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Disruption of Single-Copy Genes Encoding Acidic Ribosomal Proteins in *Saccharomyces cerevisiae*

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Using the cloned genes coding for the ribosomal acidic proteins L44 and L45, constructions were made which deleted part of the coding sequence and inserted a DNA fragment at that site carrying either the *URA3* or *HIS3* gene. By gene disruption techniques with linearized DNA from these constructions, strains of *Saccharomyces cerevisiae* were obtained which lacked a functional gene for either protein L44 or protein L45. The disrupted genes in the transformants were characterized by Southern blots. The absence of the proteins was verified by electrofocusing and immunological techniques, but a compensating increase of the other acidic ribosomal proteins was not detected. The mutant lacking L44 grew at a rate identical to the parental strain in complex as well as in minimal medium. The L45-disrupted strain also grew well in both media but at a slower rate than the parental culture. A diploid strain was obtained by crossing both transformants, and by tetrad analysis it was shown that the double transformant lacking both genes is not viable. These results indicated that proteins L44 and L45 are independently dispensable for cell growth and that the ribosome is functional in the absence of either of them.

The large ribosomal subunit from all organisms contains a set of very acidic proteins which are required for ribosome activity during protein synthesis (see references 6 and 28 for reviews). In *Escherichia coli*, the acidic L7 and L12 proteins (L7 is the N-acetylated form of L12), present as two dimers in the ribosome (44), are needed for supernatant factor-dependent reactions (3, 4, 7, 14), and it has been proposed that they are involved in the transduction of energy required for tRNA translocation (14). No functional significance for the presence of the two forms of the bacterial acidic protein L7/L12 has been found, although changes in their ratio have been detected during the cell growth cycle (35). Both forms show similar functional activities when tested in in vitro systems, and moreover, a mutation that inactivates the acetylase activity responsible for L7 formation does not affect the cell growth (11).

In eucaryotic organisms, the acidic proteins are found as a family of phosphorylated polypeptides having similar physicochemical properties but different amino acid compositions (13, 45, 46). They are encoded by independent genes (5, 19, 25, 26, 34, 36, 37, 48). A large pool of these proteins is present in the cytoplasm (24, 40, 46), and there is evidence indicating an exchange between the acidic proteins on the ribosome and those in the cytoplasm (51).

In *Saccharomyces cerevisiae*, the existence of acidic ribosomal proteins has been reported by different groups (15, 23, 25, 31, 42, 50, 51) and three different proteins, L44, L45, and L44′, have been found (13). From their gene sequences (30, 36), it has been shown that the three polypeptides have an almost identical amino acid sequence at the carboxyl end, comprising the last 30 amino acids, but are significantly different over the rest of the molecule. Nevertheless, proteins L44 and L45 are more similar to each other (about 80% similarity), including 10 identical amino acids at the amino terminal, than they are to protein L44′ (36). The previously reported *S. cerevisiae* protein A1 (12) corresponds to protein L45.

When comparing the acidic proteins from different species, proteins L44 and L45 are found to have a high degree of similarity to the acidic ribosomal protein P2 from rat and human liver (17, 37), *Artemia salina* eL12 (19), *Drosophila melanogaster* A1 (34), and *Schizosaccharomyces pombe* A1 (5). On the other hand, protein L44′ resembles protein P1 (Y.-L. Cham and I. G. Wool, personal communication), *A. salina* eL12′ (19), and *D. melanogaster* rp21c (48). A gene encoding an acidic protein that shows a predicted amino acid sequence highly similar to that of L44′ has recently been cloned from *S. cerevisiae* (25, 30); however, the protein has not been identified. It seems, therefore, that two highly conserved families of acidic proteins exist in eucaryotes but that yeasts are exceptional in having probably two members of each family.

It has been generally assumed that the acidic proteins from eucaryotic ribosomes play a role similar to that of the bacterial L7/12 (20, 29, 42), a view which is supported by the interchangeability of acidic proteins from both cell types (41). Consequently, the existence of different acidic proteins could be considered irrelevant from the point of view of ribosome function, as in bacteria. However, after seeing the notable differences in the amino acid sequences of the different acidic proteins, their functional similarity can be seriously questioned.

Taking advantage of their cloned genes (36), we succeeded in preparing *S. cerevisiae* strains lacking protein L44 or L45 using gene disruption techniques. The results of an analysis of the transformed cells are presented in this report.

MATERIALS AND METHODS

Enzymes and reagents. Restriction endonucleases were obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.), New England BioLabs, Inc. (Beverly, Mass.), and Amersham Corp. (Arlington Heights, Ill.) and were used as recommended by the suppliers. T4 DNA ligase, calf intestinal phosphatase, and DNA polymerase I Klenow fragment were from Boehringer, and DNA polymerase I and T4 DNA polymerase were from New England BioLabs.

[α-^{32}P]dCTP (3,000 Ci/mmol) was purchased from Amersham Corp.

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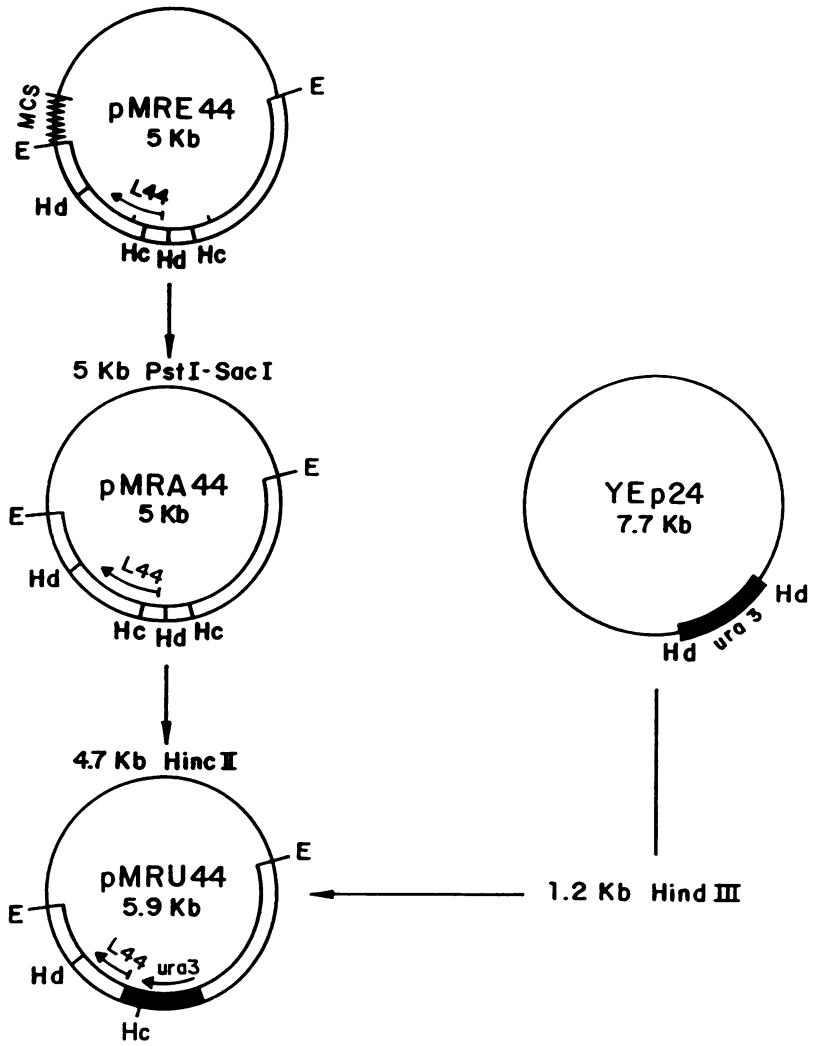

