GENETIC MECHANISMS GOVERNING THE EFFECT OF CANAVANINE ON NEUROSPORA CRASSA

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HOROWITZ and SRB (1948) first noted that wild type strains of *Neurospora crassa* may be sensitive to the amino acid canavanine. They reported three types: one sensitive, one resistant, and one of intermediate sensitivity. TEAS (1951) studied two mutant homoserine-requiring strains, 46003-R (canavanine resistant) and 46003-S (sensitive). He found that canavanine sensitivity is heritable and independent of the amino acid requirement. Canavanine inhibition of sensitive strains is competitively relieved by arginine or lysine (HOROWITZ and SRB 1948). Some resistant strains requiring homoserine are able to utilize canavanine instead, and this stimulatory effect is also competitively antagonized by arginine or lysine (TEAS 1951).

These data suggested that resistant strains have the ability to "detoxify" canavanine by converting it to a homoserine-like substance, and that canavanine reaction is governed by a pair of allelic genes. Such an explanation does not account, however, for the existence of strains of intermediate sensitivity. Although one might postulate a third allele, quantitative responses of this type are often the result of gene interaction. With the haploid Neurospora, such interaction would require the participation of two or more nonallelic genes. We have made crosses between resistant and sensitive threonineless strains and screened the progeny for segregation of the canavanine character. The results indicate that the reaction of Neurospora to canavanine is governed by two separate genes.

MATERIALS AND METHODS

The mutant strains of *Neurospora crassa* used in these experiments were supplied by MRS. S. EMERSON and MRS. M. B. MITCHELL. Two of the strains as indicated in table 1 are reisolates which are identical by all biochemical tests to the originals as reported by TEAS (1947). The threonine-less gene in strain 44105 is probably identical to that in strain 46003 (TEAS 1947). MCCLINTOCK (1945) noted a translocation in a strain of 44105. There is no evidence that this is linked with the threonine-less gene in that stock. It is not known whether this translocation is present in the stock used here.

Stock cultures were maintained on slants of complete medium (BEADLE and TATUM 1945). For crosses, a slant of Westergaard medium (WESTERGAARD and

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TABLE 1

Neurospora strains

Strain	Canavanine susceptibility	Amino acid requirement
G2-6A (44105)	sensitive	threonine, isoleucine, α-aminobutyric acid, or homoserine
44104a	resistant	threonine, isoleucine, or α-aminobutyric acid
G147-5a(51504)	resistant	threonine + methionine, or homoserine

MITCHELL 1947), supplemented with 5 mg of D,L-threonine and 1 mg of D,L-methionine per 100 ml, was inoculated with a loopful of dry spores of one of the resistant strains. After 4 days incubation at room temperature, a second inoculation was made with strain G2-6A (44105). After perithecia had formed, ascospore dissections were carried out and individual spores planted on slants of Horowitz' glycerol-tartrate medium (BEADLE and TATUM 1945) supplemented with threonine and methionine.

Growth experiments were carried out in 125 ml erlenmeyer flasks containing 20 ml of minimal medium (BEADLE and TATUM 1945) and supplement. Flasks were inoculated with one drop of a light conidial suspension and incubated 72 hours at room temperature (ca 25°C). Mycelial pads were removed, dried at 85°C, and weighed.

Canavanine flavianate was isolated from jack bean meal by the method of GULLAND and MORRIS (1935) and the flavianate decomposed according to the procedure of CADDEN (1940). The product was used as the sulfate. Other amino acids used were obtained from commercial sources.

RESULTS AND DISCUSSION

In crosses of sensitive strain G2-6A (44105) with resistant strains 44104a and G147-5a (51504), analyzable asci (*ie*, at least one spore recovered from each pair) were obtained showing the following types of segregation of the canavanine character:

RRSS (two asci)
IISS (two asci)
IRSS (one ascus)

(R = resistant; S = sensitive; I = intermediate)

Spores obtained from eleven non-analyzable asci were also tested, but only resistant (R) and sensitive (S) types were obtained. Results of typical growth experiments with the parent strains and with the strains derived from one complete ascus obtained from a cross of strains 44104a and G2-6A (44105) are presented in table 2. Replicate experiments with these and the other strains tested yielded similar results. While resistant strains are not inhibited (and are sometimes even stimulated) by canavanine, strains of intermediate phenotype consistently produce in the presence of canavanine only 50 to 75 per cent of their normal mycelial weight. Sensitive strains, on the other hand, are inhibited completely by concentrations of canavanine much lower than 1.0 mg per flask.

TABLE 2

Growth of various canavanine phenotypes in the presence and absence of canavanine

Strain	Major gene*	Dry wt of mycelium (mg) Supplement per flask of minimal medium: threonine, 1.0 mg methionine, 0.2 mg	Dry wt of mycelium (mg) Supplement per flask of minimal medium: threonine, 1.0 mg methionine, 0.2 mg canavanine, 1.0 mg	Canavanine susceptibility**
G2-6A	44105	44	0	S
44104a	44104	26	43	R
G147-5a	51504	18	18	R
K16-1***	44104	53	46	I
K16-2	44104	61	37	I
K16-3	44104	58	61	R
K16-4	44104	38	47	R
K16-5	44105	50	0	S
K16-6	44105	44	0	S
K16-7	44105	44	0	S
K16-8	44105	40	0	S

* Refers to amino acid requirement (see table 1).

