transformants were selected in which both reporter genes were expressed, and the plasmids in the cells were characterized.

Two-hybrid Assay—Two kinds of plasmids for production of LexA binding domain fusion proteins and Gal4p activation domain fusion proteins were co-transformed into L40 strain cells. Leu·Trp transformants were selected, and serial dilutions of the cell cultures were streaked on SC–Leu, Trp, His plates containing various concentrations of 3-aminotriazole and incubated at 25 °C for 4 days or 30 °C for 3 days.

Indirect Immunofluorescence—Indirect immunofluorescence microscopy was done as described previously (21, 22). The primary antibodies, anti-Myc antibody (9E10; Babco) and anti-GFP antibodies (kindly provided by Dr. P. A. Silver; Ref. 23), were diluted 1:1,000 and 1:5,000, respectively. Secondary antibodies (rhodamine-conjugated goat anti-mouse IgG, Jackson ImmunoResearch Laboratory, Inc.; fluorescein isothiocyanate-conjugated goat anti-rabbit IgG, ICN Pharmaceuticals, Inc.) were diluted 1:300. Nuclear DNA was stained with 4′,6-diamidino-2-phenylindole (DAPI, 1 μg/ml, Sigma).

Polysonye Analysis—Yeast cells were grown in 100 ml of medium to mid-log phase (optical density at 600 nm = 0.7) and harvested immediately following the addition of cycloheximide (100 μg/ml). The pellet was washed twice with CH buffer (10 mM Tris·HCl (pH 7.4), 100 mM NaCl, 30 mM MgCl<sub>2</sub>, 50 μg of cycloheximide/ml, 200 μg of heparin/ml) and suspended in 0.5 ml of CH buffer. After glass bead lysis of yeast cells, an aliquot of supernatant corresponding to 3 A<sub>260</sub> units was overlaid on top of 11 ml of a 7–40% (w/v) sucrose gradient made in 50 mM Tris·HCl (pH 7.6), 12 mM magnesium acetate, 50 mM ammonium acetate, and 1 mM dithiothreitol and centrifuged for 3.4 h at 35,000 rpm at 4 °C in a Hitachi RPS40T rotor. Gradients were collected by pumping up, using a peristaltic pump, and monitored at 254 nm. To dissociate the ribosomes into subunits, cells were lysed in 20 mM Tris·HCl (pH 7.4), 16 mM MgCl<sub>2</sub>, 1.0 mM KCl, and 0.2 mM EDTA, centrifuged over 5–25% (w/v) sucrose gradients prepared with the same buffer, and analyzed as described above.

Northern Blotting and [methyl-<sup>3</sup>H]Methionine Pulse-Chase Analyses—After glass bead lysis of yeast cells, total RNA was extracted by the hot phenol method. [methyl-<sup>3</sup>H]Methionine pulse-chase analysis was performed as described previously (24, 25). Briefly, yeast cultures in SC–Met were pulsed with [methyl-<sup>3</sup>H]methionine (10 μCi/ml) for 5 min and chased with nonradioactive methionine (500 μg/ml). Samples were taken by pouring cultures onto crushed sterile ice to prepare total RNA. Ten μg of total RNA was analyzed by electrophoresis and blotted to a Nytran membrane. The upper part of the membrane was sprayed with En<sup>3</sup>Hance (PerkinElmer Life Sciences) and exposed to film for 5 days. The lower part of the membrane was probed for U3 snRNA.

Western Blotting—Western blotting was performed following standard techniques, and signals were visualized by Enhanced Chemiluminescence (Amersham Biosciences) as instructed by the manufacturer. Anti-Myc monoclonal antibodies (9E10, Babco) and horseradish peroxidase-conjugated sheep anti-mouse IgG antibodies (NA931, Amersham Biosciences) were used.

