AN UNSTABLE ALLELE OF THE *am* LOCUS OF *NEUROSPORA CRASSA*[1]

JOHN A. KINSEY

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

AND

J. R. S. FINCHAM

Department of Genetics, University of Edinburgh, Edinburgh EH9 3JN, United Kingdom

Manuscript received June 27, 1979
Revised copy received September 4, 1979

ABSTRACT

The mutant strain am_{126} was isolated, using the direct selection procedure, after nitrous acid mutagenesis. It produced neither measurable NADP-dependent glutamate dehydrogenase (GDH) nor immunologically cross-reacting material. That the am_{126} strain produced some form of GDH product was shown by the fact that it complemented several other *am* mutant strains. The GDH formed by complementation between am_{126} and each of two other *am* mutants was relatively thermolabile, but could not be distinguished from wild-type GDH by electrophoresis in polyacrylamide gels. This, together with the relatively high yield of the complementation enzymes, suggests that the am_{126} product is a polypeptide chain not grossly abnormal in structure. The spontaneous revertant frequency was between 0.3 and 3 prototrophic revertants per 10^5 live cells. This frequency was at least 40 times greater than that for am_{19}, which had the second highest spontaneous revertant frequency among the mutants tested. Neither meiosis nor mutagenesis increased the revertant frequency, nor did incubation at elevated temperatures lower it. Sixty-eight revertant strains were examined for thermostability of their GHD. All appeared to be identical to wild type. Seven of the revertant strains were also tested for instability with regard to forward mutation to *am* auxtrophy. None was found to be unstable. Models for the genetic instability of the am_{126} mutation are discussed.

THE *am* locus of *Neurospora crassa* codes for NADP-dependent glutamate dehydrogenase (GDH) (FINCHAM 1951). In recent years, this locus and its gene product have proved particularly favorable for genetic and biochemical analysis. A fine-structure map for the gene has been reported (SMYTH 1973), and the complete amino acid sequence of the product, GDH, has been determined (WOOTTEN *et al.* 1971). Below we report on a new allele of the *am* locus that, unlike any previously described *am* mutation, is unstable. The genetic instability

[1] This investigation was supported in part by Public Health Service research grant GM 23967 and by the Science Research Council.

is expressed in mitotically dividing cells, and is unaffected by mutagenesis with UV or ethylmethane sulfonate (EMS).

MATERIALS AND METHODS

Mutant isolation and media were as described previously (KINSEY 1977). The NADP-dependent glutamate dehydrogenase (EC 1.4.1.4) assays used were those of CODDINGTON, FINCHAM and SUNDARAM (1966). CRM determination was either by the modification of the technique of ROBERTS and PATEMAN (1964) previously described (KINSEY 1977) or by rocket immuno-electrophoresis as described by LAURELL (1966). Antiserum against extensively purified wild-type GDH was prepared in rabbits by A. TAIT.

Tests for complementation between *am* mutants were made by inoculating 2 ml lots of Vogel's liquid minimal medium (VOGEL 1956) containing 0.02 M glycine with about 7×10^4 conidia of each mutant (doubly inoculated in the case of single-mutant controls). Growth was assessed by eye after three days at 25°. All strains had been made heterokaryon-compatible by repeated crossing to the wild-type ST*A* (74*A*).

Forced heterokaryons were constructed using double mutants am_{126} *inos a*, am_3 *arg-10 a* and am_{19} *arg-1*, which had been selected on the basis of heterokaryon compatibility with mutually compatible *arg-1* and *arg-10* tester strains. Heterokaryons were forced on minimal sorbose plates, supplemented with glutamate, and transferred to small 2% sucrose minimal slants, again supplemented with 5 mM glutamate. Conidia from the resulting heterokaryotic cultures were used to inoculate 50 ml lots of the same (but liquid) medium in 250 ml Erlenmeyer flasks. After 48-hr incubation without shaking at 25°, the mycelia were harvested, washed, blotted and extracted by grinding in a mortar with sand and ten times their damp weight of 0.05 M pH 8.0 sodium orthophosphate buffer containing 1 mM EDTA. The extracts were clarified by centrifugation, and 50 μl samples containing about 50 μg protein were assayed in the systems previously described (CODDINGTON, FINCHAM and SUNDARAM 1966).

