FINE-STRUCTURE MAPPING OF THE ACETAMIDASE STRUCTURAL GENE AND ITS CONTROLLING REGION IN *ASPERGILLUS NIDULANS*

M. J. HYNES

Department of Genetics, La Trobe University, Bundoora, Victoria 3083, Australia

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ABSTRACT

A large number of *amdS* mutants altered in acetamide utilization have been used to construct a fine-structure map of the *amdS* locus. The mutagen diepoxyoctane generated most of the deletion strains used for mapping. A minimum of 14 sites within the *amdS* gene were found. Biochemical analysis of *amdS* mutants defined the extent of the probable coding region. A new mutant, *amd-205*, which did not produce detectable inactive gene product, was found to be inseparable by recombination from the "up-promoter" mutation *amdI18* and was located outside of the apparent *amdS* coding region. The *cis*-dominant mutation, *amdI9*, was also located at this end of the gene. This work, therefore, provides evidence for the separation of a eukaryotic gene into controlling and structural regions.

IN *Aspergillus nidulans*, mutations in the *amdS* gene lead to loss of acetamidase enzyme activity and to inability to use acetamide as a sole source of either nitrogen or carbon (HYNES and PATEMAN 1970a; DUNSMUIR and HYNES 1973). Mutants are readily isolated by selecting for fluoroacetamide resistance in strains producing high acetamidase activities (HYNES and PATEMAN 1970b). Analysis of the regulation of acetamidase synthesis has shown that the enzyme is subject to multiple regulation by several apparently independent control mechanisms (see HYNES 1978a). The enzyme is induced by sources of acetyl-Coenzyme A (acetyl-CoA), benzoate and ω-amino acids, such as β-alanine, and repressed by good nitrogen sources, such as ammonium and L-glutamine. The *amdR* gene has the properties expected of a positive control gene involved in induction by ω-amino acids, while mutations in the *amdA* gene can lead to high acetamidase activities, but the function of this gene is not yet known. The *areA* gene is a positive control gene involved in regulation by nitrogen metabolites. None of these probable regulatory genes are closely linked to the *amdS* locus.

Cis-dominant mutations, closely linked to the *amdS* gene, have been found to affect enzyme regulation. The *amdI9* mutation results in increased induction by acetate, acetamide and other sources of acetyl-CoA (HYNES 1975, 1977, 1978a). The *amdI18* mutation causes elevated acetamidase protein levels under all growth conditions and may define a promoter site for *amdS* (HYNES 1978b). If

these mutations define *cis*-acting control sites, then it is predicted that they are located at one end of the *amdS* gene.

The mutagen diepoxyoctane, which has been found to induce a high frequency of multi-site mutations in *Neurospora crassa* (TONG-MAN ONG and DE SERRES 1974), has been used to isolate *amdS* mutants. Many of these are deletions that have been used, together with mitotic and meiotic recombination frequencies, to generate a map of the *amdS* locus. Controlling site mutations, including a new apparent extreme promoter-type mutation, were located at the centromere-distal end of all mapped structural gene mutations.

MATERIALS AND METHODS

Strains: All strains used derived originally from the Glasgow strain of PONTECORVO *et al.* (1953). The origins of *amdS* mutants used are summarized in Table 1. Strains containing the *phenA2* and *suB4-proA* markers and the *cbxA17* marker were provided by ETTA KAFER and H. N. ARST, respectively. A full list of gene symbols and linkage relationships is given by CLUTTERBUCK (1974) and CLUTTERBUCK and COVE (1976). The nomenclature for *amdS* alleles follows that recommended for *A. nidulans*, based on the bacterial system (CLUTTERBUCK 1974). This does pose some problems for distinguishing between structural gene mutations and mutation in putative separate controlling regions. The following policy has been adopted. Apparent "up-promoter" mutations have been designated *amdI9* and *amdI18*, while an apparent "down-promoter" mutation has been provisionally designated *amd-205* until such time as it can be definitively shown to lie outside the *amdS* coding region.

Mutagenesis: Nitrous acid mutagenesis was according to ALDERSON and HARTLEY (1969). Treatment with N-methyl-N'-nitro-nitrosoguanidine (NTG) was according to HYNES and PATEMAN (1970a). Mutagenesis with diepoxyoctane was performed by adding 2 ml of conidia (approximately 3.3 10^6 viable conidia per ml) to 10 ml sodium orthophosphate buffer (pH 7.2) containing 0.15 ml 1,2,7,8-diepoxyoctane (Aldrich Chemical Company). Treatment was for 45 min at room temperature. Conidia were pelleted, washed twice with sterile water and resuspended in water containing Tween 80. More than 90% of the conidia were killed by this treatment.

