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Glutamic dehydrogenase in revertants of *am* mutants in *neurospora*

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1. INTRODUCTION

It has long been known that a single alteration in a gene can result in the profound change or complete loss of its function. Since these mutations are sometimes reversible, it has been believed that the change in the gene structure must be a simple one. Recent studies of genetic fine structure and of protein products of mutant genes have tended to confirm this hypothesis. An extension of the hypothesis was that since gene function was so sensitive to slight change in structure, the wild-type configuration must be unique. This assumption would predict that the function of a mutant gene could only be restored by a specific reversal of the original change. Some observations negate this prediction. Revertants have been shown to differ from wild-type by sensitive tests of their functional (Esser, DeMoss & Bonner, 1960), mutational (Giles, 1958) and recombinational (Crick, Barnett, Brenner & Watts-Tobin, 1961) behavior. The detection of these differences is important to the interpretation of mutation rate studies, and it is crucial to the attempt to identify base pairs at specific mutant sites by response to chemical mutagens (Freese, 1963). The current study has employed Neurospora mutants of the *am* gene (the structural gene for NADP-linked glutamic dehydrogenase). Ultra-violet-induced revertants of these mutants have been compared to the original wild-type allele for the nature of their glutamic dehydrogenase. The results differ depending on the mutant employed. One of the mutants (*am*1) appears to revert only by true back mutation. Another (*am*19) can revert as the result of any one of a number of genetic alterations near the site of the original mutation, each producing an enzyme detectably different from wild-type glutamic dehydrogenase.

2. MATERIALS AND METHODS

(i) *Stocks*

Seven *am* mutants were used in these reversion experiments. All carried the inbred genetic background of the St Lawrence wild-types (74A and 3.1a) and were heterocaryon-compatible with each other. The *am*1 mutant (Fincham, 1962) was induced with ultra-violet light, and the other six mutants (*am*14–*am*19) were all induced with nitrous acid (Fincham & Stadler, 1964).

(ii) *Induction and isolation of revertants*

Conidia of the *am* mutants were suspended in water, counted, and placed under a germicidal lamp in open petri dishes for ultra-violet treatment. The treated suspension was pipetted into minimal sorbose medium (Lester & Gross, 1959) supplemented with M/50 glycine (which inhibits the slight growth of *am* mutants on minimal medium). The medium was kept liquid at 45°C. until the conidia were added, and was then poured into plates. Revertant colonies were isolated after 3–4 days' incubation at 25°C. (all colonies in an area were picked up, regardless of size, to avoid any selection).

(iii) *Enzyme assay*

Crude extracts were made as described by Fincham & Stadler (1964) by grinding mycelia in pH 8·0 phosphate buffer (0·05 M) and filtering off the debris. Protein was measured by the method of Lowry, Rosenbrough, Farr & Randall (1951), and all extracts were brought to a protein concentration of 0·2 mg./ml. by further dilution with buffer. Glutamic dehydrogenase (GDH) activity was assayed as described by Fincham & Coddington (1963 *a*) by the oxidation of $NADPH_2$ with α-oxoglutarate and ammonium chloride.

(iv) *Heat treatment of extracts*

One ml. aliquots of the extracts were placed in a water bath at approximately 65°C. for the time required to destroy about half the GDH activity of the wild-type extract. This time was generally 8–30 min., but it was very sensitive to slight differences in temperature, so it was necessary to make frequent assays of a wild-type extract with every heat stability experiment. The same procedure was used to measure the heat stability of the GDH of the revertants at pH 6·5 after adjusting the acidity of the extracts by the addition of one-half volume of 0·2 M NaH_2PO_4. The enzyme is more sensitive to heat at this pH, so the treatments were run at approximately 63°C. The altered pH in these experiments obtained only during the heat treatment, as all enzyme assays were performed at pH 8·5 in tris-HCl buffer.

