Isolation of Seventeen Proteins and Amino-Terminal Amino Acid Sequences of Eight Proteins from Cytoplasmic Ribosomes of Yeast†

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ABSTRACT: Seventeen ribosomal proteins of *Saccharomyces cerevisiae* were isolated from small ribosomal subunits and disodium ethylenediaminetetraacetate treated 80S ribosomes by chromatography on a column of carboxymethylcellulose and/or by filtration through Sephacryl S-200. The isolated proteins are YS4, YS7, YS8, YS9, YS10, YS12, YS14, YS18, YS23, YS29, YL11, YL13, YL16, YL17, YL22, YL38, and YL40 [nomenclature according to Otaka & Osawa (1981) [Otaka, E., & Osawa, S. (1981) *Mol. Gen. Genet. 181*, 176–182]]. The purification procedures and the amino acid compositions of these proteins are presented. Amino-terminal amino acid sequences of YS4, YS6, YS11, YS15, YS16, YS22, YL10, and YL31 have been determined and compared with those from rat liver [Wittmann-Liebold, B., Geissler, A. W., Lin, A., & Wool, I. G. (1979) *J. Supramol. Struct. 12*, 425–433] and *Halobacterium cutirubrum* [Matheson, A. T., Möller, W., Amons, R., & Yaguchi, M. (1980) in *Ribosomes: Structure, Function and Genetics* (Chambliss, G., et al., Eds.) pp 297–332, University Park Press, Baltimore, MD; M. Yaguchi, unpublished experiments].

In previous papers (Higo & Otaka, 1979; Itoh et al., 1979), we reported the purification and characterization of 37 cytoplasmic ribosomal proteins from yeast, *Saccharomyces cerevisiae*, and the isolated proteins were designated according to our tentative YP (yeast protein) numbering system (Otaka & Kobata, 1978; Higo & Otaka, 1979). Recently, we localized all the yeast ribosomal proteins to the small and large subunits and proposed a standard nomenclature, in which the subunit localization of each protein is indicated by YS (yeast small subunit protein) and YL (yeast large subunit protein), respectively (Otaka & Osawa, 1981).

In the present study, we have purified and characterized 17 ribosomal proteins. In addition, the previously purified proteins (Higo & Otaka, 1979; Itoh et al., 1979) have been renamed according to the standard nomenclature.

We have so far determined the complete primary structures of three of the proteins purified. Two of them (YL27 and YL35) have been structurally correlated to two rat liver ribosomal proteins (Itoh & Wittmann-Liebold, 1978; Itoh et al., 1980) and another one, an acidic protein (YL44c), to a rat liver acidic ribosomal protein and also to one ribosomal protein from *Halobacterium cutirubrum* (Itoh et al., 1980; Itoh, 1980). In this paper, amino-terminal amino acid sequences of eight yeast ribosomal proteins have been determined and compared with the known sequences of ribosomal proteins from rat liver, *H. cutirubrum*, *Escherichia coli*, *Bacillus stearothermophilus*, and *Bacillus subtilis*.

Materials and Methods

Materials and methods not presented here were previously described (Higo & Otaka, 1979).

Preparation of Ethylenediaminetetraacetic Acid (EDTA)[1] *Treated Ribosomes.* Yeast 80S ribosomes (1000 A_{260} units) suspended in 12 mL of TMD-I buffer [50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, and 1 mM dithiothreitol (DTT)] were mixed with 5 mL of 0.1 M disodium ethylenediaminetetraacetate (EDTA) and stirred for 2 h at 4 °C. The suspension was then layered on 10 mL of 0.5 M sucrose containing 50 mM Tris-HCl (pH 7.4) and 1 mM DTT in a Spinco 30 rotor tube and centrifuged at 30000 rpm at 4 °C for 20 h to pellet the EDTA-treated ribosomes.

Fractionation of Ribosomal Proteins. Details of the fractionation of proteins by carboxymethylcellulose (CMC) column chromatography and filtration by Sephacryl S-200 are shown in the legend for Figure 1.

Electrophoresis. Two-dimensional polyacrylamide gel electrophoresis of the modified Mets–Bogorad method (Mets & Bogorad, 1974; Otaka & Kobata, 1978) was used. In addition, two-dimensional polyacrylamide gel electrophoresis according to method 1 of Warner & Gorenstein (1978), a modification of the Kaltschmidt–Wittmann system (Kaltschmidt & Wittmann, 1970), was also employed.

Amino Acid Sequence. Sequence analyses by automated Edman degradation were performed in an updated Beckman 890 sequencer by using 0.5 M Quadrol buffer and a single-cleavage program [a modification of the method described by Hunkapiller & Hood (1978)]. The identification of PTH-amino acids was done by gas chromatography and amino acid analysis after back-hydrolysis with HI. Other details were described before (Higo & Loertscher, 1974; Higo et al., 1982).

Results and Discussion

Isolation of Proteins from Small Subunits. Ten proteins were purified as described below (Figure 1) from the fractions of the preparative chromatography previously described [see Figure 4 of Higo & Otaka (1979)].

