A SUPPRESSOR IN NEUROSPORA AND ITS USE AS EVIDENCE FOR ALLELISM*

BY MARY B. HOULAHAN AND HERSCHEL K. MITCHELL

WILLIAM G. KERCKHOFF LABORATORIES, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA

In Drosophila recessive mutations have been observed which suppress the effect of specific mutations at other loci. Thus an individual, homozygous for a suppressor gene in the mutant form and for the mutant gene upon which the suppressor acts, is phenotypically wild type, or nearly so. Suppressors of black,[1] purple,[2,3] sable[4] and vermilion[4,5] are among those which have been reported. In the vermilion case there is evidence[5] which shows that the action of the suppressor is to restore v^+ substance (kynurenine), the formation of which is prevented when the mutant gene vermilion is present in the homozygous condition.

A similar situation which has been found in Neurospora will be reported here. The effect of a mutation which prevents the synthesis of pyrimidine is partly suppressed by the presence of a mutant gene at a different locus. It seems logical to assume that the presence of the second mutation results in the formation of an intermediate in pyrimidine synthesis which is lacking in the pyrimidineless strain. Evidence that the new mutation is not due simply to duplication of the pyrimidineless locus will be presented.

Results of studies of combinations of the suppressor with five pyrimidineless strains have supplemented other evidence regarding relationships among the mutant genes concerned. Genetic difference is readily demonstrated in Neurospora[6] but evidence for allelism is less convincing, because neither the absence of crossing over, nor the heterocaryon test[7] is conclusive. For this reason additional evidence, obtained by use of the suppressor, that three pyrimidineless strains carry allelic genes, is welcome, particularly since these strains show marked physiological differences, and therefore constitute the first series of multiple alleles to be described in Neurospora. This series may prove useful, since one would expect biochemical studies to be simpler in Neurospora than in more complex

organisms such as corn, Drosophila and mammals, in which multiple alleles have been studied in detail.

Forty-five independent occurrences of mutation which results in pyrimidine deficiency have been recorded in Neurospora. These were obtained from x-ray and ultra-violet treatment as described by Beadle and Tatum.[8] The deficient strains utilize uracil or the nucleosides and nucleotides of uracil and cytosine, as will be reported in detail elsewhere.[9] One of the five strains to be discussed here, number 263, has been described by Loring and Pierce.[10]

Crosses to Determine Genetic Relationships.—Isolation numbers of the strains considered are 263, 38502, 37301, 37815 and 67602, but for convenience they will be referred to here as 1, 2, 3a, 3b and 3c. Each strain behaves in backcrosses as if the difference from the parent wild type were due to modification of one gene. That is, when any one is back-crossed to the parent strain, and the eight ascospores from each of a number of asci are isolated and tested using techniques reviewed by Beadle,[6] four of the eight spores always prove to be like the mutant parent and four like the wild-type parent.

TABLE 1

CROSSES BETWEEN MUTANT STRAINS

STRAINS CROSSED	NUMBER OF ASCI OBSERVED	ASCI WITH 1 PAIR OF WILD-TYPE SPORES	ASCI WITH 2 PAIRS OF WILD-TYPE SPORES	ASCI WITH 4 PAIRS OF MUTANT SPORES
1 × 3a	37	5	0	32
1 × 3b	94	8	0	86
1 × 2	17	11	0	6
1 × 3c	53	5	0	48
3a × 3b	59	0	0	59
3a × 2	17	10	0	7
3a × 3c	15	0	0	15
3b × 2	19	6	1	12
3b × 3c	100	0	0	100
2 × 3c	21	7	0	14

Table 1 gives results of intercrosses among the five strains. (In all cases here only asci are included from which at least one member of each spore pair germinated.) From these results it appears that 3a, 3b and 3c are alike genetically, while 1 and 2 are different from these three and from each other. This is evident since from crosses among the first three, no asci were observed containing wild-type spores, while all crosses involving 1 and 2 gave some asci of this type. In order to confirm evidence of genetic difference it was demonstrated that an ascus containing wild-type spores also contained spores carrying two mutant genes. This was done by outcrossing a mutant strain derived from such a spore to wild type and recovering asci containing six or eight mutant spores. If an ascus from the

intercross contains four wild-type spores, then obviously the other four spores must carry both mutant genes, but if the ascus contains two wild-type spores then only two of the six mutant spores will give rise to double mutants. If only asci of the latter type are obtained from an intercross it would be necessary to cross to wild type a strain derived from one member of each of the three pairs of mutant spores, were it not often possible to distinguish the double mutants, partly on the basis of the position of spores in the ascus and partly because of slight differences in growth habit. Results from crosses of double mutants to wild type appear in table 2.

It is interesting that the three loci represented are on the same chromosome, as can be seen from the data in tables 1 and 2. The distances from the centromere are, roughly, 12, 39 and 17 units for 1, 2 and 3, respectively.

