CYSTEINE MUTANT STRAINS OF NEUROSPORA[1]

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MUTANT strains of *Neurospora crassa* that are unable to utilize inorganic sulfur, but which are capable of growth on minimal medium supplemented with either cysteine or methionine, are obtained readily. It is surprising, therefore, that little genetic information has been available for cysteine mutants of Neurospora, other than those located in linkage group VI (STADLER 1956a,b, 1959a,b; STADLER and TOWE 1963). Recent progress in the elucidation of cysteine synthesis in *Salmonella typhimurium* (DREYFUSS and MONTY 1963) has stimulated renewed interest in the synthetic pathway in Neurospora (LEINWEBER and MONTY 1964, 1965). It therefore seemed desirable to obtain genetic information on the strains available for biochemical analyses.

In an earlier study, complementation tests demonstrated seven physiological classes among 62 cysteine mutants (MURRAY 1960). Two factors have contributed to the paucity of genetic information on these mutants. (1) Three of these groups of mutants are at loci very far out from the centromere. (2) Cysteine mutant strains apparently acquire additional mutations at other loci, leading to strains that are doubly blocked in cysteine synthesis. This problem has not been investigated systematically, but at least some originally single-mutant stocks kept by the Fungal Genetic Stock Center Collection (FGSC; Dartmouth College, Hanover, N.H.), by DR. D. R. STADLER and MRS. AGNES TOWE (personal communication), and by me, were found to be double mutants. I found a culture of *cys-5* (35001[FGSC No. 428]) to be a double mutant (*cys-5*, *cys-2*), and have isolated a probable *cys-5* allele from a culture of *cys-2* (71310[FGSC No. 968]). (Both FGSC stocks have since been replaced with bona fide single-mutant strains.) Another *cys-2* strain was shown by MRS. AGNES TOWE (personal communication) to have acquired a secondary mutation in, or close to, the *cys-5* region. The unambiguous genetic identification of each locus, provided by location on a linkage map, therefore becomes increasingly desirable.

All available cysteine mutants have now been classified and mapped. This paper presents a summary of the genetic information on eight (or possibly nine) cysteine loci. Linkage data for six of these loci have not been published previously, although some information has been made available in the Neurospora Newsletter.

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TABLE 1

Summary of data on cysteine mutants

Locus symbol and linkage group	Isolation number	Origin: treatment and strain;* reference†	Response at 25° to		
			Sulfite	Thiosulfate	Cysteine
cys-1 VIL‡	84605	X ray; L (1)	+	+	+
cys-2 VIL	38401	UV ; L (1)	—	+	+
	80702		—	+	+
	48401	UV ;A×L (1)	—	slight	+
	K36	UV ; E (2)	—	slight	+
	DS17	UV ; M (3)	—	slight	+
	71310	UV ;A×L (1)	—	slight	+
cys-3 IIL	P22	UV ; E (2)	+	slight	+(weak)
	P29	UV ; E (2)	+	slight	+(weak)
cys-4 IVR	K7	UV ; M (2)	—	slight	+
	K8	UV ; M (2)	—	slight	+
	P1	UV ; E (2)	—	slight	+
cys-5 IL	35001	UV ; L (1)	+	+	+
	NM44	UV ; E	+	+	+
	85518	NM ; M	+	+	+
	R83 R1-1-271	S ; M (4)	+	+	+
	NM86	UV ; E	+	+	+
cys-9 IR	UT156	(5)	+	+	+
cys-10 (*me-4*) IVL?	39816	UV ; L (1)	—	slight	+
cys(ox-D¹) IVR	ox-D¹	UV ; L (6)	+	+	+

* UV—Ultraviolet light. S—Spontaneous. NM—Nitrogen mustard. L—LINDEGREN. A—ABBOTT. E—EMERSON. M—Mixed.

† *References:* 1. BEADLE and TATUM 1945. 2. MURRAY 1960. 3. STADLER and TOWE 1963. 4. GROSS 1962. 5. LEINWEBER and MONTY 1965. 6. OHNISHI, MACLEOD and HOROWITZ 1962.

‡ L or R designates left or right arm, following BARRATT *et al.* (1954).

MATERIALS AND METHODS

The cysteine mutant strains used are heterogeneous in origin; their origins and characteristics are given in Table 1. [Representatives of three strains designated *cys-6* (86801) (PHINNEY 1948), *cys-7* (P120) and *cys-8* (P160) (MURRAY 1960) have apparently been lost, and it is not known whether they represented loci other than these eight. *me-4* (39816) is a cysteine mutant that was misnamed; the nomenclature is standardized in this paper by using *cys-10* as a synonym for *me-4*. It is suggested that to avoid confusion the locus symbols *me-4*, *cys-6*, *cys-7* and *cys-8* should not be used for any new mutants.]