FIG. 1. Scheme of the preparation of plasmid pMRU44 as detailed in Materials and Methods. The *Eco*RI fragment from pMRU44 was used for disruption of protein L44 gene. E, *Eco*RI; Hd, *Hin*dIII; Hc, *Hin*cII; MCS, multiple cloning sites.

Bacterial and yeast strains. *E. coli* JM83 was used for cloning steps. *S. cerevisiae* XV617 (**a** *leu2 trp1 ura3 his2*), 142 (**a** *his3 can1*), and W303-1B (α *leu2 trp1 ura3 his3 ade2 can1*) were used as required. Bacterial cells were grown in LB medium, and the yeasts were grown in either YEPD rich medium (1% yeast extract, 2% peptone, 2% glucose) or minimal SD medium (43).

Yeast genetic manipulations. Mating, sporulation, and tetrad analysis were performed by standard techniques (43).

Medium shifts in growing. Cells growing in a given medium (either YEPD or SD) were filtered through sterile Millipore nitrocellulose filters. The filters were washed with new prewarmed medium and placed in a flask containing the same prewarmed medium, and the cells were resuspended by agitation.

Bacterial and yeast transformations. *E. coli* cells were transformed by the method of Hanahan (8), and *S. cerevisiae* cells were transformed by the method of Hinnen et al. (9).

Construction of plasmids. The plasmid DNA used for L44 gene disruption was constructed as indicated in the scheme in Fig. 1. Plasmid pMRE44 (36) contains a 2.3-kilobase (kb) insert carrying the L44 gene in the *Eco*RI site of plasmid pUC19 (49). pMRA44 was derived from pMRE44 by elimination of the multiple cloning site region by digestion with *Sac*I and *Pst*I and making blunt ends with T4 DNA polymerase before ligation. The *URA3* gene was obtained as a 1.1-kb *Hin*dIII fragment from plasmid YEp24 (33) and was inserted, after blunt ends were made with the Klenow DNA polymerase I fragment, between the two *Hin*cII sites of pMRA44, forming plasmid pMRU44. The 0.3-kb *Hin*cII fragment removed from pMRA44 comprises the first 150 nucleotides of the structural gene and 149 nucleotides from the upstream region (36).

The construction for L45 gene disruption is shown in Fig. 2. The plasmid pRVE45 was obtained by inserting a 3.75-kb DNA fragment carrying the L45 gene into the *Eco*RI site of pUC18 (36). pMRA45 was derived from pRVE45 by *Hin*cII treatment to remove most of the multiple cloning site region including the *Sac*I site. Then the pMRA45 *Eco*RI-*Ban*II fragment carrying the 3′ region of the L45 gene was inserted in pUC18 to obtain pMRB45. The 1.2-kb *Pst*I fragment from pMRA45 containing the L45 5′-flanking region was cloned in the *Pst*I site of pMRB45, obtaining pMRC45. This plasmid carries a yeast DNA insert containing a partially deleted L45 gene which lacks 1 kb of the 5′-flanking sequence and the first 150 nucleotides in the coding region, introducing at the same time a multiple cloning site where the 1.7-kb *Bam*HI

