SUPPRESSOR MUTATIONS FOR THE ISOLEUCINE LOCUS IN SACCHAROMYCES¹

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A suppressor mutation is a mutation at a locus other than the primary mutant locus which restores the wild-type phenotype in the presence of the mutant gene. The present communication describes the isolation and genetic analysis of different kinds of suppressors for the isoleucine locus in yeast.

MATERIALS AND METHODS

The two diploid strains, used in these studies, were 7110Z and 7113Z. They both require isoleucine. 7110Z is heteroallelic for the two isoleucine alleles (is_{1-1}/is_{1-2}) while 7113Z is the corresponding homoallelic diploid for one of the alleles (is_{1-1}/is_{1-1}). They are both heterozygous for the linked markers histidine (hi_1) and tryptophan (tr_2) located on opposite sides of the isoleucine locus. The two haploid isoleucine mutant strains (is_{1-2}) employed for the isolation of suppressors are 7217D and 7223B, both of which require uracil (ur_1). In addition 7217D requires histidine (hi_1) and 7223B, tryptophan (tr_2). A number of other stock cultures, not listed here, were employed for making crosses for genetic analysis. These included the stocks isolated during the course of the present investigation and stocks carrying the isoleucine alleles is_{1-1} , is_{1-2} , is_{1-3} , is_{1-4} and the wild-type allele (IS). The symbol is will be used to designate the mutant isoleucine allele when a particular allele is not specified.

The different kinds of media used and the techniques employed were the same as described earlier (KAKAR 1963).

RESULTS

Suppressors from 7110Z (is_{1-1}/is_{1-2}) revertants: In four independent revertants, R-10, 11, 12 and 18, segregation ratios of 2:2, 1:3, and 0:4 for isoleucine independence (Is^+):dependence (Is^-) were obtained in asci from these diploids while the segregation of three other markers was 2:2 as expected (Table 1). The

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TABLE 1

Irregular segregations for the isoleucine requirement in 7110Z revertants

Revertant	Number of 4-spored asci	Segregation for $Is^+:Is^-$			Segregation for		
		1:3	2:2	0:4	<i>hi</i>	<i>tr</i>	<i>ur</i>
R-10	10	6	2	2	2:2	2:2	2:2
R-11	12	10	1	1	2:2	2:2	2:2
R-12	12	6	2	4	2:2	2:2	2:2
R-18	12	8	1	3	2:2	2:2	2:2

results were suggestive of the segregation of a dominant suppressor (*S*) specific for one of the two isoleucine alleles. From an examination of the asci which had the parental ditype segregation for the outside markers *hi*₁ and *tr*₂, and showed a 1:3 and 2:2 segregation for isoleucine, it could be deduced that the allele being suppressed was *is*₁₋₁. The segregation patterns indicate that the suppressor loci are distantly removed from the isoleucine locus or they are on other chromosomes.

To confirm the segregation of a suppressor, the isoleucine independent spore culture from an ascus showing 1:3 segregation was crossed to a wild-type haploid strain. Such a spore culture would be *is*₁₋₁ *S-is*₁₋₁ in genotype on the basis of above assumption and the diploid would be heterozygous for both the isoleucine and the suppressor genes. From asci of such a diploid, 3:1 and 2:2 segregation for $Is^+:Is^-$ in addition to 4:0 ratios would be proof of suppressor segregation. Table 2 shows that such ratios were obtained from crosses of this kind for each of the four revertants.

In order to verify that the allele suppressed was *is*₁₋₁, cultures from the three isoleucine dependent spores of the 1:3 type of ascus, which will be of *is*₁₋₁ *s-is*₁₋₁, *is*₁₋₂ *s-is*₁₋₁ and *is*₁₋₂ *S-is*₁₋₁ genotypes, were crossed separately with an *is*₁₋₁ and an *is*₁₋₂ strain on yeast extract peptone plates. Matings were observed on the plates and after 48 hours the plates were replicated on isoleucineless medium. Only one of the six combinations, the one resulting from a cross with the *is*₁₋₁ culture and hence *is*₁₋₂ *S-is*₁₋₁/*is*₁₋₁ *s-is*₁₋₁ in constitution, is expected to grow on isoleucineless plate. This indeed was found to be the case for each of the four revertants. Similar crosses of the *is*₁₋₂ *S-is*₁₋₁ spore cultures with two more isoleucine alleles *is*₁₋₃ and *is*₁₋₄ were isoleucine dependent, indicating that these alleles were not suppressed