Reversion studies were done using conidia from either three- or five-day cultures started as single conidial colony isolates and grown on Vogel's minimal agar supplemented with 0.01 M L-glutamate. Conidia from these cultures were harvested in sterile water and filtered through gauze to remove hyphal fragments. Conidia, either with or without mutagen treatment, were plated in molten Vogel's minimal agar (45°) containing sorbose (1.5%), glucose (0.2%), glycerol (0.2%), and 0.02 M glycine to suppress the "leaky" growth characteristic of *am* mutants. Mutagen treatments were as described by DAVIS and DE SERRES (1970).

Heat stability tests of GDH activity in crude extracts of the revertants were performed on cultures grown from a single ascospore reisolated from a cross of the revertant to an am_{126} strain of the opposite mating type. To obtain extracts of the desired pH, crude extracts were dialyzed overnight against large volumes of phosphate buffer at the appropriate pH. Small volumes (usually 0.5 ml) of extract were incubated for the indicated time in a water bath with the temperature carefully adjusted to 60°, or to 58.5° in the case of the experiment (Table 7) on heterokaryon extracts. Wild-type ST*A* (74*A*), strains were included with each set of revertants for comparison.

RESULTS

Reversion studies: The am_{126} mutant strain was isolated by the direct selective procedure (KINSEY 1977) after mutagenesis with nitrous acid. No GDH activity has ever been detected in extracts of the mutant, nor has CRM been detected either by rocket immunoelectrophoresis or by the enzyme protection technique of ROBERTS and PATEMAN (1964). Even though by these criteria we have been unable to detect a GDH product in mutant extracts, am_{126} certainly produces some form of GDH. This is evident as it is a complementing mutant that shows

TABLE 1

Complementation between am *mutants**

Mutant strain	Mutant strain 4	119	1	7	2	3	19	122	130	131	14	126	*arg-1*
4†	—												
1	—	—											
119	—	—	—										
7	—	—	—	—									
2	—	+++	+++	—	—								
3	—	+++	+++	—	—	—							
19	—	+++	+++	+	—	±	—						
122	—	+++	+++	—	—	—	±	—					
130	—	++++	+++	±	—	—	±	—	—				
131	—	+++	+++	±	—	—	±	—	—	—			
14‡	±	+++	+++	++	±	++	+	+	+	+	±		
126	—	±	±	++	+	+++	++	+	+	+	±	—	
arg-1†	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	—

* — Indicates no growth, ± indicates trace of growth, ++++ indicates growth equivalent to wild type.

† am_4 was chosen as a representative noncomplementing mutant, and *arg-1* as a nonallelic auxotroph.

‡ am_{14} is a slightly leaky mutant.

extensive allelic complementation with other *am* mutants. The complementation matrix is shown in Table 1; am_{126} complements with mutants am_2, am_3, am_7, am_{19}, am_{122}, am_{130}, and am_{131}, but not (or only very poorly) with am_1, am_{14} or am_{119}. Together, these strains comprise all of the known complementing *am* mutants. In this respect am_{126} resembles am_{14}, another CRM-negative complementing mutant, except that am_{14} complements am_1. Mutant am_{14}, however, is osmotically reparable, producing measurable GDH in the presence of 1.0 M glycerol or other suitable solutes; whereas, am_{26} is not osmotically reparable (FINCHAM 1977).

When reversion studies were performed on strains of am_{126}, the control plates (not treated with mutagen), which for most *am* strains would contain no revertants, were found to contain numerous colonies. Suitably marked strains of am_{126} were then constructed (to eliminate the possibility that contaminants were being counted as revertants) and the spontaneous revertant frequency for several substrains was determined. In each case, the substrain was derived from a single conidium. The revertant selection platings were done using conidia from the youngest possible cultures (three days at 25°).

