SUPPRESSION AND SOLUBLE PROTEINS OF YEAST

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Suppression at the level of translation has been shown in several systems (see Gorini and Beckwith, 1966; Garen, 1968). There is more information available about suppression of nonsense mutations in bacteria and bacteriophages than in eucariatic cells, however, suppressors have also been discovered in fungi which could be identified as nonsense suppressors (Hawthorne and Mortimer, 1963). The mechanism of action of this type of suppressor was proposed to be primarily related to the transfer RNA level (Magni, 1966). A characteristic feature of the mutations influencing translation is its complete or partial dominancy.

Previously we have described recessive, dominant, and semidominant supersuppressors in yeast, which affect ad_2 alleles behaving as though they contained nonsense mutations (Inge-Vechtomov, 1964; Inge-Vechtomov, et al., 1966). The purpose of this work is to compare the effect of genetically different supersuppressors of yeast Saccharomyces cerevisiae on the growth rate and protein synthesis of the suppressed strains.

Materials. Peterhoff stocks of yeast Saccharomyces cerevisiae were used in the experiments:

15V-P4 - wild type, prototrophic haploid;

6-P3 - adenine-deficient haploid which accumulates red pigment when grown

aerobically; the genotype of strain 6-P3 is: αad_{1-6} where α is mating type; ad_{1-6} is an allele of the locus ad_1 related to adenine deficiency. Mutations in this locus cause the accumulation of the poly-5-amino-4-imidazole riboside (Smirnov et al., 1967). All three types of suppressors mentioned: dominant (S), semidominant ($\overline{S}$) and recessive (s) were isolated in the course of reversion experiments with 6-P3 strain.

In the present paper data are presented for the following strains carrying supersuppressors:

R_5 genotype αad_{1-6} S_5 carries mutation of ad_I locus suppressed by dominant suppressor S_5;

R_{53} genotype αad_{1-6} $\overline{S}_{53}$ carries mutation of ad_I locus suppressed by semidominant suppressor $\overline{S}_{53}$;

R_{48} genotype αad_{1-6} s_{48} carries mutation of ad_I locus suppressed by recessive suppressor s_{48};

R_{80} genotype αad_{1-6} s_{80} carries mutation of ad_I locus suppressed by recessive suppressor s_{80}.

Results. Fig. 1 shows the growth curves of the wild type strain 15V-P4 and the strains carrying various suppressor genes. The growth rate of cells carrying dominant and semidominant suppressors (strains R_5 and R_{53}) are practically the same as that of wild type cells, whereas strains R_{48} and R_{80}, which carry recessive suppressors, grow more slowly and have a longer lag-phase. We previously pointed out that the reversion to prototrophy due to the effect of the recessive suppressor S_{48} is accompanied by a significant drop in the viability of cells (Inge-Vechtomov and Simarov, 1967).

Further comparison of the various suppressor carrying strains was made by the analysis (by electrophoresis in polyacrylamide gel) of the soluble proteins isolated from these strains. We have analyzed the soluble proteins from 10 strains carrying dominant suppressors, 4 strains carrying recessive suppressors and 2 strains carrying semidominant suppressors. The results of the electrophoretic separation, where soluble proteins from the strains carrying