The protein YS8 was obtained by filtrating the portion between the fractions S-I and S-II through a Sephacryl S-200 column. Filtration of the fraction S-VII yielded YS22, and an additional protein, which positioned slightly below YL22 on the two-dimensional polyacrylamide gel electrophoresis pattern. The amino acid composition and peptide map (data

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[1] Abbreviations: DTT, dithiothreitol; EDTA, disodium ethylenediaminetetraacetate; CMC, carboxymethylcellulose; Tris, tris(hydroxymethyl)aminomethane.

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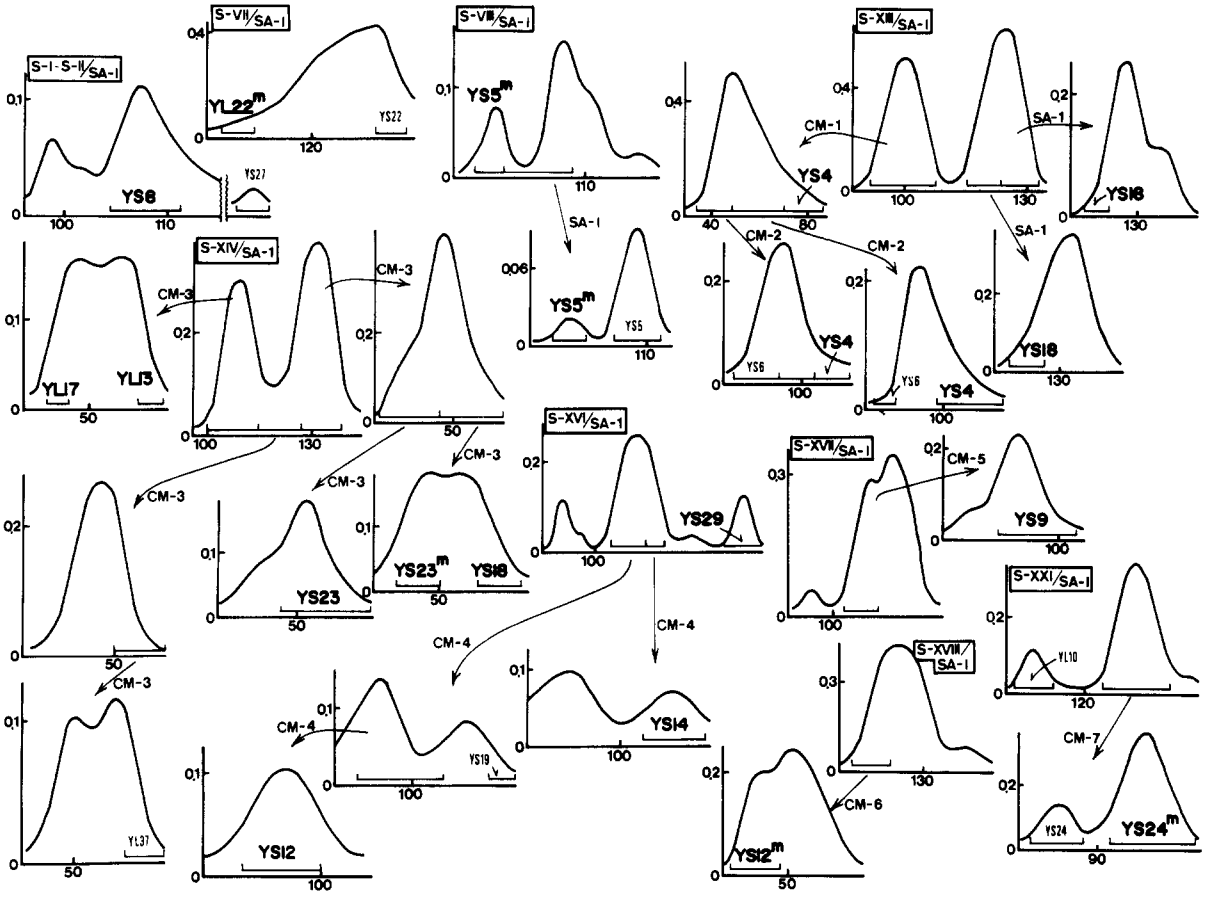