The upper section of Table 2 shows the results of five such replicate experiments. All revertants were identical in mating type and unselected marker constitution to the original strain. The revertant frequency is variable and increases with age of the culture (data not shown); however, at its lowest (0.32×10^{-5} is the lowest revertant frequency that we have ever measured), it is 40 times the revertant frequency of am_{19}, which has the second highest spontaneous revertant frequency among the *am* mutants we have tested. Table 3 shows the spontaneous revertant frequency for am_{19}, am_{126} and three other *am* mutants. All of the tests were done at the same time, starting with a culture derived in each case from a single conidium. All cultures were grown under identical conditions for the same length of time. The strain am_{100} is a non-CRM forming, noncomplementing mutant (KINSEY, unpublished results); am_{14} is a non-CRM forming, complementing missense mutant (FINCHAM and BARON 1977); am_{17}

TABLE 2

Spontaneous revertant frequencies of am_{126} *substrains*

Substrain	Total live cells plated	Total revertants	Revertants per 10^5 live cells
1	7.8×10^5*	4	0.51
2	4.5×10^5*	4	0.89
3	4.1×10^5*	5	1.2
4	3.6×10^5*	9	2.5
5	6.2×10^5*	13	2.1
1×6	1.1×10^6†	5	0.44
2×6	1.3×10^6†	11	0.82
3×6	5.1×10^6†	5	1.0

* Asexual spores (conidia).
† Sexual spores (ascospores).

TABLE 3

Spontaneous revertant frequencies of am *mutant strains*

Strain	Total live cells plated	Revertants	Revertants per 10^7 live cells
14	11.8×10^7	0	<0.08
17	5.2×10^7	3	0.58
19	7.4×10^7	6	0.81
100	8.0×10^7	0	<0.12
126	5.8×10^7	186	32.07

is a non-CRM forming, noncomplementing mutant strain with a chain terminating mutation corresponding to the codon for residue 313 of GDH (SEALE *et al.* 1977); and the am_{19} strain is a CRM-forming, complementing missense mutant in which lysine 141 of GDH is changed to methionine. Mutant am_{19} is known to "revert" by means of several intragenic suppressors (BRETT *et al.* 1976). Data for two other mutants are shown as controls in Table 4.

The lower part of Table 2 shows the spontaneous revertant frequency obtained for $am_{126} \times am_{126}$ crosses. Again, crosses were made between young subcultures that were started as single colony isolates. There was no indication that the revertant frequency increased during meiosis.

It was also apparent in the initial reversion studies that mutagen treatment had little or no effect on the revertant frequency. In order to test this carefully, reversion in genetically marked substrains of am_{126} was measured before and after treatment with UV or EMS. As before, in each case a single colony isolate was used; however, in order to have sufficient conidia to perform the larger experiments, it was necessary to grow the cultures for four to five days. As can be seen in Table 4, this resulted in a somewhat higher spontaneous revertant frequency. Data for am_{119}, another CRM-negative, complementing mutant (KINSEY unpublished), and am_6, a frame shift mutant (SIDDIG, KINSEY, KEIGHREN and FINCHAM, in preparation), are included for comparison. The am_{126} spontaneous

TABLE 4

Effect of mutagenesis on revertant frequency

Allele	Total live conidia	Mutagen	Total revertants	Revertants per 10^5 live conidia
am_{119}	8.3×10^6	none	0	0
	16.4×10^6	EMS (28)*	321	1.96
	3.7×10^6	UV (50)	15	0.43
am_{126}	0.75×10^6	none	22	2.9
	14.5×10^6	EMS (31)	202	1.4
am_{126}	0.32×10^6	none	15	4.6
	0.48×10^6	UV (98)	12	2.5
am_6	2.9×10^7	none	0	0
	3.8×10^6	UV (90)	64	1.67

* The number in parentheses represents the percent killing in each case.

control for each mutagen treatment is shown separately because of the variability in revertant frequency. It is obvious from these data that neither UV nor EMS enhances the revertant frequency; if anything, these mutagens seem to decrease the frequency with which revertants are produced.