3. RESULTS

Conidial suspensions of seven glutamic-dehydrogenase-deficient mutants (am1, 14, 15, 16, 17, 18 and 19) were treated with ultra-violet irradiation and plated for *am*+ revertants. No induced reversions of *am*15 or *am*16 were observed (in treated populations of over 10^7 cells). The rates of reversion of the other five mutants are shown in Table 1. It may be noted that *am*14, 17 and 19 all revert with similar frequencies, while the rates for *am*1 and *am*18 are an order of magnitude lower. These vegetative isolates were frequently mixtures of *am* mutant and revertant,

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Table 1. *Ultra-violet-induced reversion of* am *mutants*

Strain	Total surviving cells $\times 10^6$	Total *am*+	Per cent survival	Induced revertants per 10^6 survivors*
*am*1a	80	0	100	
	120	22	75	0·2
	16·5	8	65	0·5
	10·3	9	20	0·9
	48	60	15	1·25
	5·5	16	3·6	2·9
*am*14a	14	17	100	
	20·7	126	83	4·9
	7·5	82	15	9·7
	0·9	13	12·5	13·2
*am*15a	9·1	2	100	
	18	1	33	
*am*16a	1·5	0	100	
	5·5	0	58	
*am*17a	12	0	100	
	7·5	11	86	1·5
	7·2	30	43	4·2
	4·4	31	25	7·0
	5·25	41	10	7·8
	4·1	39	2·4	9·5
*am*18a	61	0	100	
	10·4	2	70	0·2
	1·35	1	34	0·7
	14	2	27	0·14
	6	7	20	1·2
	9·5	5	6	0·5
*am*19a	9	0	100	
	3·6	13	50	3·6
	1·7	6	17	3·5
	0·6	12	5	20

The 100% survival sample in each experiment is the untreated control.
* Frequency in the treated sample minus the frequency in the untreated control.

and genetically pure revertants were isolated by crossing the original revertant isolates back to the same *am* mutant in the opposite mating type. A single *am*+ ascospore from such a cross was cultured for the studies of the glutamic dehydrogenase produced by the revertant.

Fourteen revertants (three from *am*1, five from *am*17 and six from *am*19) were compared to wild-type (*St A*) in vegetative growth characteristics. Observations were made of growth rates (dry weight of mycelium) in liquid minimal medium, minimal plus glycine, and minimal plus alanine. (The *am* mutants grow slowly on minimal medium and are completely inhibited on minimal plus glycine; they grow like wild-type on minimal plus alanine. Wild-type grows equally well on all three media.) Comparisons were also made of size and morphology of colonies

from conidial suspensions spread on plates of minimal sorbose medium and on the same medium supplemented with glycine or with alanine. All fourteen revertants appeared identical to wild-type in all these comparisons.

Crude extracts were made from revertant mycelia and adjusted to the same total protein concentration before assay for glutamic dehydrogenase. The enzymatic activities (Tables 2 and 3) of most of these extracts were very similar to that of wild-type. However, tests of the heat stability of the extracts revealed many differences among the revertants. Fig. 1 demonstrates the pronounced difference in heat stability between an extract of wild-type and that of one of the

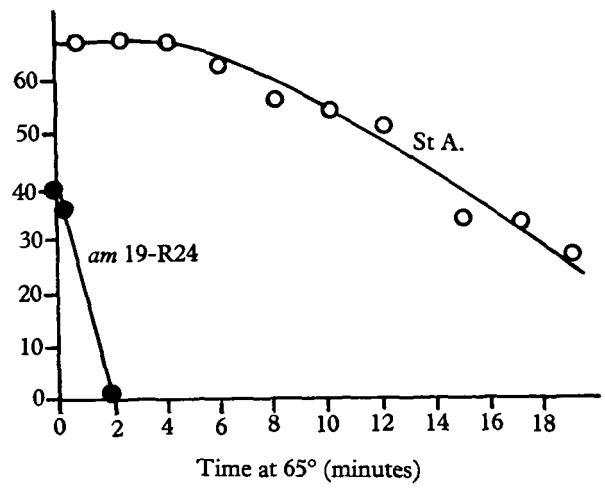

Fig. 1. Comparison of heat stability of GDH in extracts of wild-type (*St A*) and a heat-labile revertant (*am*19-R24) at pH 8·0. Samples of the extracts were withdrawn from the 65°C. bath after various periods of exposure (abcissa) and assayed for GDH activity (ordinate). Rate is expressed as change in optical density per minute × 1000.

