FINE STRUCTURE MAPPING OF THE *AM* (GDH) LOCUS OF NEUROSPORA

JOHN A. RAMBOSEK AND JOHN A. KINSEY

Department of Microbiology, University of Kansas Medical Center, Kansas City, Kansas 66103

Manuscript received April 16, 1983
Revised copy accepted June 27, 1983

ABSTRACT

Utilizing a combination of flanking marker analysis and deletion mapping we have constructed a fine structure map of the *am* locus which includes 63 point mutants and ten unique deletions. Positions of point mutants can be rapidly assigned to one of 13 segments within the gene on the basis of crosses to nine deletion strains.

THE *am*-1 locus of *Neurospora crassa* codes for NADP-specific glutamate dehydrogenase (GDH). This enzyme catalyzes a reversible reaction in which ammonium ion and α-ketoglutarate are combined to form glutamate. Thus, the enzyme plays a central role in nitrogen metabolism.

The *am*-1 locus and its gene product have been the subject of considerable genetic and biochemical analyses. The enzyme is a hexamer which consists of six identical monomers. There are 453 amino acid residues per monomer, and the complete sequence of the monomer has been determined (WOOTEN *et al.* 1974). In addition, a number of amino acid mutational replacements have been determined (BRETT *et al.* 1976; SEALE *et al.* 1977; FINCHAM and BARON 1977; KINSEY *et al.* 1980). The sequence of the mutational replacements in the polypeptide chain is colinear with the sequence of the corresponding mutational sites in the genetic map (FINCHAM 1967; *Smyth* 1973; *Kinsey et al.* 1980). The sequence of the first 17 bases of the messenger RNA has been deduced by comparing the amino acid sequence of GDH from double frameshift revertants of the mutant am_6 to that from the wild type (SIDDIG *et al.* 1980). This information has allowed the synthesis of a synthetic 17-base fragment that is complementary to the amino terminal coding region of the gene. The gene has recently been cloned and partially sequenced using this 17-mer as a probe. (KINNAIRD *et al.* 1982).

Because the *am* locus offers a particularly advantageous system for a combined genetical and molecular genetic approach to the study of recombination in a microbial eukaryote, we have constructed a detailed fine structure map of the locus which includes 63 point mutations and ten deletions. Our initial results were obtained by flanking marker analysis. However, it quickly became apparent that, in order to map the large number of mutant sites with which we were working, it would be advantageous to use deletion mapping. We, therefore, isolated a group of *am* deletion mutants which were subsequently utilized to complete the construction of the *am* locus map.

MATERIALS AND METHODS

Strains used: Mutant strains am_1 through am_{19} and *lys-1* were from the authors stock collection. Mutant strains am_{100} through am_{148} were isolated using the direct selective procedure (KINSEY 1977), and their characterization has been described (KINSEY and HUNG 1981). Mutant strain FK059 was isolated by E. Käfer using the direct selection procedure. Isolation of the *am* locus deletion mutants is described in RESULTS of this communication. Stocks used for mapping by flanking marker analysis carried the markers *cot-1* (allele C102t), *rec-3* and either *his-1* (allele K83) or *ure-2* (allele 47) which were introduced through a series of crosses. The stocks used to introduce these markers were obtained originally from the Fungal Genetics Stock Center, Humboldt State University, Humboldt, California. Stocks used for flanking marker analysis were of mixed genetic background.

Chemicals and reagents: The mutagen 1,2,7,8-diepoxyoctane (DEO) was obtained from Aldrich Chemical Company. Most other chemicals were obtained from the Sigma Chemical Company.

Crosses and cross analysis: The techniques used for crosses and for flanking marker analysis were as previously described (KINSEY *et al.* 1980). Crosses for deletion mapping were made in the same way, however, analysis involved only the measurement of recombination frequency.

Isolation of deletion mutants: *am* mutants were isolated in either a *lys-1 a* background (series 23) or *lys-1 cot-1 rec-3 a* background (series 48 and 73) using the procedure of KINSEY (1977) following mutagenesis with DEO. Previous reports had indicated that DEO causes deletions in Neurospora (ONG and DESERRES 1975) and Aspergillus (HYNES 1979). The procedure for DEO mutagenesis was as follows: Conidia were treated with 100 mM DEO for 90 min at room temperature. The incubation was carried out in a large glass centrifuge tube with continuous stirring in 0.067 M phosphate buffer, pH 7.0, with conidia adjusted to a final concentration of 6×10^6 conidia/ml. Conidia were washed three times with ice-cold Fries basal medium after the incubation period was completed.

Rapid reversion screen: *am* mutants were screened for putative deletions by characterization for reversion following ultraviolet (UV) irradiation. Conidial suspensions in sterile water were adjusted to an absorbance of 0.5 at 600 nm. One-tenth of a milliliter, containing approximately 10^6 conidia, was plated on Vogels minimal salts (VOGEL 1956) containing 1.5% sorbose, 0.2% glucose, 0.2% glycerol agar plus glycine (0.02 M) and exposed to UV (1.71×10^3 erg/mm^2). The number of revertant colonies was compared with control plates containing either am_{119}, which reverts with a relatively high frequency following UV irradiation, or am_{132}, a large deletion, which is not revertable. Mutant strains that did not revert were considered to be putative deletions and further characterized by genetic analysis.