Reversion of unstable mutants in plants sometimes shows an inverse correlation with temperature (*cf.*, HARRISON and FINCHAM 1957). This might be expected if reversion were involved with a recombinational event that requires close pairing of homologous double-stranded DNA. Reversion of am_{126} was tested at three temperatures (Table 5). Again, suitably marked, single-colony substrains were used. The cultures were maintained at the indicated temperature just until conidia could be harvested. Plating for revertants was done at 25° regardless of the temperature at which the culture had been grown. Although the revertant frequencies were quite variable, there was no indication that elevated temperature had any effect on the revertant frequency.

A characterization of the heat stability of the GDH produced by revertant strains and the ratio of glutamate formation to glutamate oxidation have previously been shown to be sensitive indicators of the nature of the original mutations (STADLER 1966; SEALE 1968). In particular, mutations with high revertant frequencies have invariably yielded a spectrum of revertants that could be readily categorized on the basis of the thermostability (or lack thereof) of their GDH when it was incubated at 55 to 65° at a variety of pH values. Thermostability at pH 6.5 has been shown to be a particularly sensitive indicator (SEALE 1968).

Consequently, the thermostability of GDH from 68 revertants of am_{126} was determined. None of the 68 revertants produced GDH that was significantly different from that of wild type with respect to heat stability at pH 6.5. The data for wild type and six representative revertants are shown in Table 6. GDH from 20 of the revertants was also checked for heat stability at pH 7.4 and 8.0. All were well within the range of values shown by wild type. The ratio of glutamate oxidation to glutamate formation was determined for 40 of the revertants. using the standard assay systems of CODDINGTON, FINCHAM and SUNDARAM (1966);

TABLE 5

Effect of temperature on revertant frequency

Temperature	Total live conidia	Revertants	Revertant frequency per 10^5 live cells
25°	1.94×10^6	29	1.49
	1.39×10^6	13	0.94
	6.5×10^6	18	0.27
33°	0.93×10^6	9	0.96
	1.27×10^6	5	0.39
	4.29×10^6	40	0.93
37°	0.36×10^6	5	1.40
	0.66×10^6	4	0.61
	10.38×10^6	37	0.36

TABLE 6

Heat stability of am_{126} *revertant strains*

Strain	Percent of the Initial Activity Remaining Minutes at 60° (pH 6.5) 2	6	12
WT	85	66	37
R1	85	64	37
R2	90	71	38
R3	88	66	30
R4	83	60	38
R5	91	60	37
R6	87	61	37

again all were essentially identical to wild type. Thus, am_{126}, unlike other alleles of the *am* locus with relatively high revertant frequencies, seems to produce only true wild-type revertants. The other *am* mutations that produce only true revertants have induced revertant frequencies one to two orders of magnitude lower than the spontaneous revertant frequency of am_{126} (STADLER 1966; SEALE 1968; KINSEY, unpublished).

BARNETT and DE SERRES (1963) reported on an unstable allele of the *ad-3* locus of Neurospora that produced unstable wild-type revertants. To determine whether or not the am_{126} revertants were unstable, seven *am* prototrophic revertants of a *lys-1* am_{126} strain were screened for spontaneous production of *am* auxotrophs, using the direct selective procedure. At least 2×10^7 live conidia were tested for each revertant. No *am* auxotrophs were found, although they would have been easily detected had they occurred. Therefore, it appears that am_{126} revertants are not themselves significantly unstable.

Nature of complementation products: The nature of the complementation products of two pairs of mutants ($am_{126} + am_3$ and $am_{126} + am_{19}$) was investigated by the use of forced heterokaryons. The specific activities given by the heterokaryons, in comparison with their component strains (grown with appropriate supplements) and with wild type, are shown in Table 7. System A, measuring glutamate synthesis, assays wild-type GDH, but does not activate the inactive GDH varieties produced by am_3 or am_{19}. System C, measuring glutamate oxidation after preincubation of the enzyme with 0.15 M glutamate at pH 8.5, measures both wild-type and am_3 (but not am_{19}) GDH. System S, which resembles C but involves a prolonged preincubation of the enzyme with 0.16 M sodium succinate at pH 8.5, activates both am_3 and am_{19} GDH varieties, as well as measuring wild-type activity. The different degrees of stability of the various activities at 58.5° are also shown in Table 7. Complementation resulted in the appearance of enzyme, active in system A and not needing any special activation treatment, amounting to about 20% and 15% respectively, of the wild-type level in ($am_{126} + am_{19}$) and ($am_{126} + am_3$). The heat-inactivation experiment showed that the complementation product was much less stable than wild-type GDH in both cases, the ($am_{126} + am_3$) product being especially labile. In the case

