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MUTATION OF A LOCUS CONTROLLING HETEROCARYON COMPATIBILITY IN *NEUROSPORA CRASSA*

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Abstract

During the isolation of tryptophan auxotrophs by irradiating the conidia of Emerson a strain of *Neurospora crassa*, a mutant, A336a, was isolated which was heterocaryon negative with representatives of both the loci tryptophan-1 and tryptophan-2. Genetical investigations have revealed that A336a is a tryp-2 mutant which is heterocaryon negative with tryp-1 mutants because a locus controlling heterocaryon formation has mutated in it. The locus which has mutated allows heterocaryon formation with strains carrying the mutated allele but inhibits heterocaryon formation with strains carrying the wild trypeallele.

Strains having the same mating-type allele formed vigorously growing heterocaryons while strains having the opposite mating-type alleles formed usually weak heterocaryons. However, there were a few exceptions where strains carrying the same mating type alleles formed vigorous heterocaryons. The variation in the vigour of heterocaryons has a pattern which suggests that besides the heterocaryon compatibility controlling loci and the mating-type locus, there are other loci which influence the growth of heterocaryons in *Neurospora crassa*.

Introduction

Beadle & Coonradt (1944) had shown that mutants belonging to the same locus do not form heterocaryons but mutants which occupy different loci do. A mutant, A336a, with an unusual behaviour in heterocaryon tests, was obtained during the classification of some freshly induced tryptophan mutants in *Neurospora crassa*. As it could utilise either anthranilic acid, indole or tryptophan for growth, it was expected to belong to either locus tryptophan-1 (tryp-1) or tryptophan-2 (tryp-2) (Ahmad & Catcheside, 1960). But on testing A336a for heterocaryosis it was found to be heterocaryon negative with the representatives of both locus tryp-1 (A106-2a) and locus tryp-2 (A60a). Therefore, it was decided to study the cause of this abnormal behaviour of A336a.

Materials and Methods

A336-a, Emerson A (EmA) (5296), Emerson a (Ema) (5297), tryp-1 (106-2a and 106-1A) tryp-2 (A60a and 75001A), adenine-1 (ad-1) (3254-a), adenine-8 (ad-8) (E56a, fifth generation), asco (37402-2a, eighth generation), histidine-1 (his-1) (K948-a), histidine-2 (his-2) (K999-a), histidine-7 (his-7) (K277-a), leucine-1 (leu-1) (33757-3a, third generation), lysine-1 (lys-1) (33933-2a, third generation), pantothenic acid-1 (pan-1) (5531-3a) and pyrimidine-3 (pyr-3) (37301-a) were used for this investigation. The number of generations entered against each strain represents the number of times a strain was crossed to Ema or EmA

A336, A60 and A106 were obtained by irradiating, with ultra-violat rays, the conidia of Ema, following the procedure of Ahmad & Catcheside (1960). His-1, his-2 and and his-7 were kindly supplied by Professor D.G. Catcheside. Cultures of asco, pyr-3

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TABLE 1. Behaviour of isolates of A336a in heterocaryon tests with the representatives of loci tryptophan-1 and tryptophan-2. The isolates were obtained by crossing A336a with EmA.

A336a X EmA Cross No.	No. of isolates tested	Mating type of isolates	Tester	Locus of the tester	Hetero-caryon negative	Haterocaryon positive, Intensity of reaction									Total
						1	2	2½	2¾	3	3¼	3½	4¼	4½	
I	16	a	A60a	Tryp-2	16	—	—	—	—	—	—	—	—	—	0
			A106-2a	Tryp-1	10	—	—	1	1	2	1	1	—	—	6
II	25	a	A60a	Tryp-2	25	—	—	—	—	—	—	—	—	—	0
			A106-2a	Tryp-1	13	4	3	1	—	1	—	3	—	—	12
III	3	a	A60a	Tryp-2	3	—	—	—	—	—	—	—	—	—	0
			A106-2a	Tryp-1	1	—	—	—	—	—	—	—	1	1	2
	4	A	A60a	Tryp-2	4	—	—	—	—	—	—	—	—	—	0
			A106-2a	Tryp-1	2	—	—	—	2	—	—	—	—	—	2

and tryp-1 were kindly sent to us by Dr. Ogata of Fungal Genetics Stock Center. Ad-8 was generously sent to us by Dr. D.R. Stadler. The remaining cultures were used from the author's stocks.

Media and methods used in these studies, were the same as reported by Ahmad *et al* (1964).

