AMINO ACID REPLACEMENTS IN RIBOSOMAL PROTEIN YL24 OF *SACCHAROMYCES CEREVISIAE* CAUSING RESISTANCE TO CYCLOHEXIMIDE

Walter STÖCKLEIN, Wolfgang PIEPERSBERG and August BÖCK
Lehrstuhl für Mikrobiologie der Universität München, Maria-Ward-Straße 1a, 8000 München 19, FRG

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1. Introduction

The glutarimide antibiotic cycloheximide blocks protein synthesis on eucaryotic 80 S ribosomes (review [1]) by binding to the 60 S ribosomal subunit in a 1:1 stoichiometry [2]. Mutants have been described recently which are resistant to this drug because of an alteration of a large ribosomal subunit protein [3–5]. For cycloheximide-resistant strains of *Saccharomyces cerevisiae* it was demonstrated that the alteration of the respective ribosomal protein YL24 (formerly L29) causes a reduction of the affinity of ribosomes for the drug [2]. The mutation responsible for resistance was localized on chromosome 7 and shown to map in the *cyh2* gene locus [6].

These results give the sequence of and the amino acid replacements in the altered tryptic peptide of protein YL24 of 2 cycloheximide resistant mutants. They show that the same amino acid of the YL24 sequence is affected in each case and also demonstrate that *cyh2* is identical with the structural gene for ribosomal protein YL24. In analogy to the gene nomenclature of ribosomal proteins in bacteria [7] we propose the new designation *rplX* for this cistron.

2. Materials and methods

2.1. *Strains and growth conditions*

The cycloheximide-resistant mutant *cy32* is a derivative of *Saccharomyces cerevisiae* A364A and has been described in [2,5]. Mutant *sp1* was obtained from B. Littlewood (Madison) and is derived from the parent A-1 (a *ade6 leu1 trp5-67 ura1*). The 3 strains were grown in a 50 l fermenter to a cell density of 4×10^7 yielding a total of 200 g cells (wet wt).

2.2. *Preparation of ribosomes and ribosomal subunits and purification of ribosomal protein YL24 from wild-type and mutants* cy32 *and* sp1

The cells were broken in a Braun homogenizer and high salt-washed ribosomes were prepared as in [5]. They were dissociated into subunits and the subunits were separated by zonal centrifugation according to [8]. Ribosomal proteins were extracted with acetic acid [9] and separated by carboxymethylcellulose column chromatography following the procedure in [10]. Fractions containing YL24 (monitored by one-dimensional SDS–polyacrylamide gel electrophoresis [5]) were pooled, dialyzed against 1% acetic acid and lyophilized. The lyophilized proteins were then chromatographed on a phosphocellulose P11 column as in [10]. This second chromatography yielded a nearly homogenous YL24 protein from all 3 strains.

2.3. *Protein chemical methods*

Tryptic digestion of YL24 proteins was performed according to [11], that with *Staphylococcus aureus* V8 protease (Miles Labs) according to [12]. Tryptic peptides were separated, isolated by extraction with 50% acetic acid and hydrolyzed as in [13]. Visualization of peptides was either with the fluorescamin [14], phenanthrenquinone [15] or ninhydrin reagents. The amino acid composition of hydrolysates was determined by means of a Biotronik LC 6001 amino acid analyzer. Amino acid sequence analysis was done as in [16].

2.4. *Other methods*

Protein concentration was determined spectrophotometrically [17]. Two-dimensional separation of ribosomal proteins was carried out as in [18].