RESULTS

Ebp2p Interacts with Assembly Factors of Both 60 S and 40 S Ribosomal Subunits—Ebp2p, an evolutionarily conserved nuclear protein, is required for pre-rRNA processing and proper assembly of 60 S ribosomal subunits in <i>S. cerevisiae</i> (14). To identify proteins that physically interact with Ebp2p, we performed a yeast two-hybrid screen on a yeast cDNA library using EBP2 as bait. As shown in Table I, among the genes isolated, BRX1, DBP9, LOC1, NOP12, and SPB1 were previously reported to encode factors required for assembly of 60 S ribosomal subunits (8, 26–30), and YKL082c was identified by copurification with Sfl1p-TAP (8) in pre-60 S subunit particles. Interaction of Ebp2p with Brx1p, Loc1p, or Ykl082c was detected by expression of the reporter gene HIS3 on SC–Leu, Trp, His plates containing 5 mM 3-aminotriazole, but interaction of Ebp2p with Dbp9p, Nop12p, or Spb1p was on plates containing as low as 0.1–1 mM 3-aminotriazole (data not shown). Interestingly, this two-hybrid screening also revealed that Ebp2p interacts with Utp11p, a component of 90 S pre-ribosomal particles (3), and Rps16a, a small ribosomal subunit protein. We also identified a previously uncharacterized gene, YIL019w, and therefore we analyzed whether it has a role in ribosome biogenesis. We named YIL019w<sub>FAF1</sub> (40 S assembly factor; see below).

<i>Faf1p Is Essential for Growth and Is Enriched in the Nucleolus</i>—<i>FAF1</i> encodes a protein consisting of 346 amino acids. A data base search revealed that DNA sequences from <i>Schizosaccharomyces pombe</i> and Neurospora crassa can encode proteins with significant similarity to Faf1p with identity of 23.1 and 21.7%, respectively (data not shown). A FAF1-null allele was created by replacing the <i>Aor51H1-EcoRV</i> fragment of <i>FAF1</i> with the <i>LEU2</i> gene. The DNA fragment containing the <i>FAF1-LEU2</i> disruption was used to transform diploid W303. Disruption was confirmed by PCR (data not shown). Thirty-three tetrad from the transformant of W303 were dissected, of which 31 tetrads yielded two viable spores, and 2 tetrads yielded only one viable spore. All viable spores were <i>Leu<sup>−</sup></i>, indicating that <i>FAF1</i> is essential for vegetative growth (data not shown).

To learn the subcellular localization of Faf1p, we constructed a plasmid to express Faf1p tagged with three repeats of the Myc tag (43.3 kDa). The subcellular localization of Myc-Faf1p was analyzed by indirect immunofluorescence microscopy (Fig. 1). Nop1p-GFP, used as a marker for the nucleolus, was detected in the region adjacent to the 4′,6′-diamidino-2-phenylindole. The morphology of the cells was observed by differential interference contrast (DIC). Arrowheads indicate the boundary between the chromatin region and the nucleolus.

**Table I**

| Ribosomal subunit | Gene | Function or ref. no. |
|-------------------|------|----------------------|
| 60 S              | BRX1 | 8, 9, 26             |
|                   | DBP9 | 27                   |
|                   | LOC1 | 28                   |
|                   | NOP12| 29                   |
|                   | SPB1 | 30                   |
|                   | YKL082c | 8              |
| 40 S              | RPS16A/B | Ribosomal protein |
|                   | UTP11 | 3                  |
|                   | YIL019w<sub>FAF1</sub> | This study |

**Fig. 1.** Faf1p is enriched in the nucleolus. Intracellular localization of Myc-Faf1p detected by indirect immunofluorescence. KM807 (myc-FAF1 NOP1-GFP) strain cells were grown in SC medium at 30 °C to early log phase. Cells were stained with anti-Myc, anti-GFP antibodies, and 4′,6-diamidino-2-phenylindole.
diamidino-2-phenylindole-stained nucleoplasm. A weak signal of Myc-Faf1p was detected throughout the cell, and a dense signal was seen in the same region as Nop1p-GFP. Thus, Faf1p is primarily localized in the nucleolus, although some Faf1p may be present in the nucleoplasm and in the cytoplasm.

**FAF1 Is Required for 40 S Subunit Assembly**—To achieve growth arrest is caused by a depletion of Faf1p. A. growth of W303a (WT) and KM804 (GAL-FAF1) on YPGal and YPD plates. B. Growth curves of W303a (WT) and KM804 (GAL-FAF1) cultured at 30 °C in SCGal medium and shifted to SC medium. The changes in optical density at 600 nm were followed after the shift. The cell cultures were diluted to keep the optical density lower than 1.0. Data are represented as log OD/OD₀, where t is the time in hours after shifting medium. C. Western blotting of KM804 (GAL-FAF1) cultured at 30 °C in SCGal medium, shifted to SC medium, and cultured for the indicated time. Cell extracts from KM804 (GAL-FAF1) (lanes 1–6) and KM806 (FAF1, lane 7) were subjected to SDS-PAGE. Western blotting using anti-Myc antibody (a) and protein staining by Coomassie Brilliant Blue (b) were shown. The positions of size markers are shown on the left.

