GENETICAL AND BIOCHEMICAL STUDIES OF HISTIDINE-REQUIRING MUTANTS OF NEUROSPORA CRASSA. IV. LINKAGE RELATIONSHIPS OF *hist-3* MUTANTS[1]

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Received September 28, 1964

THE histidine-3 (*hist-3*) mutants of *Neurospora crassa* were chosen for detailed biochemical studies, complementation mapping, and intercrosses because they were shown to exhibit deficiencies in one or the other or both of two distinguishable, though apparently closely related types of biochemical functions. Preliminary heterokaryon complementation studies (WEBBER 1959, 1960; CATCHESIDE 1960) showed that, although some pairs of *hist-3* mutants form histidine-independent heterokaryons, such *hist-3* mutants all share an inability to complement with a type of *hist-3* mutant which is recovered frequently from forward-mutation experiments. Preliminary crosses (WEBBER 1960) also demonstrated that the *hist-3* mutant sites are closely linked in the right arm of linkage group I.

On the basis of biochemical and physiological tests (WEBBER 1959, 1960, 1965; CATCHESIDE 1960), the *hist-3* mutants may be divided into three subgroups, namely, subgroup 1 mutants, deficient for histidinol dehydrogenase activity; subgroup 2 mutants, deficient for one or two reactions early in the histidine biosynthetic scheme (A. AHMED, unpublished); and subgroup 3 mutants, deficient for both histidinol dehydrogenase and one or two early reactions. Furthermore, it was shown (WEBBER 1965) that the biochemical differences detected in *hist-3* mutants are reflected rather precisely in the placement of these mutants on a detailed heterokaryon complementation map of *hist-3* mutants. Subgroup 1 mutants are restricted to one portion of the map; subgroup 2 mutants are restricted to the other portion of the map; subgroup 3 mutants cover the entire subgroup 1 portion of the complementation map and extend partially or completely across the other portion.

The present paper describes the use of intercrosses of mutants from each of the biochemical subgroups in an analysis designed to determine the correspondence of the biochemical deficiencies and complementation map positions of individual *hist-3* mutants with their genetic locations.

MATERIALS AND METHODS

The *hist-3* mutants used are listed in Table 1 with their complementation characteristics and biochemical subgroup classification (WEBBER 1965). The list includes spontaneous, X-ray induced

[1] Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

TABLE 1

Characteristics of hist-3 *mutants used*

hist-3 mutant	Biochemical subgroup	Complementation units covered
Y152–M66	3	1–14
Y226–M606	2	3
Y226–M408	2	1
Y226–M584	2	1–2
Y224–M24, Y226–M589	2	4
Y226–M216, Y226–M416, Y193–M16	2	5–6
Y226–M77	2	5
Y152–M111	3	2–14
Y226–M579, Y155–M283, Y226–M585	3	9–14
Y226–M565	3	8–14
Y152–M16	1*	9–14
Y155–M280	1	9–13
1710–12A	1	9–14
Y155–M245	1	9
Y155–M261	1	10
Y155–M234, Y226–M566	1	13–14

* Y152–M16 exhibits slight variable accumulation of histidinol and is presumed to be partially deficient for a reaction early in the biosynthesis of histidine.

and ultraviolet induced mutants (WEBBER and CASE 1960). 1710–12A is a derivative strain obtained by DR. MARY CASE by backcrossing the original T1710 (HAAS, MITCHELL, AMES, and MITCHELL 1952) to wild type 73a. Selection of the derived strain was made on the basis of heterokaryon-compatibility with mutants in 74A wild-type background. The original T1710 was reported to exhibit ascospore abortion in crosses, suggesting the presence of a chromosomal aberration. The derivative strain exhibits good ascospore viability (up to 80 percent) and normal linkage relationships with the *arg-3* and *nic-2* loci (in crosses of 1710–12A to an *arg-3 nic-2* double mutant strain) and so seems to be free of the aberration. It is the derived strain that has been used to provide the *hist-3* mutant locus referred to here as 1710.

arg-3 (30300) and *nic-2* (43002) were used as outside markers (Figure 1) six or seven units to the left and two or three units to the right of *hist-3*, respectively. Strains of mating type *A* bearing the *hist-3* and *nic-2* markers and strains of mating type *a* bearing *arg-3* and *hist-3* mark-