revertants (*am*19-R24). Fig. 2 shows the retained activity of all the revertant extracts after heat treatment at pH 8·0 compared to that of wild-type. A value of 100% indicates a revertant that has retained the same fraction of its activity after heat treatment as did the wild type extract. It may be seen that the revertants fall into two general classes: those with stability similar to that of wild-type and those which are distinctly more labile. The stable class includes all the revertants from *am*1 and some from each of the other mutants. The labile class includes nineteen revertants (six from *am*14, three from *am*17, one from *am*18 and nine from *am*19). The stable class covers a disturbingly wide range of retained activities and we should like to know whether this really represents a single type. In order to determine how much of this spread was accounted for by the limitations of the experimental technique, ten duplicate cultures of wild-type *St A* were extracted and assayed before and after heat treatments. The results (top of Fig. 2) show that all the revertants except those obviously in the labile class *may* have heat stability identical to that of the wild-type.

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Figure 3 shows the heat stability of the same revertant extracts when the pH was adjusted to 6·5 before the treatment. Under these conditions it may be seen that nearly all the revertants of *am*14 and *am*19 are more labile than wild-type, while all the revertants of *am*1 and *am*18 and nearly all of those of *am*17 again show a similar stability to that of wild-type. At pH 6·5 the stable class is quite uniform

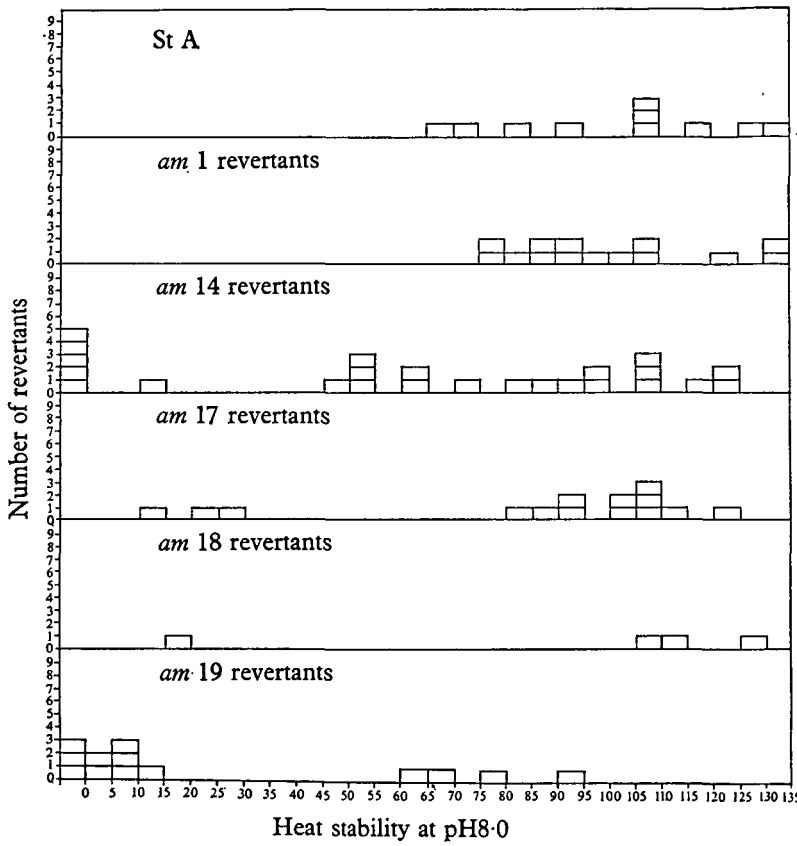

Fig. 2. Heat stability of GDH in extracts of *am* revertants at pH 8·0. See text for description. Revertant *am*19-R1 has been omitted from this figure because its retained activity at this pH is twice that of wild-type (200%).

both for the revertants and the wild-type (*St A*) controls, and the distinction between stable and labile extracts is unambiguous.

Mixing experiments were performed at both pH 8 and pH 6·5. Stable and labile extracts were mixed either before or after the heat treatment. The activities of such mixtures were always similar to the sum of the activities of the component extracts when heated and assayed separately.