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3. Results and discussion

About 3 mg YL24 protein of wild-type yeast A364A, 6 mg mutant *cy32* and 2 mg mutant *sp1* were isolated. Fig.1 shows purity and electrophoretic identity for the protein from mutant *cy32*. The amino acid composition of the wild-type and the 2 mutant proteins is given in table 1. The composition of the wild-type protein corresponds well with [10]. That of protein from mutant *cy32* would be in agreement with an exchange of a glutamic acid or glutamine residue for lysine; for the *sp1* protein no prediction is possible. The 'finger-print' of tryptic peptides of YL24 from wild-type yeast is given in fig.2; it was found that peptide T24 is altered in mutant *cy32* as well as in *sp1*. Peptide T24 from YL24 of *sp1* (T24c) attained a more electronegative charge which is in agreement with a more negative charge of YL24 in two-dimensional electropherograms (W. S., B. Littlewood, W. P., J. Davies; in preparation). In the case of mutant *cy32* tryptic peptide T24 is missing and 2 new peptides designated T24a and b appeared (fig.2).

Table 2 gives the amino acid composition of tryptic peptides from wild-type YL24 and from mutant *cy32* (T24a and b) and *sp1* (T24c) proteins. The composition of the HCl hydrolysates of wild-type and *sp1* peptides T24 is identical, the only explanation for the more electronegative migration could therefore be a glutamine/glutamic acid exchange. This is supported by analysis of peptides arising after digestion with *Staphylococcus aureus* protease (not shown). Table 2 in addition indicates that T24a and b (from mutant *cy32*) are tryptic fragments of T24 originating from an exchange of glutamine (or glutamic acid) for

Table 1
Amino acid composition of protein YL24 from wild-type A364A and mutant strains *sp1* and *cy32*

	A364A	*sp1*	Diff.	*cy32*	Diff.
Asx	5.7	6.1	+0.4	5.5	−0.2
Thr	3.4	3.5	+0.1	3.3	−0.1
Ser	3.6	4.5	+0.9	3.3	−0.3
Glx	6.5	6.6	+0.1	5.7	−0.8
Pro	5.2	5.3	+0.1	5.7	+0.5
Gly	12.8	12.4	−0.4	12.2	−0.6
Ala	9.0	9.1	+0.1	8.3	−0.7
Val	5.6	6.3	+0.7	5.7	+0.1
Met	1.6	1.6	0	1.8	+0.2
Ile	5.2	5.0	−0.2	5.0	−0.2
Leu	6.3	5.9	−0.4	6.1	−0.2
Tyr	3.3	3.0	−0.3	3.3	0
Phe	3.4	3.2	−0.2	3.6	+0.2
His	7.1	6.5	−0.6	7.1	0
Lys	13.1	13.3	+0.2	14.5	+1.4
Arg	8.2	7.6	−0.6	8.9	+0.7
Total	99.9	99.9		100.0	

Numbers represent mol% of amino acids after hydrolysis in constant boiling HCl at 110°C for 20 h