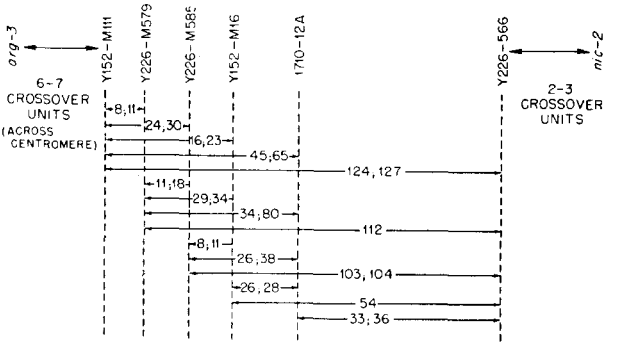

FIGURE 1.—Genetic sequence of six *hist-3* mutants. (Genetic distance is expressed as prototroph frequency $\times 10^6$).

ers were derived from crosses of the various *hist-3* mutants with the same *arg-3 nic-2* strain. Heterogeneity in genetic background was decreased by the use of *hist-3* strains most of which had been induced in 74A or a closely related wild-type strain (WEBBER and CASE 1960) and by selection for heterokaryon compatibility with 74A after backcrossing strains bearing *arg-3* and *nic-2* markers to wild type 74A several times. The derived *hist-3 nic-2* strains were then crossed with *arg-3 hist-3* strains in appropriate combinations. Crosses were made in 20 × 150 mm test tubes containing filter paper strips and the liquid medium of WESTERGAARD and MITCHELL (supplemented with 100 μg/ml of DL- histidine-HCl-H_2O and 10 μg/ml of nicotinamide) with the *hist-2 nic-2* strain serving as protoperithecial parent in each cross. The tubes were incubated for 5 to 6 weeks at 25°C and then stored at 0 to 4°C until plating. The pairs of *hist-3* mutants crossed were, in all cases, noncomplementary or only weakly complementary in heterokaryon tests; therefore, no pseudowild-type progeny were expected, and histidine-independent progeny were not tested for their presence. The absence of histidine-independent progeny from selfings of the majority of the *hist-3* mutants demonstrates that typical reversion is not involved in origin of histidine-independent progeny in these intercrosses. A sorbose overplating technique (NEWMEYER 1954) was used with slight modification. Ascospores were suspended in water containing 0.15 percent agar and heat shocked in a 60°C water bath for 1 hour; 0.02 to 0.34 ml of suspension was plated in medium supplemented with 100 μg/ml L-arginine, 75 μg/ml L-histidine, and 10 μg/ml nicotinamide to estimate the viable spores/ml of suspension (usually 3 to 25 × 10^3/ml); and 75 to 110 ml of suspension were plated in medium supplemented with arginine and nicotinamide to estimate histidine-independent progeny. For each cross the number of histidine-independent progeny is expressed as a percentage of the number of viable spores in an equal volume of suspension.

Histidine-independent colonies were grown in tubes of arginine- and nicotinamide-supplemented medium and then tested in medium supplemented with arginine or nicotinamide. An estimate of the viability in each cross was made by comparing the number of colonies obtained in arginine- nicotinamide- and histidine-supplemented plates with the number of ascospores detected by microscope counts of 0.001 ml of suspension.

RESULTS

Size of the hist-3 *locus:* The data derived from interallelic crosses of marked *hist-3* mutants are presented in Tables 2 and 3. The size of the locus is less than 0.03 conventional map units (double the largest prototroph frequency) as determined by these tests. This may be compared with a length of 0.68 map units for the *pan-2* locus (CASE and GILES 1960). Such a comparison may be of no theoretical importance, since factors other than actual physical length of the genetic material involved may affect map length as measured by recombination, but the comparison does show a 20-fold difference in the maximum prototroph frequencies.

Sequence of hist-3 *mutant sites:* The sequence of *hist-3* mutant sites is found by a comparison of frequencies of histidine-independent progeny from different *hist-3* × *hist-3* crosses. Six *hist-3* mutants were crossed in all possible combinations of pairs (as listed in Table 2) and with most combinations reciprocal with reference to markers at the *arg-3* and *nic-2* loci. In Figure 1 these six mutants are placed in the sequence which gives the best additivity of frequencies of histidine-independent progeny. The placement of additional *hist-3* mutant sites on the map is based upon prototroph frequencies (presented in Table 3) from crosses between the mutant whose site is to be detected and several of the six mutants whose locations are shown in Figure 1. The data in Table 3 do not permit unam-