RESULTS

Flanking marker analysis

We began to map *am* mutants using flanking marker analysis, utilizing the conventions developed by SMYTH (1973). These conventions have been shown to give a genetic map that is colinear with the sites of altered amino acids in mutant forms of GDH (BRETT *et al.* 1976; KINSEY *et al.* 1980). Mapping is based on two criteria which can be related to the diagram in Figure 1, in which m_1 represents the site of the more proximal and m_2 that of the more distal of two *am* alleles. P,p and D,d are general terms for markers at the centromere proximal and centromere distal ends, respectively, of the *am* locus. The first criterion is based on prototrophic recombinants between different *am* alleles in which the flanking markers are also of the recombinant type (either Pd or pD). According to this criterion the more proximal *am* allele will have its proximal flanking marker represented less frequently and its distal marker more frequently among the prototrophic progeny (*i.e.*, pD > Pd). The second criterion, or the polarity criterion, is based on *am* prototrophic recom-

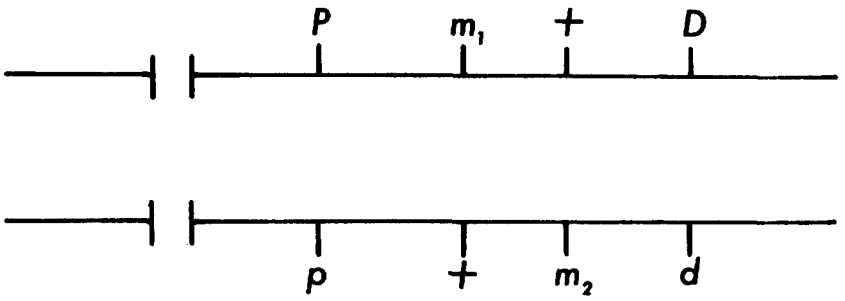

FIGURE 1.

binants which are not recombinant with respect to flanking markers. This criterion states that gene conversion occurs more frequently at the centromere proximal end of the gene than at the distal end; that is, a polarity of conversion exists within the gene. This criterion is empirically derived. Accordingly, the more proximal *am* allele is defined as that which entered the cross with the same association of flanking markers represented most frequently among the prototrophic recombinants (*i.e.*, PD > pd).

In practice, mapping of *am* mutants has depended almost exclusively on the polarity criterion, since prototrophic recombinants which are also recombinant for flanking markers generally fail to reveal significant differences between recombinant classes (*i.e.*, pD ≃ Pd) (SMYTH 1973; FINCHAM 1974; our results).

In our experiments crosses of the general constitution *ure-2* am_x; *rec-3*; *cot-1* × am_y *his-1*; *rec-3*; *cot-1* were made in which *ure-2* is the centromere proximal flanking marker, approximately 1 map unit from *am*, and *his-1* is the centromere distal flanking marker, approximately 1.5 map units from *am*. The *cot-1* marker was present in these stocks and produced very compact colonies at 33°. Prototrophic am^+ recombinants were counted, and the flanking marker constitution of the prototrophs was determined as previously described (KINSEY *et al.* 1980). The results of 90 informative crosses involving 30 previously unmapped alleles crossed to various alleles with known map positions are given in Table 1. The deduced order is shown for a cross only if the PD class was significantly (5% probability level) greater than the pd class. In addition to the data shown in Table 1, all *am* mutants used were crossed to themselves in opposite mating type and at least 5×10^6 viable spores analyzed. No prototrophic revertants were found for any of these selfing crosses (data not shown). In addition to the informative crosses shown in Table 1, more than 100 additional crosses were made that gave significant recombination frequencies (data not shown) but for which either there was no significant bias in parental flanking marker combinations or for which flanking marker analysis was not performed. In these cases, recombination frequency, if it was very low, was taken as a presumption of closeness on the genetic map when deciding which alleles to cross to particular deletion mutants.

Isolation of deletion mutants

Early in these studies we became convinced of the need for a less equivocal and a quicker method of mapping. This was particularly so since we were

TABLE 1

Frequencies and distribution of flanking markers among am *prototrophic recombinants from crosses of the general constitution* + am_x his-1 × ure-2 am_y +

Parent strains		Frequency of am^+ progeny per 10^5 live spores	Distribution of flanking markers of the am^+ progeny					Deduced order
+ am_x *his-1*	*ure-2* am_y +		*+h*	*u+*	*uh*	*++*	Total	
100	14	0.55	45	9	4	4	62	100 → 14
100	2	3.5	13	4	0	1	18	100 → 2
100	9	2.4	51	14	8	9	82	100 → 9
101	14	1.1	34	81	22	15	152	14 → 101
101	109	2.0	2	44	1	1	48	109 → 101
101	2	10.2	83	25	26	41	175	101 → 2
101	1	38.5	71	3	3	2	79	101 → 1
102	14	16.3	34	67	0	4	105	14 → 102
102	2	15.0	17	48	19	15	99	2 → 102
102	1	2.2	45	62	15	29	151	1 → 102
103	14	20.8	21	59	17	14	111	14 → 103
103	11	12.06	39	20	5	26	90	103 → 11
104	14	1.2	7	26	3	1	38	14 → 104
104	109	2.3	37	66	3	2	108	109 → 104
104	2	5.4	38	14	11	16	79	104 → 2
104	1	11.8	78	9	3	12	102	104 → 1
104	9	49.5	69	8	7	13	97	104 → 9
106	2	6.3	56	22	10	12	100	106 → 2
106	1	13.9	60	4	5	11	80	106 → 1
107	1	3.2	61	22	17	13	113	107 → 1
107	9	8.1	40	16	7	35	98	107 → 9
108	14	3.6	17	49	3	5	74	14 → 108
108	109	11.4	11	30	9	9	59	109 → 108
108	2	1.7	20	9	3	2	34	108 → 2
108	1	9.8	73	8	5	0	86	108 → 1
109	14	1.2	44	70	4	7	125	14 → 109
109	2	21.2	144	64	24	15	247	109 → 2
110	14	17.1	2	73	18	4	97	14 → 110
110	2	1.9	0	19	8	2	29	2 → 110
110	1	2.9	15	3	2	2	22	110 → 1
112	14	5.3	9	70	16	5	100	14 → 112
112	2	30.0	70	9	14	7	100	112 → 2
113	14	26.5	22	65	7	6	100	14 → 113
113	2	11.6	70	12	11	7	100	113 → 2
113	1	24.4	43	7	43	10	103	113 → 1
118	14	11.7	10	67	20	4	101	14 → 118
118	2	3.2	24	40	8	5	77	2 → 118
118	1	7.6	73	18	9	5	105	118 → 1
119	14	10.0	2	60	12	6	80	14 → 119
119	2	1.9	5	35	5	8	53	2 → 119
119	9	3.2	12	3	6	8	29	119 → 9
121	14	42.3	5	82	4	7	98	14 → 121
121	2	21.5	20	68	3	9	100	2 → 121
121	1	5.3	16	51	3	12	82	1 → 121
122	14	49.0	4	60	5	4	73	14 → 122
122	2	35.4	22	54	8	8	92	2 → 122