**Fig. 3.** Faf1p depletion causes a defect in assembly of 40S ribosomal subunits. The polysome profiles after sucrose density gradient centrifugation from wild-type W303a (WT) and KM804 (GAL-FAF1) strains cultured in SCGal and 14 h after shift to SC under low salt (100 mM NaCl) (A) and high salt (1.0 M KCl) conditions (B). The positions of 40, 60, and 80 S ribosomal particles and polysomes are indicated.
conditional expression of FAF1, we constructed a strain in which the chromosomal FAF1 gene was disrupted and expression of Myc-Faf1p was driven by the GAL1 promoter expressed in galactose but not glucose. This strain, KM804, could grow on YPG-galactose media but not on YPD-glucose media (Fig. 2A).

In liquid galactose medium, KM804 cells grow with a longer doubling time than wild-type cells (Fig. 2B), suggesting that overexpression of Faf1p results in a growth defect. When KM804 was shifted from galactose to glucose medium, cell growth slowed gradually at 10 h after the shift (Fig. 2B).

Western blot analysis using anti-Myc antibodies revealed that expression level of Myc-Faf1p decreased to wild-type levels 4 h after the shift from galactose to glucose medium and significantly decreased 8–10 h after the shift (Fig. 2C).

The effect of depletion of Faf1p on ribosome biogenesis was examined by using this strain. We performed sucrose density
gradient ultracentrifugation using cell extracts with equal \(A_{260}\) units. The polysome profile of the strain KM804 that was cultured in glucose medium for 14 h exhibited a significant accumulation of 60 S ribosomal subunits (Fig. 3A). The profile of ribosomal subunits clearly revealed that the level of 40 S subunits decreased compared with the level of 60 S subunits in the Faf1p-depleted cells (Fig. 3B). These results indicate that FAF1 is required for 40 S ribosomal subunit assembly. Interestingly, overexpression of Faf1p appeared to have little effect on the profiles of either polysomes or subunits, although it caused significant growth inhibition.

**FAF1 Is Required for Maturation of 18 S rRNA**—We performed [methyl-\(^{3}H\)]methionine pulse-chase analysis to investigate whether the depletion of Faf1p caused a defect in pre-rRNA processing. The 35 S pre-rRNA, the longest detectable precursor, is cleaved to the 27 S and the 20 S pre-rRNAs, which are further processed to the mature 25 S and 18 S rRNAs, respectively (Fig. 4A). In wild-type cells, most precursor rRNAs were processed to 25 S and 18 S after a 3-min chase in both galactose and glucose medium (Fig. 4B, lanes 1–8). On the other hand, in the Faf1p-depleted cells, the processing rate of the 35 S pre-rRNA was slower than that of the wild-type cells, and a significantly smaller amount of the mature 18 S rRNA was produced, whereas the amount of 25 S rRNA produced in the Faf1p-depleted cells appeared to be greater than that in the wild-type cells under the experimental conditions (Fig. 4B, lanes 13–16). When KM804 (GAL-FAF1) cells were cultured in galactose medium, amounts of 25 and 18 S rRNAs similar to those in wild-type cells were produced after a 20-min chase, although processing of pre-rRNA appeared to be slower than in wild-type cells (Fig. 4B, lanes 9–12). Northern analysis showed the steady state levels of the mature rRNAs and pre-rRNAs in wild-type and Faf1p-depleted cells after the shift to glucose medium for various periods of time (Fig. 4C). Following transfer of the GAL-FAF1 strain to glucose medium, abnormal 23 S RNA was detected, and the steady state levels of both 20 S rRNA and mature 18 S rRNA were reduced with time of incubation in glucose medium. On the other hand, the levels of 27 S pre-rRNA and mature 25 S rRNA were apparently unchanged by depletion of Faf1p. The amount of 18 S rRNA was reduced to 22%, and that of 25 S rRNA to 87% after 24 h culture in glucose medium (Fig. 4C, d). These results are consistent with the result of the [methyl-\(^{3}H\)]methionine pulse-chase analysis (Fig. 4B).
Utp11p supports this idea. Utp11p was identified with 16 other new proteins (Utp1–17p) by affinity purification as a complex co-immunoprecipitated U3 snoRNA (3). Utp11p was detected in the 90 S particle but not in the pre-40 S particle, like most of the factors associated with 35 S rRNA (12). However, Faflp has not been detected in any TAP complex. Faflp might be degraded during affinity purification because it appears highly unstable in vitro. The signal of Myc-Faflp is observed in the cytoplasm as well as in the nucleus, suggesting that Faflp is associated with the pre-40 S subunit until it is exported from the nucleus to the cytoplasm. It remains to be elucidated whether Faflp is required for the export of pre-40 S subunits.