TABLE 1

Origin of amdS *mutants used*

Parental genotype	Origin*	Allele numbers	Reference
biA1;amdR^{c}6	NTG	11, 17	HYNES and PATEMAN 1970b
yA1;wA3;amdR$_2^c$;pyroA4	Spont.	8	DUNSMUIR and HYNES 1973
biA1;amdI9;niiA4	Spont.	91, 120, 121, 122, 123, 125, 126, 135, 136, 142	HYNES 1975 and this paper
yA1;amdI9 areA217;riboB$_2$	Spont.	98	This paper
biA1;amdI9;amdA7;niiA4	Spont.	103, 112, 115	This paper
biA1;amdI18;niiA4	Spont.	84, 89	HYNES 1978b
yA1;amdI18;amdA7;riboB$_2$	NA Spont.	201, 205, 210 220–226	This paper
biA1;amdI18;amdA7;niiA4	DEO	311, 312, 314, 315, 316, 320, 322, 327, 347, 351, 368, 389	This paper

* Abbreviations—NTG = N-methyl-N'-nitro-nitrosoguanidine; Spont. = spontaneous; NA = nitrous acid; DEO = diepoxyoctane.

Isolation of amdS *mutants*: Mutants resistant to fluoroacetamide were isolated by adding conidial suspensions to molten media before pouring into plates, by spreading conidial suspensions onto plates, or by stab inocula and isolation of resistant sectors. The medium used contained 1.2% acetate and 1 to 3 mg per ml fluoroacetamide (ICN Pharmaceuticals Inc.) with no other added nitrogen source (see HYNES and PATEMAN 1970b).

Media: These were as described previously (see HYNES 1978a). For detection of recombinants, media contained 0.08% sodium deoxycholate to restrict colony diameter (MACKINTOSH and PRITCHARD 1963).

Genetic analysis: Standard methods of genetic analysis for *A. nidulans* were used (see CLUTTERBUCK 1974). In determining the formation of *amdS*+ recombinants in crosses between *amdS* mutants, two kinds of methods were used. As a preliminary screen, approximately 20 to 30 cleistothecia were squashed into 0.5 ml sterile water, and the resulting ascospore suspension was spread on five plates of medium containing 1% sucrose, 10 mM acetamide, 0.08% sodium deoxycholate (and required auxotrophic supplements). If more than five *amdS*+ segregants were detected, the two mutants were regarded as capable of recombination. If this test was negative, then individual cleistothecia were cleaned, squashed into 0.5 sterile water, and a loopful of the ascospore suspension was streaked on complete medium to detect hybrid cleistothecia by segregation of conidial color markers. Ascospores from hybrid cleistothecia were spread on five plates of the above sucrose-acetamide medium. Two *amdS* alleles were regarded as incapable of recombining if no recombinants were detected in at least three hybrid cleistothecia (about 5 to 30×10^4 ascospores. To detect segregants capable of growth on acrylamide, ascospores were spread on medium containing 1% glucose, 10 mM acrylamide, and 0.08% sodium deoxycholate.

For determining mitotic recombinants, conidia from diploids were scraped into water containing Tween 80, dilutions were made and plated on sucrose-acetamide medium. For accurate determinations of ascospore or conidial counts, suspensions were plated on 1% sucrose, 10 mM ammonium medium with 0.08% sodium deoxycholate.

Enyme assays: Preparation of crude extracts and assay of acetamidase were as described by HYNES (1972).

Immunological methods: Antiserum to partially purified acetamidase extras was made as described by HYNES (1978b). Protection from precipitation by antiserum of acetamidase in crude extracts of strains of genotype *amdI18*; *amdA7* (grown for 18 hr in 1% glucose, 10 mM β-alanine medium) by extracts of *amdS* mutants (grown in similar medium) was determined in the following way: 5 to 15 μl antiserum was mixed with 200 μl of sodium orthophosphate buffer (pH 7.2) in 1.5 ml microfuge tubes and 50 μl of *amdS* mutant extract added. After 10 to 30 min, 30 μl of active extract was added and the tubes held at 4° for one to two hr. The tubes were then spun for three to five min in a microfuge B (Beckman) and 50 to 100 μl supernatant assayed for acetamidase activity. Controls with no antiserum and with no *amdS* mutant extract were included. Results from tests where only more than 75% of the activity of the active extract was precipitated were used.