TABLE 2

CROSSES BETWEEN DOUBLE MUTANTS AND WILD TYPE

STRAINS CROSSED	NUMBER OF ASCI OBSERVED	ASCI WITH 1 PAIR OF WILD-TYPE SPORES	ASCI WITH 2 PAIR OF WILD-TYPE SPORES	ASCI WITH 4 PAIRS OF MUTANT SPORES
1, 3a × wild	34	8	26	0
1, 3b × wild	19	3	16	0
1, 2 × wild	12	7	5	0
1, 3c × wild	27	2	25	0
3a, 2 × wild	1	1	0	0
3b, 2 × wild	10	8	1	1
2, 3c × wild	6	4	2	0

A Suppressor of 3a.—A strain of 3a, which had begun to grow in the absence of pyrimidine as if a back mutation had occurred, was tested by crossing it to wild type. Two types of asci would be expected if back mutation had taken place, one containing eight wild-type spores, arising from nuclei carrying the back mutation, and the other, from nuclei carrying the mutant gene unchanged, containing four wild-type and four mutant spores. These two types were found, but a third type appeared as well, containing six wild-type and two mutant spores. This third type of ascus cannot be explained on the basis of back mutation at the 3a locus, but, rather, it is necessary to assume that, at a different locus, a mutation had occurred which, in some way, suppresses the effect of the mutation at the 3a locus. If this assumption were correct then all three types of asci would be expected, if there were no close linkage. The second type could result either from nuclei which did not carry the new mutation or from failure of the two mutant genes to combine in any spore pair. The first type could result from combination of the two mutations in four spores, and the third from combination in two spores.

From an ascus with eight wild-type appearing spores one member of each spore pair was crossed to wild type. Two of these crosses gave only

wild-type progeny, while from the other two, the three types of asci just described were recovered, demonstrating that the assumption made above is correct. As a further check, an ascus containing four wild-type and four mutant spores was selected from one of the above two crosses. A wild-type spore from such an ascus should carry the suppressor gene. This proved to be the case, for when a strain from one of these spores was crossed to an unchanged strain of 3a the three ascus types described above were recovered.

TABLE 3

CROSSES BETWEEN MUTANTS AND THE SUPPRESSOR[a]

STRAINS CROSSED	NUMBER OF ASCI OBSERVED	ASCI WITH 4 PAIRS OF WILD-TYPE SPORES	ASCI WITH 3 PAIRS OF WILD-TYPE SPORES	ASCI WITH 2 PAIRS OF WILD-TYPE SPORES
1 × S	19	0	0	19
3a × S	6	1	1	4
3a-S × wild	38	10	22	6
3b × S	19	2	10	7
2 × S	17	0	0	17
3c × S	11	3	2	6
3c-S × wild	14	4	7	3

[a] In some cases germination of spores from crosses between mutants and the suppressor was very poor. For this reason data from crosses of mutant-suppressor combinations to wild-type are included.

The Effect of the Suppressor on Other Pyrimidineless Mutants.—Results, given in table 3, from crosses between the suppressor strain and the remaining four mutants show that the suppressor has no effect when in combination with 1 and 2, since each ascus recovered contained four mutant and four wild-type spores. In order to show that these two mutants had not simply failed to combine with the suppressor each was crossed to a strain carrying both 3a and the suppressor gene in the mutant form. The appearance, among the progeny of both crosses, of asci containing six or eight mutant spores demonstrates that in these asci the two mutants had indeed combined with the suppressor and had not been affected by it. This follows from the fact that in a cross involving the suppressor and two mutants, both of which were capable of being suppressed, no asci could occur containing more than four mutant spores.

The data in table 3 show that 3b and 3c, as well as 3a, are suppressed. This fact is taken as evidence that mutation at the same locus has occurred in the three strains.

Growth Requirements of the Mutants.—The three mutants which behave as alleles all have much the same requirement for uridine or hydrolyzed nucleic acid if they are allowed to grow at 35°C., but at 25°C. there are striking differences (Table 4). At this temperature 3a requires nearly the same amount of uridine or hydrolyzed nucleic acid as it does at 35°C.,

while 3c needs only about one sixth as much and 3b grows normally in the absence of any source of pyrimidine. Hence it would appear that the genetic block is complete in the three strains at 35°C., and in 3a at 25°C. also, but is only partial in 3c at 25°C. and seemingly non-existent in 3b at this temperature.

TABLE 4

GROWTH REQUIREMENT OF MUTANT STRAINS

| STRAIN | HYDROLYZED NUCLEIC ACID* REQUIRED FOR 1/2 MAXIMUM GROWTH (MG.) | |
	25°C.	35°C.
3a	3.3	3.15
3b	0	2.4
3c	0.38	2.3

* Ribonucleic acid in 1 N NaOH, 24 hours at 25°C.

