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Genetic fine structure, site clustering and marker effect in the *ftr* cistron of *Coprinus*

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SUMMARY

Mutants in the *ftr* cistron of the Basidiomycete *Coprinus lagopus* have a lesion in sugar transport. Fifty-two alleles are placed in an allele map using recombination frequencies obtained from over 400 heteroallelic crosses. The mutant sites in the allele map are distinctly clustered into three approximately equally spaced regions. It is demonstrated that the clustering is not due to any mutational specificity. Evidence is presented which indicates that the clusters are functionally differentiated both within themselves and from one another. Additivity of recombination frequencies was good over the whole of the allele map and there was no overall map expansion. However, specific marker effect sites could be recognized. The data indicate that marker effect may act to enhance or reduce recombination frequency and that enhancement is equal and opposite to reduction. It is also shown that marker effect enhancement is only observed when the interval being mapped exceeds a certain minimum value, but that there was no upper limit to the size of the interval. Expression of marker effect was influenced both by the background genome and by the nature of the mutation at the second site in the heteroallelic cross. It is suggested that current models of recombination do not adequately explain these data and, more importantly, that their reliance on initial breakage of DNA strands in the formation of hybrid DNA creates grave mechanical difficulties. A model for genetic recombination is proposed in which the sequence of events is: (i) separation of intact double helices into single strands; (ii) illegitimate pairing between single strands from non-sister chromatids; (iii) strand breakage and exchange of covalent links so as to legitimize the illegitimately paired regions. The model allows for the formation of hybrid DNA either with or without coincident chiasmata. It is envisaged that the error recognized by any excision-repair system involved in recombination is the tangled strands at each end of the illegitimately paired region rather than base mismatching; and that the exchange of covalent links in stage (iii) involves the excision and replacement of segments of DNA.

1. INTRODUCTION

Mutations in the *ftr* cistron of the Basidiomycete fungus *Coprinus lagopus* can be efficiently isolated by selecting for growth on media which contain inhibitory concentrations of the glucose analogue 2-deoxy-D-glucose (deGlc). The numerous

mutants which have so far been obtained by this method have proved to be alleles of a single cistron (Moore & Stewart, 1971*a*). The mutants were also shown to be pleiotropic, for although they were initially selected only for resistance to deGlc they were found to be cross-resistant to the growth inhibitions caused by the related analogues L-sorbose and D-glucosamine. Furthermore, the *ftr* mutants were unable to utilize fructose as a sole carbon source. The inability to utilize fructose, and indeed the analogue-resistance phenotype, was demonstrated to result from a defect in sugar transport.

Quantitative functional analysis is, with the aid of isotopically labelled sugars, particularly easy in this system. The availability of a large number of alleles, and the ease with which further alleles may be selected if they are required, means that the material is at hand for a very detailed examination of the transport process and the changes brought about by different mutations. For such an investigation to be genetically meaningful it is desirable that the functional analysis be compared with an equally detailed examination of the structural relationship which exist between the alleles employed. A study of intragenic recombination is thus necessary so that an allele map can be constructed.

Allelic recombination is so rare an event that selective procedures must be used to determine the frequency of recombination. These usually favour growth of the wild-type progeny which form one of the expected recombinant classes. Studies involving resistance mutants are attractive because of the ease with which large numbers of mutants can be isolated. However, in most cases it is not possible to construct a fine structure map with resistance mutants because of the inability to devise a selective method which will favour the few sensitive wild-type recombinants which are expected to occur among the overwhelmingly resistant progeny obtained from any heteroallelic cross. In this respect, though, the pleiotropy of the *ftr* mutants offers a peculiar advantage. The mutants are, of course, resistance mutants and can be readily selected for this reason. However, they are also unable to utilize fructose whereas this sugar is one of the preferred carbon sources of the wild-type (Moore, 1969). Consequently selection for the ability to use fructose provides an effective technique for enumerating rare wild-type individuals among a large majority of mutant types. The differential is not absolute though, since the mutants are able to grow sparingly on fructose-media; but the mycelia they produce are far less massive than those produced by the wild-type (Moore & Stewart, 1971*a*). Hence there is a distinct qualitative difference between the thin barely visible mutant colonies and the densely branched colonies which originate from wild-type spores.