SDS polyacrylamide gel electrophoresis and fluorography: This was performed as described by HYNES (1978b).

RESULTS

Mapping of amdS *on linkage group III*: Previous results indicated that the *amdS* gene was on the right arm of linkage group III (HYNES and PATEMAN 1970b; CLUTTERBUCK and COVE 1976). The *amdS* gene was mapped more accurately in three-point crosses using the marker genes, *phenA*, *cbxA* and *suB-proA* (Table 2) and was found to lie between *cbxA* and *suB-proA*. The *plnA* locus was found to be unlinked to *amdS* and therefore to the left of *cbxA* (see CLUTTERBUCK and COVE 1976).

Mapping of amdI9 *in relation to* amdS: The *amdI9* mutation results in increased induction of acetamidase by acetamide and other sources of acetyl-CoA,

TABLE 2

Mapping of amdS *on linkage group III*

Cross 1. *riboA1 proA1 yA2 ; sC12 phenA2 suB4-proA*
×
proA1 biA1 ; amdS91 amdI9 ; acuD306 ; niiA4
No. progeny 227
Map *phenA2* *amdS91* *suB4-proA*
←— 23.8 ± 2.8 —→ ←— 11.5 ± 2.1 —→
←———— 35.2 ± 3.2 ————→
Cross 2. *proA1 yA2 ; phenA2 amdS91 amdI9 suB4-proA*
×
pabaA1 ; cbxA17
No. progeny 92
Map *phenA2* *cbxA17* *amdS91* *suB4-proA*
← 16.3 ± 3.9 →← 7.6 ± 2.8 →←— 8.3* ± 7.6 →
←———— 23.9 ± 4.4 ————→
←———— 15.4* ± 10.0 ————→

* Based on only 13 *proA1* progeny.

is closely linked to *amdS* and is *cis*-dominant with respect to *amdS* (HYNES 1975, 1977). Random ascospores from cross 1 of Table 2 were spread on sucrose-acetamide medium, and ten *amdS+I9* recombinants were selected and scored for the *phenA2* and *suB4-proA* outside markers. Six were of genotype *phenA2 suB-proA+*, one of genotype *phenA2 suB4-proA* and three of genotype *phenA+ suB-proA+*, giving the order *phenA2-amdS91-amdI9-suB4-proA*.

Other crosses used to orient *amdI9* involved the marker *actA1*, which is about 20 map units to the left of *amdS* (Table 3). These results were consistent with the *amdI9* mutation being to the right of *amdS*. Previous results have shown that *amdI9* and *amdI18* are approximately 0.13 map units apart (HYNES 1978b), and it is shown below that *amdI18* lies to the right of *amdS*.

Mapping of reference point mutations: Strains of genotype *amdI18;amdA7* grow extremely strongly on medium containing acetamide as a sole nitrogen or

TABLE 3

Mapping of amdI9 *using* actA1 *as the outside marker*

Relevant genotypes in cross	No. of *amdSI9* recombinants *actA1*	*actA+*
amdS135 I9 × actA1	14	1
amdS120 I9 × actA1	13	7
amdS126 I9 × actA1	8	0
amdS121 I9 × actA1	8	0
*amdS I9 × actA1**	101	36

* Pooled data from crosses involving 20 different *amdS* alleles in which only a few *amdS+I9* recombinants were scored.

carbon source and on medium with acrylamide as the sole nitrogen source. Acrylamide medium can be used to distinguish between segregants of genotype *amdI18*;*amdA7* from *amdI18* or *amdA7* segregants, since the single-mutant strains grow more weakly (HYNES 1978b). This observation was exploited to look for mutations abolishing growth on amide media, which were very close to or had deleted the *amdI18* site.