On the other hand, Ebp2p was identified in pre-60 S complexes purified using TAP-Ssf1p (8) or TAP-Nop7p (28) but not in 90 S particle. Whether Faf1p is required for the export of pre-40 S subunits.

It remains to be elucidated why the cells grow very slowly when Faflp is overproduced.

Interestingly, a defect in 40 S synthesis by depletion of Faflp had little effect on 60 S synthesis. This indicates that the synthesis of the 60 S subunits is not regulated by concentration of the 40 S subunits. In contrast, defects in 60 S biogenesis severely affect 40 S biogenesis. For example, we demonstrated that cells depleted with either Ebp2p or Rrs1p has decreased levels of not only 25 S rRNA but also 18 S rRNA, although the steady state level of 25 S rRNA declined more rapidly than that of 18 S rRNA, indicating that the decreased level of 25 S rRNA leads to degradation of 18 S rRNA (14, 22). This difference suggests that the accumulation of free 40 S subunits, but not 60 S subunits, is harmful to the cell.

We have shown that Rps16p, ribosomal protein S16, interacts with both Utp11p and Faflp, suggesting that these proteins recruit Rps16p to the ribosomal particle. We previously proposed that Rrs1p and Rps2p recruit Rpl11p to pre-60 S ribosomal particle (24, 33). Ebp2p interacts with Rrs1p but not with either Rps2p or Rpl11p (33). Interestingly, a cryo-electron microscopy reconstruction of the translating 80 S ribosome suggested that both Rpl11p and Rps16p interact with the P site-bound peptidyl-tRNA (34). The T loop of tRNA at the P-site interacts with Rpl11p, and Rps16p is likely to interact with the anticodon stem-loop of tRNA. It is possible that Ebp2p coordinates 40 S and 60 S biogenesis via interactions with the Faflp

Utp11p complex and Rrs1p-Rpf2p complex, both of which recruit important ribosomal proteins.