This technique has been used in the study reported here to place 52 of the *ftr* alleles in an allele map of that cistron. The map obtained is sufficiently detailed to serve as a reliable basis for the structure/function studies which are envisaged for the future. In addition a great deal of data have been obtained which are of more general significance in that they may contribute to an understanding of the recombination process itself. A model of recombination is proposed which takes account of these data.

2. MATERIALS AND METHODS

(i) *Organism.* The strain used for the original induction of mutants was the haploid wild-type number BC9/6,6 (mating type A_6B_6). Mating-type recombinants of *ftr* mutants were isolated from crosses made between the original mutants and the wild-type numbered ZBw601/40,40 (mating type $A_{40}B_{40}$). The techniques used in the induction of mutants with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) and in the isolation, characterization aud complementation testing of *ftr* mutants have been described previously (Moore & Stewart, 1971*a*). All *ftr* mutants isolated after treatment with NTG have their isolation numbers prefixed with the letter Z. A number of spontaneous mutants were isolated for the present study by selecting deGlc-resistant variants on a medium containing 5 mM fructose + 0·5 mM deGlc. Untreated asexual spores (oidia) were used for this selection procedure but it was otherwise carried out as previously described (Moore & Stewart, 1971*a*). Spontaneous mutants were given isolation numbers prefixed with the letter S.

(ii) *Media.* For general purposes the *Coprinus* complete medium (CM) (Moore, 1968) was used. Selection media used in scoring recombination and reversion frequencies were based on the NCM medium described by Moore (1969) but with the addition of 0·5 mM-$MgSO_4$. NCM is a basal medium which completely lacks carbon sources; these were prepared as separate solutions and sterilized independently of other medium constituents. All crosses were fruited on horse dung, which was collected locally and sterilized by autoclaving at 15 p.s.i. for 30 min.

(iii) *Determination of recombination frequencies.* Crosses were done by first making a dikaryon from haploid strains of appropriate mating type. After about 5 days incubation (on CM) at 37 °C the colony had grown to sufficient size for inoculation to horse dung. The dung culture was first incubated for 3 days at 37 °C in the dark and was then transferred to a Saxcil Growth Environmental Cabinet operating at a temperature of 27 °C, with a nominal light intensity of 2000 lumens/ft^2 and a relative humidity of about 80 %. Under these conditions mature fruit bodies were produced in approximately 10 days.

Basidiospore suspensions were prepared initially, and subsequently diluted, in sterile water. All suspensions were filtered through cotton before being counted visually with the aid of a haemacytometer. Counts of viable spores were done by adding quantities of an appropriately diluted spore suspension to bottles containing 100 ml of molten (50–55 °C) NCM-agar medium which contained 5 mM glucose. The medium was then poured into four plastic Petri dishes. Prototroph frequencies were also determined using pour plates; here, though, quantities of the undiluted spore suspension were added to NCM + 5 mM fructose and NCM + 5 mM fructose + 2 mM sorbose. Benzylpenicillin, to a final concentration of 80–100 i.u./ml, was routinely added to all media. The same cultural techniques were used for determining reversion frequencies of oidia.

All plates were incubated at 37 °C. After 48 (basidiospores) or 72 h (oidia) the glucose plates carried distinct colonies whereas the selection plates (fructose and fructose + sorbose) carried a complete hazy background of mutant growth; any