FIGURE 1: Purification of several yeast ribosomal proteins from carboxymethylcellulose column chromatographic fractions. The fractions S-I~S-II, S-VII, S-VIII, S-XIII, S-XIV, S-XVI, S-XVII, S-XVIII, and S-XXI [see Higo & Otaka (1979)] were further fractionated according to the isolation system indicated in the figure by SA-1, SA-2, and CM-1–CM-8, and the names of the original fractions, e.g., S-I~S-II/SA-1, are given in boxes. The arrows in the figure with their corresponding symbols indicate the process to which a fraction was subjected: SA-1, Sephacryl S-200 (2.5 × 140 cm) in 10% HCOOH, 3 mL/tube was collected in 10 min; SA-2, Sephacryl S-200 (1.5 × 140 cm) in 10% HCOOH, 1.5 mL/tube was collected in 10 min. For CM-1–CM-8, a carboxymethylcellulose column (0.8 × 45 cm) equilibrated with 0.05 M sodium acetate buffer (pH 4.7) containing 6 M urea, 0.5 mM DTT, and 0.04% methylamine was used. A 2-mL fraction was collected in 7.5 min with a NaCl gradient of 0.1–0.17 M (CM-1), 0.08–0.175 M (CM-2), 0.1–0.225 M (CM-3), 0.1–0.2 M (CM-4), 0.12–0.22 M (CM-5), 0.13–0.23 M (CM-6), 0.15–0.255 M (CM-7), and 0.06–0.15 M (CM-8) in the buffer mentioned above in a total volume of 300 mL. Ordinate: Protein concentration by absorption at 280 nm. Abscissa: Fraction (tube) number. The inserted boldfaced numbers indicate newly isolated protein species.

not shown) of this protein were almost the same as those of YL22 (see next section). Therefore, this appears to be a modified form of YL22 and was named YL22^m. Similar procedures for naming were also adopted for other proteins irregularly positioned on the two-dimensional polyacrylamide gel electrophoresis electrophoretogram. The filtration of S-VIII through Sephacryl S-200 gave three peaks. The first one consisted of YS5^m, and from the left half of the second peak, YS5 and YS5^m were recovered. The fraction S-XIII gave two main peaks by Sephacryl S-200 filtration. The proteins YS4 and YS6 were isolated from the first peak and YS18 from the second peak by the subsequent CMC column chromatography at pH 4.7. The pH 4.7 CMC system was also applied to fraction S-XIV for purification of YL17, YL13, YS23, and YS23^m, to fraction S-XVI for YS29, YS14, and YS12, to fraction S-XVII for YS9, to fraction S-XVIII for YS12^m, and to fraction S-XXI for YS24^m, respectively. The yields were between 2 and 20 mg by dry weight.

Isolation of Proteins from EDTA-Treated Ribosomes. When the 80S ribosomes are treated with EDTA, specific ribosomal proteins corresponding to about 20% of the total proteins were selectively removed. One hundred milligrams of proteins from the EDTA-treated ribosomal pellet was chromatographed on a 0.8 × 45 cm CMC column at pH 5.6 (Figure 2). From fractions A, B, C, D, and R, several proteins were purified as follows.

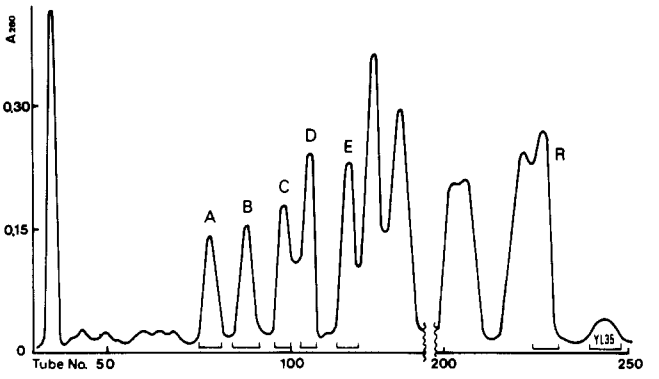

FIGURE 2: Carboxymethylcellulose column chromatography of EDTA-treated ribosomal proteins from yeast. About 100 mg of proteins loaded on a carboxymethylcellulose column (0.8 × 45 cm) was eluted with a linearly increasing concentration of sodium acetate buffer (pH 5.6), from 0.05 to 0.65 M, containing 6 M urea, 0.1 mM DTT, and 0.04% methylamine in a total volume of 500 mL. 2 mL/tube was collected in 7.5 min. The inserted letters indicate the fractions used for further purification of the proteins for Figure 3.

Fraction A from several column chromatographic runs was combined and filtrated through Sephacryl S-200 to obtain YS8^m and YL38 (Figure 3). Similarly, YS10 and YL38 were purified from fraction B, YL11 and YL22 from fraction C, YS5 mainly from fraction D, YS7 and YL16 from fraction