TABLE 2

Suppressor segregation in 7110Z revertants

Cross		Number of 4-spored asci	Segregation for $Is^+:Is^-$			Segregation for			Suppressor designation
Source of Is^+ spore			4:0	3:1	2:2	<i>hi</i>	<i>tr</i>	<i>ur</i>	
R-10	× wild type	10	1	8	1	2:2	2:2	2:2	<i>S</i> ₁ - <i>is</i> ₁₋₁
R-11	× wild type	15	5	7	3	2:2	2:2	2:2	<i>S</i> ₂ - <i>is</i> ₁₋₁
R-12	× wild type	9	0	8	1	2:2	..	2:2	<i>S</i> ₃ - <i>is</i> ₁₋₁
R-18	× wild type	6	3	3	0	2:2	2:2	2:2	<i>S</i> ₄ - <i>is</i> ₁₋₁

either. The four specific suppressors of is_{1-1} from the revertants R-10, 11, 12 and 18 have been respectively designated S_1-is_{1-1} , S_2-is_{1-1} , S_3-is_{1-1} and S_4-is_{1-1} .

Suppressors from 7113Z (is_{1-1}/is_{1-1}) revertants: Five different revertants from this homoallelic diploid gave 2:2 segregations for $Is^+ : Is^-$ as expected from either back mutation at the isoleucine locus or from suppressor mutation. However, the segregation for isoleucine independence in relation to that of the linked markers was suggestive of suppressor rather than reverse mutation.

To test for the presence of a suppressor gene, an isoleucine-independent segregant from four of the five cases was crossed to a wild-type culture. Deviations from 4:0 segregation were encountered in the asci obtained from each cross, confirming that reversion in all of the four revertants was due to mutation at a suppressor locus (Table 3).

The suppressors from 7113Z revertants were tested for their specificity in crosses of the suppressed strains with strains carrying the alleles is_{1-2} and is_{1-3} . The results of such an analysis are given in Table 4. In the crosses X-144, X-145, X-194, X-160 and X-151 segregations other than 2:2 were realized for isoleucine, suggesting that the suppressors of R-1, 2 and 5 are specific for the allele is_{1-1} and do not suppress the alleles is_{1-2} and is_{1-3} . These specific suppressors have been designated S_5-is_{1-1} , S_6-is_{1-1} and S_7-is_{1-1} respectively. There is, however, one case (R-4) in which the crosses with is_{1-2} and is_{1-3} regularly gave a 2:2 segregation

TABLE 3

Suppressor segregation in 7113Z revertants

Cross		Number of 4-spored asci	Segregation for $Is^+ : Is^-$			Segregation for		
Source of Is^+ spore			4:0	3:1	2:2	<i>hi</i>	<i>tr</i>	<i>ur</i>
R-1	× wild type	4	0	4	0	2:2	2:2	..
R-2	× wild type	5	3	2	0	2:2	2:2	..
R-4	× wild type	3*	1	0	2	2:2	2:2	..
R-5	× wild type	4	0	4	0	2:2	2:2	2:2

* There were three more asci with three spores growing in which two spores were isoleucine independent and one spore was isoleucine dependent.

TABLE 4

Specificity of the suppressors from 7113Z revertants

Cross			Number of 4-spored asci	Segregation for $Is^+ : Is^-$			Segregation for			Suppressor designation
Source of Is^+ spore		Diploid		0:4	1:3	2:2	<i>hi</i>	<i>tr</i>	<i>ur</i>	
R-1	× is_{1-2}	X-144	6	1	5	0	2:2	2:2	..	S_5-is_{1-1}
R-2	× is_{1-2}	X-145	5	3	1	1	2:2	2:2	2:2	S_6-is_{1-1}
R-4	× is_{1-2}	X-146	4	0	0	4	2:2	2:2	2:2	S_1-is
R-5	× is_{1-2}	X-194	15	1	14	0	2:2	2:2	2:2	S_7-is_{1-1}
R-1	× is_{1-3}	X-160	6	2	3	1	2:2	2:2	2:2	S_5-is_{1-1}
R-2	× is_{1-3}	X-151	5	1	2	2	2:2	2:2	2:2	S_6-is_{1-1}
R-4	× is_{1-3}	X-152	6	0	0	6	2:2	2:2	..	S_1-is