TABLE 2

Genetic data from crosses involving six hist-3 *mutants (Procedures are described in "Materials and Methods")*

hist-3 mutant in parents		Ascospore viability (Percent)	Estimated viable spores plated $\times 10^{-6}$	Histidine-independent colonies		Total colonies tested	Marker distribution			
							Nonparental		Parental	
Mating type *A* *hist-3 nic-2*	Mating type *a* *arg-3 hist-3*			Number	Frequency per 10^6		*arg*$^+$*nic*$^+$	*arg*$^-$*nic*$^-$	*arg*$^-$*nic*$^+$	*arg*$^+$*nic*$^-$
Y152–M111	Y226–M579	100	2.017	17	8	17	4	2	6	5
Y226–M579	Y152–M111	58	0.929	10	11	10	2	1	4	3
Y152–M111	Y226–M585	39	2.028	60	30	60	21	4	24	11
Y226–M585	Y152–M111	90	1.787	43	24	43	14	4	18	7
Y152–M111	Y152–M16	67	2.966	46	16	46	10	8	15	13
Y152–M16	Y152–M111	91	3.447	80	23	80	22	9	38	11
Y152–M111	1710–12A	40	0.379	17	45	17	1	12	2	2
1710–12A	Y152–M111	47	2.444	96	65	90	43	5	29	13
Y152–M111	Y226–M566	91	1.994	247	124	230	27	69	48	86
Y226–M566	Y152–M111	59	2.673	339	127	283	101	33	117	32
Y226–M579	Y226–M585	35	1.110	12	11	11	1	4	2	4
Y226–M585	Y226–M579	31	0.926	17	18	17	6	3	2	6
Y226–M579	Y152–M16	81	1.670	57	34	57	8	9	18	22
Y152–M16	Y226–M579	73	0.717	21	29	21	9	4	6	2
Y226–M579	1710–12A	85	0.540	43	80	43	5	13	13	12
1710–12A	Y226–M579	61	0.264	9	34	9	4	0	3	2
Y226–M566	Y226–M579	51	1.435	161	112	145	40	24	55	26
Y226–M585	Y152–M16	81	1.880	24	11	24	7	0	11	6
Y152–M16	Y226–M585	75	3.571	27	8	27	9	1	6	11
Y226–M585	1710–12A	27	1.584	41	26	41	19	2	14	6
1710–12A	Y226–M585	64	2.119	81	38	81	60	3	9	9
Y226–M585	Y226–M566	26	1.184	123	104	114	19	19	27	49
Y226–M566	Y226–M585	26	2.250	232	103	188	80	19	63	26
Y152–M16	1710–12A	67	1.135	30	26	30	8	7	11	4
1710–12A	Y152–M16	63	2.700	75	28	71	45	4	9	13
Y226–M566	Y152–M16	58	2.997	161	54	148	49	20	33	46
1710–12A	Y226–M566	53	2.479	86	36	86	14	41	2	29
Y226–M566	1710–12A	81	2.654	86	33	84	75	0	4	5

TABLE 3

Partial genetic data (prototroph frequency per 10^6 viable ascospores) from selected hist-3 × hist-3 *crosses and position of mutants*

Genotype of one parent	Genotype of other parent					Tentative placement of mutants
	Y152–M111	Y226–M579	Y226–M585	Y152–M16	1710	
Y226–M606	7	56; 62	42	..	..	Near Y152–M111
Y226–M408	7; 0	12; 24	31; 14	..	..	Near Y152–M111
Y226–M584	0.2; 0.4	27; 10	43; 15	..	..	Near Y152–M111
Y224–M24	1	16	23	..	..	Near Y152–M111
Y226–M589	1	6	23	..	..	Near Y152–M111
Y226–M216	2; 1	4; 4	19; 22	..	..	Between Y152–M111 and Y226–M579
Y226–M77	5	1	20	..	..	Near Y226–M579
Y155–M283	4; 14	1; 0	17; 31	19; 19	75; 64	Near Y226–M579
Y226–M565	64	36	0	..	..	Near Y226–M585
Y155–M280	57; 41	38; 45	14; 9	10	..	Between Y152–M16 and 1710
Y155–M245	86; 43	71; 63	40; 30	25; 21	..	Distal to 1710
Y155–M261	85	75; 83	46; 54	20; 36	..	Distal to 1710
Y155–M234	122	108	95	37; 103	..	Distal to 1710
Y226–M416	0	0	3	..	..	Near Y152–M111 and Y226–M579
Y193–M16	18; 24	11; 26	44; 34	..	..	Ambiguous
Y152–M66	3	26; 27	34; 15	8; 7	..	Ambiguous

biguous placement of all mutants, but Table 4 lists in sequence those mutants which have been assigned to a site.