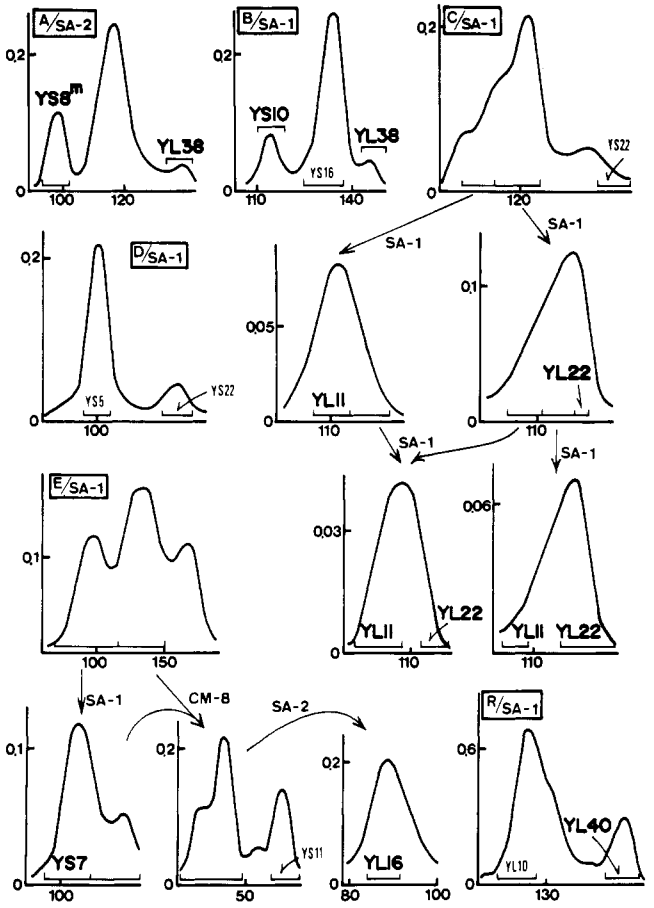

FIGURE 3: Purification of several proteins from carboxymethylcellulose column chromatographic fractions in Figure 2. The fractions A–E and R (Figure 2) were rechromatographed according to the isolation system indicated in the figure which is explained in the legend of Figure 1.

E, and YL40 from fraction R. The yields were between 1.5 and 15 mg by dry weight.

All the proteins newly isolated in this paper are indicated by boldface type in Figures 1–3 and by solid circles in Figure 4.

Amino Acid Composition. Amino acid compositions of the isolated proteins are presented in Table I. Examination of the amino acid compositions of YS4, YS7, YS8, YS9, YS10, YS12, YS14, YS18, YS23, YS29, YL11, YL13, YL16, YL17, YL22, YL38, and YL40 suggested the uniqueness of these proteins. The proteins with superscript m, namely, YS5^m, YS8^m, YS12^m, YS23^m, YS24^m, and YL22^m, revealed an amino acid composition very similar to that of YS5 (Higo & Otaka, 1979), YS8, YS12, YS23, YS24 (Higo & Otaka, 1979), and YL22, respectively. The results of amino acid composition, peptide, and two-dimensional polyacrylamide gel electrophoresis analyses suggested that they were either modified products or aggregates of the respective genuine proteins.

The molecular weights of the genuine proteins mentioned above were already given in a previous paper (Otaka & Kobata, 1978).

The number of genuine protein species so far isolated and characterized is 46, out of 73 species recognized in the 80S ribosomes (Otaka & Osawa, 1981). The remaining proteins were found as complicated mixtures in several chromatographic fractions. Purification of these proteins may therefore require different procedures.

Redesignation of the Previously Isolated Proteins. All of the previously isolated ribosomal proteins, that were reported

Table I: Amino Acid Composition[a] of Proteins Isolated from Yeast Small Ribosomal Subunits and EDTA-Treated Ribosomes