TABLE 1—Continued

Parent strains		Frequency of am^+ progeny per 10^5 live spores	Distribution of flanking markers of the am^+ progeny					Deduced order
$+\ am_x$ *his-1*	*ure-2* am_y +		$+h$	$u+$	uh	$++$	Total	
124	14	18.2	11	83	5	1	100	14 → 124
124	2	21.2	21	58	12	8	99	2 → 124
124	1	7.2	0	49	3	11	63	1 → 124
124	9	1.2	18	47	12	10	87	9 → 124
125	14	40.5	4	87	0	9	100	14 → 125
125	2	15.0	0	93	1	6	100	2 → 125
125	1	1.3	2	30	8	2	44	1 → 125
125	9	0.5	12	4	5	4	25	125 → 9
128	14	3.9	25	53	8	14	100	14 → 128
128	2	15.3	58	9	7	21	95	128 → 2
128	1	15.2	68	9	6	26	109	128 → 1
130	14	2.5	12	51	9	11	83	14 → 130
130	2	7.3	34	11	9	2	56	130 → 2
130	1	5.3	25	4	9	3	41	130 → 1
130	9	30.0	59	9	1	7	76	130 → 9
131	2	4.2	7	49	7	9	72	2 → 131
131	9	18.9	69	14	8	9	100	131 → 9
133	2	4.7	12	62	5	21	100	2 → 133
133	1	6.2	37	3	3	1	44	133 → 1
135	14	5.0	3	32	6	7	48	14 → 135
135	2	5.5	11	33	6	10	60	2 → 135
135	1	24.4	79	6	3	20	108	135 → 1
135	9	3.4	34	18	6	7	65	135 → 9
138	14	0.7	1	18	1	6	26	14 → 138
138	2	19.1	14	1	1	1	17	138 → 2
138	9	75.0	22	8	4	10	44	138 → 9
139	14	20.0	7	47	5	4	63	14 → 139
139	1	2.3	10	29	2	1	42	1 → 139
139	9	0.7	1	9	1	0	11	9 → 139
140	14	3.7	11	65	13	6	95	14 → 140
140	2	1.6	15	31	4	9	59	2 → 140
140	1	10.9	65	12	9	14	100	140 → 1
140	9	7.3	52	5	8	15	72	140 → 9
141	2	9.3	7	69	9	15	100	2 → 141
141	9	5.4	32	9	3	0	44	141 → 9
142	14	1.3	3	19	0	6	28	14 → 142
142	2	>20	59	11	6	18	94	142 → 2
142	9	19.8	54	10	9	25	98	142 → 9
143	14	7.1	1	18	4	1	24	14 → 143
143	2	45.2	10	1	2	3	16	143 → 2
143	9	10.3	65	10	6	18	99	143 → 9
144	14	9.6	2	40	4	5	51	14 → 144
144	2	3.7	3	33	1	4	41	2 → 144
144	1	9.2	20	0	5	5	30	144 → 1

dealing with large numbers of mutant strains and any given flanking marker cross could order only two alleles and those only with respect to one another. We also found that many flanking marker crosses failed to give any significant

mapping data, especially when both markers were in the middle of the gene. Therefore, we have isolated and characterized deletion mutants of the *am* gene and utilized these in the manner devised by BENZER (1959, 1961) to map mutant sites.

Most of the deletion mutants we have mapped were found among DEO-induced mutants that failed to revert following UV irradiation. The exceptions are *Δ73-SG1* and *Δ73-SG2*, which are spontaneous mutants, and am_{111}, am_{128} and am_{132}, which have been previously described (KINSEY and HUNG 1981). The frequency of *am* mutants following DEO mutagenesis was approximately $1/10^6$ surviving conida. This figure is about tenfold higher than the frequency of spontaneous *am* mutants in the strains used. One observation worth noting is that DEO stored at room temperature for several months ceases to be mutagenic for Neurospora conidia while still remaining toxic. We have noted this phenomenon with several lots of DEO.

Of 108 DEO-induced mutants that we have analyzed, 59 failed to revert following UV treatment. These 59 *am* mutants, as well as four spontaneous mutants with similar UV reversion characteristics, were crossed to a series of 11 point mutants that together span the *am* locus (SMYTH 1973; KINSEY *et al.* 1980). As a control the DEO mutants were also crossed to am_{132} which deletes the entire *am* locus.