Distribution among histidine-independent progeny of markers at adjacent loci: The strains used in this series of crosses contained contrasting alleles at the *arg-3* locus and at the *nic-2* locus. The distribution of markers among prototrophs from any particular cross was expected to provide evidence about the sequence in the linkage group of the two *hist-3* mutants involved in that cross. It was anticipated that if histidine-independent progeny arose by recombination between *hist-3* mutant sites, then these histidine-independent progeny should bear predominantly either arg^+ and nic^+ or *arg-3* and *nic-2*, depending upon the relative positions in the linkage group of the two *hist-3* mutant sites. Reciprocal crosses were expected to yield approximately reciprocal results with respect to marker combinations. These expectations were not confirmed in the majority of pairs of reciprocal crosses.

Mutant 1710 stands out in Table 2 as an exceptional mutant that frequently yields from crosses a large percentage of prototrophs marked with either arg^+ and nic^+ or with *arg-3* and *nic-2* depending upon the relative positions of the two *hist-3* mutant sites. From crosses involving 1710, 814 histidine-independent progeny were obtained and tested and 526 of these (65 percent) were marked either with arg^+ and nic^+ or with *arg-3* and *nic-2*. From crosses not involving 1710, only 44 percent (3433/7804) of the histidine-independent progeny exhibited nonparental (either arg^+ and nic^+ or *arg-3* and *nic-2*) marker combina-

TABLE 4

Genetic sequence of some hist-3 *mutants*

Mutant	Subgroup	Complon coverage
Y226–M606	2	3
Y226–M408	2	1
Y226–M584	2	1–2
Y224–M24	2	3
Y152–M111*	3	2–14
Y226–M589	2	4
Y226–M216	2	5–6
Y226–M77	2	5
Y226–M579*	3	9–14
Y155–M283	3	?
Y226–M565	3	8–14
Y226–M585*	3	9–14
Y226–M497	3	9–13
Y152–M16*	1	9–14
Y155–M280	1	9–13
1710*	1	9–14?
Y155–M245	1	9
Y155–M261	1	10
Y155–M234	1	13–14
Y226–M566*	1	13–14

* Mutants which are included in Figure 1.
Brackets enclose mutants whose positions relative to one another are probably but not certainly as shown.
Y226–M497 and Y155–M280 were not tested with a complon 14 mutant, but may be presumed to cover complon 14 as well as 13.

tions. The production of reciprocal marker results (one nonparental combination favored in one cross and the other nonparental combination favored in a reciprocal cross) from reciprocal crosses involving 1710 permits one to fix the relative positions of the *hist-3* mutants in the linkage group. The subgroup 2 and 3 mutants (e.g., Y226–M606 and Y152–M111) are nearer to *arg-3* (closer to the centromere) than the subgroup 1 mutants. Y226–M566 and Y155–M234 are considered the farthest from the centromere.

Marker distributions in prototrophs from crosses involving Y226–M566 indicate that this site segregates as though more closely linked to the *nic-2* locus than to the *arg-3* locus. This favors the placement of the Y226–M566 end of the map to the right (farthest from the centromere).

DISCUSSION

Relative genetic positions of hist-3 *mutants: hist-3* mutants were assigned to sites on a genetic map based on the assumption that frequency of histidine-independent progeny provides an estimate of genetic distance separating two *hist-3* mutants crossed. Not all mutants for which crossing data are available can be unambiguously assigned to a position in the linkage map. Mutant Y226–M416, for instance, gave a low frequency of histidine-independent progeny with four

mutants at different sites. Mutants Y193–M16 and Y152–M66 gave low frequencies of histidine-independent progeny with tester mutants at the end and middle of the map and higher frequencies with intermediate testers. However, the majority of mutants could be assigned to sites at or near one of eight positions scattered along a genetic map approximately 0.03 conventional map units in length.

Distribution of adjacent markers: The majority of the *hist-3* interallelic crosses provide data which are similar to those described by PATEMAN (1960) for the *am* locus. There is an excess of parental marker combinations among histidine-independent progeny, suggesting that many of these progeny result from an exchange between the two *hist-3* sites involved in the cross as well as an exchange adjacent to the *hist-3* locus. There is also an apparent nonreciprocity of marker combination frequencies in reciprocal crosses. The present data differ from those of CASE and GILES (1960) on the *pan-2* locus, where nonparental marker combinations are in excess and reciprocal crosses result in reciprocity for adjacent marker combinations. The *hist-3* data contrast (less strongly) with the data of MURRAY (1963) where parental classes of methionine-independent progeny of interallelic *me-2* crosses are in slight excess, but reciprocity in reciprocal crosses is indicated.