Media, and methods of crossing, ascospore isolation and linkage detection were as described by PERKINS (1959). Precursor utilization tests were made by auxanograms.

The linkage data are presented in Table 2 using the format of PERKINS (1959). In crosses of *cys-3* strains only the *cys*$^+$ isolates were scored, since *cys*$^-$ ascospores generally fail to germinate. In crosses of *cys-10* by *fi pdx-1*, the morphological marker fissure (*fi*) could be scored with only a fair degree of precision in the *cys*$^+$ class, and the *cys*$^-$ class was ignored.

RESULTS

Eight cysteine loci have been identified genetically. Linkage data are presented in Table 2 and summaries of the information available on each of the eight cysteine loci are given below in the text.

The early literature presents some discrepancies in the information on the

TABLE 2

Linkage data of cysteine loci

Zygote genotype and recombination percent	Parental combinations	Recombination: Singles region 1	Recombination: Singles region 2	Recombination: Doubles regions 1 and 2	Total, and percent germination	Marker isolation numbers
+ + *thr-3* *cys-3* *pyr-4* + 17.7 29.6	76 –	24 –	41 –	1 –	142 80%	P22 36601 44104
+ + *cys-4* *pan-1* *mat* + 11.5 9.8	48 41	6 7	5 6	0 0	113 75%	5531 B57 K7
+ *cys-5* *A* *leu-3* + *a* 7.8 5.2	40 27	2 4	1 3	0 0	77 83%	47313 35001 sex
+ + + *cys-10* *fi* *pdx-1* 18.1 16.8	58 –	16 –	14 –	3 –	101 50%	39816 M155–2 37803
+ + *cot* *cys-10* *col-4* + 36.6 13.0	33 33	21 20	5 7	2 2	123* 82%	39816 70007 C102
A + *cys-9* + *a* *cr* + *os* 3.4 40.9	26 23	2*A* 1*a*	20 16	0 0	88 63%	B122 UT156 B135
+ *thi-1* *ad-9* *cys-9* + + 13.0 15.2	34 33	7 5	10 4	0 0	92 92%	UT156 56501 Y154M37
+ *tryp-4* *pan-1* *cys(ox-D¹)* + + 14.6 5.6	88 90	12 18	9 3	0 0	220 95%	*ox-D¹* Y2198 5531

The left-hand number of each pair of complementary classes represents the genotype that contains the wild-type allele of the leftmost markers.

Gene symbols: *A*—mating type A, *a*—mating type a; *ad*—adenine; *col*—colonial; *cot*—colonial temperature sensitive; *cr*—crisp (a morphological); *fi*—fissure (a morphological); *leu*—leucine; *mat*—mat (a morphological); *os*—osmotic; *pan*—pantothenic acid; *pdx*—pyridoxine; *pyr*—pyrimidine; *tryp*—tryptophan.

* Data of D. D. PERKINS.

utilization of inorganic sulfur by cysteine mutants; the instability of inorganic sulfite (POSTGATE 1963) is the cause of at least some of the anomalies. The present information on precursor utilization (Table 1) is in agreement with the detailed investigations of LEINWEBER and MONTY (1964, 1965). These authors used filter-sterilized supplements and obtained dry-weight assays.

cys-1. (linkage group VIL) The one mutant at this locus, strain 84605, responds to either sulfite or thiosulfate, and at 25°C (but not 34°) it also has a partial requirement for tyrosine (HOROWITZ and SHEN 1952).

cys-2. (VIL) Alleles at this locus are heterogeneous in their response to thiosulfate, but in contrast to *cys-1* (84605) do not respond to sulfite. No interallelic

complementation of *cys-2* mutants has been observed (STADLER and TOWE 1963) but those *cys-2* strains tested (38401 [PITTENGER 1954], 48401, K36, DS17) complement *cys-1*, as demonstrated by the formation of pseudowild-type progeny. *cys-2* alleles have been used extensively in recombination and fine-structure analyses (STADLER 1959a,b; STADLER and TOWE 1963).