The idea behind these crosses was that any substantial deletion would fail to show recombination with one or more of these point mutants. Past experience with point mutant crosses had indicated that virtually all point mutant × point mutant crosses (except selfing crosses) yield some recombinants. On the basis of the results of these screening crosses, further crosses of the putative deletions would be made to other point mutants that mapped in the indicated area. Since these crosses were intended only for rapid screening, ascospore concentrations were estimated on the basis of optical density measurements of the spore suspensions. Except as specifically noted an estimated 10^6–10^7 spores were tested for each cross. It should be pointed out that some crosses that gave no recombinants in this test subsequently were shown to give a few recombinants when more spores were analyzed (compare Table 2 with Table 3). Also inherent in this scheme would be the loss of many of the possible small deletions which did not overlap one of the 11 point mutants.

Seventy-one percent (45 of 63) of the nonreverting mutants failed to show recombination with at least one of the 11 point mutants. However, only 21 of these could subsequently be demonstrated to be deletions based on the criterion that a deletion must fail to recombine with two or more mutants capable of recombination with each other. Ten of these 21 failed to recombine with any of the strains used, and presumably each deletes the entire *am* locus. The results of crosses between these 21 deletion mutants and the 11 point mutants and am_{132} are shown in Table 2.

Crosses of confirmed deletions to each other and to relevant point mutants

Those strains deleted for part of the *am* locus were crossed to other *am* strains, based on the results of flanking marker analyses or recombination frequency. The deletion mutants were also crossed to other deletion mutants

TABLE 2

Crosses of putative deletions to point mutants

Deletions	Point mutants[a]											
	6	14	15	130	4	2	131	103	1	11	9	132
23-10	0	0	0	0	0	0	ND	ND	0	0	0	0
23-44	ND	+	+	+	+	+	ND	−	0	+	+	0
23-57	0	0	0	0	0	0	ND	0	0	0	0	0
23-12	0	0	0	0	0	0	ND	0	0	0	0	0
23-70	0	0	0	0	0	0	0	0	0	0	0	0
23-81	+	+	+	+	+	+	ND	0	−	−	−	ND
23-82	0	0	0	0	0	0	ND	−	+	+	+	0
23-84	0	0	0	0	0	0	ND	−	+	+	+	0
23-21	0	0	0	0	0	0	0	0	0	0	0	0
48-12	ND	0	0	ND	0	0	ND	ND	0	0	0	0
48-5	0	0	0	ND	0	0	ND	ND	0	0	0	ND
48-22	0	0	0	ND	0	0	ND	ND	0	0	0	ND
48-2	+	+	+	+	+	+	0	+	+	+	+	0
48-35	ND	0	0	0	0	0	0	+	+	+	+	0
39-A4	0	0	0	0	0	0	0	0	0	+	+	0
39-A11	0	0	0	0	0	0	0	0	0	0	0	0
73-G4	0	0	0	0	0	0	0	0	0	0	0	0
73-40	+	+	−	−	0	0	0	0	0	0	0	0
73-38	0	0	0	0	0	0	0	+	+	+	+	0
73-SG1	+	+	+	0	0	+	+	+	+	+	+	0
73-SG2	+	+	+	0	0	+	+	+	+	+	+	0

ND = not done, + = prototrophic recombinants, 0 = no prototrophic recombinants, − = no prototrophic recombinants but fewer viable spores than needed for conclusive test.

[a] The order of the tester strains is centromere am_6, am_{14}, am_{15} ... am_{11}, am_9. Mutant am_{132} is a complete deletion of the *am* locus.

that could potentially overlap them. For example, the data in Table 2 indicate that Δ*23-82* is a deletion in which approximately half of the gene corresponding to the amino terminus of GDH is missing, with the right end point of the deletion lying between am_2 and am_1. Consequently, all of the alleles mapping in the vicinity of, or in the interval between, am_2 and am_1 were crossed to Δ*23-82* in order to define the right end point of the deletion. Similarly, it can be seen from Table 2 that Δ*73-40* is a deletion in which all of the gene except that corresponding to the amino terminus of GDH is deleted, with the left end point of the deletion lying between am_{14} and am_4. Thus, all of the alleles that mapped near or between am_{14} and am_4 were crossed to Δ*73-40* in order to define the left end point of this deletion. The data in Table 2 also show Δ*73-SG2* to be a small deletion with both end points in the gene and mapping in the vicinity of the left end point of Δ*73-40*. Subsequent crosses to Δ*73-40* and alleles mapping near the deleted region of Δ*73-SG2* were used to show the overlapping nature of Δ*73-40* and Δ*73-SG2* and to define the end points of Δ*73-SG2*. Employing similar strategy with the other partial deletions shown in Table 2 and with am_{128} and am_{111} (described later), we were able to define the end point(s) and overlapping or nonoverlapping nature of these deletion mutants. In so doing we were able to assign *am* alleles to one of 13 regions of