When crosses failed in both Westergaard's and Suyama's media, the following media were tried: Westergaard's medium with 50 mg. tryptophan per 100 ml and Westergaard's medium with 4, 6 and 8 drops, per crossing tube, of mycelial extract from a highly fertile cross of A637a X ad-2 Stl-2 12A (Ahmad *et al*, (1967).

Growth of heterocaryons was assessed in arbitrary, units where ½ denotes minimum growth and 5 denotes maximum growth i.e. almost as good growth as the wild type.

Experiments and Results

1. BEHAVIOUR OF THE ISOLATES OF A336a.

A336a was corssed with EmA. Of the 120 mutant spores isolated from this cross, 100 of them showed vigorous growth and good conidiation. When these were tested on V.M. plates for leakiness, 63 single-spore cultures were non-leaky and they were crossed with EMA and Ema for determining their mating-type. Only 16 cultures possessed 'a' mating-type and they were tested for heterocaryosis with A60a of tryp-2 locus and 106-2a of tryp-1 locus. All the 16 isolates of A336 were heterocaryon negative with A60 but with 106-2a, 6 were positive and 10 negative (Table 1, Cross I).

A second cross of A336a was made with EmA. Of the 150 mutant spores isolated, 100 of them gave vigorously growing and good conidiating cultures. When these were tested on V.M. plates, 88 were non-leaky. The non-leaky cultures were crossed with EmA and EmA for datermining their mating-type. Out of 88, 25 possessed 'a' mating type. When these 25 single-spore cultures were tested for heterocaryosis, all of them were negative with A60a but 12 were positive and 13 negative with A106-2a (Table 1, Cross II). A similar behaviour was shown by 7 isolates from a third corss of A336a with EmA (Table 1, Cross III).

It thus appeared that A336a was probably allelic to A60 and belonged to locus tryp-2, and that it was haterocaryon negative with A106-2a of locus tryp-1 because a locus controlling heterocaryon formation had mutated during irradiation of the conidium which gave rise to A336a culture.

2. CONFIRMATION OF LOCATION OF A336a AT LOCUS TRYPTOPHAN-2 BY LINKAGE STUDIES.

Locus tryp-1 is located in linkage group III while locus tryp-2 is located in linkage group VI (Ahmad *et al*, 1968). A336a was crossed with tryp-1 and tryp-2 to see whether it was allelic to tryp-1 or to tryp-2. A106-1A and 75001-A were used in these crosses as representatives of loci tryp-1 and tryp-2 respectively. A count of the progeny from the two crosses was undertaken. It is seen from the data (Table-2) that A336a is located at locus tryp-2. The failure of A336a to form heterocaryon with A60, a representative of locus tryp-2, was thus due to its allelism with A60.

TABLE 2. Data showing that A336a is located at locus tryp-2.

Cross	Allele of the marker used	Classification of spores: Mutant	Wild-type recombinants: Number	Wild-type recombinants: Percentage	Linkage	Location of A336
A336a X Tryp-1	A106-A	773	251	24.5	No Linkage	Not Located at tryp-1
A336a X Tryp-2	75001	5244	0	0	Linked	Located at tryp-2

3. FURTHER ANALYSIS OF HETEROCARYON INCOMPATIBILITY FACTOR IN A336a

If the heterocaryon negative nature of A336a followed from a mutation of a single locus in Ema, then crosses of heterocaryon positive and heterocaryon negative isolates of A336 should show a 1:1 segregation of heterocaryon positive and heterocaryon negative cultures in the progeny. Also a cross of two heterocaryon positive isolates together should yield no heterocaryon negative progeny, while a cross of two heterocaryon negative isolates together should yield no heterocaryon positive progeny with the tester 106-2a.

The above crosses were made by using 'A' and 'a' heterocaryon positive and heterocaryon negative isolates obtained by corssing A336a with EmA.

(i) Analysis of the progeny from a cross of heterocaryon positive isolate with a heterocaryon negative isolate of A336a.

A336—7A X A336—21a
Heterocaryon positive Heterocaryon negative

From this cross 101 mutant spores were isolated. All of them were crossed with EmA and Ema to determine their mating-typesand all of them were tested for heterocaryosis with A106-2a. 58 of them were heterocaryon negative and 43 of them were heterocaryon positive (Table 3). The growth of heterocaryons formed by the heterocaryon positive isolates was not the same in the case of every isolate. It ranged from 1/4 to 2-1/2 units. Majority of isolates with 'A' mating-type formed weak heterocaryons, whose growth did not exceed 1 unit. On the other hand majority of isolates

TABLE 3. Heterocaryon tests of 101 isolates from a cross of a heterocaryon positive X a heterocaryon negative isolate of A336a using 106-2a as a tester. A and a indicate mating-type of isolates.