indicating this is a nonspecific suppressor. This could be verified in another way. In the segregants of X-146 and X-152 one should be able to recover the allele is_{1-1} in some asci among the two isoleucine requiring segregants. It was indeed found that the allele is_{1-1} was segregating in some instances. The nonspecific suppressor has been designated S_1-is .

Tests for allelism of isoleucine suppressors of 7110Z and 7113Z: Crosses were made between two haploid isoleucine independent clones carrying suppressors from different sources, and the asci obtained from these crosses were dissected for analysis of segregation pattern. A 4:0 segregation for $Is^+ : Is^-$ is expected if the two suppressors are allelic, while if the suppressors are not allelic, 3:1 and 2:2 ratios are expected in addition. The dissection results of Table 5 show that in intercroses of S_1-is_{1-1} , S_2-is_{1-1} , S_3-is_{1-1} , S_5-is_{1-1} , and S_7-is_{1-1} isoleucine requiring spores were obtained, suggesting that these suppressors are at different loci. The 4:0 segregation pattern for $Is^+ : Is^-$ was realized only in intercroses of S_1-is_{1-1} , S_4-is_{1-1} and S_6-is_{1-1} , suggesting that these three suppressors are allelic to one another. However, since the number of asci analyzed is small, there may be some doubt of allelism here, and the suppressors might be closely linked. Thus seven of the

TABLE 5
Allelism between suppressors of 7110Z and 7113Z

Cross	Number 4-spored asci	Segregation for $Is^+ : Is^-$			Segregation for			Other asci	No. spores growing
		4:0	3:1	2:2	<i>hi</i>	<i>tr</i>	<i>ur</i>		
$S_1-is_{1-1} \times S_2-is_{1-1}$	11	3	5	3	2:2	2:2	..	1	3*
$S_1-is_{1-1} \times S_3-is_{1-1}$	9	2	7	0	2:2	..	2:2	7	2-3*
$S_1-is_{1-1} \times S_4-is_{1-1}$	4	4	0	0	2:2	2:2	2:2	2	2†
$S_1-is_{1-1} \times S_5-is_{1-1}$	0	..	..	..	..	..	..	5	3*
$S_1-is_{1-1} \times S_6-is_{1-1}$	3	3	0	0	2:2	2:2	2:2	2	3†
$S_1-is_{1-1} \times S_7-is_{1-1}$	0	..	..	..	..	..	..	8	1-3*
$S_2-is_{1-1} \times S_3-is_{1-1}$	4	0	4	0	2:2	2:2	2:2	4	2-3*
$S_2-is_{1-1} \times S_4-is_{1-1}$	4	1	3	0	..	2:2	2:2	1	3*
$S_2-is_{1-1} \times S_5-is_{1-1}$	2	2	0	0	2:2	2:2	..	3	2-3*
$S_2-is_{1-1} \times S_6-is_{1-1}$	2	2	0	0	2:2	2:2	..	3	1-3*
$S_2-is_{1-1} \times S_7-is_{1-1}$	1	1	0	0	2:2	2:2	..	4	2-3*
$S_3-is_{1-1} \times S_4-is_{1-1}$	10	1	6	3	2:2	..	2:2	1	3*
$S_3-is_{1-1} \times S_5-is_{1-1}$	2	2	0	0	2:2	2:2	..	3	2*
$S_3-is_{1-1} \times S_6-is_{1-1}$	2	0	0	2	2:2	2:2	..	4	1-3*
$S_3-is_{1-1} \times S_7-is_{1-1}$	0	..	..	..	..	..	..	7	2-3*
$S_4-is_{1-1} \times S_5-is_{1-1}$	0	..	..	..	..	..	..	5	1-3*
$S_4-is_{1-1} \times S_6-is_{1-1}$	3	3	0	0	2:2	2:2	2:2	0	..
$S_4-is_{1-1} \times S_7-is_{1-1}$	2	2	0	0	2:2	2:2	2:2	3	2-3*
$S_5-is_{1-1} \times S_6-is_{1-1}$	4	3	0	1	2:2	2:2	..	2	2-3*
$S_5-is_{1-1} \times S_7-is_{1-1}$	0	..	..	..	..	..	..	5	1-3*
$S_6-is_{1-1} \times S_7-is_{1-1}$	1	1	0	0	2:2	2:2	..	9	2-3*
$S_1-is \times S_5-is_{1-1}$	6	0	6	0	2:2	2:2	..	0	..
$S_1-is \times S_6-is_{1-1}$	6	2	4	0	2:2	2:2	..	0	..
$S_1-is \times S_7-is_{1-1}$	5	0	4	1	2:2	2:2	..	2	3*