TABLE 3

Crosses of am *alleles to deletion strains*

Mapping region	*am* allele	Δ*128*	Δ73-SG2	Δ73-40	Δ23-82	Δ48-2	Δ111	Δ23-81	Δ39-A4	Δ23-44
1	6	3.5	6.7	5.6	$0/8.6 \times 10^6$	–	–	0.62	$0/5.8 \times 10^6$	–
	100	3.52	–	7.6	–	–	–	–	–	–
	14	7.2	4.0	4.4	$0/7.3 \times 10^6$	–	–	–	–	–
	138	1.9	1.5	5.6	–	–	–	–	–	–
	109	2.0	3.9	2.38	$0/6 \times 10^6$	–	–	–	–	–
	106	0.95	3.0	2.0	–	–	–	–	–	–
	101	6.2	3.3	4.1	–	–	–	–	–	–
	FK059	0.18	1.8	4.3	$0/6.1 \times 10^6$	12.6	4.5	–	–	–
	123	0.7	6.1	13.6	$0/1.7 \times 10^6$	14.5	–	–	$0/1.9 \times 10^6$	–
	120	2.0	5	3.3	$0/1.2 \times 10^6$	13.4	–	–	$0/2 \times 10^6$	–
2	15	$0/6.4 \times 10^6$	1.5	1.5	$0/5.4 \times 10^6$	–	–	3.5	–	–
	142	$0/3 \times 10^6$	2.8	1.0	–	–	–	–	$0/4.2 \times 10^6$	–
	112	$0/3.4 \times 10^6$	0.38	0.23	–	–	–	–	–	–
	104	$0/3.4 \times 10^6$	0.625	0.28	–	–	–	–	–	–
	130	$0/3.0 \times 10^6$	0.10	0.22	$0/1 \times 10^6$	–	–	11.5	–	–
	23-6	$0/6 \times 10^6$	–	1.2	–	–	–	–	–	–
	23-30	$0/5.4 \times 10^6$	–	0.9	–	–	–	–	–	–
	23-58	$0/8.6 \times 10^6$	–	2.2	–	–	–	–	–	–
	73-46	$0/3.2 \times 10^6$	–	1.5	–	–	–	–	–	–
3	148	$0/3.2 \times 10^6$	$0/2 \times 10^6$	$0/8.2 \times 10^5$	–	–	–	–	–	–
4	129	1.41	$0/2.4 \times 10^6$	$0/4.6 \times 10^6$	$0/2.3 \times 10^6$	8.5	–	–	$0/3.3 \times 10^6$	–
	137	2.0	$0/2.4 \times 10^6$	$0/6 \times 10^5$	$0/3.3 \times 10^6$	1.9	–	–	–	–
	143	4.8	$0/2.5 \times 10^6$	$0/1.6 \times 10^6$	–	–	–	–	–	–
	127	4.7	$0/1.3 \times 10^6$	$0/2.4 \times 10^6$	$0/5.2 \times 10^6$	3.9	–	–	–	–
	108	0.9	$0/1.9 \times 10^6$	$0/2 \times 10^6$	–	–	–	–	–	–
	107	4.0	$0/1.4 \times 10^6$	$0/2.3 \times 10^6$	$0/1.7 \times 10^6$	6.25	–	2.8	–	–
	4	0.75	$0/2.3 \times 10^6$	$0/4.4 \times 10^6$	$0/2.1 \times 10^6$	–	–	6.0	–	–
	113	15.7	$0/2.2 \times 10^6$	$0/2.4 \times 10^6$	–	–	–	–	–	–

5	2	5.2	0.27	$0/5.2 \times 10^6$	$0/4.5 \times 10^6$	2.8	–	2.1	–	–
	16	–	2.1	$0/3.1 \times 10^6$	$0/2.2 \times 10^6$	2.1	0.65	–	$0/2.4 \times 10^6$	–
	136	–	1.2	$0/2.5 \times 10^6$	$0/1.7 \times 10^6$	0.48	0.89	–	$0/8.3 \times 10^6$	–
	140	–	0.36	$0/5.1 \times 10^6$	$0/6.1 \times 10^6$	1.7	0.92	–	–	–
	146	–	1.7	$0/3.7 \times 10^6$	$0/4 \times 10^6$	0.11	1.4	–	–	–
	145	–	0.17	$0/8.5 \times 10^5$	$0/4.1 \times 10^6$	0.54	0.86	–	–	–
	131	–	0.38	$0/2.5 \times 10^6$	$0/2.6 \times 10^6$	0.5	1.6	1.47	–	–
	110	–	2.6	$0/1.4 \times 10^6$	$0/2.3 \times 10^6$	1.6	0.35	–	–	–
	119	–	4.4	$0/4.6 \times 10^6$	$0/2.1 \times 10^6$	0.9	1.7	–	–	–
	144	–	1.2	$0/3.8 \times 10^6$	$0/3.8 \times 10^6$	0.7	–	–	–	–
	133	–	0.71	$0/2.3 \times 10^6$	$0/6.9 \times 10^6$	0.37	0.69	–	–	–
6	118	–	–	–	$0/1.7 \times 10^6$	$0/2.4 \times 10^6$	0.65	–	–	–
	135	–	–	–	$0/3.4 \times 10^6$	$0/3.3 \times 10^6$	0.44	–	–	–
7	134	–	–	$0/1.3 \times 10^6$	$0/6.1 \times 10^6$	$0/1.1 \times 10^7$	$0/2.6 \times 10^6$	–	–	1.8
	5	–	–	–	$0/2.6 \times 10^6$	$0/8.5 \times 10^6$	$0/2.3 \times 10^6$	–	–	–
8	17	–	–	–	0.3	1.3	$0/6.4 \times 10^6$	0.52	–	–
9	147	–	–	–	0.31	–	$0/1.8 \times 10^6$	$0/8.9 \times 10^6$	–	–
10	103	–	1.3	$0/2.6 \times 10^6$	0.26	0.67	0.9	$0/5.6 \times 10^6$	$0/3.9 \times 10^6$	1.1
	141	–	–	–	0.23	–	8.5	$0/1 \times 10^6$	$0/2.8 \times 10^6$	2.8
11	1	–	–	–	1.0	0.9	4.7	0.01	$0/4.5 \times 10^6$	$0/3.4 \times 10^6$
	105	–	–	–	0.22	–	0.9	$0/3.4 \times 10^6$	$0/6.4 \times 10^6$	$0/5 \times 10^6$
12	7	–	–	–	–	–	4.1	0.04	0.3	0.2
	11	–	–	–	0.8	1.7	5.5	0.08	0.9	4.2
	117	–	0.98	$0/6 \times 10^6$	0.3	2.0	–	$0/6 \times 10^6$	0.053	–
	125	–	–	–	–	–	–	$0/2.2 \times 10^6$	0.66	–
	116	–	–	–	–	–	5.0	$0/3.4 \times 10^6$	1.5	1.46
	3	–	–	–	–	–	1.4	$0/7.4 \times 10^6$	0.05	–
	122	–	–	–	0.1	–	4.44	$0/7.4 \times 10^6$	0.4	–
	102	–	–	–	–	–	–	0.65	1.4	1.39
	9	–	–	$0/1.3 \times 10^6$	0.3	–	8.0	0.1	1.3	–