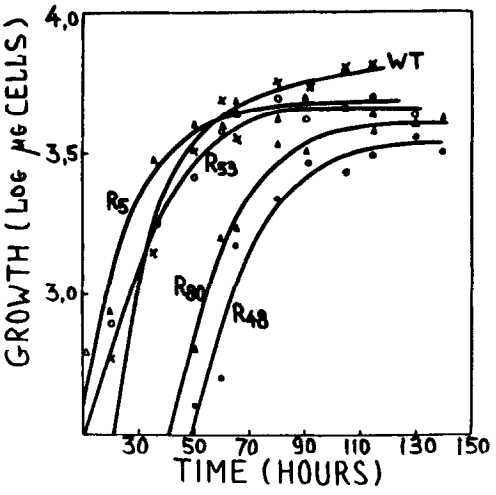

Fig. 1. Growth rates of various strains of Saccharomyces cerevisiae

Cells of each strain were inoculated into 12 identical 10 ml quantities of Rider's medium with vitamins and grown without aeration at 30°C. The inoculum per tube was approximately 3.5×10^6 cells. In 5-10 hours intervals suspensions were poured on weighed filters N5 (0.5µ pore size), washed with water (2 x 5 ml), ethanol (2 x 1.5 ml), dried under a lamp and weighed again. The difference in weight between loaded and empty filter was taken as the net weight of cells in a given suspension.

three types of suppressors were analyzed simultaneously, are presented in Fig. 2. No significant differences were observed in the number of fractions or the electrophoretic mobility between soluble proteins of wild type strain 15B-P4, adenine-deficient strain, 6-P3 and strains carrying dominant (R_5) and semi-dominant (R_{53}) suppressors. It should be noted that all strains analyzed, carrying dominant and semidominant suppressors, reveal a similar distribution of the soluble proteins in the gel.

Certain differences from this distribution were found, however, among soluble proteins of the strains carrying recessive suppressors (R_{48} and R_{80}). These differences, as the separation shows, were seen mainly as the increase in the electrophoretic mobility of protein bands in addition to other small changes in the number of proteins. Since the soluble proteins from strains

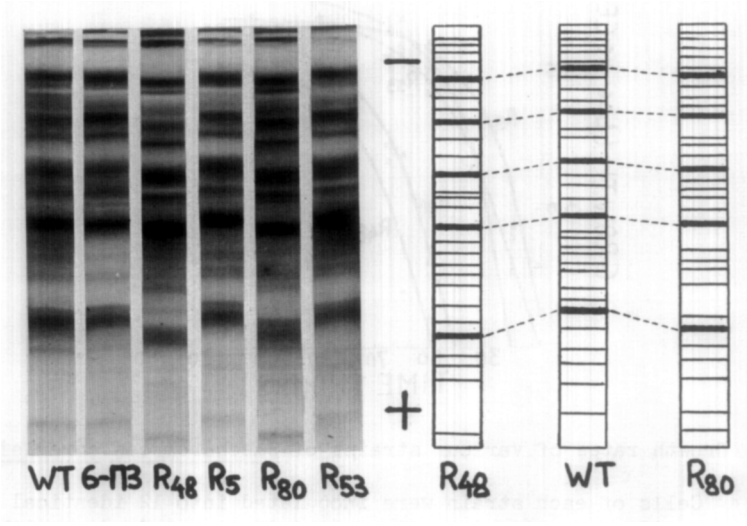

Fig. 2. The separation of soluble proteins isolated from various strains of Saccharomyces cerevisiae by electrophoresis in polyacrylamide gel

Yeasts were grown without aeration in Rider's medium with vitamins (adenine was added in the case of 6-P3 strain) at 30° from 72-hours inoculum. Cells were harvested at stationary phase by centrifugation, washed twice with water, suspended in 10^{-2}M Mg Tris-HCl buffer pH 7.4 with 10^{-3}M Mg^{++} and broken in a Hughes cell. Cell debris and ribosomes were removed by 120 min centrifugation of homogenate at 10,5000 x g. Supernatant aliquots (0.3 ml) were mixed with 2M sucrose (0.7 ml) and the resultant solutions were used for electrophoresis. 30 µl aliquots of these solutions used for separation contained 200-300 µg of protein. The separation was carried out in 9.5% gel with vertical flat-bed technique using apparatus of our own construction and continuous buffer system of Peacock et al. (Peacock et al., 1965). The details of the method are given in the paper (Uspenskaja et al., 1967).