* Isoleucine dependent spores were present among these.

† All spores were isoleucine independent.

different is_{1-1} suppressors represent at least five different loci. The nonspecific suppressor has been tested against specific suppressors S_5-is_{1-1} , S_6-is_{1-1} , and S_7-is_{1-1} and is not allelic to any of these.

Suppressors from 7217D (is_{1-2}) revertants: Since in the previous analysis is_{1-1} specific suppressors were so common, a search was made to obtain specific suppressors of the allele is_{1-2} . Approximately 10^7 cells of this culture were plated on isoleucineless plates and irradiated with an ultraviolet dose which gave roughly 50 percent survival. Twenty revertants were crossed to a wild-type strain to see whether the reversion was due to back mutation or to suppressor mutation. In 11 out of 20 cases, isoleucine-requiring segregants were recovered, suggesting the segregation of a suppressor gene (Table 6). These suppressors have been designated S_2-is to $S_{12}-is$.

To check the specificity of the suppressors with respect to other alleles, asci were obtained from crosses of ten of the suppressor revertants with an is_{1-1} strain. As is seen in Table 7, only 2:2 segregations for $Is^+ : Is^-$ were found and among the isoleucine dependent spores, both the alleles is_{1-1} and is_{1-2} were segregating. Therefore none of the suppressors is specific for the allele is_{1-2} . Crosses of three of the revertants, carrying the suppressors S_2-is , S_3-is , and S_5-is with is_{1-3} strains also indicated nonspecificity for the is_{1-2} allele:

TABLE 6

Reversion in the haploid strain 7217D

Diploid	Number of 4-spored asci	Segregation for $Is^+ : Is^-$			Segregation for			Suppressor designation
		4:0	3:1	2:2	<i>hi</i>	<i>tr</i>	<i>ur</i>	
X-87	6	0	0	0	2:2	2:2	..	
X-88	4†	4	0	0	2:2	2:2	..	
X-89	5	5	0	0	2:2	2:2	..	
X-90	2*	0	1	1	2:2	2:2	2:2	S_2-is
X-91	3†	3	0	0	2:2	2:2	2:2	
X-92	5	5	0	0	2:2	2:2	2:2	
X-93	5	5	0	0	2:2	2:2	2:2	..
X-94	6	6	0	0	2:2	2:2	2:2	
X-95	2*	1	1	0	2:2	2:2	2:2	S_3-is
X-96	5	2	3	0	2:2	2:2	2:2	S_4-is
X-97	4†	4	0	0	2:2	2:2	2:2	
X-98	3†	3	0	0	2:2	2:2	2:2	
X-99	5	0	4	1	2:2	2:2	2:2	S_5-is
X-100	5	3	2	0	2:2	2:2	2:2	S_6-is
X-101	4	0	4	0	2:2	2:2	2:2	S_7-is
X-102	4	1	3	0	2:2	2:2	2:0	S_8-is
X-103	4	1	1	2	2:2	2:2	2:2	S_9-is
X-104	4	1	3	0	2:2	2:2	2:2	$S_{10}-is$
X-105	3*	0	2	1	2:2	2:2	2:2	$S_{11}-is$
X-106	4	0	3	1	2:2	2:2	2:2	$S_{12}-is$

The revertants were crossed to wild-type strain and the diploids dissected.

* There were more asci with 2 to 3 spores growing, and these contained Is^- segregants.

† There were more asci with 2 to 3 spores growing, and all of these were Is^+ .