TABLE 3—Continued

Mapping region	*am* allele	Δ*128*	Δ73-SG2	Δ73-40	Δ23-82	Δ48-2	Δ111	Δ23-81	Δ39-A4	Δ23-44
13	139	–	–	$0/1.9 \times 10^6$	–	–	–	1.1	0.38	–
	114	–	–	$0/2.1 \times 10^6$	–	–	–	1.9	0.64	–
	124	–	–	$0/2.2 \times 10^6$	–	–	–	0.75	2.5	–
	121	–	–	$0/1.7 \times 10^6$	–	–	–	1.9	1.6	1.8

Results are the numbers of prototrophic recombinants per 10^5 viable spores. Where no prototrophic recombinants are found, a 0 is followed by the number of viable spores tested. – = not done.

the gene by deletion analyses. The mapping crosses of *am* alleles crossed to deletion strains are given in Table 3, and the crosses of deletions by deletions are given in Table 4.

During this mapping process it was discovered that the previously described mutants am_{128} and am_{111} (KINSEY and HUNG 1981) are also deletion mutants. am_{111}, which in a previous communication (KINSEY and HUNG 1981) was mistakenly reported to revert, was found to map in two nonoverlapping deletions, *Δ23-81* and *Δ48-2* (Table 4), and to cover the allele am_{17} which maps between *Δ23-81* and *Δ48-2* (Table 3). am_{128} was found to map in *Δ73-40* and *Δ73-SG2* (Table 4) and to cover several alleles that did not map in these deletions (Table 3). Thus, am_{128} and am_{111} are small deletions, deleting portions of the gene corresponding to the amino terminal and carboxy terminal ends of GDH, respectively. Four DEO-induced mutants—*Δ23-82*, *Δ23-84*, *Δ48-35* and *Δ73-38*—are identical on a genetic basis; all delete approximately half of the gene corresponding to the amino terminus of GDH. Only results for *Δ23-82* are listed in Tables 2 and 3. Two spontaneous deletions, *Δ73-SG1* and *Δ73-SG2*, are also identical on a genetic basis; each deleted a small region of the gene in the vicinity of and including am_4. Only results for *Δ73-SG2* are listed in Tables 2 and 3.

By using a set of nine of these deletion mutants—am_{128}, am_{111}, *Δ73-SG2*, *Δ73-40*, *Δ23-44*, *Δ23-82*, *Δ23-81*, *Δ48-2* and *Δ39-A4*—we can easily map any *am* allele to one of 13 discrete regions of the gene. We have used this set of deletion strains to map 7 alleles (am_{16}, *am*FK059, am_{117}, am_{120}, am_{123}, am_{129} and am_{136}) for which we previously had no mapping information (Table 3), as well as to complete the mapping of the 49 partially mapped alleles.

DISCUSSION

Flanking marker analysis

We have utilized flanking marker analysis primarily to place mutants sites in one of four segments of the gene. This placement was subsequently corroborated in a few cases by peptide sequence information obtained from analysis of mutant GDH (KINSEY *et al.* 1980), in one case by DNA sequence analysis from a cloned mutant version of the *am* gene (J. A. RAMBOSEK and *J. A. Kinsey*, unpublished results), and in all cases by deletion mapping analysis.

In each case we were able to confirm independently the assignment made on the basis of conversion polarity, thus confirming the usefulness of the polarity criterion for mapping at the *am* locus. Additional analysis of the various classes of prototrophic recombinants, with respect to flanking markers, are also consistent with the previous observations of FINCHAM (1964, 1967) and SMYTH (1973). For example, if all of the crosses are taken together, the mean value for total prototrophs that are also recombinant for flanking markers is 22 ± 0.11%. This compares with a value of 29.5% obtained by SMYTH (1973) and once again indicates that only about 25% of conversion events at the *am* locus are associated with exchange of flanking DNA regions.