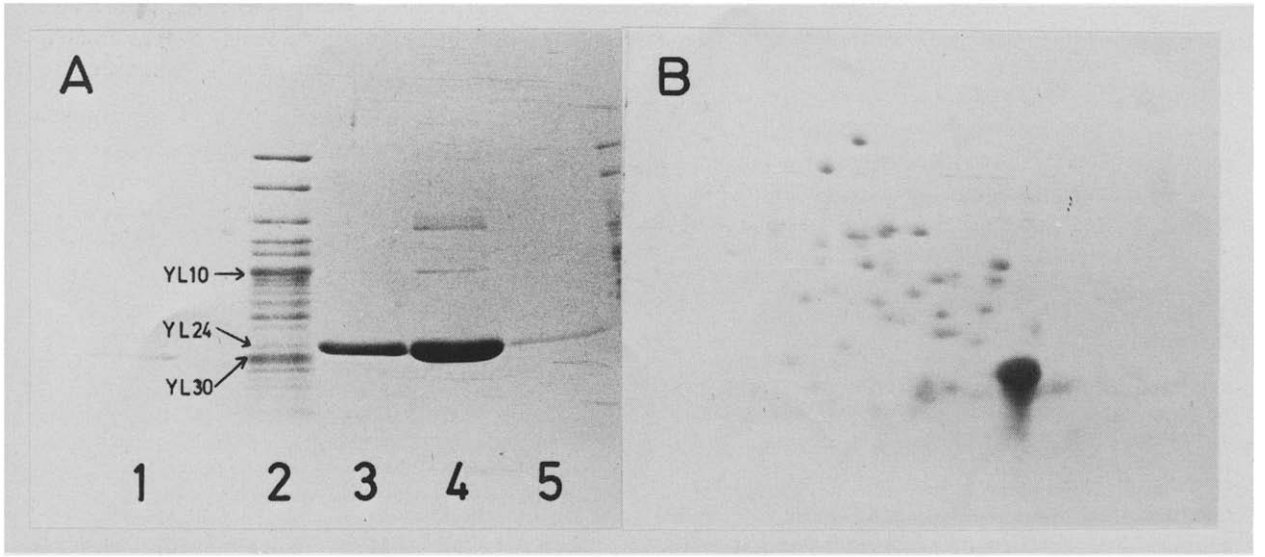

Fig.1. (A) SDS gel electrophoresis of fractions from the phosphocellulose column (lanes 1,3–5 corresponding to fraction 27, 51, 57 and 64, respectively) and of total protein from the 60 S subunit (lane 2). The proteins are numbered according to [19]. (B) Co-electrophoresis of 25 μg 80 S ribosomal proteins with 30 μg purified YL24 from strain *cy32*.

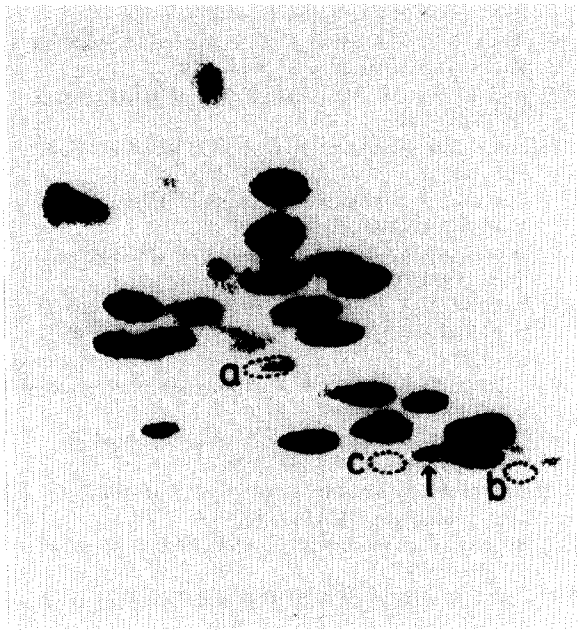

Fig.2. 'Fingerprint' of tryptic peptides (stained by ninhydrin) from wild-type YL24. The arrow indicates the migration position of T24. The migration position of mutationally altered T24 in mutants *spl* (c) and *cy32* (a,b) is given by circles. The faint stain in position (a) of the wild-type fingerprint is due to an incomplete tryptic digestion product. Its amino acid content which is between 9% and 15% of that of T24a from strain *cy32* was subtracted from that of T24a (table 2). T24a and T24b give a yellow stain with ninhydrin; T24b and T24c are stained red with phenanthrenquinone.

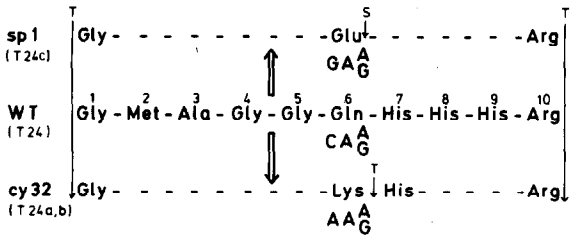

Fig.3. Amino acid sequence of tryptic peptides T24 from wild-type A364A and mutants *spl* (T24c) and *cy32* (T24 a,b). The position of cleavage by trypsin (T) and *Staphylococcus aureus* protease (S) is indicated.

lysine, thereby introducing an additional trypsin-sensitive site.