protein	YS4	YS5^m	YS7	YS8	YS8^m	YS9	YS10	YS12	YS12^m	YS14	YS18	YS23	YS23^m	YS24^m	YS29	YL11	YL13	YL16	YL17	YL22	YL22^m	YL38	YL40
Asp	8.4	8.4	9.5	10.8	9.5	5.8	6.5	6.5	6.9	6.9	8.5	5.5	4.7	7.3	9.7	12.6	8.6	7.3	6.3	10.6	10.3	10.0	11.0
Thr	4.2	6.8	5.6	6.9	6.0	5.7	4.8	7.6	7.0	3.5	4.3	3.4	2.9	5.2	2.2	5.9	3.1	5.2	5.2	6.0	5.7	5.0	6.2
Ser	3.4	4.7	4.5	3.6	4.5	7.6	5.5	5.5	5.1	5.2	4.6	7.3	7.9	4.2	6.3	4.1	4.7	4.2	8.3	4.9	4.9	5.9	3.1
Glu	12.6	10.5	9.9	14.0	13.5	11.7	15.4	7.5	6.4	8.9	10.1	10.3	10.1	3.1	8.0	9.5	8.9	11.7	12.8	9.8	10.6	10.2	7.3
Pro	4.1	5.6	5.2	4.3	4.7	2.3	5.7	5.3	5.3	4.0	3.4	2.8	2.7	3.2	2.2	2.5	4.5	4.9	2.8	3.4	3.1	4.4	4.6
Gly	7.0	9.6	4.7	4.7	5.0	7.8	3.9	6.2	6.6	8.2	6.4	5.2	4.9	7.2	10.6	6.5	6.5	4.6	8.5	8.3	6.9	7.9	2.0
Ala	7.5	8.6	12.7	11.8	10.4	11.7	6.8	6.2	7.3	8.7	9.2	12.5	13.6	8.7	3.3	4.1	10.7	10.4	15.1	5.5	5.3	10.3	9.8
Val	7.5	8.4	7.4	9.4	8.5	4.3	8.9	10.8	10.3	9.6	9.4	6.2	5.8	9.9	5.4	13.4	7.7	10.7	6.1	9.1	9.4	8.3	1.4
Met	0.8	0.8	0.8	1.0	0.8	0.0	0.2	0.6	0.6	0.2	1.0	0.8	1.0	0.0	0.0	1.5	1.3	0.1	0.1	1.4	1.4	1.5	2.9
Ile	4.6	6.0	4.5	6.9	6.9	5.4	6.0	6.0	6.3	3.4	3.2	6.4	6.8	1.6	4.6	8.3	4.0	2.8	3.9	5.8	6.5	4.6	6.1
Leu	8.9	8.9	9.2	6.7	7.8	5.5	10.8	4.1	3.5	7.1	7.6	8.8	8.9	6.6	4.2	6.0	8.0	10.8	5.4	7.0	8.1	8.0	4.7
Tyr	2.4	2.3	3.7	1.3	1.9	3.5	2.0	3.6	4.3	2.7	3.4	4.1	4.1	6.8	6.2	2.7	4.2	2.9	3.1	3.1	2.9	3.3	3.2
Phe	4.3	3.6	3.0	3.5	3.3	4.1	4.9	5.5	5.2	5.7	5.0	1.0	0.5	1.7	8.0	4.2	4.0	4.0	3.0	4.5	4.7	3.6	2.8
His	1.2	1.6	1.4	1.1	1.4	2.2	2.1	3.4	3.3	3.1	1.6	3.0	3.0	6.4	5.7	2.5	2.5	1.3	2.3	1.8	2.2	1.3	1.7
Lys	11.8	8.2	13.3	6.8	8.6	11.5	10.1	13.4	13.8	15.3	12.3	15.3	16.3	22.3	9.1	10.5	11.1	14.1	8.6	10.0	10.1	9.6	17.0
Arg	11.6	6.6	5.0	7.4	7.8	11.2	7.0	8.2	8.8	7.9	10.4	7.9	7.2	6.1	14.9	6.2	10.6	5.6	8.8	9.1	8.1	6.4	16.6

[a] Values are in mole percent.

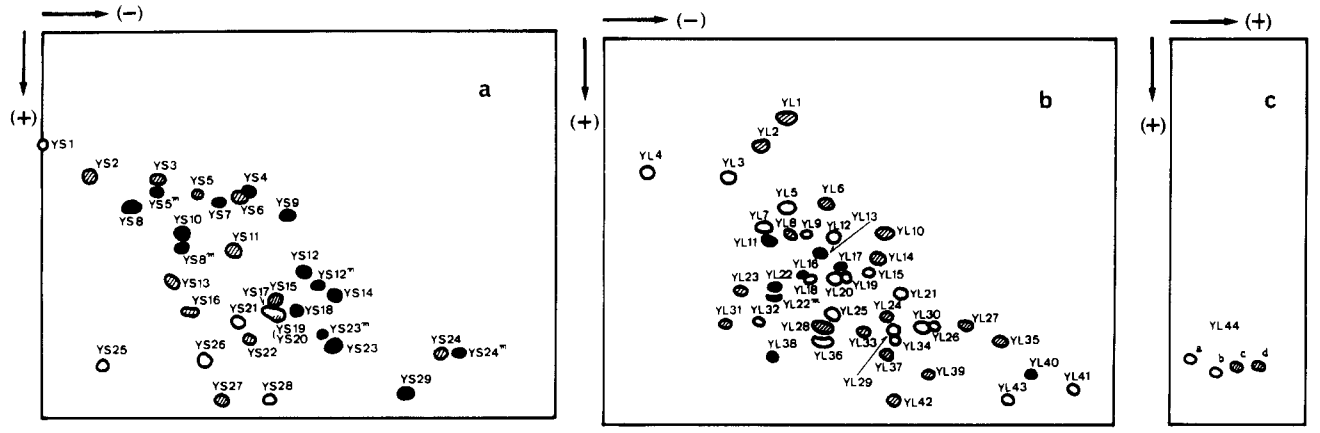

FIGURE 4: Schematic presentation of two-dimensional polyacrylamide gel electrophoretogram of yeast ribosomal proteins indicating the newly isolated protein species with solid circles. Electrophoresis was according to Otaka & Osawa (1981): (a) small subunit proteins; (b and c) large subunit proteins. The hatched circles indicate the genuine proteins previously isolated (Higo & Otaka, 1979; Itoh et al., 1979).