Mutants of apparent genotype *amdS amdI18*;*amdA7* were isolated (Table 1), outcrossed to a strain of genotype *amdA7* and ascospores from hybrid cleistothecia spread on acrylamide medium to detect segregation of *amdS*+ *amdI18*;*amdA7* segregants. The mutant strains containing the *amdS* alleles *210,220,221,222, 224,225* and *226* clearly retained the *amdI18* site, while the *amdS223* and *amd-205* mutants did not. Subsequent analysis showed that the *amdS223* lesion deletes nearly all known *amdS* sites (see below). The *amd-205* lesion did not contain a large deletion. A further 25 hybrid cleistothecia from outcrosses of the type *amd-205*;*amdA7* × *amdA7* were isolated and the ascospores (approx. 1 to 2×10^6) spread on acrylamide medium without yielding any *amdI18*-containing recombinants. Additional screening of large numbers of ascospores from squashes of random cleistothecia failed to yield recombinants. The *amd-205* lesion is therefore extremely tightly linked to (or more probably deleted) the *amdI18* site. Attempts to revert *amdS223* and *amd-205* with NTG have failed.

With the expectation that *amd-205* was at one end of the *amdS* locus, a series of *amdS* mutants was crossed to an *amd-205* strain and approximate relative frequencies of *amdS*+ recombinants determined. This yielded a provisional ordering of sites: *amd-205—amdS224 221 226 220 225 222*. Frequencies of mitotic recombination between *amd-205* and these mutant sites were determined (Table 4). The two methods gave similar map orders, except for the closely linked *amdS221* and *224* mutations.

Mapping of potential deletion mutants: Preliminary screening of diepoxyoctane-induced *amdS* mutants indicated that this group contained many potential multi-site mutations. A selected number of these were crossed to the set of reference point mutants (Table 5). Results for two additional multi-site mutants, *amdS223* (spontaneous) and *amdS11* (NTG-induced) are included in Table 5, as well as the apparent point mutant *amdS135*. The results were consistent with the multi-site mutants containing continuous deleted segments of the *amdS* gene

TABLE 4

Mitotic mapping of reference point mutations

Diploids of genotype:

pabaA1 ; *cbxA17 amd-205* ; *amdA7*

yA1 ; *amdS amdI18* ; *amdA7* ; *riboB2*

amdS allele	*223*	*221*	*224*	*226*	*220*	*225*	*222*
Frequency of acetamide positive recombinants (per 10^6 conidia)	0	0.3	0.8	2.1	3.1	4.3	6.6

TABLE 5

Presence of amdS+ *recombinants in crosses between reference point mutants and probable deletion strains*

amdS deletion strains	Reference point *amdS* mutations							
	205	*224*	*221*	*226*	*220*	*225*	*222*	*135*
223	0	0	0	0	0	0	0	+
322	0	—	0	0	—	—	0	—
368	0	—	0	—	—	—	0	—
316	0	0	0	0	0	0	+	+
351	0	—	0	0	+	+	+	—
320	+	+	+	0	0	0	0	0
314	+	+	+	+	0	0	+	+
11	+	+	+	+	0	0	+	+
347	+	+	+	+	+	0	0	—
311	+	+	+	+	+	+	0	+

(+) = recombinants; (0) = no recombinants found; (—) = not tested.

and, furthermore, were entirely compatible with the order of reference point mutations derived from recombination frequencies.

Crosses between deletion mutants confirmed and extended the relative positions of deleted segments (Table 6). In these analyses, it was noted that recombination near deletion end-points was often very low.

Mapping of additional amdS *mutations*: Mutants derived from a variety of genetic backgrounds, and, in some cases, chosen because of their growth and biochemical properties, were mapped against relevant deletion strains and point mutants to further refine the fine-structure *amdS* map. This map, defining at least 14 sites, is shown in Figure 1. Other *amdS* mutations have been located in this region but not mapped precisely.

Orientation of the amdS *gene on linkage group III*: The *cbxA17* outside marker was used to orient the *amdS* gene (Table 7). These crosses showed that *amd-205* and *amdI18* lie at the extreme end of *amdS*, distal to the centromere, the order being centromere—*cbxA*—*amdS*—*amd-205* (*amdI18*). It was also confirmed

TABLE 6

Presence of amdS *recombinants in crosses between deletion strains*

	223	*311*	*314*	*316*	*320*
347	—	0	0	—	0
351	—	+	0	—	—
11	0	+	0	0	0
320	0	0	0	0	
316	0	+	0		
314	0	+			
311	0				

Symbols as for Table 5.