If the prototrophs that are recombinant for flanking markers are themselves

TABLE 4

Selected crosses between deletions

Strains crossed	Prototrophs per viable spores	Prototrophs per 10^5 viable spores
128 × *73-SG2*	$0/2.5 \times 10^6$	0.0
128 × *73-40*	$0/2 \times 10^6$	0.0
73-SG2 × *73-40*	$0/4.2 \times 10^6$	0.0
48-2 × *111*	$0/3.1 \times 10^6$	0.0
23-81 × *111*	$0/6.2 \times 10^6$	0.0
48-2 × *23-81*	$29/1.2 \times 10^6$	2.4
23-81 × *39-A4*	$0/1.6 \times 10^6$	0.0
23-81 × *23-82*	$9/4.3 \times 10^6$	0.21
111 × *23-82*	$0/8.4 \times 10^6$	0.0
48-2 × *23-82*	$0/2.5 \times 10^6$	0.0
73-SG2 × *48-2*	$2/7.6 \times 10^5$	0.26

analyzed, again combining the data from all of our crosses, we find no significant difference between the two classes. This is in contrast to the results of SMYTH (1973) in which he found a slight, but significant, excess of one class over the other. It was on this basis that SMYTH decided that am_6 represents the centromere proximal end of the gene. Although we have utilized this convention throughout this study, there is no direct support for it in our data.

Deletion analysis

Our early efforts to obtain deletions were not very successful, and it was only when we started to use DEO as a mutagen (ONG and DESERRES 1975) that we began to obtain deletions in significant numbers. DEO is a bifunctional alkylating agent that is thought to crosslink guanosine residues in adjacent positions on complementary strands (ONG and DESERRES 1975), thus promoting double strand breaks and excision. Altogether, 21 of 108 DEO-induced mutations were shown to be deletions; this may represent only a fraction of the actual induced deletions because our method of screening would allow many small deletions to go undetected.

It is interesting to note that about half of the proven deletions cover the entire *am* locus; however, none of these large deletions extend into the nearest known gene, *gul-1* (0.3 map units distal to *am*). This fact, coupled with our repeated failure to obtain *am* through *gul-1* deletions among mutants selected simultaneously for the *gul* and *am* phenotypes, suggests to us that there is indispensible material between the two sites.

We have isolated five proven deletions not induced by DEO. Two of these were spontaneous, and both of these, *Δ73-SG1* and *Δ73-SG2*, appear to be identical and probably represent repeat isolations of a preexisting deletion mutation present in the stock used. One of the others, *Δ111*, was isolated after UV mutagenesis, and two, *Δ128* and *Δ132*, were isolated after nitrous acid mutagenesis. All of these non-DEO mutants are small internal deletions except *Δ132*, which removes the entire *am* locus and considerable material on either side but, once again, not extending to the *gul-1* locus (J. A. RAMBOSEK and J. A. KINSEY, unpublished results).

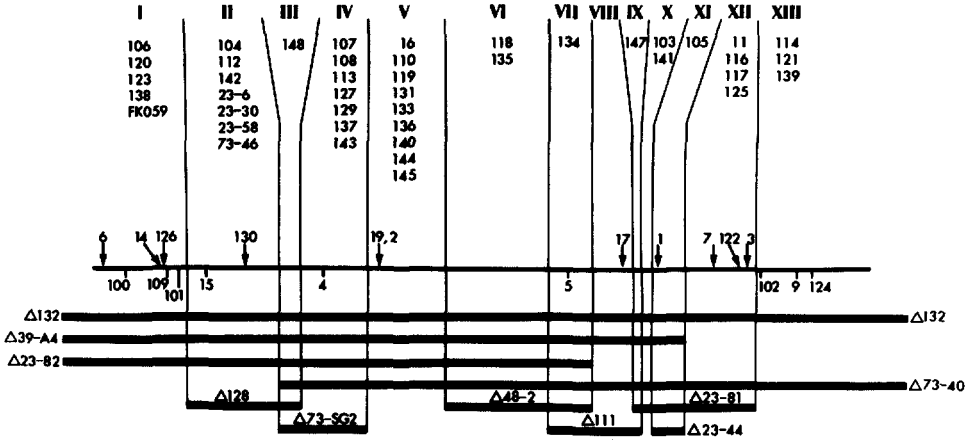

FIGURE 2.—A fine structure map of the *am* locus. See text for detailed explanation.

For the purpose of these studies we have chosen to include mutant *23-81* as a deletion, however, its behavior is somewhat anomalous. On the basis of mutant peptide analysis one can assign certain alleles in this region to an unequivocal order (reference to Figure 2 will aid the following discussion). That order is, left to right, am_{17}, am_1, am_7, am_{122}, am_3. The deletion, *Δ111*, covers am_{17} but none of the other alleles and, therefore, has a righthand end point between am_{17} and am_1. The deletion, *Δ39-A4*, by the same kind of reasoning, has an end point between am_1 and am_7. The small deletion, *Δ23-44*, has its left end point between am_{17} and am_1 and its right end point between am_1 and am_7. By analysis of their behavior in crosses to these three deletions, new *am* mutations can now be oriented on this map. The putative deletion *23-81* does not cover am_{17} nor does it appear to cover am_1, yet it overlaps Δ111 and clearly covers am_{122} and am_3. Moreover, it also covers am_{147} which is in turn covered by *Δ111*. Thus, *23-81* has the appearance of failing to recombine with left hand markers *Δ111* and am_{147}, as well as am_{105} which maps within *Δ23-44* (as does am_1). It then recombines with central markers am_1, am_7 and am_{11} and then fails to recombine with righthand markers am_{122} and am_3 as well as am_{116}, am_{117} and am_{125}, all of which map to the right of *Δ39-A4*. It then recombines with all of the remaining markers that map to the right of *Δ39-A4*. We have shown *23-81* in our map as if it were a continuous deletion, however, it is possible that it represents two small neighboring deletions or that it represents an inverted region. We are currently cloning the *23-81* version of the *am* gene to sort out the answer to this question.