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                    5                   10                  15                  20                  25                  30
YS 4    Ala-Lys-Leu-Asn-Ile-Ser-Tyr-Pro-Val-Asx-Gly-Ala-Gln-Leu-Thr-Phe-Glu-Ile-Asp-Asp-Glu-His-Arg-Ile- X -Val-Phe-Phe- X -Lys-
RS 6    Met-Lys-Leu-Asn-Ile-Ser-Phe-Pro-Ala-Thr-Gly-Ser-Gln-Lys-Leu-Leu-Glu-Val-Asp-Asp-Glu-Arg-Lys-Leu-Arg- X -Phe-Tyr-Glu-Lys-

                    5                   10                  15                  20                  25
YS 6    Ala-Arg-Gly-Pro-Lys-Lys-His-Leu-Lys-Arg-Leu-Ala-Ala-Pro-His-His- X -Leu-Leu-Leu-Asx-Leu-Leu-Gly-Gly-Tyr-Ala-Pro-
RS 4    Ala-Arg-Gly-Pro-Lys-Lys-His-Leu-Lys-Arg-Val-Ala-Ala-Pro-Lys-His-Trp-Met-Leu-Asp-Lys-Leu-Leu-Gly-Phe-Val-Ala-Pro-

                    5                   10                  15                  20
YS11    Pro-Arg-Ala-Pro-Arg-Thr-Tyr-Ser-Lys-Thr-Tyr-Ser-Thr-Pro-Lys-Arg-Pro-Tyr-Gln-Ser-Asx-Arg-Leu-
H-S9        Ala-Leu-Pro-Gly-Glu-Asn-Thr-Lys-Phe-Tyr-Glu-Thr-Pro-Asn-His-Pro-Tyr-Gln-Gly-Glx-Arg-Ile-

                    5                   10                  15                  20                  25                  30
YS15    Gly-Arg-Met-His-Ser-Ala-Gly-Lys-Gly-Ile-Ser-Ser-Ser-Ala-Ile-Pro-Tyr-Ser-Arg-Asn-Ala-Pro-Ala-Gly-Phe-Lys-Leu-Ser-Ser-Glu-
H-S11   Ala-Arg-Met-His-Thr-Arg-Arg-Arg-Gly-Ser-Ser-Gly-Ser-Asp-Arg-Pro-Thr-Ala-Asp-Glu-Pro-Pro-Glx-Glx-Ser-Asx-Val-Asx-Glx-Asx-

                   35                  40
        Cys-Val-Ile-Glu- X -Ile-Val-Lys-Tyr-Ala-

                    5                   10                  15                  20                  25                  30
YS22    Thr-Arg-Ser-Ser-Val-Leu-Ala-Asp-Ala-Leu-Asn-Ala-Ile-Asn-Asn-Ala-Glu-Lys-Thr-Gly-Lys-Arg-Gln-Val-Leu-Ile-Arg-Pro-Ser-Ser-
H-S20   Thr-Ala-Asn-Asp-Pro-Leu-Ser-Asp-Glu-Leu-Ser-Glx-Ile-Asp-Asp-Ala-Glu-Ser-Val-Gly-His-Leu-Thr-His-Thr-

                   35                  40
        Lys-Val-Ile-Ile-Lys-Phe-Leu-Gln-Val- X -Glx-Lys-His-

                    5                   10                  15                  20                  25                  30
YS16    Ala-Gly-Val-Ser-Val-Arg-Asp-Val-Ala-Ala-Gln-Asp-Phe-Ile-Asn-Ala-Tyr-Ala-Ser-Phe-Leu-Gln-Arg-Gln-Gly-Lys-Leu-Glu-Val-Pro-

                   35                  40                  45
        Gly-Tyr-Val-Asp-Ile-Val-Lys-Thr-Ser-Ser-Gly-Asn-Glu-Met-Pro-Pro-Gln-Asx-Ala-

                    5                   10                  15                  20                  25                  30
YL10    Gly-Ala-Tyr-Lys-Tyr-Leu-Glu-Glu-Leu-Gln-Arg-Lys-Lys-Gln-Ser-Asp-Val-Leu-Arg-Phe-Leu-Gln-Arg-Val-Arg-Val-Gly-Glu-Tyr-Arg-

                   35                  40
        Gln-Lys-Asn-Val-Ile-His-Arg-Ala-Ala-Arg-Pro-Thr-Arg-

                    5                   10                  15                  20                  25                  30
YL31    Ala-Pro-Asn-Thr-Ser-Arg-Lys-Gln-Lys-Ile-Ala-Lys-Thr-Phe-Thr-Val-Asx-Val-Ser-Ser-Pro-Thr-Glu-Asx-Gly-Val-Phe-Asx-Pro-Ala-

                   35                  40                  45                  50
        Ser-Tyr-Ala-Lys-Tyr-Leu-Ile-Asx-His-Ile-Lys-Val-Glx-Gly-Ala-Val-Gly-Asx-Leu-Gly-Asx-
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FIGURE 5: Amino-terminal amino acid sequences of eight ribosomal proteins from yeast and their most probable counterparts of rat liver and *Halobacterium cutirubrum*. Rat liver and *Halobacterium cutirubrum* sequences were taken from B. Wittmann-Liebold, H. G. Wittmann, A. Lin and I. G. Wool, as cited in Wool (1979), and from M. Yaguchi, L. P. Visentin, A. T. Matheson, C. Roy, and A. R. Strøm, as cited in Matheson et al. (1980), respectively. Identical amino acid residues were boxed in a solid line, while the substitution that could have arisen by a single base mutation was boxed in a broken line.