TABLE 7

Specificity of the suppressors from 7217D revertants

Suppressor	Crossed to	Number of 4-spored asci	Segregation for $Is^+:Is^-$			Segregation for			Allele segregating
			2:2	1:3	0:4	<i>hi</i>	<i>tr</i>	<i>ur</i>	
S_2-is	is_{1-1}	4	4	0	0	2:2	2:2	2:2	is_{1-1} , is_{1-2}
S_3-is	is_{1-1}	4	4	0	0	2:2	2:2	..	is_{1-1} , is_{1-2}
S_4-is	is_{1-1}	2	2	0	0	2:2	2:2	..	is_{1-1} , is_{1-2}
S_5-is	is_{1-1}	4	4	0	0	2:2	2:2	2:2	is_{1-1} , is_{1-2}
S_6-is	is_{1-1}	6	6	0	0	2:2	2:2	..	is_{1-1} , is_{1-2}
S_8-is	is_{1-1}	3	3	0	0	2:2	2:2	2:2	is_{1-1} , is_{1-2}
S_9-is	is_{1-1}	2	2	0	0	2:2	2:2	2:2	is_{1-1} , is_{1-2}
$S_{10}-is$	is_{1-1}	3	3	0	0	2:2	2:2	2:2	is_{1-1} , is_{1-2}
$S_{11}-is$	is_{1-1}	4	4	0	0	2:2	2:2	2:2	
$S_{12}-is$	is_{1-1}	6	6	0	0	2:2	2:2	..	is_{1-1} , is_{1-2}

The nonspecific suppressors S_2-is , S_3-is and S_5-is were tested for allelism with the nonspecific suppressor obtained from 7113Z (R-4), i.e., S_1-is . The dissection results of various crosses gave 4:0 segregation for $Is^+:Is^-$ (Table 8). Thus the four nonspecific suppressors tested are at the same locus, in contrast with the widespread distribution of the specific suppressors reported above.

Suppressors from 7223B (is_{1-2}) revertants: An attempt was also made to obtain specific spontaneous suppressors of is_{1-2} . Approximately 200 cells of 7223B were plated on complete medium and allowed to grow for seven days. The colonies were then streaked on isoleucineless plates to select the revertants. In this way

TABLE 8

Allelism between nonspecific suppressors

Cross	Number of 4-spored asci	Segregation for $Is^+:Is^-$			Segregation for		
		4:0	3:1	2:2	<i>hi</i>	<i>tr</i>	<i>ur</i>
$S_1-is \times S_2-is$	6	6	0	0	2:2	2:2	2:2
$S_1-is \times S_3-is$	3*	3	0	0	2:2	2:2	..
$S_1-is \times S_5-is$	5	5	0	0	2:2	2:2	..

* There were four more asci with three spores growing, all of which were isoleucine independent.

TABLE 9

Reversion in the haploid strain 7223B

Diploid	Number of 4-spored asci	Segregation for $Is^+:Is^-$			Segregation for			Allele segregating	Suppressor designation
		2:2	1:3	0:4	<i>hi</i>	<i>tr</i>	<i>ur</i>		
X-185	9	9	0	0	2:2	2:2	2:2	is_{1-1} , is_{1-2}	$S_{13}-is$
X-178	4	4	0	0	2:2	2:2	2:2	is_{1-1} , is_{1-2}	$S_{14}-is$
X-189	6	6	0	0	2:2	2:2	2:2	is_{1-1} , is_{1-2}	$S_{15}-is$
X-192	5	5	0	0	2:2	2:2	2:2	is_{1-1} , is_{1-2}	$S_{16}-is$

The revertants were crossed to an is_{1-1} strain and diploids dissected.

four independent revertants were found. These were crossed to haploid is_{1-1} strains for evidence of suppressors and specificity. Only 2:2 segregations for $Is^+ : Is^-$ were found in asci from these crosses (Table 9) and among the isoleucine-requirers, some carried is_{1-1} and others is_{1-2} . Thus the four revertants were due to a nonspecific suppressor mutation. These suppressors have been designated S_{13-is} , S_{14-is} , S_{15-is} , and S_{16-is} . Allelic tests between these and other nonspecific suppressors have not yet been made.

