JOURNAL OF BACTERIOLOGY, Feb. 1977, p. 1165-1167

Vol. 129, No. 2
Printed in U.S.A.

Genetics of Expression of Asparaginase II Activity in *Saccharomyces cerevisiae*

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Received for publication 27 August 1976

Expression of asparaginase II activity in *Saccharomyces cerevisiae* requires the participation of the products of at least two cistrons, *asp2* and *asp3*, which are unlinked on the yeast genetic map.

Because of its ability to inhibit several biological functions, the enzyme L-asparaginase (L-asparagine amidohydrolase [EC 3.5.1.1]) has been studied extensively over the past few years (3, 8). The study of asparaginases from yeast recently was given impetus by the discovery that *Saccharomyces cerevisiae* can synthesize two distinctly different enzymes that are capable of hydrolyzing L-asparagine. One of these enzymes has been studied both biochemically (6) and genetically (4, 5). This enzyme is synthesized constitutively, is little affected in synthesis or activity by the products of its function, and is capable of deamidating L-asparagine only when the amino acid is inside the cell (1, 6). It is coded for by a single structural gene called *asp1*, and mutants lacking activity of the enzyme have been found and characterized (4, 5). This enzyme has been named L-asparaginase I by Dunlop et al. (2), who discovered the second asparaginase in this organism. The second enzyme (asparaginase II) has been extensively biochemically characterized by its discoverers (1, 2): it exists in only very small quantities in cells growing in medium containing ammonium ion as a nitrogen source, but the enzyme is strongly derepressed when cells are incubated in medium containing an energy source but no nitrogen supply. Derepression of this enzyme is affected by a variety of nitrogenous compounds, and the enzyme is capable of hydrolyzing both L- and D-asparagine; both amino acid isomers can by hydrolyzed when they are external to the cell. Biochemical evidence presented by Dunlop and Roon (1) and Dunlop et al. (2) suggests that L-asparaginase I and asparaginase II probably are not closely related. In this paper, genetic evidence is presented that strongly suggests that the two forms of the enzyme are synthesized independently. Additionally, these data show that at least two cistrons are involved in the expression of asparaginase II.

The yeast *S. cerevisiae* was used in the study. All strains were obtained or derived from those harbored in the Yeast Genetics Stock Center, University of California, Berkeley. Genetic symbols have been described (5). Cells were grown in YEPD or MV medium, which have been described (6). L-Tryptophan, when needed, was added to MV medium to a final concentration of 20 μg/ml. Solid media were prepared by adding 2% Bacto-agar (Difco) to liquid media. Yeast crosses and segregational analyses were carried out as previously described (7). L-Asparaginase I activity was assayed in whole cells as described by Jones and Mortimer (6). Asparaginase II was assayed in whole cells that had been grown in MV or MV plus tryptophan essentially as described by Dunlop and Roon (1), using approximately 10^7 cells/ml incubated for 30 min at room temperature with a substrate concentration of 1.15 mM. Under these conditions, evolution of ammonia was proportional to time and cell concentration. Ammonia determination was by direct nesslerization (6). When strains requiring tryptophan for growth were to be assayed for asparaginase II, L-tryptophan (20 μg/ml) was required during derepression for activity to appear, apparently because de novo protein synthesis is required for derepression of this enzyme (1).

Measurement of asparaginase II activity in several strains in my collection showed that the enzyme is present in many of them (averaging about 82 nmol of ammonia per min·mg [dry-weight]) but is absent in strain XE101-1A, which also lacks L-asparaginase I. To determine whether the presence of active L-asparaginase I is necessary for the presence of active asparaginase II and vice versa, strain XE101-1A was crossed with wild-type strain S288C, and haploid segregants from the cross were assayed for both asparaginase activities. This cross was numbered XE155. These experiments clearly showed that the presence or absence of

active L-asparaginase I is irrelevant to the presence or absence of active asparaginase II and vice versa. For instance, in several tetrads among the 18 analyzed, the four segregant haploid progeny individually expressed a different one of the four possible combinations of the two enzyme activities.