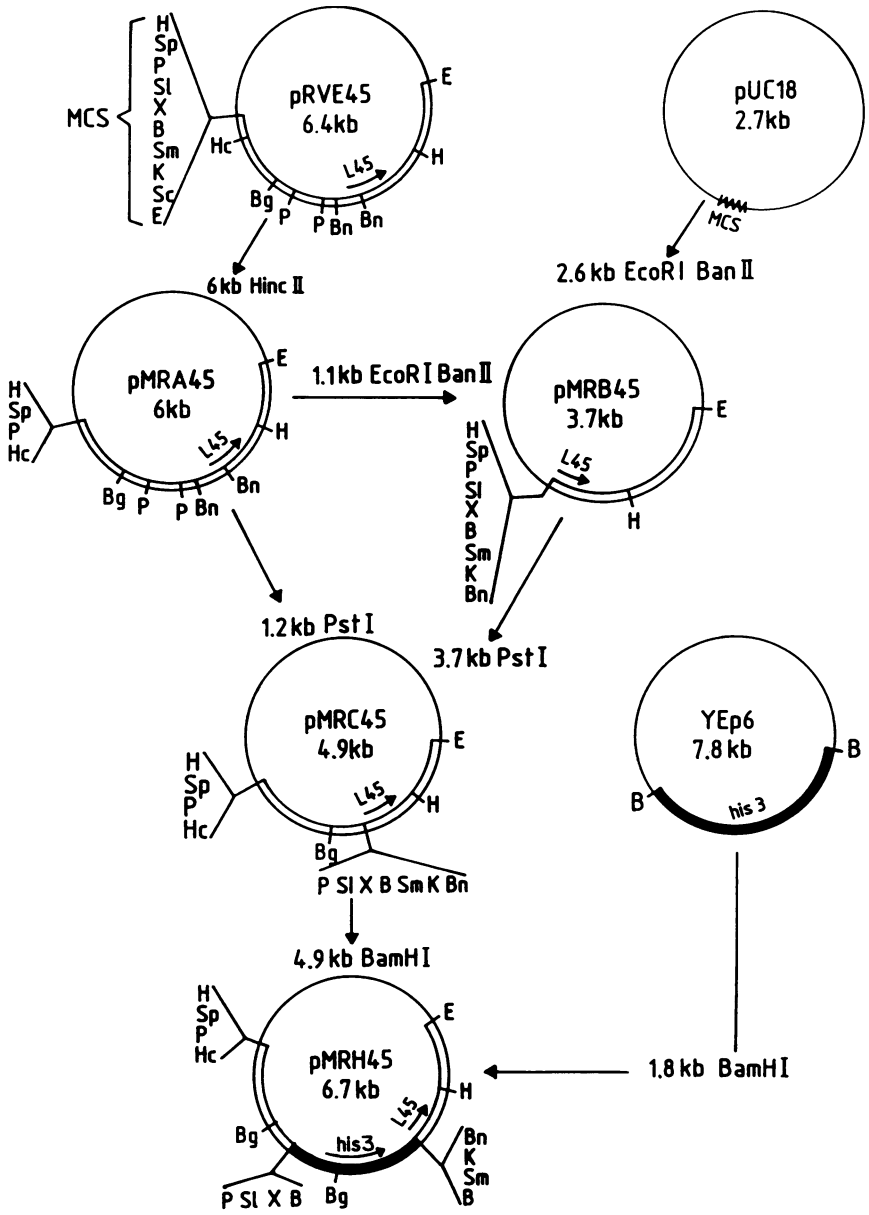

FIG. 2. Scheme of the preparation of plasmid pMRH45 as detailed in Materials and Methods. The *Eco*RI-*Sph*I fragment from pMRH45 was used for disruption of protein L45 gene. B, *Bam*HI; Bn, *Ban*II; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; Hn, *Hin*cII; D, *Dra*I; P, *Pst*I; Sp, *Sph*I; Sl, *Sal*I; X, *Xba*I; Sm, *Sma*I; K, *Kpn*I; Sc, *Sac*I.

fragment from YEp6 (33) containing the *HIS3* gene was cloned, yielding PMRH45.

Gene disruption method. *S. cerevisiae* transformants having a disrupted L44 gene were obtained by the method of Rothstein (39) with 4 μg of either the 3.1-kb *Eco*RI fragment from plasmid pMRU44 (Fig. 1) or the 4-kb *Eco*RI-*Sph*I fragment from pMRH45 (Fig. 2).

Southern blots. Total DNA from yeasts was prepared as previously described (10). DNA (3 μg) was digested with different restriction enzymes, fractionated in 0.8% agarose gels, and transferred to nylon membranes (Amersham Corp.). Hybridization was done by standard procedures (22).

Preparation of ribosomes, supernatant fractions, and ribosomal acidic proteins. Ribosomes and supernatant fractions (S-100) were obtained from cells growing exponentially in YEPD medium as previously described (42). The acidic proteins were extracted from the ribosomes by washing with 1.5 M NH_4Cl–50% ethanol, providing an $SP_{1.5}$ fraction which contains the total acidic protein from the particles (42). In some cases, total cell extracts obtained after centrifugation at 10,000 rpm for 10 min (in a Sorvall S-34 rotor) of a cell suspension broken with glass beads were used for estimation of the total acidic protein content of the cells.

Immunological techniques. The presence of acidic proteins in cell extracts was detected with monoclonal antibodies (MAbs) specific for each one of the acidic proteins (M. D. Vilella, M. Remacha, and J. P. G. Ballesta, unpublished data) by inhibition enzyme-linked immunosorbent assay techniques. $SP_{1.5}$ fractions containing the three acidic proteins were adsorbed to plastic plates and made to react with a fixed amount of antibody previously treated with increas-

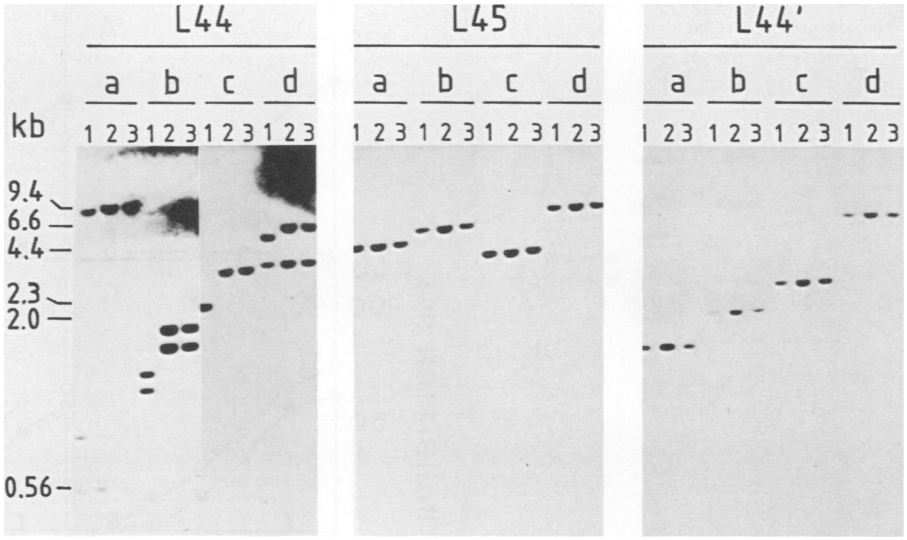

FIG. 3. Southern blot of DNA from *S. cerevisiae* XV617 (lanes 1) and transformants XVΔ44.2 and XVΔ44.5 (lanes 2 and 3) digested with *Hin*dIII (a), *Hin*cII (b), *Eco*RI (c), and *Pvu*II (d). ^{32}P-labeled DNA inserts carrying the genes for L44, L45, and L44′ were used as probes as indicated. The molecular marker positions are indicated on the left.

ing dilutions of the sample to be tested. The specific protein in the sample cross-reacted with the antibody, diminishing the amount of immunoglobulin G available to react with the plastic-bound protein. The samples inhibited cross-reaction with the bound protein proportional to their acidic protein content. The bound immunoglobulin G was afterward estimated with a peroxidase-linked second antibody.

Electrophoretic methods. Acidic proteins were resolved on 5% polyacrylamide electrofocusing gels with ampholytes (Pharmacia, Uppsala, Sweden) in the 2.5 to 5.0 pH range (13). Proteins were detected by silver staining.

RESULTS

Transformation of *S. cerevisiae* strains. Using linearized DNA from pMRU44 (Fig. 1) carrying a partially deleted form of the gene encoding protein L44 as well as the *URA3* gene as a genetic marker, *S. cerevisiae* XV617 was transformed. Three Ura$^+$ colonies were obtained when the transformed cells were grown on uracyl-deprived medium. Similarly, five His$^+$ colonies were obtained when *S. cerevisiae* 142 was transformed with DNA carrying a partially truncated L45 gene and *HIS3* from plasmid pMRH5 (Fig. 2).