R_{48} and R_{80} differ from that of wild type mainly in mobility of the bands, it was supposed that this change was caused by the increase in the number of negatively charged, and/or decrease in the amount of positively charged, groups in the proteins. To check this suggestion the amino acid composition of soluble proteins from the wild type strain 15V-P4 and strains R_{48} and R_{80}

Table I
Amino acid composition of soluble proteins for some strains of Saccharomyces cerevisiae*

Amino acid \ Strain	Wild type	Strain R_{48}	Strain R_{80}
Lysine	10.2	9.7	9.8
Histidine	2.2	2.8	3.3
Arginine	4.4	4.7	4.5
Proline	5.9	5.7	6.3
Alanine	11.6	11.2	11.0
Valine	9.4	9.3	9.5
Leucine	10.6	10.5	10.5
Tyrosine	3.6	3.8	3.8
Phenylalanine	5.2	5.3	5.3
Glycine	9.4	9.7	9.4
Threonine**	4.2	5.3	4.7
Serine**	2.2	4.2	3.6
Isoleucine	7.4	7.5	7.4
Aspartic acid	14.0	13.6	13.7
Glutamic acid	12.7	12.8	12.1
Amide NH_3*** (total Asp. NH_2 + Glu NH_2)	21.3	14.2	14.7
Total Aspartic + Glutamic acids in protein****	5.4	12.2	11.1

* The following procedure was adopted to prepare the soluble proteins for amino acid analysis. Supernatant after high speed centrifugation (see legend for Fig. 1) was made 5% in TCA and nucleic acids were removed by hydrolysis at 90° for 15 min (Schneider, 1945). The residue was washed twice with 5% TCA, ethanol, ethanol-ether (3:1), chloroform-methanol (2:1), ether and dried at room temperature. Protein preparations were hydrolyzed with 6 N HCl at 110° for 24 hours. Determinations of amino acid composition were carried out by routine procedure on an "Hitachi" amino acid analyzer. The relative molar content of each amino acid is expressed as a percentage of the amino acid content (molar) over the sum of "stable amino acids: lysine, histidine, arginine, aspartic acid plus asparagine, glutamic acid and glutamine, proline, alanine, valine, leucine, tyrosine, phenylalanine and glycine (Sueoka, 1961). The values given are the average of two experiments for each strain.

** No correction was made for the destruction during hydrolysis.

*** Expressed as percentage over the sum of "stable" amino acids. NH_3 formed as a result of partial destruction of serine and threonine during hydrolysis and present in reagents is not taken into account.

**** Calculated by subtraction of amide NH_3 content from the sum of asp-NH_2 plus glu-NH_2 and expressed as percentage over the sum of "stable" amino acids.

were determined. The results are presented in Table I. It is evident that the relative content of amide-containing amino acids, judged by the amount of amide NH_3, is lower in strains R_{48} and R_{80} than in the wild type strain.

Discussion. Since suppression is the result of a single step mutation, the change in the value of amide NH_3 in the soluble proteins R_{48} and R_{80} should be ascribed only to the increase in asparagine or glutamine content. The values of Asp-NH_2 (asparagine plus aspartic acid) and Glu-NH_2 (glutamine plus glutamic acid) are similar in all three strains studied (see Table I). This can be observed only if the decrease in asparagine is accompanied by an increase in the content of aspartic acid or a decrease in glutamine would be compensated for by the increase in the glutamic acid content of proteins.

We may explain variations in the amino acid composition of proteins as a result of change in the translation mechanism whereby codons normally conding for glutamic and aspartic acid are miscoded as glutamine and asparagine. If we accept this explanation we have to remember that the mutations under investigation are recessive, as was shown in the large scale experiments on suppression of ad_2 mutations by s_{48} (Inge-Vechtomov, Simarov, 1967).

Recessiveness of s_{48} suggests that possibly the gene may be a structural gene for an enzyme participating in the translation process. For the same reason we suggest that the recessive supersuppressors are not likely to be genes coding for transfer RNA or ribosomes.

However, the low viability of the R_{48} strain must also be taken into account. The population of R_{48} and R_{80} strains contained a large proportion of non-viable cells at the time of harvesting and changes in the amino acid composition of their proteins could be attributed to differences between the viable and nonviable cells.

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