Table 2 gives the complete results of the heat stability tests on the fourteen revertants of *am*17, and Table 3 gives a summary of the results on all seventy-two revertants studied. They are grouped in classes with Class I representing those extracts which are not distinguishable from wild-type by these tests. [All fourteen

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revertants of *am*1 fall into this class, and ten of the fourteen revertants of *am*17 do also. But only one of the twenty-three revertants of *am*14 is in Class I and none of the seventeen revertants of *am*19. The two revertants of *am*19 listed as Class Ia were stable to heat treatment at either pH, but at pH 6·5 they showed a

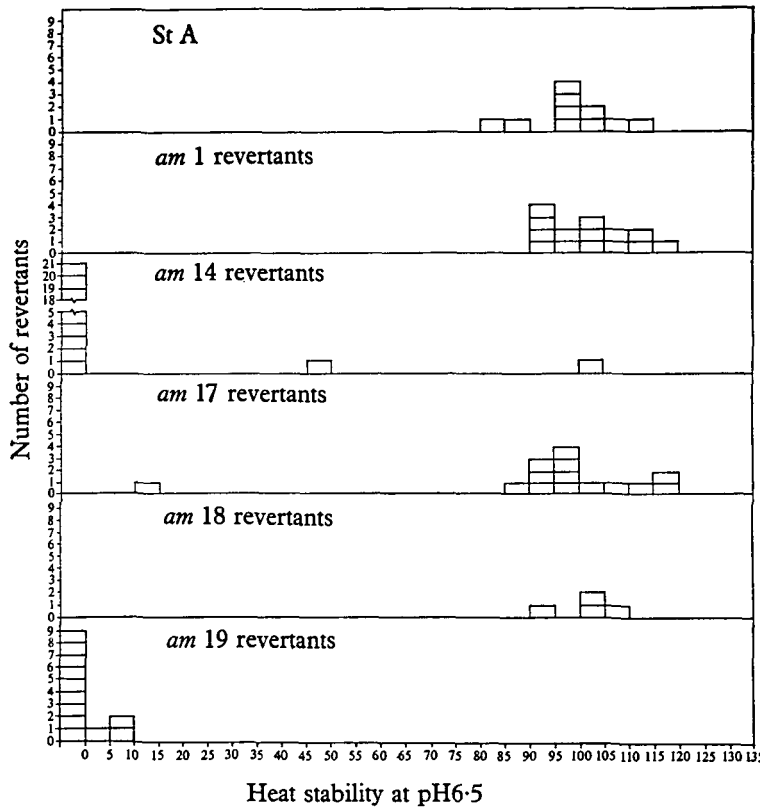

Fig. 3. Heat stability of GDH in extracts of *am* revertants at pH 6·5. See text for description. Revertants *am*19-R5 and *am*19-R23 have been omitted from this figure because they show a delayed activation in the reaction mixture (see Fig. 4); when compared on the basis of maximum rate, they show heat stabilities similar to that of wild-type.

delayed activation in the reaction mixture (see Fig. 4) which was clearly different from the wild-type extracts. The one revertant of *am*14 in Class IIIa showed an intermediate stability at pH 6·5, and repeated tests showed this to be a consistent difference from wild-type. Only one revertant showed distinctly greater stability than wild-type in any of these tests. This was the *am*19 revertant designated as Class V. Heat treatment sufficient to lower the activity of wild-type extracts at pH 8·0 by half did not diminish the activity of this revertant at all. However, at pH 6·5 it is very labile.

The heat-stability tests on the glutamic dehydrogenase activity of the revertant extracts permitted us to place the revertants in several distinct classes on the basis

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Table 2. *GDH of* am*17 revertants*

Revertant	Activity*	Heat stability** pH 6·5	Heat stability** pH 8·0
1	1·17	45/42 (107%) S	75/61 (123%) S
2	0·89	50/42 (120%) S	16/61 (28%) L
		21/17	12/40
3	1·04	40/42 (95%) S	52/61 (85%) S
4	1·25	45/42 (117%) S	57/61 (94%) S
		25/17	38/40
		33/29	40/42
5	0·95	44/42 (105%) S	67/61 (110%) S
6	1·45	41/42 (100%) S	59/61 (88%) S
		18/17	30/40
7	1·02	38/42 (90%) S	57/61 (93%) S
8	0·84	33/30 (97%) S	100/74 (108%) S
		27/32	24/41
9	1·11	38/30 (115%) S	86/74 (108%) S
		33/32	38/41
10	1·09	32/30 (97%) S	90/74 (104%) S
		28/32	30/41
11	0·72	33/30 (87%) S	20/74 (23%) L
		21/32	6/41
12	0·48	0/30 (11%) L	90/74 (113%) S
		7/30	40/41
13	0·67	36/30 (95%) S	14/74 (15%) L
		23/32	3/41
14	1·09	33/30 (98%) S	83/74 (103%) S
		28/32	35/41

* Relative to wild-type, without heat treatment.