Evidence for a suppressor which permits the utilization of threonine as a substitute for isoleucine requirement for growth: From a cross between a revertant segregant ($is_{1-1} S_7-is_{1-1}$) and an is_{1-2} parent, in every ascus there were one or two spores which did not grow on plates lacking both isoleucine and threonine, but which grew on plates that contained threonine (D or L, $300\mu\text{g/ml}$) and not isoleucine. All the spores grew on plates containing isoleucine ($60\mu\text{g/ml}$) but no threonine, indicating that none of the spores required threonine. In other words, some of the segregants had the capability of using threonine as a substitute for isoleucine requirement for growth. The capacity to utilize threonine segregated independently of the isoleucine alleles and the suppressor gene.

An examination of is_{1-1} , is_{1-2} and is_{1-3} haploid stocks at hand showed that this property to utilize threonine existed in some but not in all of the stocks. Replication on plates containing different concentrations of L-threonine (ranging from 10 to $300\mu\text{g/ml}$) gave a variable growth response. Nothing grew on plates containing $10\mu\text{g}$ threonine/ml and the best concentration was $300\mu\text{g/ml}$.

In order to study the genetics of the threonine utilization, a cross was made between a threonine nonutilizing strain and a threonine utilizing strain. The diploid did not grow on plates containing threonine but lacking isoleucine, indicating that capability to use threonine is recessive. Among asci dissected from this diploid, 11 out of 14 four-spored asci showed that the capability to utilize threonine segregated 2:2, whereas three segregated 1:3 for utilization *vs.* non-utilization.

The ability to use threonine as a substitute for isoleucine was verified by growth experiments in liquid medium. The results further indicated that threonine utilization is a slow process and suggested that a long lag period (about 18 to 20 hours) is required for adaptation. It was found that the growth in this medium was not due to the selection of isoleucine revertant cells.

Growth experiments with strains containing suppressor genes: It was of interest to see if any differences in growth rates existed in the revertants due to nonspecific or specific suppressor mutations and the wild-type strain with the possibility of understanding the role of suppressor mutations. For this purpose haploid strains of various genotypes with or without specific and nonspecific suppressor genes were selected: ($is_{1-1} S_1-is_{1-1}$, $is_{1-2} S_1-is_{1-1}$, $IS S_1-is_{1-1}$, $is_{1-1} S_1-is_{1-1} S_2-is_{1-1}$, $is_{1-1} S_1-is$ and IS). The growth experiments were carried out in test tubes containing 5.0 ml of synthetic liquid medium without or with $60\mu\text{g/ml}$ DL-isoleucine. The starting inoculum was 10^6 cells/ml. Growth was measured by turbidity changes as a function of time in a Klett-Summerson Photoelectric colorimeter using a green filter. The cultures were grown on a shaker at 29°C .

The results of such a growth experiment in the absence and in the presence of isoleucine are shown in Figures 1 and 2. As expected, strain $is_{1-2} S_1-is_{1-1}$ does not grow in the absence of isoleucine. A noteworthy feature of the results is that the presence of the specific suppressor gene S_1-is_{1-1} stimulates growth regardless of which alleles are present at the isoleucine locus or the suppressor locus S_2-is_{1-1} . Such a difference was noticed in both kinds of media, i.e., with and without isoleucine. The growth of the strain carrying the nonspecific suppressor is similar to that of the wild-type strain, and does not show the stimulation effect. Similar experiments with other specific and nonspecific suppressors have not yet been carried out.

DISCUSSION

The present study has revealed that suppressor mutations play an important role as a mechanism of reversion to prototrophy of the isoleucine auxotrophic mutants of yeast. This is true both for the diploid (hetero- or homo-allelic) and the haploid strains, and in the latter case, for the revertants which occurred spontaneously or following ultraviolet treatment.

In general two kinds of suppressors have been encountered in the two types of diploids. All four of the suppressors from the heteroallelic diploid and three of the suppressors from the homoallelic diploid are of one kind and specifically suppress the allele is_{1-1} . One of the suppressors from the homoallelic diploid is of another kind and is nonspecific in its suppressive effect as determined from tests with the three alleles is_{1-1} , is_{1-2} and is_{1-3} . None of the suppressors appears to be linked to the isoleucine locus. The seven specific suppressors represent at least five different genes. Also, the nonspecific suppressor was not allelic to the five specific suppressors against which it was tested. They are all dominant.