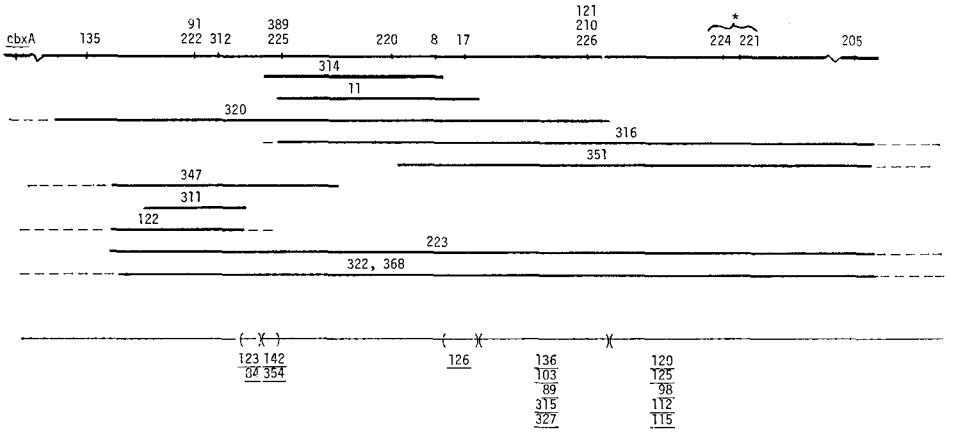

FIGURE 1.—Fine-structure map of the *amdS* locus. Heavy lines represent deletion segments and dashed lines indicate uncertainties in the end points of deletions. Allele numbers placed on top of each other represent lack of separation by recombination and hence must be either small deletions, extremely closely linked or mutations at the same site. Allele numbers in italics at the bottom of the figure represent assignment to the indicated bracketed regions defined by deletions and recombination with other point mutations in that region, but recombination between members of the group has not been tested.

* *amdS221* and *amdS224* recombine with each other, but the order of sites is not known.

that *amdS135* is located to the left of *amdS222* and therefore is the site closest to the centromere end of the gene.

Determination of the map length of the amdS *region*: Frequencies of *amdS*+ recombinants in crosses between an *amdS135* strain and strains containing *amdS222*, *amdS221* and *amd-205* were accurately determined as 0.17, 1.55 and 2.99 per 10^3 ascospores, respectively. Assuming equal frequencies of undetected double mutant reciprocal recombinants, this yielded the map distances 0.034, 0.310 and 0.598, respectively. A cross between *amdS224* and *amd-205* strains gave a map distance of 0.230. Therefore, *amd-205* is 0.2 to 0.3 map units from the nearest *amdS* sites (*224* and *221*)—a distance similar to that between *amdS135* and *amdS221*, the two most extreme *amdS* sites.

Biochemical characteristics of amdS *mutants*: A previously characterized temperature-sensitive allele (*amdS8*; DUNSMUIR and HYNES 1973) and a new temperature-sensitive allele, *amdS98*, showed temperature-sensitive growth on acetamide media and had thermolabile acetamidase activity. These mutations were mapped at two different sites within the *amdS* gene (Figure 1).

The *amdS115* mutant was found to have interesting properties relevant to the mechanism of indirect induction by acetamide. Strains containing *amdS115* were much more affected in utilization of acetamide as a nitrogen source in the presence of glucose or sucrose than in their use of acetamide as a sole carbon and nitrogen source. Acetamidase activity could not be detected in crude extracts of *amdS115* strains, but these extracts did contain antigenic cross-reacting material (see below), suggesting that the *amdS115* mutation resulted in an altered

TABLE 7

Mapping of amdS *mutations using* cbxA17 *as the outside marker*

(a) In relation to *amdI18*:

Relevant genotypes in cross	No. of *amdS*+ *amdI18* recombinants	
	cbxA17	*cbxA*+
amdS84 amdI18 × *cbxA17*	23	8
amdS89 amdI18 × *cbxA17*	36	6

Order: *cbxA17* — *amdS* — *amdI18*

(b) In relation to *amd-205*:

Relevant genotypes in cross	No. of *amdS*+ *amdI18* recombinants	
	cbxA17	*cbxA*+
cbxA17 amd-205 × *amdS311*	15	0
cbxA17 amd-205 × *amdS312*	10	2
cbxA17 amd-205 × *amdS320*	16	1
cbxA17 amd-205 × *amdS222*	34	2
cbxA17 amd-205 × *amdS220*	37	0
cbxA17 amd-205 × *amdS315*	32	3
cbxA17 amd-205 × *amdS17*	12	1
cbxA17 amd-205 × *amdS11*	18	0
cbxA17 amd-205 × *amdS8*	4	0
cbxA17 amd-205 × *amdS115*	55	12
cbxA17 amd-205 × *amdS98*	6	1