** Each result is expressed as a fraction of which the numerator is the percentage activity retained by the revertant extract after heat treatment, and the denominator is the percentage activity retained by wild-type (*St A*) after the same heat treatment. These fractions are represented as percentage values in the brackets. In those cases in which duplicate experiments were performed, an average percentage is given. S = stable, L = labile.

of these properties. There are two possible schemes for the genetic control of these differences. It may be that the gene involved in the reversion event itself governs the heat stability properties of the enzyme. In this case the different classes must represent genetically different reversion events. On the other hand, it may be that the reversion event was identical in all cases (perhaps the precise reversal of the original *am* mutation), but the heat stability properties are determined by other genes segregating in the genetic background. Each revertant was crossed back to its parent *am* mutant before the enzyme studies in order to get it genetically pure, so there is a possibility of some variation in genetic background arising in the progeny of these crosses. In order to determine which type of genetic control applied, several more *am*+ ascospores were isolated from the crosses of three of the *am*19 revertants (representing three different classes) to *am*19, and these were compared in their enzymatic properties to several different genetic reisolates of

Table 3. *Summary of GDH analyses of* am *revertants*

Mutant	Class of revertant	Number of revertants	Heat stability pH 6·5	Heat stability pH 8·0	Activity*
*am*1 (14 revertants)	I	14	S (92–118%)	S (77–135%)	0·64–1·25
*am*14 (23 revertants)	I	1 (No. 21)	S (105%)	S (63%)	0·93
	III	16 (Nos. 3, 4, 6, 7, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 22)	L (0)	S (50–123%)	0·73–1·18
	IIIa	1 (No. 23)	L (46%)	S (118%)	0·98
	IV	5 (Nos. 1, 2, 5, 8, 12)	L (0)	L (0)	0·33–1·04
*am*17 (14 revertants)	I	10 (Nos. 1, 3, 4, 5, 6, 7, 8, 9, 10, 14)	S (90–117%)	S (85–123%)	0·84–1·45
	II	3 (Nos. 2, 11, 13)	S (87–120%)	L (15–28%)	0·67–0·89
	III	1 (No. 12)	L (11%)	S (113%)	0·48
*am*18 (4 revertants	I	3 (Nos. 1, 2, 4)	S (102–106%)	S (107–130%)	0·92–1·05
	II	1 (Nos. 3)	S (94%)	L (19%)	1·08
*am*19 (17 revertants)	Ia	2 (Nos. 5, 23)	S	S (78–93%)	0·62–1·10
	III	2 (Nos. 4, 6)	L (0–5%)	S (60–70%)	1·20–1·51
	IV	9 (Nos. 2, 3, 7, 8, 9, 10, 12, 21, 24)	L (0–10%)	L (0–12%)	0·55–1·15
	V	1 (No. 1)	L (0)	XS (200%)	1·22
	VI	3 (Nos. 26, 27, 28)	(No activity)	(No activity)	(No activity)

Heat stability is calculated by dividing the retained activity of the revertant by the retained activity of wild-type after the same heat treatment. S = stable, L = labile, XS = extra-stable.
* Relative to wild-type, without heat treatment.

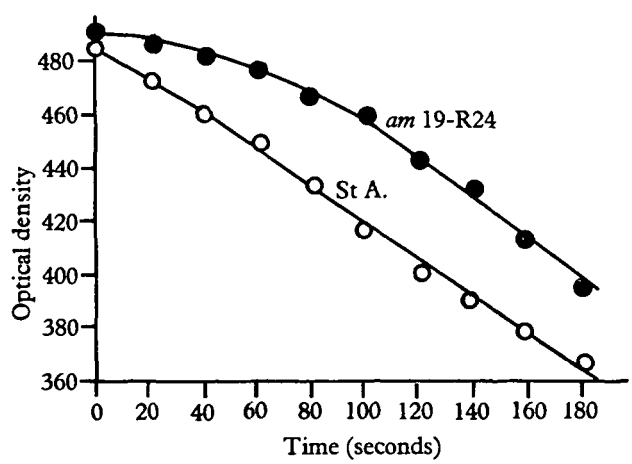

Fig. 4. Delayed activation of GDH of revertant *am*19-R5 in extract at pH 6·5. Extracts were added to the reaction mixture at pH 8·5 at time zero.