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REFERENCES

1. Warner, J. R. (1989) Microbiol. Rev. 53, 256–271
2. Woolford, J. L., Jr., and Warner, J. R. (1991) in The Molecular and Cellular Biology of the Yeast Saccharomyces: Genome Dynamics, Protein Synthesis, and Energetics (Braich, J. R., Pringle, J. R., and Jones, E. W., eds) pp. 587–626, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
3. Dragon, F., Gallagher, J. E. G., Compagnone-Post, P. A., Mitchell, B. M., Prowancher, K. A., Wehner, K. A., Wormsley, S., Settleig, R. E., Shabanowitz, J., Osheim, Y., Beyer, A. L., Hunt, D. F., and Baserga, S. J. (2002) Nature 417, 967–970
4. Udenn, S. A., and Warner, J. R. (1972) J. Mol. Biol. 65, 227–242
5. Cusack, R., Dencher, V., Chen, J. P., Petkalski, E., Straus, D., Marzich, M., Schäfer, T., Kuster, B., Tschochner, H., Tellervog, D., Gavrin, A.-C., and Hurt, E. (2002) Mol. Cell 10, 105–115
6. Kressler, D., Linder, P., and de la Cruz, J. (1999) Mol. Cell. Biol. 19, 7897–7912
7. Venema, J., and Tollervey, D. (1999) Annu. Rev. Genet. 33, 261–311
8. Fatica, A., Cronshaw, A. D., Dlamic, M., and Tollervey, D. (2002) Mol. Cell 9, 341–351
9. Wehner, K. A., and Baserga, S. J. (2002) Mol. Cell 9, 329–339
10. Baier, J., Grandi, P., Gadal, O., Lessmann, T., Petkalski, E., Tollervey, D., Lechner, J., and Hurt, E. (2001) Mol. Cell 8, 517–529
11. Nunn, T. A., Baier, J., Petkalski, E., Tollervey, D., and Hurt, E. (2002) EMBO J. 21, 5539–5547
12. Schäfer, T., Strauss, D., Petkalski, E., Tollervey, D., and Hurt, E. (2003) EMBO J. 22, 1370–1380
13. Tschochner, H., and Hurt, E. (2003) Trends Cell Biol. 13, 255–263
14. Tsujii, R., Miyoshi, K., Tsuno, A., Matsu, Y., Toh-e, A., Miyakawa, T., and Mizuta, K. (2000) GeneS Cells 3, 543–553
15. Mager, W. H., Planta, R. J., Ballesta, J.-P. G., Lee, J. C., Mizuta, K., Suzuki, K., Warner, J. R., and Woolford, J. L., Jr. (1997) Nucleic Acids Res. 25, 4872–4875
16. Sinko, R. S., and Hieter, P. (1989) Genetics 122, 19–37
17. Kasser, C., Michaelis, S., and Mitchell, A. (1994) Methods in Yeast Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
18. Ito, H., Fukuoka, Y., Murata, K., and Kinuma, A. (1993) J. Bacteriol. 175, 163–168
19. Hollenberg, S. M., Sternaglans, R., Cheng, P. F., and Weintraub, H. (1995) Mol. Cell. Biol. 15, 3813–3822
20. Durfee, T., Becherer, K., Chen, P. L., Yeh, S. H., Yang, Y., Kilburn, A. E., Lee, W. H., and Elledge, S. J. (1993) Genes Dev. 7, 555–569
21. Pringle, J. R., Adams, A. E. M., Drubin, D. G., and Haarer, B. K. (1991) Methods Enzymol. 194, 565–602
22. Tsuno, A., Miyoshi, K., Tsuno, A., Matsu, Y., Toh-e, A., Miyakawa, T., and Mizuta, K. (2000) Mol. Cell. Biol. 20, 2096–2074
23. Seedorf, M., Danem, M., Kahan, J., Taura, T., and Silver, P. A. (1999) Mol. Cell. Biol. 19, 1547–1557
24. Miyoshi, K., Tsuno, R., Yoshida, H., Maki, Y., Wada, A., Matsu, Y., Toh-e, A., and Mizuta, K. (2002) J. Biol. Chem. 277, 18334–18339
25. Miyoshi, K., Miyakawa, T., and Mizuta, K. (2001) Nucleic Acids Res. 29, 3297–3303
26. Kaser, A., Bogen, E., Haallegger, M., Deppner, E., Lepper, G., Jantsch, M., Breitenbach, M., and Kreil, G. (2001) Biol. Chem. 382, 1637–1647
27. Daugeron, M. C., Kressler, D., and Linder, P. (2001) RNA 7, 1317–1334
28. Harnpichcharn, P., Jakoljevic, J., Horsey, E., Miles, T., Roman, J., Rout, M., Meagher, D., Imai, B., Gao, Y.,.where, C. J., Shabanowitz, J., Hunt, D. F., and Woolford, J. L., Jr. (2001) Mol. Cell 8, 505–515
29. Wu, K., Wu, P., and Ariy, J. P. (2001) Nucleic Acids Res. 29, 2938–2949
30. Pintard, L., Kressler, D., and Lapuente, B. (2000) Mol. Cell. Biol. 20, 1370–1381
31. Mizuta, K., and Warner, J. R. (1994) Mol. Cell. Biol. 14, 2493–2502
32. Mougey, E. B., O’Reilly, M., Osheim, Y., Miller, O. L., Jr., Beyer, A., and Sullner-Weib, B. (1993) Genes Dev. 7, 1609–1619
33. Morita, D., Miyoshi, K., Matsu, Y., Toh-e, A., Shinakawa, H., Miyakawa, T., and Mizuta, K. (2002) J. Biol. Chem. 277, 28780–28786
34. Spahn, C. M. T., Beckmann, R., Ewar, N., Penczek, P. A., Sali, A., Bloedel, G., and Frank, J. (2001) Cell 107, 373–386

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