Order: *cbxA17* — *amdS* — *amd-205*

(c) In relation to *amdS222*:

Relevant genotypes in cross	No. of *amdS*+ recombinants	
	cbxA17	*cbxA*+
cbxA17 amdS222 × *amd-205*	8	40

Order: *cbxA17* — *amdS222* — *amd-205*

cbxA17 amdS222 × *amdS135*	43	20

Order: *cbxA17* — *amdS135* — *222*

acetamidase protein that was unstable *in vitro*. For full induction of the acetamidase by acetamide, the acetamide must be metabolized to acetyl-CoA (HYNES 1977; KELLY and HYNES 1977). Formation of acetyl-CoA from acetate and acetamide is restricted in the presence of glucose or sucrose because of catabolite repression of acetyl-CoA synthetase and also, perhaps, acetamidase (KELLY and HYNES 1977). A partial block in acetamidase activity would therefore restrict induction to a greater extent when glucose or sucrose is present than in their absence. Like *amdS115*, other *amdS* strains have shown a greater reduction in acetamide utilization in the presence than in the absence of sucrose or glucose.

Extended incubation on acetamide media of *amdS* mutants showed slightly leaky growth for the point mutants *amdS312* and *315* and for the small deletion strain *amdS311*, but not for the deletion strains *amdS314*, *316* and *320*.

SDS polyacrylamide gel electrophoresis of crude extracts of mycelium labelled with ^{14}C-amino acids has allowed identification of the *amdS* gene product (HYNES 1978b). The molecular weight of this polypeptide was estimated as 55 58,000 daltons, based on the standards phosphorylase-a (95,000), bovine serum albumin (68,000) and phospho-glycerate-kinase (47,000). The *amdS* polypeptide was detected in extracts of the *amdS224* mutants, but not in extracts of some other mutants including *amd-205* (Figure 2). In at least some of these cases, absence of *amdS* polypeptide may have been due to protease action on unstable mutant proteins. Induction conditions were likely to result in high protease levels (see COHEN 1977), and it has been observed that extracts of strains known to produce high protease levels show a reduction in high molecular weight labelled bands (HYNES unpublished). The protease inhibitor, phenyl-methyl-sulfonyl-fluoride, did not prevent this breakdown. For some extracts, precipitation of labelled proteins with anti-serum followed by electrophoresis did reveal the presence of *amdS* polypeptide, but results were variable.

Results of immunological tests on extracts of *amdS* mutants for material capable of protecting wild-type enzyme from precipitation by anti-serum raised against partially purified acetamidase (cross-reacting material CRM) are shown in Table 8. Three classes of mutants were apparent—those clearly CRM-positive