Southern analysis of DNA of transformed strains. DNA prepared from Ura$^+$ and His$^+$ colonies (strains XVΔ44.2 and 142Δ45.1, respectively) as well as from the corresponding parental strains was treated with different restriction enzymes and resolved by agarose gel electrophoresis. Nylon blots of the gels were hybridized with the 2.3-kb *Eco*RI-*Eco*RI fragment from pMRE44, the 3.4-kb *Eco*RI-*Hin*cII fragment from pMRA45, and the 1.9-kb *Hin*dIII-*Hin*dIII fragment from pMRH46 (36) containing the individual protein L44, L45, and L44′ genes, respectively. The hybridization pattern was different for the disrupted genes (Fig. 3 and 4), indicating the presence of an altered physical map in this region of the DNA. The sizes of the new hybridizing DNA fragments agreed with bands expected from the restriction map of the inserted DNA. There were no differences in the hybridization patterns of the parental and the transformed strains when the nondisrupted gene probes were used.

Analysis of acidic proteins. The acidic proteins in the ribosomes from the parental and the transformed strains were extracted and resolved by isoelectrofocusing. Proteins L44 and L45 were not detected in the particles from the corresponding transformed strains (Fig. 5). Interestingly, a compensating increase of the other acidic proteins seemed not to take place in the ribosomes from the disrupted strains. In fact, in both cases a decrease of protein L44′ could be detected (Fig. 5).

The absence of these proteins was confirmed by using MAbs specific for each acidic protein. The anti-protein L45 MAb recognizes an epitope located between amino acid

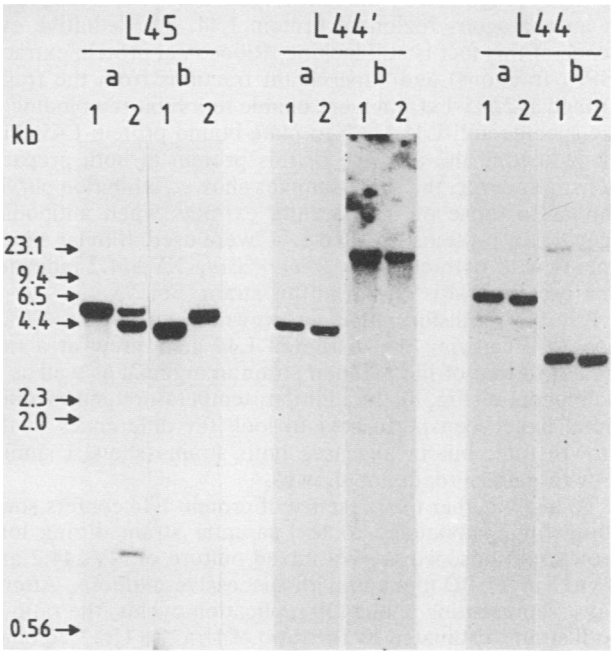

FIG. 4. Southern blot of DNA from *S. cerevisiae* 142 (lanes 1) and transformant 142Δ45.1 (lanes 2) digested with *Bgl*II (a) and *Eco*RI (b). The DNA probes used are described in the legend to Fig. 3.

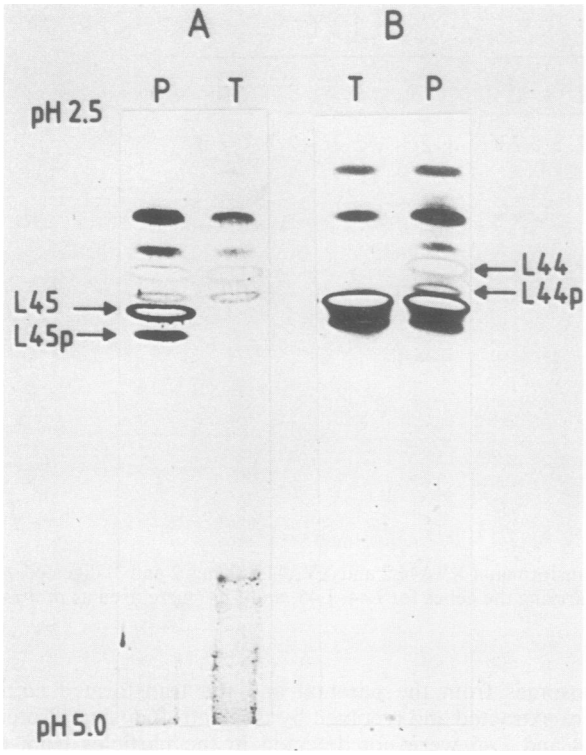

FIG. 5. Polyacrylamide isoelectrofocusing gels (pH range 2.5 to 5.0) of acidic ribosomal proteins from cells having the protein L45 gene (A) and the protein L44 gene (B) disrupted. Proteins were extracted from ribosomes of the transformed (T) and the corresponding parental (P) strains. L44p and L45p indicate the phosphorylated form of the proteins.

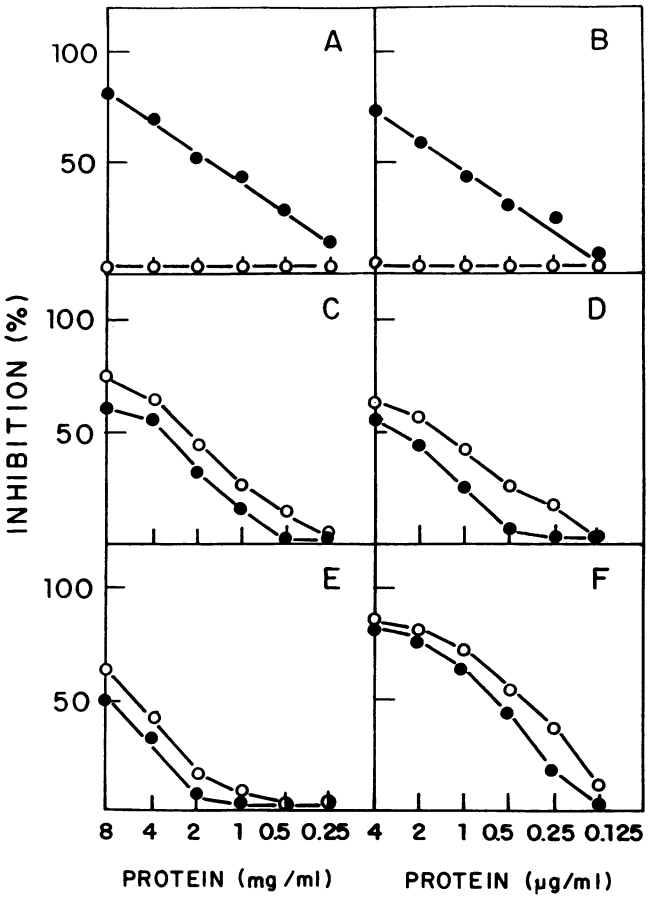

FIG. 6. Detection of acidic ribosomal proteins in protein L45 gene-disrupted strain 142Δ45.1 by enzyme-linked immunosorbent assay. A fixed amount of MAb specific for protein L45 (A and B), protein L44 (C and D), and protein L44′ (E and F) was treated with increasing concentrations of either S-100 cell supernatant (A, C, and E) or $SP_{1.5}$ ribosomal fractions (B, D, and F) from the parental strain (●) and the transformant strain 142Δ45.1 (○). The treated antibodies were then incubated with a fixed amount of plastic-bound acidic proteins, and the immunoglobulin G retained in the plate was estimated by using peroxidase-labeled goat anti-mouse immunoglobulin G as the second antibody. The percent inhibition, taking the untreated antibody as a control, is proportional to the amount of acidic protein in the tested sample.