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Table 4*a*. *Heat stability of glutamic dehydrogenase from genetic reisolates of two* am*19 revertants compared to genetic reisolates of wild-type*

		pH 6·5			pH 8·0		
		No heat	Heat	Retained activity	No heat	Heat	Retained activity
EXPT. 1							
*am*19R24 ×	24–2				84	0	0
*am*19	24–3				85	0	0
	24–4				73	2	3%
	24–5				70	1	1%
	24–6				70	1	1%
ST × *am*19	ST 2				183	95	52%
	ST 3				171	100	59%
	ST 5				159	72	45%
	ST 6				196	88	45%
EXPT. 2							
*am*19R1 ×	1–2	94	0	0	80	67	84%
*am*19	1–3	129	1	1%	85	97	114%
	1–4	120	2	2%	82	97	118%
	1–5	82	0	0	61	76	124%
	1–6	120	1	1%	89	95	107%
ST × *am*19	ST 2	75	29	39%	71	29	41%
	ST 3	80	31	39%	74	24	32%
	ST 4	67	29	43%	67	32	48%
	ST 5	62	25	40%	53	23	43%
	ST 6	72	27	38%	64	28	44%

Rates are recorded as change in optical density per minute × 1000.

Table 4*b*. *Activation delay of GDH activity in pH 6·5 extracts (not heated) of genetic reisolates of* am*19R5 compared to genetic reisolates of wild-type*

		Reaction rate		Ratio $\frac{\text{minute 1}}{\text{minute 3}}$
		1st minute in reaction mixture	3rd minute in reaction mixture	
*am*19R5 × *am*19	5–2	13	45	0·29
	5–3	12	43	0·28
	5–4	11	38	0·29
	5–5	11	40	0·27
	5–6	10	40	0·25
ST × *am*19	ST 2	35	38	0·92
	ST 3	51	44	1·16
	ST 5	51	44	1·16
	ST 6	46	41	1·12

Rates are recorded as change in optical density per minute × 1000.

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am+ from the cross of wild-type to *am*19. In this experiment the material was selected for the *am*+ gene (revertant or wild-type), while the potential differences in genetic background are identical in all four crosses and should be randomized in the progeny studied. The results (Table 4) show that the enzymatic properties are consistent in the *am* progeny of any given revertant, and thus these properties must be determined by the gene involved in the *am* reversion event.

The experiment just described showed that the gene involved in reversion controlled the properties of the revertant glutamic dehydrogenase, but did not elucidate the relationship between the site of reversion and the locus of the original *am* mutation. The results are compatible with reversion by back-mutation at the *am* locus or by a suppressor at any locus in the genome. If reversion were accomplished by a suppressor, the original *am* mutation would still be present and might be revealed by recombination. To test for this possibility, twenty-two revertants (one from *am*1, seven from *am*14, five from *am*17, one from *am*18 and eight from *am*19), representing all of the enzyme classes, were crossed to wild-type. Ascospores from these crosses were germinated on minimal sorbose medium plus glycine. (Although vegetative cultures of *am* mutants do not grow at all on this medium, *am* ascospores form very characteristic thin, unbranched germ tubes.) 200–500 germinated spores from each cross were scanned. There were no spores that looked exactly like the *am* controls, and the few doubtful ones were isolated and grown for subsequent nutritional tests; all proved to be *am*+. Thus none of these twenty-two crosses yielded any *am* recombinants in the sample observed. This result shows that none of these revertants resulted from a suppressor mutation at a locus unlinked or loosely linked to *am*. However, there is no evidence against reversion by an 'interal suppressor' (second site alteration within the *am* locus). The samples examined were too small to reveal the low frequency of *am* recombinants expected from such a revertant.