TABLE 7

GDH activities in complementing heterokaryons

Culture	Assay system	Specific activity	% activity remaining at 58.5° after 5′	15′
am_{126} *inos*†	A	0	—	—
am_{126} *arg-1*	C	0	—	—
am_{126} *arg-1**	A	0	—	—
	C	0	—	—
	S	0	—	—
am_3 *arg-10**	A	40	—	—
	C	1270	—	70
am_{19} *arg-1**	A	0	—	—
	C	0	—	—
	S	1420	0	0
am_{126} *inos*†	A	250	27	4
am_3 *arg-10*	C	570	63	45
am_{126} *inos*†	A	400	35	15
am_{19} *arg-1*	C	420	60	45
	S	470	80	65
Wild-type 74 *A*	A	1920	—	75
	C	870	—	80

† Expressed in O.D. units (Δ O.D.$_{340}$/min/mg protein $\times$ 100).

‡ All grown in 50 ml lots of Vogel's minimal supplemented with 5 mM glutamate and with 2 mM L-arginine where indicated by *.

(—) Indicates not tested.

Note: am_3 is *glu 393* → *gly* and am_{19} is *lys 141* → *met* (BRETT *et al.* 1976).

of ($am_{126} + am_{19}$), a part of the activity assayed in System C that must have been due to complementation, since am_{19} GDH is not active in this system, was considerably more stable. Perhaps this indicates heterogeneity of the complementation product. By analogy with the analysis of CODDINGTON, FINCHAM and SUNDARAM (1966) ($am_1 + am_{19}$) hybrids, we would speculate that this reflects relatively stable hybrid oligomers containing a preponderance of am_{19} monomers and responding to System C activation, while being inactive (or poorly active) in System A. Similarly, we would speculate that the System C activity in the ($am_{126} + am_3$) heterokaryon presumably includes a major contribution from the stable homoligomeric am_3 product and that the System S activity in ($am_{126} + am_{19}$) is attributable in part to the stable am_{19} homoligomer.

DISCUSSION

There are now reports in the literature of unstable mutations in many organisms including the previously mentioned one at the *ad-3* locus of Neurospora. The *ad-3* mutation differs from am_{126} in that *ad-3*+ revertants themselves appear to be unstable and to segregate *ad-3* mutants at high frequency. In the case of am_{126}, the revertants appear to be stable.

There are three primary models that have been proposed, or demonstrated, to explain unstable loci. The first is based upon the Bar Locus in Drosophila

(STURTEVANT 1925). This model presumes that the original event was a duplication of existing genetic material, which in the case of am_{126} would presumably be a relatively small block of DNA near the centromere-proximal end of the gene. This would then allow precise removal of the duplicated segment by "illegitimate" pairing, which in the case of am_{126} would have to take place in nuclei dividing by mitosis, as am_{126} reversion occurs in vegetatively dividing cells and meiosis does not appear to increase the revertant frequency. The duplication would have to end in proper translational phase to allow translation to continue, and presumably would result in a monomer that contained a repeat of some of the amino acid sequence near the amino-terminus. This might serve to explain the instability of the am_{126} monomer and our failure to find any CRM. Mapping data (KINSEY, unpublished) suggests that am_{126} maps near am_{14}, which is known to produce GDH altered in residue 20 (*leu—his*). Leucine 20 is thought to be important in stabilizing monomer-monomer contact (FINCHAM and BARON 1977).

A second model for unstable alleles is based upon the Salmonella H1-H2 flagellar antigen phase transitions that have recently been shown to involve an invertable sequence (ZIEG 1978). It is conceivable that am_{126} represents an inversion of DNA in the *am* locus and that this inversion is somewhat unstable, reverting readily to its normal orientation. The observation that the revertants are not themselves unstable makes this possibility seem unlikely, as it is difficult at this point to see why one orientation of an inversion should be more stable than the other.