residues 64 and 86 (Vilella et al., unpublished data). The epitope recognized by the anti-L44 MAb is probably located in an analogous region of protein L44, but definitive evidence of this fact is still lacking. Ribosomal protein extracts ($SP_{1.5}$ fractions) and supernatant fractions from the transformed 142Δ45.1 strain were unable to inhibit the binding of the specific anti-L45 MAb to plate-bound protein L45 (Fig. 6), indicating the absence of this protein in both preparations. However, the same samples showed inhibition curves similar to those of the parental extracts when antibodies specific for proteins L44 and L44′ were used. Similar experiments with extracts from *S. cerevisiae* XVΔ44.2 indicated that protein L44 is absent in this strain (Fig. 7).

Effect of transformation on growth rate of cells. Strain XVΔ44.2 carrying the disrupted L44 gene grew at a rate similar to that of the parental strain in minimal as well as in rich medium (Fig. 8). In addition, temperature and medium shifts have been performed to look for differences in the growth rate, but in all cases both strains show a similar growth pattern (data not shown).

To test whether the presence of protein L44 confers some competitive advantage to the parental strain during long growth periods, we grew a mixed culture of XVΔ44.2 and XV617 in YEPD medium with successive dilutions. After 8 days, representing about 130 replication cycles, the ratio of both strains estimated by the ratio of Ura^- to Ura^+ colonies present in the culture was unchanged.

On the other hand, *S. cerevisiae* 142Δ45.1, lacking protein L45, grew at a slower rate than the parental strain in either rich or minimal medium (Fig. 8).

Genetic analysis of transformants. *S. cerevisiae* XVΔ44.2 was crossed with wild-type *S. cerevisiae* W303, giving a D4 diploid strain that was grown and sporulated. Seven asci were dissected, and the segregation of the Ura3 marker was checked. As expected, 50% of the total D4 spores were $Ura3^+$. These spores were also deficient in protein L44 as measured with specific MAbs and showed the same growth rate as the wild-type strain.

A similar analysis was performed with *S. cerevisiae* 142Δ45.1. This strain was crossed with *S. cerevisiae* W303, and the resulting D5 diploid was sporulated. Dissection of eight asci indicated that 50% of the spores were $His3^+$ and grew at a slower rate than those having the $His3^-$ phenotype. Using the anti-L45 MAb, it was confirmed that protein L45 was absent from all the His^+ colonies (data not shown).

Analysis of a double transformant. *S. cerevisiae* D4.33 (**a** *rpL44*::*URA3*) and D5.72 (α *rpL45*::*HIS3*) were crossed, producing a diploid strain that did not show any growth alteration. After sporulation, a tetrad analysis of 10 asci was

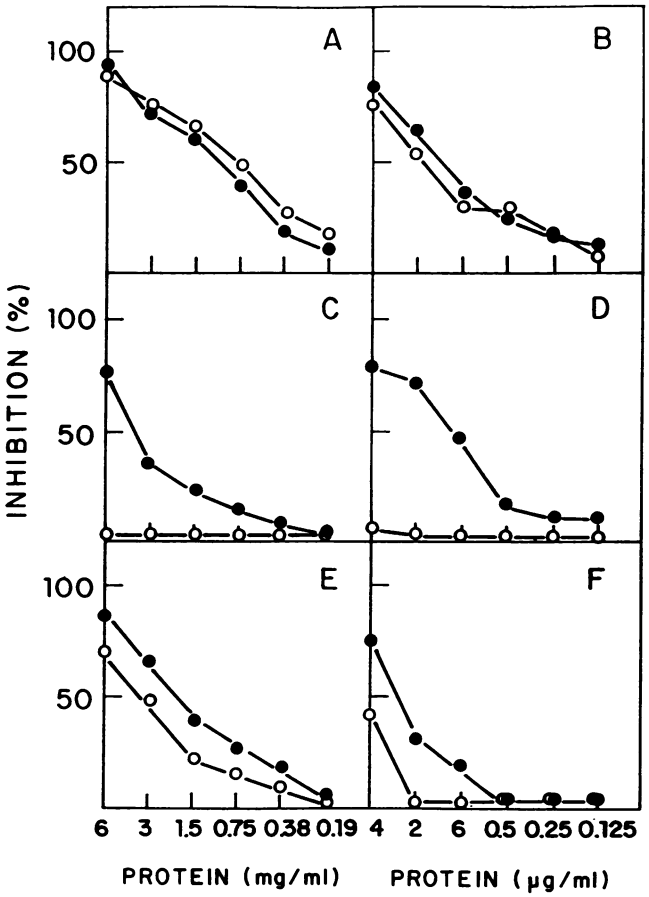

FIG. 7. Detection of acidic proteins in protein L44 gene-disrupted strain XVΔ44.2 by enzyme-linked immunosorbent assay. Procedure and symbols are as described in the legend to Fig. 6.

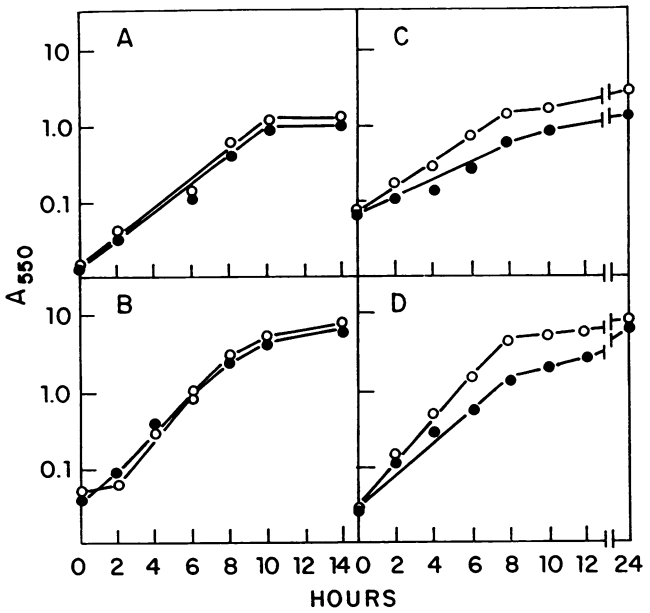

FIG. 8. Growth curves of transformants *S. cerevisiae* XVΔ44.2 (A and B) and 142Δ45.1 (C and D) and their corresponding parental strains XV617 and 142, respectively, in minimal (A and C) and in rich (B and D) media. Symbols: ●, transformant; ○, parental strains.

performed. As summarized in Fig. 9, segregation of the genetic markers corresponded to the expected pattern. The Ura3$^+$ phenotype was coincident with the absence of L44 (27% of the spores), and the His3$^+$ phenotype was coincident with the absence of L45 (23% of the spores). A total of 25% of the spores were Ura3$^-$ and His3$^-$ and contained both L44 and L45 proteins as determined by enzyme-linked immunosorbent assay. A total of 25% of the spores did not grow, and they are thought to correspond to those having the Ura3$^+$ and His3$^+$ phenotype, which would be deficient in both L44 and L45 genes.