with YP number (Higo & Otaka, 1979; Itoh et al., 1979), were reexamined by the two kinds of two-dimensional polyacrylamide gel electrophoresis [see Otaka & Osawa (1981)] and redesignated according to the standard nomenclature (Otaka & Osawa, 1981) (Table II). No designations were given for YP14′, YP14″ (Higo & Otaka, 1979), YP24′, and YP47′ (Itoh et al., 1979), since they could not be correlated with the genuine ribosomal proteins so far isolated.

Amino-Terminal Amino Acid Sequences. Figure 5 shows the amino-terminal amino acid sequences of eight proteins, YS4, YS6, YS11, YS15, YS16, YS22, YL10, and YL13, out of yeast ribosomal proteins so far isolated. In YS16, both proline and alanine were detected at the ratio of 1 to 2 as amino terminal, indicating microheterogeneity of this protein.

Among the analyzed proteins, two (YS4 and YS6) could be structurally correlated to rat liver ribosomal proteins (R-S6 and R-S4) and three (YS11, YS15, and YS22) to *Halobacterium* ribosomal proteins (H-S9, H-S11, and H-S20). YS16, YL10, and YL31 could not be correlated when compared with 11 rat liver sequences and 16 *Halobacterium* sequences (see the legend to Figure 5 for references; M. Yaguchi, unpublished experiments). The extent of sequence similarity between the

Table II: Redesignations of the Previously Isolated Proteins from Yeast Small and Large Ribosomal Subunits

YP name[a]	standard name[b]	YP name[c]	standard name[b]
YP6	YS2	YP1	YL1
YP7	YS3	YP2	YL2
YP9	YS5	YP9	YS5
YP12	YS6	YP11	YL6
YP28	YS11	YP13′	YS7[m g]
YP38	YS13	YP16	YL8
YP45	YS16	YP18	YL10
YP50	YL31	YP26	YL14
YP52	YS20	YP39	YL23
YP58	YS22	YP41	YL24
YP63	YS24	YP42	YS15
YP70	YS27	YP44[d]	YL27
		YP45	YS16
		YP52a	YL28
		YP53	YL33
		YP55[e]	YL35
		YP59	YL37
		YP62	YL39
		YP68	YL42
		YPA1[f]	YL44c

[a] Higo & Otaka (1979). [b] Otaka & Osawa (1981). [c] Itoh et al. (1979). [d] Used in Itoh & Wittmann-Liebold (1978). [e] Used in Itoh et al. (1980). [f] Used in Amons et al. (1977), Itoh et al. (1980), and Itoh (1980). [g] YS7[m] denotes a modified form of the genuine protein YS7 (see Results and Discussion).

Table III: Correlation and Sequence Similarity of Some Ribosomal Proteins from Yeast, Rat Liver, and *Halobacterium cutirubrum*

part	*Halobacterium*	sequence similarity	yeast	sequence similarity	rat liver
A			YS4	54 (14/26)[a]	R-S6
			YS6	74 (20/27)	R-S4
	H-S9	33 (6/18)	YS11		
	H-S11	26 (6/23)	YS15		
	H-S20	32 (8/25)	YS22		
B	H-L13	52 (11/21)	YL4[b]		
	H-L20	31 (18/58)	YL44c	50 (15/30)	R-P2
			YL35	53 (16/30)	R-L37

[a] Sequence similarity values are given in percent, and identical residues per examined residues are given in parentheses. [b] YL3 of Nazar et al. (1979). For details, see the text.

corresponding ribosomal proteins is summarized in Table III together with the correlation data so far available (shown in part B) on YL4 ["YL3" of Nazar et al. (1979)], YL35 (Wittmann-Liebold et al., 1979; Itoh et al., 1980), and YL44c (Itoh et al., 1980; Itoh, 1980). The yeast ribosomal proteins reveal 50–74% similarity to rat liver ribosomal proteins and 26–52% similarity to *Halobacterium* ribosomal proteins. Although the data available are still limited, it seems to us that the ribosomal proteins can be structurally correlated by their amino-terminal sequences to each other among eukaryotes and that many of the eukaryotic ribosomal proteins have possible structural counterparts among *Halobacterium* ribosomal proteins. On the other hand, the amino-terminal sequences of yeast ribosomal proteins do not show any significant homology to those of *E. coli*, *B. stearothermophilus*, and *B. subtilis* ribosomal proteins (Higo et al., 1978, 1980, 1982). A similar comparison of 10 *Halobacterium* 30S ribosomal proteins with all the 30S ribosomal proteins from typical bacteria so far sequenced also did not reveal any detectable similarity (M. Yaguchi, unpublished experiments). The resemblance of eukaryotes and *Halobacterium* [metabacteria group (Osawa & Hori, 1980)] in ribosomal proteins described here is consistent with the rather eukaryotic nature of *Halobacterium* 5S RNA (Hori & Osawa, 1979) and RNA polymerase (Zillig & Stetter, 1980).