The difference between crosses involving 1710, a mutant obtained in a stock (derived from E5256A and E5297a) probably not closely related to 74A, and crosses not involving 1710 is not surprising. DE SERRES (1958) was able to demonstrate in interallelic *ad-3* crosses that the segregation of the closely linked marker *hist-2* among adenine-independent progeny (as well as the frequency of adenine-independent progeny) varied quantitatively depending on whether the two *ad-3* mutants crossed had been obtained in stocks of the same genetic background or of different genetic backgrounds. It may be possible to attribute certain unexpected features of the present data (e.g., failure of reciprocal crosses to give reciprocal marker distributions among *hist*$^+$ progeny) to variation in the genetic background of the mutants used. Such variation may have been introduced by the use of *arg-3* and *nic-2* markers which did not originate in wild type 74A.

Genetic basis for biochemical heterogeneity among hist-3 *mutants:* There is clearly a correspondence among the three different methods of classifying *hist-3* mutants. The close correspondence between biochemical subgroup classification of mutants and complon coverage was previously pointed out (WEBBER 1965). It is now also evident that subgroup 2 and 3 mutants are mixed together in one portion of the linkage map, while subgroup 1 mutants occupy an adjacent portion farther from the centromere. Furthermore, certain of the subgroup 1 mutants (i.e., Y155–M234 and Y226–M566) cover complons 13 and 14 (at one side of the complementation map) and occupy positions on the linkage map farthest from the centromere.

The *hist-3* locus is divided into at least two parts. Genetic alterations in the distal portion result in subgroup 1 mutants which are deficient for histidinol dehydrogenase and occupy one or more of complons 9 through 14. Genetic alterations in the proximal portion are of two different classes. Genetic alterations

of one class result in subgroup 2 mutants which are deficient for one or two reactions early in the biosynthetic pathway for histidine and occupy one or more of complons 1 through 8. The other class of genetic alterations in the proximal portion of the map produce subgroup 3 mutants which are deficient for one or more early reactions as well as for histidinol dehydrogenase. Subgroup 3 mutants extend from some point in the complon 1–9 region through complon 14.

The evidence indicates a separate genetic position for subgroup 1 mutants, but a location of subgroup 2 and 3 mutants in the same genetic region. DE SERRES (1964) has presented evidence from studies with the *ad-3B* mutants of Neurospora suggesting that base-pair substitutions result in mutants with nonpolarized complementation patterns while base-pair insertions or deletions produce noncomplementing mutants or mutants with polarized complementation patterns. If one assumes, further, that the genetic code in the *hist-3* region is read from left to right, then the difference between subgroup 2 and 3 mutants could be attributed to a difference in type of genetic alteration. On this hypothesis, subgroup 2 mutants would result from base-pair substitutions, whereas subgroup 3 mutants would result from base-pair insertions and deletions. Subgroup 1 mutants should result from both classes of alteration.

The author is grateful for the encouragement and criticism of DR. FREDERICK J. DE SERRES during the progress of this crossing program and manuscript preparation, and for the review and criticism of the manuscript by DR. NORMAN H. GILES and DR. MARY E. CASE.

SUMMARY

Intercrosses of mutants from the three biochemical subgroups of *hist-3* mutants have been analyzed to determine the correspondence between their characteristic biochemical deficiencies and their positions on the complementation and genetic maps. These crosses demonstrated a striking correspondence between genetic and complementation map positions of mutants with nonpolarized complementation patterns. Subgroup 1 mutants are located in the right side of both maps while subgroup 2 mutants are in the left side of both maps. Subgroup 3 mutants are located among subgroup 2 mutants in the left side of the genetic map.

A possible interpretation of these results is that subgroup 3 mutants result from base-pair insertions and deletions while subgroup 2 mutants result from base-pair substitutions. Subgroup 1 would include mutants resulting from both classes of alterations.

Contrasting markers at the *arg-3* and *nic-2* loci were incorporated into the parents and observed in the progeny. The two following different types of distribution of markers occurred. Crosses not involving mutant 1710 showed more than 50 percent parental combinations of markers; reciprocal crosess not involving 1710 failed to yield reciprocal marker combination frequencies, and crosses involving 1710 (a mutant obtained in a wild-type strain other than 74A) usually exhibited more than 50 percent nonparental combinations of markers and good reciprocity in most pairs of reciprocal crosses.

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