NUMBER OF SINGLE-SPORE CULTURES						
Heterocaryon negative			Heterocaryon positive			
A	a	TOTAL	Growth of Heterocaryon	A	a	TOTAL
36	22	58	1/4	1	0	1
			1/2	8	0	8
			3/4	5	0	5
			1	8	2	10
			1-1/4	1	0	1
			1-3/4	0	1	1
			2	0	3	3
			2-1/4	0	3	3
			2-1/2	2	9	11
Total: 36	22	58		25	18	43

with 'a' mating-type formed vigorous heterocaryons and attained heterocaryotic growth of at least 2 units. However, heterocaryons of 2 'A' isolates with 106-2a showed a growth of 2-1/2 which is the maximum which has been attained by any 'a' isolate heterocaryon (Fig. 1).

(ii) Analysis of the progeny from a cross between two heterocaryon positive isolate of A336a.

A336—7A X A336—10a
Heterocaryon positive Heterocaryon positive

From this cross 100 mutant spores were isolated. Of these 22 of them which grew vigorously and conidiated well, possessed 'a' mating-type and were non-leaky. They were tested for heterocaryosis with A106-2a. All 22 of them were heterocryon positive.

This cross was repeated and another 100 spores were isolated. Of the good growing, good conidiating and non-leaky single-spore cultures, only 7 had 'a' mating type. When they were tested for haterocaryosis with 106-2a, all 7 of them were heterocaryon positive.

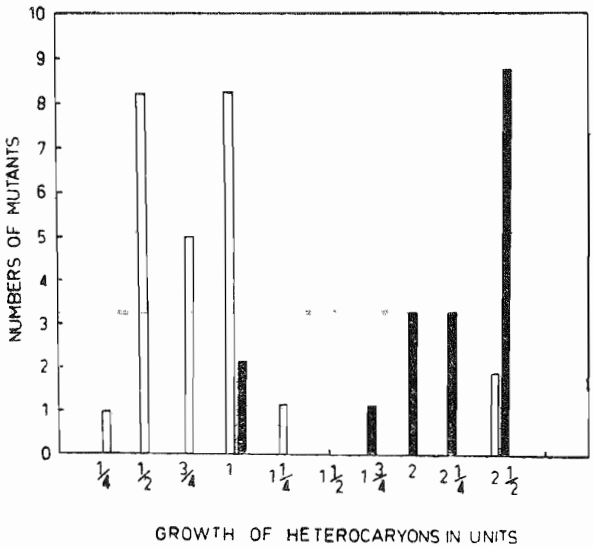

Fig. 1. Showing that 'A' isolates of A336 usually form much weaker heterocaryons than its 'a' Isolates with 106-2a. Empty column=A isolates; shaded column=a isolates.

(iii) Analysis of the progeny from a cross between two heterocaryon negative isolates of A336a.

A336—21a X A336—32A
Heterocaryon negative Heterocaryon negative

This cross remained sterile inspite of repeated attempts to improve fertility by employing various techniques outlined under media and methods. So no progeny could be analysed from this cross.

4. NATURE OF HETEROCARYON INCOMPATIBILITY FACTOR IN A336a

An attempt was next made to see whether the allele carried by A336a was an inhibitor of heterocaryon formation as such and inhbited the formation of heterocaryon with all the strains or it inhibited heterocaryon formation with some of the strains but not with the other.

Therefore, A336a was tested for heterocaryosis with the representatives of loci ad-1, ad-8, asco, his-1, his-2, his-7, leu-1, lys-1, pan-1 and pyr-3. A336a was heterocaryon negative with pyr-3, it formed very weakly growing heterocaryons with the representatives of loci ad-1, ad-8, asco, his-1, his-7, leu-1 and pan-1 but with lys-1 it formed a vigorously growing heterocaryon.

Discussion

Beadle & Coonradt (1944) demonstrated that mutants belonging to different loci form heterocaryons whereas mutants which occupy the same locus do not. Accordingly, A336a was expected to be heterocaryon positive with either tryp-1 or tryp-2 or both

but in actual tests it did not form a heterocaryon with the representatives of either of these two loci.