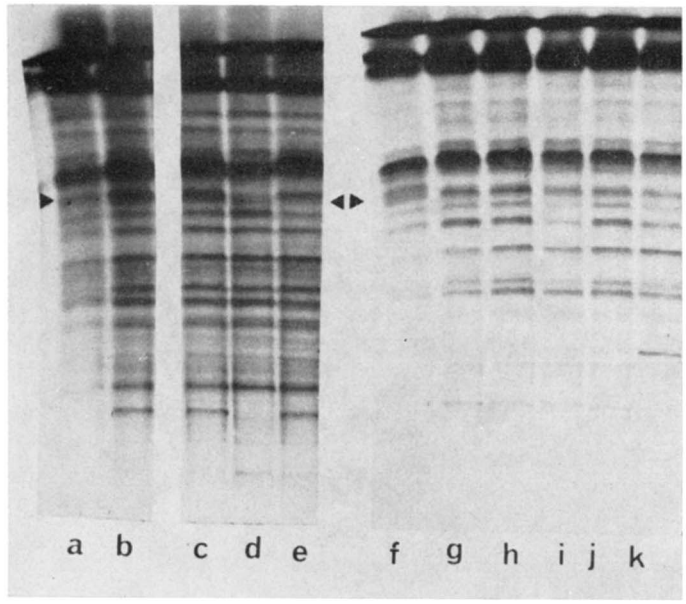

FIGURE 2.—SDS polyacrylamide gel electrophoresis of ^{14}C-labelled crude extracts. Acrylamide concentration was 7.5%. The *amdS* polypeptide is indicated by arrows. Incubation was for three hrs with 5 μCi (tracks a–e) or 2.5 μCi (tracks f–k) ^{14}C-labelled amino acids added to 4 ml medium lacking added carbon on nitrogen sources. The inducer used was β-alanine (5 mM): (track a) *amdI18*; *amdA7* not induced; (track b) *amdI18*; *amdA7* induced; (track c) *amdS224* induced; (track d) wild type not induced; (track e) wild type induced; (track f) *amdI18*; *amdA7* not induced; (tracks g, h, i, j and k) *amdS223*, *220*, *221*, *222* and *amd-205* induced.

TABLE 8

Immunological (CRM) tests on amdS *mutants*

Mutant strain (*amdS*)	CRM test
8, 17, 98, 115, 135, 224, 226, 312, 315	Positive
11, 89, 91, 205, 223, 314, 315, 320, 368	Negative
84, 122, 142, 220, 221, 222, 225, 226, 311	Variable results

or negative and those which gave variable, inconsistent results. The latter class probably produce unstable *amdS* products that react only partially with the anti-serum. All of the CRM-positive class were those that behaved as point mutations in the mapping studies; this class included *amdS224* and *amdS135*, which are located at either end of the *amdS* region (Figure 1). All deletion strains were CRM-negative with the exception of *amdS311*, which contains a small deletion. The *amd-205* mutant was clearly CRM-negative, and only two apparent point mutants, *amdS89* and *amdS91*, were CRM-negative. Apart from *amdS311*, all of the mutants giving variable results were apparent point mutants.

Double mutants involving amd-205: Because of the special properties of the *amd-205* mutation, double-mutant strains with the regulatory mutations, *areA102* and *amdR^{c}104*, were constructed and tested for growth on amide media. Presence of the *amd-205* lesion abolished amide utilization in these strains. Thus, *amd-205* is epistatic to the *amdA7*, *amdR^{c}104* and *areA102* regulatory mutations, indicating that this mutation affects all the control mechanisms for acetamidase. It should also be noted that *amd-205* does not complement *amdS* mutations in heterozygous diploids.

DISCUSSION

The ordering of mutant sites within the *amdS* gene has been accomplished by approximate frequencies of meiotic recombination, mitotic recombination and by deletion mapping. The results of these three methods are compatible with each other. A minimum of 14 sites within *amdS* has been defined. No attempt to determine strict additivity of recombination frequencies between sites has been made.

The *cis*-dominant regulatory mutations, *amdI9* and *amdI18*, have been mapped at the centromere-distal end of the cluster of *amdS* sites, using outside markers. The *amd-205* lesion was found to be inseparable from *amdI18* and was clearly located at this end of *amdS*. No distortions due to marker-specific effects (HASTINGS 1975) were apparent. Conversion events could not be distinguished from crossover events in the analysis.

amdS mutations throughout the *amdS* map produce inactive enzyme protein, including sites at each end (*amdS135*, *amdS221* and *amdS224*—see Figure 1). These sites therefore probably define the approximate extent of the *amdS* coding region. The length of this region is about 0.3 map units, a value consistent with results for other fungal genes (THURIAUX 1977).

In contrast, the *amd-205* mutant does not produce detectable inactive gene product and is located approximately 0.2 to 0.3 map units from the nearest *amdS* sites. Since *amd-205* is inseparable from *amdI18*, which causes increased levels of *amdS* gene product (HYNES 1978b), the hypothesis that both of these mutations define a promoter-type site (or small region) determining the level of *amdS* expression is attractive.

The molecular weight of the *amdS* coded polypeptide is approximately 55,000 to 58,000 daltons. Assuming no post-translational processing of the polypeptide, the length of the *amdS* coding region is therefore about 1.5 kilobases (using the method of calculation of CHOVNICK, GELBART and MCCARRON 1977). From the estimated map distances, the *amd-205* lesion is 1.2 to 1.5 kilobases from the *amdS221* and *224* sites. This of course assumes that recombination is equally frequent throughout the region and that no site-specific effects occur. CHOVNICK, GELBART and MCCARRON (1977) have concluded that a probable control-site mutation for the rosy gene in *Drosophila melanogaster* is approximately 3 kilobases from the coding region, which is estimated to be 4.4 kilobases long.