The third model is based upon insertional mutations of the type that occurs in the *gal-3* mutant of *E. coli* (*cf.*, AHMAD 1977), with which am_{126} shares several properties. However, any hypothesis involving a long insertion, similar to an *E. coli* IS element, is made difficult by the complementation data. The relatively good yield of electrophoretically normal GDH formed by complementation between am_{126} and other *am* alleles suggests that am_{126} must produce a missense chain not too dissimilar to the wild type in size and capacity for forming tertiary structure. On the other hand, the relative instability of the complementation products, together with the failure of am_{126} by itself to produce CRM, suggests a gross defect in capacity to form stable quaternary associations, rather like that of the closely linked am_{14}, which carries the substitution leu 20—his. A relatively small insertion of one or a few amino acids in an N-terminal portion of the chain important for inter monomer contact is perhaps as good a hypothesis as any for the nature of the am_{126} lesion. If such a duplication occurred in a sequence of repeated bases, both the origin and the instability of the mutant sequence might be explained as either recombinational or replicational error.

The recent elucidation by frame-shift analysis of the nucleotide sequence corresponding to the five amino-terminal amino acid residues of Neurospora GDH (SIDDIG, KINSEY, KEIGHREN and FINCHAM, in preparation) gives us hope that we shall soon be in a position to look directly at the alteration in the DNA sequences involved.

LITERATURE CITED

AHMAD, A., 1977 The *gal-3* mutation of *E. coli*. pp. 31–48. In: *DNA Insertion Elements, Plasmids and Episomes*. Edited by A. I. BUKHARI, J. A. SHAPIRO and S. L. ADHYA. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

BARNETT, W. E. and F. J. DE SERRES, 1963 Fixed genetic instability in *Neurospora crassa*. Genetics **48**: 717–723.

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

CODDINGTON, A., J. R. S. FINCHAM and T. K. SUNDARAM, 1966 Multiple active varieties of *Neurospora* glutamate dehydrogenase formed by hybridization between two inactive mutant proteins *in vivo* and *in vitro*. J. Mol. Biol. **17**: 503–512.

DAVIS, R. H. and F. J. DE SERRES, 1970 Genetic and microbiological research techniques for *Neurospora crassa*. Methods Enzymol. **17**: 79–143.

FINCHAM, J. R. S., 1951 The occurrence of glutamic dehydrogenase in Neurospora and its apparent absence in certain mutant strains. J. Gen. Microbiol. **5**: 793–806. ——, 1977 Allelic complementation reconsidered. Carlsberg Res. Commun. **42**: 421–430.

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.

HARRISON, B. J. and J. R. S. FINCHAM, 1957 Instability at the *PAL* locus in *Antirrhimun majus*. I. Effects of environment on frequencies of somatic and germinal mutation. Heredity **19**: 237–258.

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.

LAURELL, C., 1966 Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal. Biochem. **15**: 45–52.

ROBERTS, D. B. and J. A. PATEMAN, 1964 Immunological studies of amination deficient strains of *Neurospora crassa*. J. Gen. Microbiol. **34**: 295–305.

SEALE, T., 1968 Reversion of the *am* locus in Neurospora: Evidence for nonsence suppression. Genetics **58**: 85–99.

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

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.

STADLER, D. R., 1966 Glutamic dehydrogenase in revertants of *am* mutants in *Neurospora*. Genet. Res. **7**: 18–31.

STURTEVANT, A. H., 1925 The effect of unequal crossing-over at the Bar locus in Drosophila. Genetics **10**: 117–147.

VOGEL, H. J., 1956 A convenient growth medium for *Neurospora*. 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, 1971 Amino acid sequence of NADP-specific glutamate dehydrogenase of *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S. **71**: 4361–4365.

ZIEG, J., 1978 Regulation of gene expression by site-specific inversion. Cell **15**: 237–244.

Corresponding editor: C. SLAYMAN