Fine structure map

The current fine structure map of the *am* locus based upon previous mapping experiments (SMYTH 1973; KINSEY *et al.* 1980) as well as flanking marker analysis and deletion mapping (reported here) is shown in Figure 2. The scale of the map is based on recent nucleotide sequencing data (*J. H. Kinnaird* and *J. R. S. Fincham*, personal communication; J. A. Rambosek and J. A. Kinsey, unpublished results). Mutant sites indicated by the symbol just above the line

are accurately placed based on sequencing of mutant peptides (BRETT *et al.* 1976; KINSEY *et al.* 1980) or in the case of am_{126} on DNA sequencing data from a cloned am_{126} version of the *am* gene (J. A. Rambosek and J. A. Kinsey, unpublished results). Mutant sites placed just below the line are placed in the correct order, based on flanking marker analysis, but the actual distance between such sites is an estimate, based on recombination frequency. Allele designations placed well above the line are mapped only with respect to deletion end points as indicated by the vertical bars. Deleted regions are indicated by heavy bars below the line. The actual end points of the deletions are only estimates, however, the relationship of the end points to defined point mutants are accurate with respect to relative order.

Many of the mutant sites that have been placed only within a given interval as defined by deletion end points are separable by recombination and/or distinguishable on the basis of reversion frequency, complementation, cross-reacting material production, temperature sensitivity or suppressibility.

We are currently using this more detailed fine structure map to reassess the effects of *rec-3* on recombination at the *am* locus (SMYTH 1973), utilizing stocks that have been extensively backcrossed to the standard IVA/ORSa reference stocks.

We thank J. R. S. FINCHAM and DAVID STADLER for reading the manuscript and making suggestions. We also thank MALCOLM LUKER who suggested to us that we use DEO as a mutagen. This project was supported by grants BRSG S07 RR 05373 and GM 23967 from the National Institutes of Health.

LITERATURE CITED

BENZER, S., 1959 On the topology of the genetic fine structure. Proc. Natl. Acad. Sci. USA **45:** 1607–1620.

BENZER, S., 1961 On the topography of the genetic fine structure. Proc. Natl. Acad. Sci. USA **47:** 403–415.

BRETT, M., G. K. CHAMBERS, A. A. HOLDER, J. R. S. FINCHAM, and J. C. WOOTTON, 1976 Mutational amino acid replacements in *Neurospora crassa*. J. Mol. Biol. **106:** 1–22.

FINCHAM, J. R. S., 1967 Recombination within the *am* gene of *Neurospora crassa*. Genet. Res. **9:** 49–62.

FINCHAM, J. R. S., 1974 Negative interference and the use of flanking markers in fine-structure mapping in fungi. Heredity **33:** 116–121.

FINCHAM, J. R. S. and A. J. BARON, 1977 The molecular basis of an osmotically reparable mutant of *Neurospora crassa* producing unstable glutamate dehydrogenase. J. Mol. Biol. **110:** 627–642.

HYNES, M. J., 1979 Fine structure mapping of the acetamidase structural gene and its controlling region in aspergillus nidulans. Genetics **91:** 381–392.

KINNAIRD, J. H., M. A. KEIGHREN, J. A. KINSEY, M. EATON and J. R. S. FINCHAM, 1982 Cloning of the *am* (glutamate dehydrogenase) gene of *Neurospora crassa* through the use of a synthetic DNA probe. Gene **20:** 387–396.

KINSEY, J. A., 1977 Direct selective procedure for isolating *Neurospora* mutants defective in nicotinamide adenine dinucleotide phosphate-specific glutamate dehydrogenase. J. Bacteriol. **132:** 751–756.

KINSEY, J. A., J. R. S. FINCHAM, M. A. M. SIDDIG and M. KEIGHREN, 1980 New mutational variants of Neurospora NADP-specific glutamate dehydrogenase. Genetics **95:** 305–316.

KINSEY, J. A. and B. S. T. HUNG, 1981 Mutation at the *am* locus of *Neurospora crassa*. Genetics **99:** 405–414.

ONG, T.-M. and F. J. DESERRES, 1975 Mutation induction by difunctional alkylating agents in *Neurospora crassa*. Genetics **80:** 475–482.

SEALE, T. W., M. BRETT, A. J. BARON and J. R. S. FINCHAM, 1977 Amino acid replacements resulting from suppression and missense reversion of a chain-terminator mutation in Neurospora. Genetics **86:** 261–274.

SIDDIG, M. A. M., J. A. KINSEY, J. R. S. FINCHAM and M. KEIGHREN, 1980 Frameshift mutations affecting the N-terminal sequence of *Neurospora* NADP-specific glutamate dehydrogenase. J. Mol. Biol. **137:** 125–135.

SMYTH, D. R., 1973 A new map of the amination-1 locus of *Neurospora crassa* and the effect of the recombination-3 gene. Aust. J. Biol. Sci. **26:** 1355–1370.

VOGEL, H. J., 1956 A convenient growth medium for *Neurospora* (medium N). Microbiol. Genet. Bull. **13:** 42–43.

WOOTTON, J. C., G. K. CHAMBERS, A. A. HOLDER, A. J. BARON, J. G. TAYLOR, J. R. S. FINCHAM, K. M. BLUMENTHAL, K. MOON and E. SMITH, 1974 Amino acid sequence of NADP-specific glutamate dehydrogenase of *Neurospora crassa*. Proc. Natl. Acad. Sci. USA **71:** 4361–4365.

Corresponding editor: R. C. ULLRICH