Overall, this analysis provides support for the view that the *amdS* locus consists of a coding region and a separate control region. Recently, a new mutation specifically affecting control by the *amdR* gene has been found to be very close to the *amdI18* mutation (HYNES, in preparation). Only direct analysis of the amino acid sequence of the *amdS* gene product in wild-type and mutant strains and the nucleotide sequence of the *amdS* region can confirm this. Attempts to clone the *amdS* gene by recombinant DNA techniques are currently in progress.

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LITERATURE CITED

ALDERSON, T. and M. J. HARTLEY, 1969 Specificity for spontaneous and induced forward mutation at several gene loci in *Aspergillus nidulans*. Mutation Res. **8**: 255–264.

CHOVNICK, A., W. GELBART and M. MCCARRON, 1977 Organization of the rosy locus in *Drosophila melanogaster*. Cell **11**: 1–10.

CLUTTERBUCK, A. J., 1974 *Aspergillus nidulans* genetics. pp. 447–510. In: *Handbook of Genetics* Edited by R. C. KING. Plenum Press. New York.

CLUTTERBUCK, A. J. and D. J. COVE, 1976 The genetic loci of *Aspergillus nidulans*. pp. 00–00. In: *CRC Handbook of Microbiology* Vol. IV. Edited by A. I. LASKIN and H. LECHEVALIER. Chemical Rubber Publishing Co., Cleveland, Ohio.

COHEN, B. L., 1977 The proteases of *Aspergilli*. pp. 281–292. In: *Genetics and Physiology of* Aspergillus. Edited by J. E. SMITH and J. A. PATEMAN. Academic Press, London.

DUNSMUIR, P. and M. J. HYNES, 1973 Temperature sensitive mutants affecting the activity and regulation of the acetamidase of *Aspergillus nidulans*. Molec. Gen. Genet. **123**: 333–346.

HASTINGS, P. J., 1975 Some aspects of recombination in eukaryotic organisms. Ann. Rev. Genet. **9**: 129–144.

HYNES, M. J., 1972 Mutants with altered glucose repression of amidase enzymes in *Aspergillus nidulans*. J. Bact. **111**: 717–722. ——, 1975 A *cis*-dominant regulatory mutation affecting enzyme induction in the eukaryote *Aspergillus nidulans*. Nature **253**: 210–212. ——, 1977 Induction of the acetamidase of *Aspergillus nidulans* by acetate metabolism. J.

Bacteriol. **131**: 770–775. ——, 1978a Multiple independent control mechanism affecting the acetamidase of *Aspergillus nidulans*. Molec. Gen. Genet. **161**: 59–65. ——, 1978b An "up-promoter" mutation affecting the acetamidase of *Aspergillus nidulans*. Molec. Gen. Genet. **166**: 31–36.

HYNES, M. J. and J. M. KELLY, 1977 Pleiotropic mutants of *Aspergillus nidulans* altered in carbon metabolism. Molec. Gen. Genet. **150**: 193–204.

HYNES, M. J. and J. A. PATEMAN, 1970a The genetic analysis of regulation of amidase synthesis in *Aspergillus nidulans*. I. Mutants able to use acrylamide. Molec. Gen. Genet. **108**: 95–106. ——, 1970b The genetic analysis of regulation of amidase synthesis in *Aspergillus nidulans*. II. Mutants resistant to fluoroacetamide. Molec. Gen. Genet. **108**: 107–116.

KELLY, J. M. and M. J. HYNES, 1977 Increased and decreased sensitivity to carbon catabolite repression of enzymes of acetate metabolism in mutants of *Aspergillus nidulans*. Molec. Gen. Genet. **156**: 87–92.

MACKINTOSH, M. E. and R. H. PRITCHARD, 1963 The production and replication of microcolonies of *Aspergillus nidulans*. Genet. Res. **45**: 320–322.

PONTECORVO, G., J. A. ROPER, L. M. HEMMONS, K. D. MACDONALD and A. W. BUFTON, 1953. The genetics of *Aspergillus nidulans*. Advan. Genet. **5**: 141–238.

THURIAUX, P., 1977 Is recombination confined to structural genes on the eukaryotic genome? Nature **268**: 460–462.

TONG-MAN ONG and F. J. DE SERRES, 1974 Mutagenicity of chemical carcinogens in *Neurospora crassa*. Cancer Res. **32**: 1890–1893.

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