Strain 2 has not been thoroughly tested, but the data available indicate that its requirements are similar to those of 3c. Strain 1 has much the same requirement for uridine as 3b, but it differs from the other four strains in that it can utilize orotic acid as a substitute for uridine. Further studies along these lines will be reported elsewhere.[9]

Growth Characteristics of the Suppressed Mutant.—Preliminary experiments have shown that growth of a strain carrying the mutant gene 3a and the suppressor is not completely normal in the absence of pyrimidine. The dry weight of mycelium obtained after four days growth at 25°C. in 20 ml. of medium is about 40 mg. while that obtained using a wild-type strain under the same conditions is about 80 mg. Normal growth of the suppressed mutant takes place if any one of the following supplements is added to the medium: 0.5 mg. of uridine, 3 mg. of hydrolyzed nucleic acid, 10 mg. of lysine or 10 mg. of histidine. If the medium contains 0.5 microgram of arginine no growth takes place, although arginine in concentrations as high as 10 mg. in 20 ml. does not affect the growth of the wild type. Citrulline and ornithine are also inhibitory but are far less so than arginine. The quantities of these three compounds which, in 20 ml. of medium will allow half-maximum growth are as follows: arginine 0.15 microgram, citrulline 230 micrograms and ornithine 400 micrograms. Inhibition by arginine can be overcome by hydrolyzed nucleic acid or lysine, but not by histidine. The five mutant strains without the suppressor, when grown on a medium containing a quantity of hydrolyzed nucleic acid which allows half-maximum growth, or less, are not affected by the addition of 5 micrograms of arginine, but show a slight inhibition if 10 mg. of histidine are added.

No interpretation which is consistent with all of these facts has been made as yet, but further experiments are in progress. In connection with the lysine-arginine interaction reported here it should be mentioned that

Doermann[11] has found the lysine-requiring mutants of Neurospora to be inhibited if the molar concentration of arginine in the medium exceeds that of lysine.

Discussion.—Although it has not been proved directly, there is much evidence which is consistent with the hypothesis that modification of a gene results in a direct change in the activity of one enzyme.[12] On this basis the differences in growth characteristics of the three mutants which behave as alleles would make it appear that different modifications of the same gene are possible, each of which results in a distinct difference in the activity of the enzyme. This view agrees with observations on multiple alleles in other organisms.[13] A study of the properties of the enzyme affected in the three pyrimidineless strains may prove to be useful, but such a study will not be possible until further knowledge of the reaction involved is available.

It would be of interest to know the former condition of the gene which, by mutation, became able to take over the function of the inactive gene at the 3a locus. Perhaps the most obvious possibility is that the wild-type parent carries an inactive duplicate of this locus which was made active by mutation. This possibility would seem to be ruled out, however, by the fact that arginine suppresses the suppressor but does not affect wild type, in which the 3a locus must be active. Also the fact that growth of the mutant strains without the suppressor, on limiting quantities of hydrolyzed nucleic acid, is not affected by arginine would seem to indicate that arginine inhibition of the suppressed mutant is not simply a result of an insufficient supply of pyrimidine.

Two alternative possibilities that have been suggested are, first, that an entirely new gene has arisen from inactive genic material, and, second, that a gene corresponding to an enzyme which promotes a reaction in another synthesis has been modified so that the enzyme now catalyzes the required reaction in pyrimidine synthesis as well. No basis of choice between these two possibilities seems apparent at present.

Summary.—A suppressor, comparable to those found in Drosophila, has occurred in Neurospora. This suppressor acts upon three physiologically different pyrimidine-requiring strains which behave in genetic tests as if they carry allelic genes. It has no effect in combination with two other pyrimidineless strains which are genetically different from these three.

The characteristics of one of the suppressed mutants are sufficiently different from those of wild type to make it unlikely that suppression is due to duplication of the suppressed locus.

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X-RAY AND ULTRAVIOLET STUDIES ON POLLEN TUBE CHROMOSOMES. II. THE QUADRIPARTITE STRUCTURE OF THE PROPHASE CHROMOSOMES OF TRADESCANTIA*

BY C. P. SWANSON

DEPARTMENT OF BIOLOGY, THE JOHNS HOPKINS UNIVERSITY

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Most x-ray data dealing with induced chromosome breakage are interpretable on the basis of a divided or an undivided chromosome. The extensive studies on induced breaks in the microspore chromosomes of *Tradescantia*[1] indicate that chromatid and chromosome breaks result, respectively, from changes occurring within a divided or an undivided chromosome. Data from pollen tube chromosomes in the same plant,[2] where the split nature of the chromosomes can be determined at the time of treatment, agree with the above view, and, at the same time, invalidate beyond a doubt the interpretation of Darlington and LaCour[3] as to the origin of induced aberrations of the isochromatid type. The relative lack of mosaics in x-rayed Drosophila[4] suggests that, for the most part, the chromosomes in the sperm head are undivided at the time of treatment, although chromatid breaks are by no means rare in the treated salivary gland chromosomes. It appears, therefore, that, as a general rule, the smallest subdivision of the chromosome to be involved in breakage and rearrangement is the chromatid. This interpretation explains very well similar breakage results obtained from chromosomes treated with ultraviolet,[2] as well as the extensive data of Stadler[5] on endospermal deficiencies in treated maize.

That the chromatid is not the smallest subdivision of the chromosome, however, has long been known (see Nebel[6, 7] and Kuwada[8] for reviews). In addition to earlier observational studies there is much supporting data