DISCUSSION

The results showed that it was possible to obtain yeast strains lacking either the protein L44 gene or the protein L45 gene by transforming the parental cells with linear DNA carrying partially deleted genes which lack part of the 5′-flanking region as well as part of the amino-terminal-coding sequence. Transformants lacking the protein L44 gene were perfectly viable, while the absence of the protein L45 gene moderately inhibited cell growth. Double transformants lacking both genes were not viable.

Using Southern blots, it was shown that in both cases the genome is altered in the region of the gene for the corresponding protein, while the genes for the other acidic proteins remained unaffected.

The absence of the proteins from the ribosomes was confirmed directly by isoelectrofocusing and immunological techniques with monoclonal specific antibodies.

There are data indicating that an interrupted gene coding for ribosomal protein rp51 which conserves an intact 5′-flanking region but encodes approximately 75% of the protein amino acid sequence is sufficient to preserve cell viability, albeit at a very low growth rate (2). Although our results do not totally exclude the possibility that the remaining 3′ regions of the genes coding for the highly conserved carboxyl end of both proteins are transcribed, the probability that they are in addition translated in the correct phase is indeed low. For protein L45, this possibility can in fact be excluded and probably for protein L44 as well, since the peptides would be recognized by the corresponding MAbs. Moreover, unpublished data from our laboratory with β-galactosidase-fused acidic protein fragments suggest that the amino terminal of the acidic proteins, which is missing in the truncated genes, is critical for interaction with the ribosome (M. Remacha, S. Zinker, J. M. Payo, and J. P. G. Ballesta, unpublished data). Finally, the comparatively small effect of disrupting individual genes on cell growth and especially the inviability of the double transformant makes it highly improbable that, as for protein rp51, the remaining fragments of genes L44 and L45 are responsible for the phenotypes of the corresponding transformants.

The fact that the double-gene disruption is lethal while the interruption of only one of the genes has no dramatic effect on cell growth might indicate that proteins L44 and L45 play the same role in protein synthesis and ribosome structure. However, an identical role for L44 and L45 working like functionally duplicated genes is unexpected considering the differences in their amino acid sequences (30, 36) since, although the yeast ribosomal proteins are usually encoded by duplicated genes, they show identical coding sequences (16, 27).

Alternatively, proteins L44 and L45 might have a cooperative effect on the ribosome activity. If so, the absence of

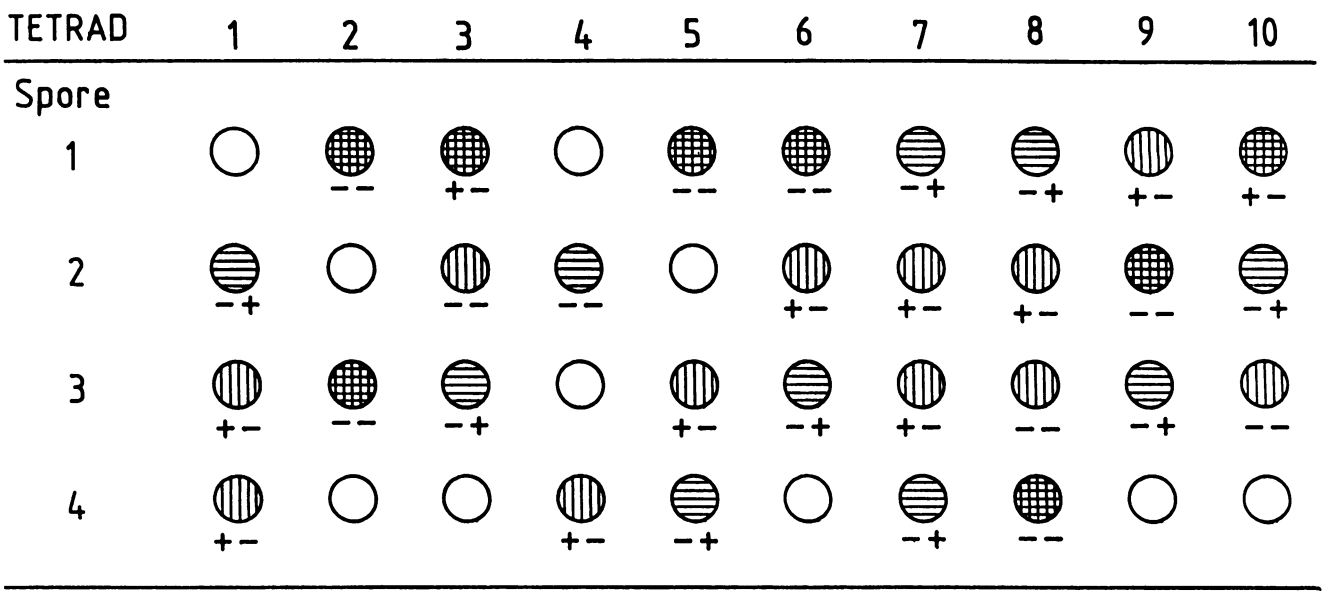

FIG. 9. Tetrad analysis of diploid strain obtained by crossing protein L45 gene-disrupted (*S. cerevisiae* D5.72) and protein L44 gene-disrupted (*S. cerevisiae* D4.33) strains. Each circle corresponds to one spore. Empty circles, Lack of growth; vertical bars, only protein L44 present; horizontal bars, only protein L45 present; crossed bars, both proteins present. The signs below the circles indicate the genetic characteristic of the spores related to the His (left) and Ura (right) markers.

each protein alone would only slightly affect the structure of the particles, having no or a weak effect on the cell growth, but the absence of both could cause lethal structural changes. The fact that the absence of either L44 or L45 in the ribosomes of the disrupted strains (Fig. 5) was not compensated by an increase of the other protein is compatible with this interpretation.