All the isolates, from three independent back crosses of A336a with EmA, were heterocaryon incompatible with A60a belonging to locus tryp-2 but roughly half of them were heterocaryon positive with 106-2a, a representative of locus tryp-1 (Table 1).

These results suggested the possibility that A336a was heterocaryon negative with A60a because it was allelic to it while it was heterocaryon negative with A106-2a of locus tryp-1 because it carried a heterocaryon incompatibility factor which inhibited the formation of heterocaryon with A106-2a.

That A336 belonged to locus tryp-2, was confirmed by linkage studies. When it was crossed with the representatives of loci tryp-1 and tryp-2, it yielded 24.5% wild-type recombinants with the representative of locus tryp-1 but it yielded no wild-type recombinant, in a count of 5244 ascospores, from its cross with the representative of locus tryp-2 (Table 2). It was thus confirmed that A336a failed to form heterocaryon with A60a because it was located at locus tryptophan-2.

If the lack of heterocaryon formation by A336a with A106-2a of locus tryp-1 was due to the presence of a heterocaryon incompatibility factor in it, then the cross of a heterocaryon positive isolate with a heterocaryon negative isolate of A336 should give a 1:1 segregation of heterocaryon positive and heterocaryon negative cultures and a cross of two heterocaryon positive isolates of A336 should give only heterocaryon positive isolates. These expectations were indeed realised when these experiments were undertaken (Table 3 and section 3i and 3ii under Experiments and Results).

It thus seems that during U.V. irradiation of the condium of Ema which gave rise to the strain A336a two mutations occurred, one at locus tryp-2, making it a tryptophan tauxotroph and a second one at another locus controlling the formation of heterocaryons with other strains.

Investigation of the nature of the heterocaryon incompatibility factor carried by A336a showed that it did not inhibit heterocaryon formation with all the *Neurospora crassa* strains. With lys-1, it formed a heterocaryon growing as vigorously as the wild type.

As a result of the investigation of genetics of heterocaryosis, Holloway (1955) concluded that *Neurospora crassa* carried a locus W which controls the initiation of heterocaryon. The allele W initiates the formation of a heterocaryon, while w inhibits it. It is obvious that A336a does not carry w because it is able to form a vigorously growing heterocaryon with lys-1.

Garnjobst (1953) on the other hand affirmed that the formation of heterocaryons in *Neurospora crassa* is controlled by two loci C and D, each with two alleles, Cc and Dd. Formation of heterocaryon is inhibited if the two strains differ in alleles of C or D or alleles of both C and D. Thus a C D strain is heterocaryon compatible with another C D strain but is incompatible with cD, Cd, or cd strains. The behaviour of A336a suggests that a locus like C or D mutated in the conidium that gave rise to it, so that it is unable to form heterocaryons with strains carrying the normal allele but forms heterocaryons with strains carrying the mutated allele. Thus if the wild type strain carries alleles CD, the mutated strain, A336a, may carry Cd. A336a will, therefore be

heterocaryon incompatible with any strain carrying the wild type alleles CD but will be compatible with any mutated strain carrying Cd alleles.

Beadle & Coonradt (1944) reported that in *Neurospora crassa*, heterocaryons formed by strains of same mating-type grow vigorously like wild type while heterocaryons formed by strains of opposite mating-type grow more slowly. These observations of Beadle & Coonradt were confirmed by Gross (1950, 1952).

Experiments reported in the preceding pages showed that as a general rule strains of the same mating-type formed more vigorous heterocaryons than strains of opposite mating-type (Table 3 and Figure 1). However, this was not true universally. Two 'A' single-spore cultures formed as vigorous heterocaryons with 106-2a as the most vigorous heterocaryon forming 'a' single-spore cultures. Secondly, the vigour of heterocaryons formed by the 'A' single-spore cultures varied from 1/4 to 2-1/2 while the vigour of heterocaryons formed by 'a' single-spore cultures varied from 1 to 2-1/2. These variations in the vigour of heterocaryons suggest that besides the mating-type locus and the primary heterocaryon controlling loci, C and D, there are other loci which modify the growth of heterocaryons. Some alleles of these loci seem to accelerate growth as in the case of the two 'A' single-spore cultures while other alleles retard the growth of heterocaryons. The latter is exemplified by those 'a' single-spore cultures whose heterocaryons with 106-2a showed a growth of less than 2-1/2 units (Table 3).

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