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SHORT COMMUNICATIONS

Distribution of alleles of diverse mutagenic origins in the allele map of the *ftr* cistron

The *ftr* cistron of the Basidiomycete fungus *Coprinus lagopus* controls a function involved in transport of the sugar fructose[7]. A great many transport-negative mutants have been isolated; they are all alleles of this one gene.

Coprinus wild-type grows equally well whether fructose or glucose is given as the carbon source. The *ftr* mutants grow as well as wild-type on glucose, but when given fructose the amount of growth they make depends on the concentration of that sugar in the medium. Since they have to rely on diffusive entry of fructose *ftr* mutants produce very little growth at low concentrations. Thus on medium containing 5 m*M* fructose there is a very clear distinction between the dense growth of wild-type and sparse growth of *ftr*. Use can be made of this test to undertake genetic fine structure analysis, and over 50 alleles have been positioned in the fine structure map of the gene[5]. The map has an intriguing feature, for instead of being more or less randomly spread over the whole map the recombinable sites fall into three distinct clusters. It is tempting to assume that this clustering has some significance in terms of protein function, but there are other possibilities. In particular it is possible that clustering of the mutant sites is related to some mutational peculiarity.

Almost all of the mutants so far mapped were selected after treatment with the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG), so the clusters could represent regions of the gene which were unusually sensitive to this agent. This does not seem very probable as a number of spontaneous mutants have also been mapped and these too fall into the same clusters. More recently, though, mutants have been obtained after treatment with the ethyl ester of methanesulphonic acid (EMS) and with hydroxylamine (HA), and these allow a more stringent test of the possibility of mutagen specificity. Mutants caused by EMS occur more often at GC sites than at AT sites[2], and HA too predominantly causes GC to AT transitions[1,3]. On the other hand NTG is thought to cause AT to GC transitions[4]. Any suggestion that the clusters in the existing map result from differential mutagenesis must rely on the assumption that those regions of the gene are (either actually or effectively) AT-enriched. A corollary of such an argument is that the regions between the observed clusters would have to be relatively GC-enriched. So mutants induced by EMS or HA would be expected to map between the existing clusters. This point has been tested by investigating the distribution of the new mutants within the cistron.

The media and techniques used in experiments described here were the same as those used previously[5]. Spontaneous mutants (code numbers prefixed with the letter S) and NTG-induced alleles (code numbers prefixed by the letter Z) have also been described before[5,7]. Hydroxylamine was used for mutagenesis at a final concentration of 1.0 *M*. Oidiospores of the BC9/6,6 wild-type were suspended to a density of $1.6 \cdot 10^8$ per ml and treated for 70 min at 22°. Treatment was terminated by filtration through

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Abbreviations: EMS, ethyl methanesulphonate; HA, hydroxylamine; NTG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

TABLE I

RECOMBINATION FREQUENCIES OBTAINED IN CROSSES INVOLVING ETHYL METHANESULPHONATE-INDUCED ALLELES[a]

Reference alleles	*S24*	*Z463*	*Z492*	*Z15*	*Z505*	*Z500*	*Z154*	*Z435*	*S31*	*Z215*	*Z197*	*Z77*
Map distance[b]		2.0	0.5	1.5	0.9	26.4	2.0	8.5	1.9	21.3	1.8	2.8
EMS1	7.2		4.7	0.8	4.6		33.1				42.7	
EMS2		54.5	28.5	40.7			9.2	66.9	29.2		22.8	
EMS3				39.0		21.7	6.6	6.6	6.6	21.5	17.6	
EMS4				41.1			26.6			5.5	1.7	0.0
EMS5	0.3		2.7	11.1			39.7				107.3	
EMS6		1.6	0.7	4.3			20.7				29.3	
EMS7			30.7	22.1	32.1	8.9	1.2				17.7	
EMS8				63.0			34.4			0.0	4.3	7.1
EMS9			28.0			6.6	5.2	3.9	0.2		29.2	
EMS11				48.2			27.1			1.7	4.1	
EMS12				66.0			55.3			0.0	2.2	
EMS13				38.4			34.7			0.2	1.0	4.5
EMS14				31.5			30.8			5.8	5.4	0.0
EMS15				38.0			48.0			0.0	4.3	7.9
EMS17				27.7		15.5	5.2	0.5	1.0		14.7	
EMS18		5.7	0.4	3.9			30.9				39.2	
EMS19	0.0		5.7	6.7	23.7		29.2				33.0	
EMS20				15.3		14.5	4.0	0.0	0.4		73.9	
EMS21			0.0	5.1			23.4					
EMS22				77.7		88.0	40.9			21.6	17.8	34.1

[a] The recombination frequencies recorded in the body of the Table are expressed as "wild-type recombinants/10^5 viable progeny analysed".
[b] The map distances between adjacent reference alleles (arranged here in their correct order) are also in units of "wild-types/10^5 progeny analysed".