The lack of a compensating increase of the nondisrupted proteins in the ribosome, despite their being present in the cytoplasmic pool, seems to indicate that the interaction site in the particle is very specific for each protein and that they are not structurally interchangeable.

On the other hand, since the acidic proteins have been shown to exchange during protein synthesis (51), the negative effect on the cell growth caused by the absence of protein L45 might reflect the higher concentration of this protein in the cytoplasmic pool which has been observed with the specific MAb (Vilella et al., unpublished data). The lower amount of L44 is probably not sufficient to keep the protein-synthesizing machinery at the required speed for a maximum growth rate. The cell seems unable to compensate for the lack of protein L45 by producing higher amounts of protein L44, indicating that both genes seem to be independently controlled.

An analogous situation has been reported for other ribosomal proteins encoded by duplicated genes in which both gene copies are controlled independently and usually expressed to a different extent (32, 47). Thus, no intergenic or intragenic dosage compensation has been detected after deleting one of the two genes coding for protein rp51 (1), L16 (38), or L2 (18), which usually causes a negative effect on the growth rate. Moreover, for rp28, the deletion of the most abundantly expressed copy seems to be a lethal event for the cell (Pearson, cited in reference 21).

A very interesting consequence of our results is that the third acidic protein, L44′, as well as the protein encoded by the fourth reported gene (25, 30) seem not to be able to functionally substitute for proteins L44 and L45. Although alternative explanations are possible, the most obvious conclusion is that these proteins have a different functional role. This conclusion is supported by the low concentration of L44′ detected in the cytoplasmic pool (Vilella et al., unpublished data), suggesting that contrary to L44 and L45, protein L44′ does not exchange during protein synthesis (51). Moreover, the overall structure of the L44′ gene, having a 300-nucleotide-long intron and a consensus UAS_{rpg}, resembles closely the standard ribosomal protein genes, while the L44 and L45 genes are clearly exceptional (36). These data suggest that protein L44′ has a more static role, being permanently bound to the ribosomal particle. In support of this view is the observation that, unlike L44 and L45, protein L44′ does not form dimers in solution (13).

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LITERATURE CITED

1. **Abovich, N., L. Gritz, L. Tung, and M. Rosbach.** 1985. Effect of RP51 gene dosage alterations on ribosome synthesis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **5**:3429–3435.
2. **Abovich, N., and M. Rosbach.** 1984. Two genes for ribosomal protein 51 of *Saccharomyces cerevisiae* complement and contribute to the ribosomes. Mol. Cell. Biol. **4**:1871–1879.
3. **Armstrong, I. L., and W. P. Tate.** 1980. The requirement for the *Escherichia coli* ribosomal proteins L7 and L12 in release factor-dependent peptide chain termination. FEBS Lett. **109**: 228–232.
4. **Ballesta, J. P. G., and D. Vazquez.** 1972. Reconstitution of 50S ribosome subunit. Role of proteins L7 and L12 in GTPase activities. Site of action of thiostrepton. FEBS Lett. **28**:337–342.
5. **Beltrame, M., and M. E. Bianchi.** 1987. Sequence of the cDNA for one acidic ribosomal protein of *Schizosaccharomyces pombe*. Nucleic Acids Res. **15**:9089.
6. **Bielka, H.** 1982. The eukaryotic ribosome. Springer-Verlag KG,