The three revertants of *am*19 designated as Class VI are like the other revertants in that they have recovered the ability to grow on minimal medium or minimal plus glycine, but the crude extracts of these strains show no GDH activity, and the question arises as to whether these are really *am*+ revertants. One of these strains was among those crossed to wild-type in the test for suppressors. The absence of *am* recombinants among the progeny of this cross shows that the ability of this revertant strain to grow on minimal medium was regained through a genetic event at or near the *am* locus. It suggests that the revertant has recovered the ability to make functional GDH, but of a type that is too unstable for demonstration in crude extracts.

4. DISCUSSION

Complementation studies of all the mutants used in this work along with other *am* mutants (Fincham & Stadler, 1964) have revealed that *am*15, 16, 17 and 18 do not complement with any *am* mutant yet isolated. Any pairwise combination of *am*1, *am*14 and *am*19 gives complementation, and these three mutants com-

plement with certain other *am* mutants. Comparisons of the GDH from extracts of various complementing pairs show that those pairs which include *am*1 produce a more stable enzyme than those which do not. An altered GDH has been purified from extracts of *am*1 (Fincham, 1962); it appears identical to the wild-type enzyme in several physical characteristics, but it has no enzyme activity. It has been suggested (Fincham & Coddington, 1963 *b*) that the protein product of the *am*1 allele has the stable folding properties of wild-type GDH but is altered in its active site. This hypothesis leads to the prediction that the only genetic change which could restore activity in this strain would be the precise reversal of the original mutation event. On the other hand, a GDH-like protein has been purified from *am*19 which has slight enzyme activity but is unstable. Sundaram & Fincham (1964) have studied the GDH of *am*19 and of one of its revertants (*am*19-R24), and they have found that these proteins shift back and forth readily between two electrophoretically different configurations. It appears that the GDH of *am*19 has its active site intact but is changed at some other site which alters its folding properties. One might predict that such a mutant could revert to *am*+ either by the reversal of the original mutation event or by a change at some other site in the structural gene leading to a second alteration which would 'balance' the effect of the first on the conformation of the protein product. The observations reported above on revertants of these mutants are consistent with the predictions.

The several distinct classes of *am*19 revertants indicate that second changes of several different kinds can restore stability to the same altered protein. It should be noted that a revertant not identical to wild-type may sometimes be produced by a second alteration at the same site as the original mutation. Such an interpretation has been offered for certain revertants of adenine mutants in Neurospora (Barnett & DeSerres, 1963), and revertants for tryptophan synthetase in *E. coli* have been shown to result from a second change in the same codon (affecting the same amino acid residue) as the original mutation (Henning & Yanofsky, 1962). It is probable that at least some of the revertants result from events which are not at the site of the original mutation. However, the genetic tests show that the sites of the two events, if separate, are closely linked, and presumably both are within the *am* gene. Such second-site reversions have been convincingly demonstrated in *E. coli* (Yanofsky, Helinski & Maling, 1961) and in phage (Jinks, 1961; Crick, Barnett, Brenner & Watts-Tobin, 1961).

Pateman & Fincham (1964) have recently studied revertants of *am*3, another mutant that produces an unstable GDH with some enzyme activity. The results are similar to those reported here for *am*19. Examination of the GDH of revertants of *am*3 reveals at least seven classes which are different from wild-type. The authors suggest that these several classes of revertants represent different ways of modifying the folding of the protein so as to restore an enzymatically active configuration.

Studies of reversion of Neurospora mutants at two other loci known to control specific enzymes have indicated that a mutant gene may revert by taking any of a number of allelic forms not identical to wild-type. Giles, Partridge & Nelson

(1957) examined fifteen revertants of a mutant which was lacking in adenylosuccinase activity; the revertants all had some enzyme activity but less than wild-type. Giles (1958) carried some of these revertants through another round of mutation and reversion. He found that they were more restricted than wild-type in their subsequent reversion potential; different primary revertants exhibited different ceiling levels of adenylosuccinase activity in the secondary revertants. Esser, DeMoss & Bonner (1960) studied twenty-five revertants of a mutant deficient for tryptophan synthetase; the revertants fell into four classes with respect to the ratio of active enzyme to immunologically related protein (CRM) in extracts.