Similarly, in 11 out of 20 ultraviolet induced revertant and in all four of the spontaneously arising revertants of is_{1-2} haploid strains, the reversion was found to be due to suppressor mutation. Fourteen of those tested suppressed the allele is_{1-1} also, and were thus nonspecific in nature. Three of these suppressors were analysed further and shown to suppress the allele is_{1-3} . They are allelic to the nonspecific suppressor obtained from the homoallelic diploid and are consequently allelic to one another.

It must be pointed out that all four cases of spontaneous revertants analysed from the homoallelic diploid and all four from the haploid strain were found to be revertants due to suppressors. GILES (1951) found that in a methionineless strain of *Neurospora* reversions were obtained perhaps exclusively as a result of suppressor mutation. Whether the same is true for the isoleucine cases cannot be stated definitely from the few cases studied.

Besides the two kinds of suppressors discussed above, there is a third kind of suppressor which is interesting because it permits the utilization of threonine as

FIGURES 1 and 2.—Growth of the revertants that are due to suppressor mutation in isoleucineless medium (Figure 1, left), and in medium containing isoleucine (Figure 2, right).

a substitute for isoleucine for growth. This suppressor is also nonspecific; it is shown to be present in the stocks of various isoleucine allelic mutants and to segregate independently of these alleles. The fact that the various isoleucine mutant stocks do not require threonine means that although they can make their own threonine they cannot use it for isoleucine substitution in the presence of this suppressor gene (or genes). For the latter purpose, threonine has to be supplied externally at a certain threshold level. Genetic analysis has revealed that the capacity to use threonine to fulfill the requirement for isoleucine is recessive in behaviour, and is perhaps under the control of two linked and complementary genes. The latter conclusion is based on 1:3 segregation for utilization *vs.* non-utilization.

In *Neurospora* (YANOFSKY 1952; GILES and PARTRIDGE 1953), *Salmonella* (YURA 1956; HOWARTH 1958), *Escherichia coli* (STADLER and YANOFSKY 1959) and yeast (PARKS and DOUGLAS 1957), it has been observed that the presence of a suppressor in an otherwise wild-type strain has the effect of appreciably retarding the growth rate. Similar preliminary growth analysis in yeast with the specific suppressor S_1-is_{1-1} suggests, on the contrary, that the incorporation of this suppressor gene into the genome of other strains which carry is_{1-1} , is_{1-2} , IS or $is_{1-1} S_2-is_{1-1}$, stimulates the growth as compared to the growth of the strain lacking this suppressor. The growth of the strain carrying the nonspecific suppressor ($is_{1-1} S_1-is$) is, however, similar to that of wild type and does not show the stimulation effect. Such a difference in growth was noticed in both kinds of media, i.e., with and without isoleucine. This suggests that the difference in growth rates is not due to isoleucine in the medium being made available to the cell more readily in the presence than in the absence of S_1-is_{1-1} . Slight variations in the starting inoculum cannot account for this difference in growth either, since the strains containing S_1-is_{1-1} start to level off at a higher level of growth than the ones lacking S_1-is_{1-1} . Otherwise, it was expected that the latter would reach the same amount of growth as the former before leveling off.

It must be pointed out, however, that in the tests for allelism between different suppressors, poor viability of spores was encountered from certain suppressor $\times$ suppressor crosses (Table 7). It is possible that a spore having a combination of two suppressors is inhibited in growth, producing minute colonies on the dissection slabs (400 to 500 cells). Since a few exceptions were encountered, this idea is not well supported. Such a behaviour was noticed in only certain suppressor crosses and not in all.

Although attempts at the biochemical analysis of the action of suppressors have not yet been made, some speculation is possible from the results of the present investigation. The studies in yeasts, *Neurospora*, *E. coli* and *Salmonella* show that threonine appears to be a precursor in the synthesis of isoleucine by these organisms (ADELBERG 1955; UMBARGER 1955; STRASSMAN and WEINHOUSE 1955 and WAGNER and BERGQUIST 1960). WHITE, HANDLER, SMITH, and STETTEN (1959) have pointed out that in yeasts the pathway of isoleucine synthesis is probably through α -aminobutyrate to α -ketobutyrate to isoleucine.