These experiments also showed that segregational patterns for the expression of asparaginase II activity are not typical of that expected for the segregation of two alleles of a single cistron (two wild-type and two mutant haploid progeny per tetrad). For example, several tetrads contained one wild-type and three mutant progeny, and other tetrads contained no wild-type and four mutant progeny, suggesting that two alleles of each of two separate cistrons were segregating in this cross, each cistron of which contributed to the expression of asparaginase II. These cistrons were provisionally called *asp2* and *asp3*.

This conclusion was confirmed by crossing each haploid segregant in a tetratype tetrad (XE155-1) with a strain wild type for both asparaginase activities and analyzing the segregational patterns of progeny from the crosses. If two alleles of each of two unlinked cistrons contribute to the expression of asparaginase II, then in a tetratype tetrad such as XE155-1, one haploid strain should be of genotype *asp2 asp3*, one should be *asp2* +, one should be + *asp3*, and the fourth should be of genotype + +. When each of these strains is crossed with a wild-type strain, progeny from the first such cross should be distributed in tetrads of all three types in proportions of one parental ditype (PD):one nonparental ditype (NPD):four tetratype (T). Progeny of the second and third crosses should be distributed in tetrads as two wild type:two mutant, and all the progeny from the fourth cross should be wild type. As shown in Table 1, these expectations were met. Pooling data from a total of 31 tetrads analyzed in crosses XE155 and XE163, in which both mutant alleles were segregating, showed that 3 were PD, 7 were NPD, and 21 were T tetrads. These values do not differ significantly from those expected from the segregation of two alleles of each of two unlinked cistrons ($\chi^2 = 1.56$; df = 2; $P > 0.25$).

The segregational patterns of *asp2* and *asp3* and the fact that a mutation in each cistron is expressed as lack of enzyme activity suggest that the presence of active asparaginase II should be dominant to its absence. To test this prediction, haploid strains of appropriate genotype were crossed with a wild-type strain of opposite mating type, and diploid strains from the crosses were tested for asparaginase II activity. All diploids heterozygous for either *asp2* or *asp3* exhibited activity of this enzyme, averaging 46 ± 5 nmol of ammonia per min · mg (dry weight) among the eight strains tested. Therefore, the lack of asparaginase II activity is recessive to its presence.

TABLE 1. *Segregation of asp2 and asp3 in haploid progeny from cross XE155 tetrad 1*

Cross no.	Parental strains	No. of tetrads	No. of tetrads exhibiting segregational pattern[a]:			
			0:4	2:2	1:3	4:0
XE162	XE155-1A × XE59-8B	9	0	9	0	0
XE163[b]	XE155-1B × XE59-8B	13	4	0	9	0
XE164	XE155-1C × S288C	8	0	8	0	0
XE165	XE155-1D × S288C	8	0	0	0	8

[a] Wild type:mutant.

[b] Tetrad proportions in this cross do not differ significantly from the 1:1:4 ratios expected if two alleles of each of two unlinked cistrons were segregating in the cross. $\chi^2 = 3.72$; df = 2; $P > 0.1$.

To confirm functionally that two cistrons are involved in the expression of asparaginase II, complementation tests were performed by assaying the enzyme in diploid cells heterozygous for both *asp2* and *asp3*. Complementation did occur in diploid cells heterozygous for the two cistrons, but did not occur in cells homozygous for either of the mutant alleles. Among the former, asparaginase II activity averaged 22 ± 2 nmol of ammonia per min · mg (dry weight). Among the latter, activity averaged 5 ± 2 nmol/min · mg (dry weight), which is not significantly different from zero under these conditions of assay.

All of these results strongly suggest that the appearance of active asparaginase II in this species depends upon the products of at least two cistrons that are unlinked on the yeast genetic map. The results also strongly suggest that L-asparaginase I and asparaginase II are functionally independent to the extent that strains lacking one of the enzymes can possess active enzyme of the other variety. The simplest interpretation of the data is that asparaginase II is a polymeric enzyme consisting of at least two different classes of monomers. An alternative explanation is that one of the cistrons is a structural gene coding for a core protein molecule that is inactive unless modified by the activity of a second enzyme coded for by the second cistron. That such might be the

case has been suggested by Dunlop and Roon (1).

I thank E. Cooperrider, C. Hanson, and R. Rose for excellent technical assistance and W. Belser for reading the manuscript.

This study was supported by a special grant from the American Cancer Society, California Division, Inc., to whom appreciation is expressed.

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