It has been known that the number of ribosomal proteins from various bacteria including *Halobacterium* is approximately the same and that the eukaryotic ribosomes contain a greater number of ribosomal proteins than the prokaryotic ones (Wool, 1979, 1980). Thus, the correlation of all the *Halobacterium* ribosomal proteins to the eukaryotic-type ribosomal proteins, when established, may be helpful to identify the eukaryote-specific "extra proteins".

Since both eukaryotic and *Halobacterium* ribosomal proteins have much less, if any, similarity to the ribosomal proteins of typical bacteria, a simple comparison of their partial sequences may not reveal any convincing correlation even if it exists. Indeed, yeast YL44c and *Artermia salina* eL12, which appears to play a similar function as bacterial L7/L12, were completely sequenced, and some common structural features among acidic proteins from eubacterial, *Halobacterium*, and eukaryotic ribosomes were recognized (Itoh et al., 1980; Amons & Möller, 1980; Yaguchi et al., 1980).

Protein YS4 is one of the major phosphoproteins in yeast ribosomes (Otaka & Kobata, 1978), and the sequence revealed 50% similarity to that of R-S6, the major phosphoprotein of rat liver ribosomes (see Figure 5 and Table III). These two sequences have identical serine residues at position 6, whereas serine at position 12 of R-S6 is replaced by alanine in YS4. This common serine residue at position 6 may be one of the phosphorylation sites in these proteins. Although the physiological significance of the phosphorylation of these proteins is still not clear, it is of interest to examine whether there is a similar phosphoprotein in *Halobacterium*.

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Supplementary Material Available

Eight tables summarizing the basis for amino acid assignments in the proteins (15 pages). Ordering information is given on any current masthead page.

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Effect of Protease Binding by α_2-Macroglobulin on Intrinsic Fluorescence†

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ABSTRACT: We have evaluated intrinsic protein fluorescence as a method for investigating the reactions of α_2-macroglobulin (α_2M) with proteases and amines. Changes in fluorescence intensity of α_2M in the presence of proteases and amines were shown to correlate with structural and functional changes in the α_2M molecule. By intrinsic fluorescence we found that 2 mol of trypsin bound to 1 mol of α_2M whereas thrombin and plasmin each bound in a stoichiometry closer to 1:1. Studies showed that changes in fluorescence caused by ammonium ion paralleled the loss of the ability of α_2M to protect trypsin from soybean trypsin inhibitor. The exposure of sulfhydryl groups on α_2M by a small organic amine (methylamine) also correlated with fluorescence change that could be quantitatively eliminated by prior reaction of α_2M with trypsin. Cleavage of α_2M by four serine proteases (plasmin, thrombin, trypsin, and elastase) as determined by sodium dodecyl sulfate gel electrophoretic analyses and the binding of plasmin and thrombin as measured by macromolecular inhibitor assays corresponded to the increase in fluorescence intensity. In addition, the rate of thrombin inhibition for clotting fibrinogen was the same as the rate of fluorescence change observed when thrombin was incubated with α_2M. Our results indicate that intrinsic protein fluorescence is an easy and rapid technique for assessing both qualitative and quantitative aspects of protease–α_2M interactions.

While the physiological function of α_2M[1] is unknown, its ability to bind nearly all proteases (Barrett & Starkey, 1973) and its affinity for macrophages (Debanne et al., 1975; Kaplan & Nielsen, 1979a) and fibroblasts (Maxfield et al., 1978) make this large [M_r ~725 000 (Jones et al., 1972)], tetrameric glycoprotein the object of considerable current interest. In addition to the functional importance suggested by the binding, internalization, and subsequent degradation of α_2M–protease complexes by cells (Debanne et al., 1976; Kaplan & Nielsen, 1979b; Van Leuven et al., 1979; Mosher & Vaheri, 1980), α_2M has a number of other interesting and unusual characteristics. When α_2M binds an enzyme, the degree to which the activity of that enzyme is inhibited depends on the molecular size of the substrate (Rinderknecht et al., 1975; Mehl et al., 1964). Another property that distinguishes α_2M is its ability to bind and inhibit two protease molecules at a time (Ganrot, 1966; Barrett et al., 1979; Swensen & Howard, 1979a). Recent data suggest that α_2M contains a reactive site on each of its four subunits. Each of these sites, like the one present in the complement proteins C3 and C4, is sensitive to

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[1] Abbreviations: α_2M, α_2-macroglobulin; $NaDodSO_4$, sodium dodecyl sulfate; TLCK, 1-chloro-3-(tosylamido)-7-amino-L-2-heptanone; SBTI, soybean trypsin inhibitor; *p*-NPGB, *p*-nitrophenyl *p*-guanidinobenzoate; PEG, poly(ethylene glycol).

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