A GENETIC AND BIOCHEMICAL ANALYSIS OF MUTANTS TO TRYPTOPHAN INDEPENDENCE IN SACCHAROMYCES¹

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IT has been shown that reversion to growth factor independence in auxotrophic strains of *Neurospora* may result from mutation of the defective locus itself, or mutation at a different locus (WAGNER and MITCHELL 1955). Mutant genes of the latter type have been designated as suppressors, and biochemical studies of suppressed mutants have indicated that the metabolic pathway concerned may be different from or similar to that of wild type (LEIN and LEIN 1952; YANOFSKY 1952a).

Suggestive evidence that at least two classes of mutants to tryptophan independence occurred in *Saccharomyces* was obtained when it was observed that two kinds of clones developed when auxotrophic stocks were plated on tryptophanless medium. The genetic and biochemical properties of these mutants are the subject of this paper.

MATERIALS AND METHODS

The tryptophan requiring haploids as well as the tester stocks were obtained from Drs. D. C. HAWTHORNE and H. L. ROMAN. Clones containing the genes *tr-1* and *tr-2* were obtained originally from Drs. SEYMOUR POMPER (POMPER and BURKHOLDER 1949) and CAROLINE RAUT. The results of crosses indicate that *tr-1* and *tr-2* are not allelic and that *tr-1* is closely linked to its centromere (HAWTHORNE 1955a, b). Indole or tryptophan can fulfill the tryptophan requirement of both *tr-1* and *tr-2* clones, while *tr-2* can use anthranilic acid as well. Clones with *tr-1* accumulate both anthranilic acid and N-fructosyl-anthranilic acid in their growth medium (PARKS and DOUGLAS 1956) which suggests that *tr-1* clones are blocked in their conversion of these compounds to indole. Stocks that are doubly recessive (*tr-1*, *tr-2*) accumulate neither of these compounds, indicating that *tr-2* controls a step in tryptophan biosynthesis which occurs prior to the formation of anthranilic acid.

Indole synthesis from anthranilic acid was determined by using 0.25 gm dry weight of a 48-hour culture of the test organism grown in complete medium. Each assay flask contained 5 ml of yeast cells in water, 500 μ g anthranilic acid, 150 μ g hydroxylamine (YANOFSKY 1952b), and 4.4 ml of M/10 phosphate buffer at pH 7.0 to give a total volume of 10 ml. The reaction mixtures were incubated at room temperature on a rotary shaker for five hours. Indole was extracted in toluene and determined by

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the method of STANLEY and SPRAY (1941). The BRATTON-MARSHALL (1939) test for diazotizable amines was employed in the analysis for anthranilic acid.

Tryptophan synthetase activity was determined with identical yeast suspensions and buffer concentration as above but with 500 μ g indole, 2 mgs serine, and 20 mgs of glucose as substrates. Tryptophan was measured by the method of HORN and JONES (1945).

The synthetic medium used and the techniques for genetic analysis and scoring of segregants have been described by ROMAN (1956).

Cross feeding was demonstrated on agar plates by mixing cells of an indicator strain in the medium and spotting cells of the clones to be tested on the surface.

RESULTS AND DISCUSSION

Two types of tryptophan independent mutants were observed when dense suspensions (4×10^7 cells/ml) of *tr-1* stocks were plated on tryptophanless medium (fig. 1). One type consisted of cells that had the capacity to feed the auxotrophic

FIGURE 1.—Feeder (F) and nonfeeder (NF) revertants of *tr-1* on tryptophanless medium.

TABLE 1

Tests of segregants from a cross of an "F" mutant (#3732A-F) with a tryptophan independent tester (stock 3701B)

Phenotypic ratios	Number of asci			
	<i>Tr:tr</i>	<i>Hi:hi</i>	<i>Ur:ur</i>	<i>a:α</i>
2:2	0	10	9	10
3:1	0	0	1	0
4:0	10	0	0	0

The cross was heterozygous for the gene pairs controlling histidine independence: dependence (*Hi:hi*); uracil independence: dependence (*Ur:ur*); and mating type (*a:α*). *Tr:tr* refers to tryptophan independence: dependence.