** R = resistant; S = sensitive, I = intermediate.

*** Cultures K16-1 to K16-8 were derived from a single ascus obtained in a cross between strains G2-6A and 44104a.

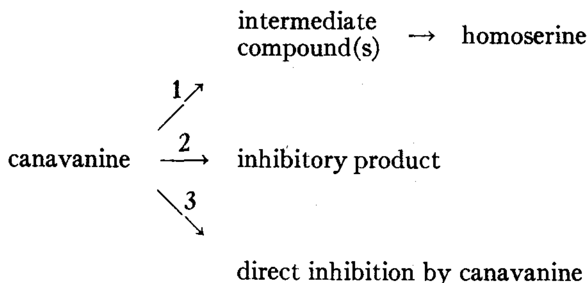
It is apparent that the phenotypes R, S and I are not governed by a set of alleles at a single locus, since the intermediate type appears in the progeny of crosses between resistant and sensitive strains. The only new genetic type possible under these circumstances would be a recombination of non-allelic genes present in the parents. Therefore the new intermediate phenotype must contain both the genes which, when alone, determine resistance or sensitivity in the parent strains.

Designating the genes conferring resistance or sensitivity as *R* and *S* respectively, and their alleles as *r* and *s*, the possible segregations (other than simple recovery of the parent types) may be represented as follows:

P_1	*Rs* (resistant)	×	*rS* (sensitive)
F_1	*Rs RS rS rs*	or	*RS RS rs rs*

From the phenotypic segregations noted earlier it may be seen that if *Rs* and *rS* are the parent genotypes and if the genotype *RS* is intermediate, the progeny containing the alleles of both the *R* and *S* genes (genotype *rs*) must be sensitive in phenotype.

At the present time the nature of the difference between the *R* and *S* genes and their alleles is not known. Although we have assumed in the discussion which follows that the genes *r* and *s* are simply "inactive", it must be recognized that resistance or sensitivity to a drug can be attained by a variety of mechanisms other than the blocking of a reaction. As the simplest explanation suitable for a working hypothesis, however, the following reaction scheme is tentatively proposed:



Reaction 1 would lead to a homoserine precursor, but not directly to homoserine itself, since not all resistant strains can utilize canavanine in place of homoserine (TEAS 1951). The product of reaction 2 is inhibitory to growth. These alternative reactions would explain TEAS' observation that arginine and lysine can competitively antagonize this substance either in inhibition or in growth promotion. Canavanine may also inhibit directly, or be slowly converted to still another inhibitory product (reaction 3).

Synthesis of the enzyme mediating reaction 1 is controlled by the *R* gene, of the enzyme for reaction 2 by the *S* gene. The respective alleles *r* and *s* are considered inactive, either through inability to elaborate the enzymes or because of a quantitative or qualitative change in the enzymes produced. The genotype *Rs* would be resistant because the reaction product is either inert or promotes growth upon conversion to homoserine (depending on whether there is also present a gene mutation which blocks the homoserine synthesis beyond this point). The genotype *rS*, conversely, would be sensitive. Type *RS* is intermediate in sensitivity, both the inhibitory and stimulatory products being formed from canavanine at about the same rate. The genotype *rs* is sensitive, since reaction 3 (which may be so slow as to be quantitatively insignificant in the presence of reactions 1 or 2) can still take place.

If this is true, two genetically different strains could appear equally sensitive to canavanine. From ascus K16 (table 2), for example, strains 5 and 6 should differ from strains 7 and 8, one pair having the genotype *rs* while the other has the genotype *rS*. Genetic evidence on this point would be essentially negative in character (crosses of the *rs* type with resistant strains should never yield progeny of intermediate sensitivity). However, if sensitivity to canavanine may be the result of two biochemically different processes one would expect to find physiological differences between the two sensitive types. This was confirmed by growth experiments in which these strains were grown in the presence of independently varied concentrations of both canavanine and arginine. The results indicate an arginine:canavanine molar ratio of 3:1 for complete relief of inhibition of strains K16-5 and 6, and a ratio of 7:1 for complete relief with strains K16-7 and 8. If the amino acids compete for different enzymes in the two cases, it is not surprising that different molar ratios are required for competitive relief of inhibition of the two sensitive types. We have also found that strains K16-5 and 6 are sensitive to α-aminobutyric acid at concentrations above .05 mg per flask, while strains K16-7 and 8 are not inhibited even by much higher concentrations of this compound.

There is no evidence that canavanine is a normal intermediate in Neurospora

metabolism; the enzymes which attack it may have as their usual substrate some chemically similar substance. Homoserine and its precursors seem to be involved in these relationships. It should be possible to identify the immediate products of canavanine metabolism by the various genetic types, and thus to help elucidate the metabolic pathways by which homoserine is formed.

SUMMARY

Experiments are reported which support the contention that in Neurospora, resistance and sensitivity to the amino acid canavanine are determined by two nonallelic genes. In crosses between resistant and sensitive strains, types of intermediate sensitivity occur in the progeny. The observed segregations suggest that the intermediate phenotype contains both these genes and that two genetically different sensitive types may occur. A tentative metabolic scheme is presented in explanation of these data.

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