The sequence of T24 from YL24 protein from the wild-type and the cycloheximide-resistant mutant *spl* (T24c) was determined (fig.3). It was found that indeed a glutamine residue is replaced by glutamic acid in *spl* T24c. The sequence of T24a and b was not analyzed; however, their amino acid composition (table 2) allows the conclusion that a glutamine/lysine exchange had taken place in this case. In conclusion, the results give the first example for the protein–chemical basis of antibiotic resistance at the eucaryotic 80 S ribosome and show that mutations providing a cycloheximide-resistant phenotype to yeast are in the structural gene for ribosomal protein YL24. Similar to streptomycin-resistant mutations in eubacteria [20] a single amino acid may be replaced in different ways to confer resistance. In analogy to gene nomenclature in bacteria [7] we propose the designation *rplX* for the YL24 cistron.

Table 2
Amino acid composition of the tryptic peptides T24 from wild-type, T24a and b from mutant *cy32* and T24c from mutant *spl*

	T24 (A364A)	T24c (*spl*)	T24a (*cy32*)	T24b (*cy32*)
Glx	1.0	1.0	–	–
Gly	2.4	2.6	2.5	–
Ala	1.0	1.0	1.0	–
Met	0.9	0.5	1.1	–
His	2.6	2.6	–	2.5
Lys	–	–	0.8	–
Arg	0.8	1.0	–	1.0
Total	8.7	8.7	5.4	3.5

Numbers represent the molar amount of amino acids relative to that of alanine or arginine (underlined). The composition of T24a was corrected for the amount of amino acids present in the overlapping peptide (see legend to fig.2)

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References

[1] Vazquez, D. (1979) in: Molecular biology, biochemistry and biophysics (Kleinzeller, A. et al. eds) vol. 30, Springer, Berlin, New York.
[2] Stöcklein, W. and Piepersberg, W. (1980) Antimicrob. Agents Chemother. 18, 863–867.
[3] Coddington, A. and Flury, R. (1977) Mol. Gen. Genet. 158, 93–100.
[4] Begueret, J., Perrot, M. and Crouzet, M. (1977) Mol. Gen. Genet. 156, 141–144.
[5] Stöcklein, W. and Piepersberg, W. (1980) Curr. Genet. 1, 177–183.
[6] Mortimer, R. K. and Schild, D. (1980) Microbiol. Rev. 44, 519–571.
[7] Bachmann, B. J. and Low K. B. (1980) Microbiol. Rev. 44, 1–56.
[8] Van der Zeijst, B. A. M., Kool, A. J. and Bloemers, H. P. J. (1972) Eur. J. Biochem. 30, 15–25.
[9] Hardy, S. J. S., Kurland, C. G., Voynow, P. and Mora, G. (1969) Biochemistry 8, 2897–2905.
[10] Itoh, T., Higo, K. and Otaka, E. (1979) Biochemistry 18, 5787–5793.
[11] Itoh, T. and Wittmann, H. G. (1973) Mol. Gen. Genet. 127, 19–32.
[12] Houmard, J. and Drapeau, G. R. (1972) Proc. Natl. Acad. Sci. USA 69, 3506–3509.
[13] Piepersberg, W., Böck, A., Yaguchi, M. and Wittmann, H. G. (1975) Mol. Gen. Genet. 143, 43–52.
[14] Fishbein, J. C., Place, A. R., Ropson, I. J., Powers, D. A. and Sofer, W. (1980) Analyt. Biochem. 108, 193–201.
[15] Yamada, S. and Itano, H. A. (1966) Biochim. Biophys. Acta 130, 538–540.
[16] Chang, J. Y., Brauer, D. and Wittmann-Liebold, B. (1978) FEBS Lett. 93, 205–214.
[17] Ehresmann, B., Imbault, P. and Weil, J. H. (1973) Analyt. Biochem. 54, 454–463.
[18] Mets, L. J. and Bogorad, L. (1974) Analyt. Biochem. 57, 200–210.
[19] Otaka, E. and Osawa, S. (1981) Mol. Gen. Genet. 181, 176–182.
[20] Wittmann, H. G. and Wittmann-Liebold, B. (1974) in: Ribosomes (Nomura, M. et al. eds) pp. 115–140, Cold Spring Harbor Laboratory, New York.