TABLE 2

Tests of segregants from a cross of an "NF" mutant (3732-A-NF) and a tryptophan independent tester stock (3701 B)

Phenotypic ratios	Number of asci			
	<i>Tr:tr</i>	<i>Hi:hi</i>	<i>Ur:ur</i>	<i>a:α</i>
2:2	6	21	22	22
3:1	10	1	0	0
4:0	6	0	0	0

The cross was heterozygous for the gene pairs listed in Table 1.

parent as evidenced by satellite growth around the colonies. Clones of this type produced colonies 24–36 hours after plating and were designated as feeder ("F") mutants. The second mutant type did not feed *tr-1* and appeared a day or two later on the plates. These were designated as nonfeeder ("NF") mutants. A total of 130 colonies of both types were found on 21 plates; 19 percent of these were "F" mutants. Several mutants of both types were selected from the primary plates and, after purification by several replatings on tryptophanless medium, were used in the subsequent experiments.

The results of crosses indicate that the "F" and "NF" mutants are genetically different with respect to the mutant genes conferring tryptophan independence. When an "F" mutant was crossed with a tryptophan independent tester stock, all of the 40 segregants from ten asci were tryptophan independent, while the expected 2:2 segregations in each ascus were obtained for the gene pairs controlling mating type, histidine independence or dependence and, with the exception of one ascus, uracil dependence or independence (table 1). From the fact that no tryptophan requiring segregants emerged from the cross it can be concluded that the "F" mutant originated either as a consequence of back mutation at the *tr-1* locus, or through mutation at another locus closely linked to *tr-1*.

A cross similar to the one above but employing an "NF" mutant as one of the parents gave the expected monohybrid segregations in 21 asci for mating type, uracil independence, and (with one exceptional ascus) histidine independence. However, the 2:2 and 3:1 segregations for tryptophan independence (table 2) indicate

that the "NF" parent is a suppressed mutant since it carries a gene for tryptophan independence at a new locus. The proportion (25 percent) of tryptophan dependent segregants recovered in the cross is that expected on the basis of independent segregation of the *tr-1* locus and its suppressor. The suppressor gene is designated as *tr-1-Su* since it exhibits dominance in a diploid prepared by mating a *tr-1* haploid with an "NF" mutant.

It is known that some suppressor genes are highly specific in their effects, (YANOF-SKY and BONNER 1955), while others are nonspecific, in that a number of nonallelic genes may be affected (MITCHELL and MITCHELL 1952). To test whether *tr-1-Su* was capable of suppressing *tr-2* as well as *tr-1* a cross was made between a tryptophan independent suppressed clone (*Tr-2, tr-1, tr-1-Su*) and a tryptophan dependent *tr-2* clone (*tr-2, Tr-1, tr-1-su*). On the basis of independent segregation of the three genes one would expect the following percentages of the segregants to be tryptophan independent: 25 percent if neither *tr-1* nor *tr-2* were suppressed, 37.5 percent if only *tr-1* were suppressed, and 62.5 percent if both *tr-1* and *tr-2* were suppressed. The observed value of 34 percent (table 3) indicates that only *tr-1* is affected. It is also apparent from the data in tables 1, 2, and 3 that *tr-1-Su* has no observable effect on the genes conferring the requirements for uracil, histidine and adenine.

It was pointed out earlier that *tr-1* and *tr-2* stocks utilize indole in place of tryptophan, and, in the case of *tr-2* stocks, anthranilic acid as well. These facts suggested that tryptophan biosynthesis in yeast follows the same pathway as in *Neurospora* (TATUM and BONNER 1944) and *Escherichia coli* (YANOF-SKY 1955). To obtain additional information on this point, and also to examine the nature of the blocked reaction affected by *tr-1-Su*, all of the various stocks were tested for their ability to carry out two key reactions of this pathway, the synthesis of indole from anthranilic acid, and of tryptophan from indole and serine. The results (table 4) showed that all of the clones possessed tryptophan synthetase activity, and that all but the un-suppressed *tr-1* stocks converted anthranilic acid to indole. The suppressed *tr-1* clones, however, synthesized indole at a rate considerably less than that of wild type or *tr-2* stocks. It can be concluded from these observations that the pathway of biosynthesis of tryptophan in yeast is in fact similar to that of *Neurospora* and *Escherichia coli* and that *tr-1-Su* repairs a defect in this pathway affecting the conversion of anthranilic acid to indole.