Many of the revertants studied here produce glutamic dehydrogenase which is different from that of wild-type, but they suffer no disadvantage in growth in laboratory conditions. It would be of interest to know whether these same kinds of mutational alterations occur in natural populations, and, if so, whether strains carrying such altered glutamic dehydrogenase can compete successfully with the wild-type from which they were derived. In this connection, extracts were made from five strains of Neurospora of independent origin, isolated in widely separate parts of the world and compared in heat stability properties to the extracts of St Lawrence wild-type (Table 5). It may be seen that all these strains produce glutamic dehydrogenase with the full stability of our original wild-type. The absence of partially stable enzymes among the strains from nature could mean that they have a selective disadvantage in nature, or it may be that the sequence of mutational changes giving rise to these types does not occur with appreciable frequency under natural conditions.

Table 5. *Heat stability of glutamic dehydrogenase of diverse wild-type strains*

	pH 6·5			pH 8·0		
	No heat	Heat	Retained activity	No heat	Heat	Retained activity
St Lawrence	96	59	61%	108	58	54%
Fiji	70	25	36%	73	36	49%
New Zealand	93	62	67%	120	52	43%
Java	126	56	44%	144	77	53%
North Africa	131	85	65%	153	80	52%
Liberia	106	45	42%	114	62	54%

Rates are recorded as change in optical density per minute × 1000.

The number of classes of revertants for a given mutant represents a minimum estimate of the number of different possible genetic changes at or near the *am* locus which can restore glutamic dehydrogenase activity to that mutant. The five classes for *am*19 reversion represent at least six different types, because one of the nine revertants of Class IV produces glutamic dehydrogenase with a different electrophoretic mobility from that of the other eight (Sundaram & Fincham, 1964). This means there are at least six different genetic alterations (none of which is a true reverse mutation) which can restore activity to the *am*19 gene product. For *am*1 the evidence suggests there is only a single genetic event which can result

in reversion. If ultra-violet irradiation were a non-specific mutagen causing all kinds of single-site genetic changes with similar frequencies, we might expect reversion of *am*19 (and *am*14) to be much more frequent than that of *am*1. The rates (Table 1) show a rough fit with this prediction. However, the different revertant classes from the same mutant do not occur with equal frequencies (Table 3). The majority classes from *am*14 (Class III) and *am*19 (Class IV) could each represent a grouping of several different genetic types. This seems less likely for the majority class from *am*17, which appears identical to wild-type. If these revertants represent the true reverse mutation, then this is occurring more frequently than the other genetic events (at least two) which can restore activity. Such results are compatible with the findings of Drake (1963) on ultra-violet-induced mutations in phage; he concluded that ultra-violet was a partially-specific mutagen causing different types of mutation events with markedly differing frequencies.

A method has been developed in recent years to attempt to identify the bases at particular genetic sites by observing the revertability of mutants with specific mutagens (Freese, 1963). If a mutagen known to cause the misreplication of a particular base is effective in the reversion of a particular mutant, it is concluded that the susceptible base was present at the mutant site. A necessary assumption is that the induced revertants are true back-mutations. If such an analysis were undertaken with the *am* mutants of Neurospora, it is clear that the success of the method would depend on the type of mutants employed. Mutants of the *am*1 type should show a distinct pattern of response to specific mutagens depending on the base constitution at the mutant site. But such a clear-cut result could hardly be expected in reversion of mutants like *am*14 and *am*19. One might expect all mutagens to be effective in producing revertants of one of these mutants, but the spectrum of types produced by a given mutagen might be specific.

SUMMARY

Five allelic mutants of Neurospora which lack glutamic dehydrogenase (*am* mutants) were induced to revert with ultra-violet. The glutamic dehydrogenase produced by the revertants was compared to that of wild-type. Several distinct classes of revertants could be distinguished by these tests. However, genetic analysis showed that all the reversions resulted from events at or near the site of the original *am* mutation. The spectrum of reversion types depended on the nature of the *am* mutant employed. One mutant, which produces an *am* protein believed to be altered in its active site, yielded revertants which were all indistinguishable from wild-type. Another mutant, which produces a protein with a functional active site but altered folding properties, gave at least six classes of revertants which were different from wild-type.

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