Berlin.
7. **Hamel, E., M. Koka, and T. Nakamoto.** 1972. Requirement of an *Escherichia coli* 50S ribosomal protein component for effective interaction of the ribosome with T and G factors and guanosine triphosphate. J. Biol. Chem. **246**:805–814.
8. **Hanahan, H. D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166**:557–580.
9. **Hinnen, A., J. B. Hicks, and G. R. Fink.** 1978. Transformation of yeast. Proc. Natl. Acad. Sci. USA **75**:1929–1933.
10. **Holm, C., D. W. Meeks-Wagner, W. L. Fangman, and D. Botstein.** 1986. A rapid efficient method for isolation of DNA from yeast. Gene **42**:169–173.
11. **Isono, S., and K. Isono.** 1981. Ribosomal protein modification in *Escherichia coli*. III. Studies of mutants lacking an acetylase specific for protein L12. Mol. Gen. Genet. **183**:473–477.
12. **Itho, T.** 1981. Primary structure of an acidic ribosomal protein YPA1 from *Saccharomyces cerevisiae*. Isolation and characterization of peptides and complete amino acid sequence. Biochim. Biophys. Acta **671**:16–24.
13. **Juan-Vidales, F., M. T. Saenz-Robles, and J. P. G. Ballesta.** 1984. Acidic proteins of the large ribosomal subunit in *Saccharomyces cerevisiae*. Effect of phosphorylation. Biochemistry **23**:390–396.
14. **Kisha, K., W. Moller, and G. Stöffler.** 1971. Reconstitution of GTPase activity of 50S ribosomal protein from *E. coli*. Nature (London) New Biol. **233**:62–63.
15. **Kruiswijk, T., and R. J. Planta.** 1975. Further analysis of the protein composition of yeast ribosomes. FEBS Lett. **58**:102–105.
16. **Leer, R. J., M. M. C. van Raamsdonk-Duin, C. M. T. Molenaar, M. A. Witsenboer, W. H. Mager, and R. J. Planta.** 1985. Yeast contain two functional genes coding for ribosomal protein S10. Nucleic Acids Res. **13**:5028–5039.
17. **Lin, A., B. Wittmann-Leibold, J. McNally, and I. G. Wool.** 1982. The primary structure of acidic phosphoprotein P2 from rat liver 60S ribosomal subunits. J. Biol. Chem. **257**:9189–9197.
18. **Lucioli, A., C. Presuti, S. Ciafre, E. Caffarelli, P. Fragapane, and I. Bozzoni.** 1988. Gene dosage alteration of L2 ribosomal protein genes in *Saccharomyces cerevisiae*: effects on ribosome synthesis. Mol. Cell. Biol. **8**:4792–4798.
19. **Maasen, J. A., E. N. Schop, J. H. G. Brands, F. J. van Hemert, J. A. Lenstra, and W. Möller.** 1985. Molecular cloning and analysis of cDNA sequences for two ribosomal proteins from *Artemia*: the coordinate expression of genes for ribosomal and elongation factor 1 during embryogenesis of *Artemia*. Eur. J. Biochem. **149**:609–616.
20. **MacConell, W. P., and N. O. Kaplan.** 1980. The role of ethanol extractable proteins from the 80S rat liver ribosome. Biochem. Biophys. Res. Commun. **92**:46–52.
21. **Mager, W. H.** 1988. Control of ribosomal protein gene expression. Biochim. Biophys. Acta **949**:1–15.
22. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Michel, S., R. R. Traut, and J. C. Lee.** 1983. Yeast ribosomal proteins: electrophoretic analysis in four two-dimensional gel systems; correlation of nomenclatures. Mol. Gen. Genet. **191**: 251–256.
24. **Mitsui, K., M. Motikuzi, Y. Endo, S. Yokota, and K. Tsurugi.** 1987. Characterization of a novel acidic protein of 38 kDa, A0, in yeast ribosomes which immunologically cross-reacts with the 13 kDa acidic ribosomal proteins A1/A2. J. Biochem. **102**: 1565–1570.
25. **Mitsui, K., and K. Tsurugi.** 1989. Identification of A1 protein as the fourth member of the 13 kDa-type acidic ribosomal protein family in yeast *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. **161**:1001–1006.
26. **Mitsui, K., and K. Tsurugi.** 1988. cDNA and deduced amino acid sequence of acidic protein A2 from *Saccharomyces cerevisiae*. Nucleic Acids Res. **16**:3575.
27. **Molenaar, C. M. J., L. P. Woudt, A. E. M. Jansen, W. H. Mager, R. J. Planta, D. M. Donovan, and N. J. Pearson.** 1984. Structure and organization of two linked ribosomal protein genes in yeast. Nucleic Acids Res. **12**:7345–7358.
28. **Möller, W., and J. A. Maassen.** 1986. On the structure, function and dynamics of L7/L12 from *Escherichia coli* ribosomes, p. 309–325. *In* B. Hardesty and G. Kramer (ed.), Structure, function and genetics of ribosomes. Springer-Verlag, New York.
29. **Möller, W., L. I. Slobin, R. Amons, and D. Richter.** 1975. Isolation and characterization of two acidic proteins of 60S ribosomes from *Artemia salina* cysts. Proc. Natl. Acad. Sci. USA **72**:4744–4748.
30. **Newton, C. H., J. Yee, L. C. Shimmin, and P. P. Dennis.** 1990. A family of genes encode the multiple forms of the *Saccharomyces cerevisiae* ribosomal proteins equivalent to the *Escherichia coli* L12 protein and a single form of the L10-equivalent protein. J. Bacteriol. **172**:579–588.
31. **Otaka, E., and S. Osawa.** 1981. Yeast ribosomal proteins. V. Correlation of several nomenclatures and proposal of a standard nomenclature. Mol. Gen. Genet. **181**:176–182.
32. **Planta, R. J., W. H. Mager, R. J. Leer, L. P. Woudt, H. A. Raue, and T. T. A. L. El-Baradi.** 1986. Structure and expression of ribosomal protein genes in yeast, p. 699–712. *In* B. Hardesty and G. Kramer (ed.), Structure, function and genetics of ribosomes. Springer-Verlag, New York.
33. **Pouwels, P. H., B. E. Enger-Valk, and W. J. Brammer.** 1985. Cloning vectors. A laboratory manual. Elsevier Science Publishers bv, Amsterdam.
34. **Qian, S., J.-Y. Zhang, M. A. Kay, and M. Jacobs-Lorena.** 1987. Structural analysis of Drosophila rpA1 gene, a member of the eukaryotic "A" type ribosomal protein family. Nucleic Acids Res. **15**:987–1003.
35. **Ramagopal, S., and A. R. Subramanian.** 1974. Alteration in acetylation level of ribosomal protein L12 during growth cycle of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **71**:2136–2140.
36. **Remacha, M., M. T. Saenz-Robles, M. D. Vilella, and J. P. G. Ballesta.** 1988. Independent genes coding for three acidic proteins of the large ribosomal subunit from *Saccharomyces cerevisiae*. J. Biol. Chem. **263**:9094–9101.
37. **Rich, B. E., and J. A. Steitz.** 1987. Human acidic ribosomal phosphoproteins P0, P1, and P2. Analysis of cDNA clones, in vitro synthesis, and assembly. Mol. Cell. Biol. **7**:4065–4074.
38. **Rotenberg, M. O., M. Moritz, and J. L. Woolford, Jr.** 1988. Depletion of *Saccharomyces cerevisiae* ribosomal protein L16 causes a decrease in 60S ribosomal subunits and formation of half-mer polyribosomes. Gen. Dev. **2**:160–172.
39. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
40. **Sanchez-Madrid, F., F. Juan-Vidales, and J. P. G. Ballesta.** 1981. Effect of phosphorylation on the affinity of acidic proteins from *Saccharomyces cerevisiae* for the ribosome. Eur. J. Biochem. **114**:609–613.
41. **Sanchez-Madrid, F., F. Juan-Vidales, and J. P. G. Ballesta.** 1981. Functional role of acidic ribosomal proteins. Interchangeability of proteins from bacteria and eukaryotic cells. Biochemistry **20**:3263–3266.
42. **Sanchez-Madrid, F., R. Reyes, P. Conde, and J. P. G. Ballesta.** 1979. Acidic ribosomal proteins from eukaryotic cells. Effect on ribosomal functions. Eur. J. Biochem. **98**:409–416.
43. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1983. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
44. **Subramanian, A. R.** 1975. Copies of proteins L7 and L12 and heterogeneity of the large subunit of *Escherichia coli* ribosome. J. Mol. Biol. **95**:1–8.
45. **Tsurugi, K., E. Collatz, K. Todokoro, N. Ulbrich, H. N. Lightfoot, and I. G. Wool.** 1978. Isolation of eukaryotic ribosomal proteins: purification and characterization of 60S ribosomal subunit proteins La, Lb, Lf, P1, P2, L13′, L14, L18′, L20 and L38. J. Biol. Chem. **253**:946–955.
46. **van Agthoven, A. J., J. Kriek, R. Amons, and W. Möller.** 1978. Isolation and characterization of acidic phosphoproteins of 60S ribosomes from *Artemia salina* and rat liver. Eur. J. Biochem. **91**:553–565.
47. **Warner, J. R., E. A. Elion, M. D. Dabeva, and W. F. Schwin-**

dinger. 1986. The ribosomal genes of yeast and their regulation, p. 719–732. *In* B. Hardesty and G. Kramer (ed.), Structure, function and genetics of ribosomes. Springer-Verlag, New York.

48. **Wigboldus, J. D.** 1987. DNA and deduced amino acid sequence of Drosophila rp21C, another "A"-type ribosomal protein. Nucleic Acids Res. **15**:10064.
49. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.
50. **Zinker, S.** 1980. P5/P5′, the acidic ribosomal phosphoproteins from *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **606**: 76–82.
51. **Zinker, S., and J. R. Warner.** 1976. The ribosomal proteins of *Saccharomyces cerevisiae*. J. Biol. Chem. **251**:1799–1807.