TABLE II

RECOMBINATION FREQUENCIES OBTAINED IN CROSSES INVOLVING HYDROXYLAMINE-INDUCED ALLELES

Reference alleles	*S24*	*Z463*	*Z492*	*Z15*	*Z505*	*Z500*	*Z154*	*Z435*	*S31*	*Z197*
Map distance		2.0	0.5	1.5	0.9	26.4	2.0	8.5	1.9	23.1
HA1			1.3	2.9			14.4			51.6
HA2		0.3	0.8	6.3			46.6			56.0
HA3	1.0		3.4	2.6	5.0		2.3			8.8
HA4			10.3	3.2	2.0		7.6			23.7
HA5				12.1			8.3			
HA6			2.0	0.6			33.3			43.0
HA7	11.7	3.1	3.2	3.7	10.0		19.7			90.0
HA8		0.0	0.5	6.7			28.9			91.4
HA9			7.2	0.5			14.1			45.6
HA10				20.2			0.0			26.5
HA15				48.8			62.1			0.0
HA16			2.3	2.1			29.9			46.8
HA17				21.2		16.1	11.2	7.4	4.1	23.9
HA19				0.2			15.8			46.4
HA21		0.1	1.0	9.2			45.7			44.6
HA22				0.0			13.4			29.8

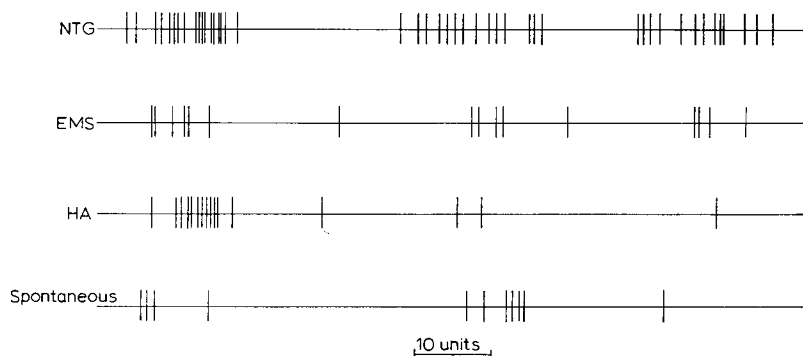

Fig. 1. Distributions of alleles of different mutational origin in the *ftr* fine structure map. In each case the horizontal line represents the long axis of the chromosome while the vertical lines indicate the positions of the recombinable sites. Positions of NTG-induced, EMS-induced, HA-induced and spontaneous mutants are shown to approximate scale (distances between close sites are exaggerated for graphical convenience).

Whatman GF/A glass-fibre and the water-washed spores were resuspended in water and then plated in NCM medium containing 5 m*M* fructose and 0.5 m*M* 2-deoxy-D-glucose. Resistant mutants were isolated from this medium after about 5 days incubation at 37° and given code numbers prefixed by the letters HA. Spore viability after hydroxylamine treatment was 0.4%. Mutation with EMS was done similarly; an oidiospore suspension was made 0.1 *M* with respect to EMS and treated for 70 min at 22°, post-treatment spore viability being 4.2%. Resistant mutants were again selected on NCM+5 m*M* fructose+0.5 m*M* 2-deoxy-D-glucose and were given stock numbers prefixed by the letters EMS.

The general strategy employed was to cross the HA and EMS test mutants with reference alleles of known position in the existing allele map; results of the crosses are given in Tables I and II. Since the aim was to determine general distribution rather than exact position the minimum number of crosses required to place a test mutant within about 5 units of a reference allele were undertaken. In fact most of the test alleles can be positioned far more closely than this even after allowance is made for the variability of recombination frequencies[6]. The distributions of the various mutants can thus be established in the form illustrated in Fig. 1. This makes the clustering of NTG-induced mutants quite clear and also shows that neither HA- nor EMS-induced mutants are found in unusual numbers between those clusters. Rather, whatever the mutational origin of the mutants they mostly fall into the same three clusters. Clustering of NTG-induced alleles must therefore be related to the functional differentiation of the cistron and result from some effect which operates at the selection step rather than the mutation step. There is, however, a difference between the distributions of HA and EMS mutants, the former being almost entirely restricted to the left-hand end of the gene. Since the two mutagens cause the same chemical change to the DNA coding this difference in distribution is most probably an illustration of the way in which the coding pattern surrounding potential mutant sites can influence the reaction between mutagen and sensitive site. In this case some aspect of gene primary structure makes the leftward cluster relatively more sensitive to hydroxylamine than to ethyl methanesulphonate. Thus a degree of mutagen specificity can be recognised from the distributions of the different mutants, but it is a relatively minor feature of the total

allele map. The major structural peculiarity—clustering of NTG-induced alleles—does not seem to be due to differential mutagenesis.

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