Early genetic information (STADLER 1956a) suggested that *cys-1* (84605) and *cys-2* (38401 and 80702) were separate, but closely linked, genes in the left arm of linkage group VI. (Prototrophs occur from intercrosses with a frequency of about 0.5%.) Subsequent evidence supports this view, but is inconclusive. Cysteine-independent spores from a cross of *lys-5* (DS6-85) *cys-2* (DS17) × *cys-1* (84605) *ylo* (Y30539y) were isolated and tested with respect to the outside marker genes, with the following results:

Cysteine prototrophs recombinant with respect to marker genes	*Cysteine prototrophs parental with respect to marker genes*
40 *ylo*+ *lys*+: 1 *ylo*⁻ *lys*⁻	25 *ylo*+ *lys*⁻: 26 *ylo*⁻ *lys*+

Thirty of the *ylo*+ *lys*+ isolates were tested and 26 were shown to be true wild types. The large asymmetry between the two classes with markers recombined is characteristic of a cross between mutants at closely linked but separate gene loci, and would also indicate that *cys-1* is proximal to *cys-2*. However, the incidence of cysteine prototrophs with parental combinations of marked genes is higher than previously reported for crosses between mutants located in closely linked genes, e.g. *ad-3A* and *ad-3B* (DE SERRES 1958).

cys-3. (IIL) This is the leftmost marker in linkage group II. As indicated in Table 1, *cys-3* mutants are able to utilize sulfite but *not* thiosulfate. Mutants at this locus have been shown to be deficient in a "permease" which facilitates the uptake of both sulfate and thiosulfate (F. J. LEINWEBER, personal communication). The very poor response of these mutants to cysteine is not understood.

When crosses are made on an unsupplemented medium, using a *cys-3* strain as fertilizing parent, the *cys*⁻ ascospores fail to darken. The addition of methionine to the crossing medium promotes darkening of the ascospores, but fails to give good allele ratios of *cys*⁻ : *cys*+.

cys-4. (IVR) This is the rightmost marker in group IV. Interallelic crosses are fertile and yield fewer than 0.1% prototrophs.

cys-5. (IL) Mutants 35001 and 85518 both respond to sulfite, and 85518 was known to be linked to mating type (N. H. HOROWITZ, unpublished). The present data (see Table 2) show that *cys-5* (35001) is located between *leu-3* and mating type. The following intercrosses demonstrated close linkage to 35001: 35001 by 85518 (0 prototrophs in 111 isolates); 35001 by R83R1-1-271 (0 in 200); 35001 by NM44 (0 in approximately 16,000 viable ascospores); and 35001 × NM86 (13 in approximately 15,000 viable ascospores). However the heterocaryon-compatible strains NM44 and NM86 were found to complement each other. Further complexity in the *cys-5* region was suggested by the finding (F. J. LEINWEBER, personal communication) that 35001 and 85518 have different phenotypes at the enzyme level. Since NM86 gave prototrophs when crossed to 35001,

TABLE 3

Data from crosses between mutants in the cys-5 *region*

Cross	Cysteine prototrophs					
	Number	Frequency per 100 viable spores	Classification of cysteine prototrophs			
			leu⁻ A	*leu⁺ a*	*leu⁻ a*	*leu⁺ A*
leu-3 cys-5(35001)*A* × *cys*(NM86)*a*	72*	0.49	5	1	0	66
leu-3 cys(NM86)*a* × *cys*(35001)*A*	102*	0.35	92	0	6	4

* The prototrophs were not tested for pseudowild types.

further crosses were analysed using *leu-3* (R156) and mating type as markers flanking the cysteine region. The results are shown in Table 3. The absence of negative interference is compatible with the suggestion that NM86 is not a *cys-5* allele. Further genetic and enzymic information is needed to clarify the genetic organization in the *cys-5* region.

cys-9. (IR) This locus is in linkage group IR between crisp (*cr*) and *thi-1*, but much closer to *cr*. DR. H. B. HOWE (personal communication) also showed that *cys-9* (UT156) is probably in linkage group IR.

cys-10. (IVL?) Linkage of *cys-10* (39816) has been sought in all seven linkage groups in both arms wherever possible, and evidence was obtained of linkage to *pdx-1*, *col-4* and *chol-1* in linkage group IV. *cys-10* showed no linkage to *cys-4*, the rightmost marker in IV, but in a number of crosses linkage was demonstrated to fissure (*fi*), a morphological mutant believed to be located in IVL (PERKINS, GLASSEY and BLOOM 1962). (Scorability of *fi* is not completely reliable.) No other markers are known in the left arm of group IV. It is suggested *tentatively* that *cys-10* is the first biochemical marker to be located there.