On the other hand, UMBARGER and BROWN (1957) have demonstrated a direct conversion of threonine to α -ketobutyrate in *E. coli*. More recently, ATRAMSKY, ROWLAND and SHEMIN (1962) have suggested the possibility of α -ketobutyrate formation from α -methyl aspartate rather than from threonine in *E. coli*.

The feeding experiments, not reported here, indicate that isoleucine requirement of various isoleucine mutants used in present investigations can be satisfied with α -aminobutyrate. It seems likely, therefore, that in yeast the normal route of conversion of threonine to α -ketobutyrate is through α -aminobutyrate, and the function of the nonspecific suppressor is to open up the alternative pathway which ordinarily is not operative (say α -methyl aspartate to α -ketobutyrate). Such a mechanism will explain the nonspecificity of the suppressor since it does not involve the repair of the original defect (YANOFSKY and ST. LAWRENCE 1960).

As regards the action of the specific suppressors, the effect may be indirect, i.e., through some kind of inhibition relief, or the effect may be direct and lead to the formation of an active protein (WAGNER and MITCHELL 1963). One of the specific suppressors does seem to modify the environment and make it more favorable for growth. Whatever mechanism is involved, it has to be flexible enough to cover an apparently identical effect of at least five different specific suppressor genes for the isoleucine locus.

Similarly, a simple explanation for the action of the threonine utilizing genes appears to be an alternative pathway, perhaps a direct conversion of threonine to α -ketobutyrate. This follows from the fact that the threonine has to be supplied externally and that the threonine which is being made internally cannot be used by this suppressor. It could also be that a certain threshold amount of threonine is needed for the suppressor action and this threshold level is not attained internally, but can be achieved by furnishing this metabolite from outside.

Another finding which demands an explanation is the fact that whereas all of the suppressors obtained from is_{1-2} haploids are nonspecific, most of the ones obtained from is_{1-1}/is_{1-1} or is_{1-1}/is_{1-2} diploids are specific for the allele is_{1-1} . One of the possibilities is that is_{1-2} allele results in a major alteration of the enzyme so that a repair of this enzyme is not easily possible. Consequently, a majority (and in the present instances all) of the suppressors will be of the kind which do not repair the enzyme, but bypass this reaction through an alternative route. Such suppressors will then be nonspecific. On the other hand, the allele is_{1-1} could result in a minor alteration in the structure of the protein so that either this alteration can easily be repaired or the function of the protein restored by modifying the internal environment of the cell. Any suppressor mutation which can repair this defect or suitably alter the environment will turn out to be specific for this particular allele. Some of the ways by which this kind of repair can be achieved have been discussed by YANOFSKY and ST. LAWRENCE (1960) and YANOFSKY, HELINSKI, and MALING (1961).

This will account for a large number of suppressor mutations specific for one

allele. This, however, does not exclude the possibility of occurrence of non-specific suppressors in the present instance.

SUMMARY

It has been shown that a large number of isoleucine revertants result from suppressor mutation. Three types of suppressors of isoleucine requirements have been encountered. One kind of suppressor specifically suppresses the allele is_{1-1} and none of the other three alleles. The second type is nonspecific and suppresses all the alleles. Both of these kinds of suppressors are dominant. Whereas mutation at any one of the five loci indicated can give rise to specific suppressors, all of the four nonspecific suppressors analyzed are the result of mutation at a single locus. The third type of suppressor, which is also nonspecific, permits the utilization of threonine as a substitute for the isoleucine requirement for growth. This property seems to be under the control of two recessive but complementary and linked genes.

Growth studies indicate that the presence of specific suppressor (S_1-is_{1-1}) in a haploid culture with any one of the isoleucine alleles (is_{1-1} , is_{1-2} or IS) stimulates the growth to some extent. There is also some evidence, although not conclusive, that in some cases a haploid strain possessing two suppressor genes gives poor growth.

It is suggested that the nonpecific suppressors act by making available an alternative pathway of synthesis for isoleucine, and that the specific suppressors act by repairing the primary defect in some way or by controlling some environmental factor.

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