TABLE 3

Tests of segregants from a cross of an "NF" mutant (7432-NF-12-ID) with a tryptophan dependent (*tr-2*) tester stock (#1169-2a)

Phenotypic ratios	Number of asci				
	<i>Tr:tr</i>	<i>Hi:hi</i>	<i>Ur:ur</i>	<i>a:α</i>	<i>Ad:ad</i>
2:2	4	11	11	11	11
1:3	7	0	0	0	0
0:4	0	0	0	0	0

This cross was heterozygous for the gene pairs controlling adenine independence: dependence (*Ad:ad*) in addition to the gene pairs listed in Table 1.

TABLE 4
Indole and tryptophan synthesis by clones of different genotypes

Clone	Genotype	μg indole formed	μg tryptophan formed
3701B	<i>Tr-2, Tr-1, tr-1-su</i>	240	130
1040-7c	<i>Tr-2, Tr-1, tr-1-su</i>	130	160
"F" mutant # 19	<i>Tr-2, Tr-1, tr-1-su</i>	120	130
"F" mutant # 20	<i>Tr-2, Tr-1, tr-1-su</i>	160	150
1138-2B	<i>tr-2, Tr-1, tr-1-su</i>	160	110
3732A	<i>Tr-2, tr-1, tr-1-su</i>	0	150
1071-3B	<i>Tr-2, tr-1, tr-1-su</i>	0	80
"NF" mutant # 11	<i>Tr-2, tr-1, tr-1-Su</i>	40	160
"NF" mutant # 12	<i>Tr-2, tr-1, tr-1-Su</i>	36	170

TABLE 5
Cross-feeding relations of tryptophan dependent and independent clones

Tryptophan genotype	Capacity to feed clones of tryptophan genotype	
	<i>Tr-2, tr-1, tr-1-su</i>	<i>tr-2, Tr-1, tr-1-su</i>
<i>Tr-2, tr-1, tr-1-Su</i>	—	+
<i>Tr-2, Tr-1, tr-1-su</i>	+	—
<i>Tr-2, tr-1, tr-1-su</i>	—	+
<i>tr-2, Tr-1, tr-1-su</i>	—	—

Failure of the suppressor to restore completely the block imposed by *tr-1* probably accounts for the fact that chromatographic tests showed that "NF" mutants continue to secrete anthranilic acid and N-fructosyl-anthranilic acid into their growth medium, while "F" mutants do not. This might be expected if the rate of synthesis of these compounds exceeded their rate of conversion to subsequent intermediates.

The incomplete removal of the *tr-1* block in the "NF" mutants also accounts in part for some of the cross-feeding relations summarized in table 5. The "NF" mutants, like their parental auxotrophs, retain their capacity to feed *tr-2* by virtue of their secretion of anthranilic acid. They are, however, unable to feed their parental auxotrophs. "F" mutants display the inverse reaction, since they are unable to feed *tr-2* clones, but feed *tr-1* clones. This situation can probably best be explained by the assumption that "F" mutants convert the anthranilic acid supplied by the cells of the *tr-1* clone to indole and tryptophan, some of which must then be excreted into the medium. "NF" cells apparently do not do this under the conditions of the cross-feeding tests, or do so too slowly to elicit a reaction within the time limits of the test.

SUMMARY

1. Tryptophan independent clones that arise spontaneously in tryptophan dependent *tr-1* haploid *Saccharomyces* were shown to consist in most cases of suppressed mutants.

2. The pathway of tryptophan biosynthesis in *Saccharomyces* is similar to that in

Neurospora and *Escherichia coli*. Clones of the *tr-1* genotype are unable to convert anthranilic acid to indole. The suppressor gene *tr-1-Su* effects a partial restoration of the blocked reaction.

3. The suppressor of *tr-1* has no effect on another nonallelic locus concerned in tryptophan biosynthesis.

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