A surprising interaction was observed when *cys-10* (39816) was crossed to choline mutants and the double mutants were isolated. Double mutants of *cys-10* (39816), *chol-2* (47904) were difficult to distinguish from the *cys-10* strain, whereas double mutants of *cys-10* (39816) *chol-1* (34486) grew very slowly on minimal medium supplemented with methionine and choline, but grew somewhat better on minimal supplemented with methionine.

cys(ox-D¹). A strain (ox-D¹) was shown by HOROWITZ, OHNISHI and WATANABE (1960) to have a requirement for sulfite and also to lack the enzyme D-amino acid oxidase (see OHNISHI, MACLEOD and HOROWITZ 1962). These authors were unable to separate the two defects by recombination, although other *ox-D* mutants had no lesion in cysteine synthesis. It was therefore suggested that ox-D¹ had a lesion, possibly a deficiency, extending into two adjacent loci, one of which is *ox-D* and the other is a cysteine locus. OHNISHI *et al.* (1960) mapped *ox-D¹* close to *pdx-1* in linkage group IV. Further evidence places *ox-D* between *pdx-1* and *pyr-3* (BARRY 1960).

DISCUSSION

Distribution of genes: The strikingly uneven distribution of genes among the linkage groups of *N. crassa* has been discussed by PERKINS (1959). One of the

most interesting examples of this is in linkage group IV, shown by ST. LAWRENCE (1952) to correspond to chromosome 2. Chromosome 2 has one long arm and a short arm that carries the nucleolus organizer. Linkage group IV has a long, well mapped, right arm and a left arm marked only tentatively by *fi* and *cys-10*. Does the cytologically long arm correspond to the genetically long arm? If so, the nucleolar arm would represent a region in which few mutations have been detected.

Gene clustering: Genetic analyses place the available cysteine mutations in seven discrete regions of the linkage maps. However, the present evidence is consistent with two of these regions comprising two closely linked cysteine loci. The convincing demonstration of two closely linked cistrons requires at least several heterocaryon-compatible strains representing each of the two postulated cistrons. These requirements are not met in the present investigation, and hence it is tentatively suggested that of the nine cysteine loci, four consist of two closely linked pairs. A comparable situation exists with respect to mutants blocked between cysteine and methionine. Four of the nine methionine loci may occur as two closely linked pairs. The loci *me-6* (35809) and *mac* (65108) are closely linked, but could be allelic, whereas *me-7* (K79) and *me-9* (NM43) are nonallelic, closely linked loci (MURRAY, unpublished observations).

If each of these four complex regions is equivalent to one cysteine (or methionine) locus, then physiological heterogeneity in at least three of the four loci, indicates that these would provide further examples of enzymes possessing more than one function (cf. AHMAD and CATCHESIDE 1960; CATCHESIDE 1960; WEBBER 1960; DAVIS and WOODWARD 1962). Alternatively, if each region comprises two closely linked cistrons, a nonrandom distribution of genes is indicated.

Adjacent genes and recombination analyses: Systems involving adjacent genes should find important application for recombination studies. Most of the current models for the mechanism of recombination postulate that crossing over occurs by breakage and reunion at fixed points, but that sites between such points may be subject to conversion (HOLLIDAY 1964; STADLER and TOWE 1963; WHITEHOUSE and HASTINGS 1965). Do the fixed points of breakage coincide with the ends of cistrons, possibly with the ends of *each* cistron? The predicted characteristic of crosses between two mutations that map in adjacent recombination regions is the absence of recombinants whose flanking markers are of the minority nonparental combination. On this criterion, *cys-1* and *cys-2* (STADLER, TOWE and MURRAY 1965), *cys-5* (35001) and *cys*(NM86), *me-6* (35809) and *mac* (65108) respectively, each represent two recombination regions. (The appropriate crosses of *me-7* × *me-9* have not been analysed). Further genetic and biochemical investigation of these four complex regions is suggested. Should each of the four regions comprise two cistrons in different regions of recombination, the question would remain whether or not the cistrons are adjacent.

Accumulation of secondary mutations: The apparent acquisition, by cysteine mutant strains, of secondary mutations in the cysteine biosynthetic pathway, suggests that the double mutants have a selective advantage over the singly mutant strains. Such a situation has been reported by MITCHELL and MITCHELL (1950) for adenine mutants of Neurospora.

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SUMMARY

Genetic analyses place the available cysteine mutations of *N. crassa* in seven discrete regions, two of which may each comprise two cistrons. Linkage data are provided for six previously unmapped loci. An interaction between a cysteine mutant and choline mutants is reported. Some cysteine mutants appear to accumulate secondary lesions in the biosynthesis of cysteine.

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