colonies arising from wild-type spores formed very localized (usually of the order of 1–3 mm diameter) areas of dense mycelial growth embedded in this hazy background. These wild-type colonies were clearly distinct from the background and no difficulty was experienced in counting them. At the start of this project, of course, it was necessary to prove that these small, densely growing regions were indeed wild-type colonies and not just areas where the density of mutant growth was for some reason greatly increased. To do this a medium which supported only the growth of wild-type was required. It was found that *ftr* mutants are characteristically more sensitive than wild-type BC9/6,6 to inhibition by methylene blue (MB) when growing on a nutritionally stringent medium (i.e. any medium containing fructose as sole carbon source). On a medium consisting of NCM + 5 mM fructose + 6 μg/ml MB the growth of *ftr* strains was completely inhibited while the wild-type was able to grow, although with a much reduced growth rate. This medium was used to isolate over 100 wild-type recombinants from 11 heteroallelic crosses. All of the isolates proved to be phenotypically and genotypically wild-type. About 50 % of the isolates were found to be heterokaryotic when first isolated. This does not reflect on the accuracy of recombination frequencies since *ftr* is completely recessive and recombination was so infrequent that the probability of a wild-type + wild-type heterokaryon is extremely low. In addition, since the MB technique can be combined with the previously described (Moore & Stewart, 1971*b*) deGlc technique for resolving dikaryons (providing fructose is used as the normal carbon source), no difficulty was experienced in preparing wild-type monokaryons from the heterokaryotic isolates. To minimize complication in routine procedures the MB technique was not used for the analysis of crosses.

3. RESULTS AND DISCUSSION

(i) *Sources of error.* This analysis is based on the determination of the frequencies with which wild-type spores are found among the progeny of heteroallelic crosses, and on the assumption that these are direct measures of recombination frequencies. Possible sources of error, apart from experimental variation, are pseudo-wild-types generated by reversion, suppression, disomy, dikaryosis, etc.

All of the NTG-induced alleles used in this work are non-complementing (Moore & Stewart, 1971*a*) and this applies also to the spontaneous alleles which complement neither among themselves nor in combination with any of the NTG-induced alleles. Thus no combination of parental genotypes (whether in heterokaryon, diploid or aneuploid) can generate a pseudo-wild-type colony. As indicated in Methods, all of the wild-type colonies which were individually isolated from allelic crosses proved to be genotypically wild-type in subsequent tests, so that suppression is not likely to seriously affect the results. Table 1 shows the reversion frequencies of a representative sample of *ftr* mutants. Oidia were used in these experiments because homoallelic crosses were rarely fertile, and even when they did fruit frequently produced sterile or semi-sterile sporophores so that basidiospores could not be obtained in sufficient number. However, the data of Table 1 show that reversion

Table 1. *Reversion frequencies of representative NTG-induced* ftr *strains*

Isolation number	Mating type	Oidia tested ($\times 10^{-6}$)	Wild-types observed	Reversion frequency ($\times 10^{8}$)
88	A_6B_6	2·06	0	77·70*
98	A_6B_6	18·65	0	14·60*
154	A_6B_6	57·10	2	3·50
154	$A_{40}B_{40}$	14·10	0	19·30*
163	A_6B_6	27·16	73	290·00
169	A_6B_6	57·20	3	5·24
169	$A_{40}B_{40}$	5·18	0	45·10*
182	A_6B_6	48·92	2	4·09
183	A_6B_6	11·61	0	22·10*
197	A_6B_6	8·74	0	28·40*
211	$A_{40}B_{40}$	8·44	0	31·20*
215	A_6B_6	44·20	2	4·52
361	A_6B_6	10·50	1	9·52
435	A_6B_6	61·50	0	4·70*
435	$A_{40}B_{40}$	17·70	2	11·29
463	A_6B_6	89·10	2	2·24
492	A_6B_6	27·61	2	7·25
496	A_6B_6	57·75	11	19·04
496	$A_{40}B_{40}$	41·80	1	2·39
505	A_6B_6	34·30	2	5·83

* Calculated values for the 95 % upper limit of expectation.

is not likely to be a significantly disturbing factor unless the conditions which affect reversion in basidiospores are very different indeed from those which operate in oidia.

Experimental variation is thus left as the most probable source of error. Approximately 39 % of the crosses performed were duplicated, either as identical repeats or as reciprocal crosses. Other duplications arose from those cases where different alleles were shown to occupy the same site in the allele map. On the whole there was a remarkably good correspondence between recombination frequencies derived from different trials. It became clear, though, that the range of acceptable variation must extend to at least ± 50 % of the value determined from any one cross. Although this seems to be a very large error, in the context of the allele map it is not at all restrictive. Providing the analysis is sufficiently extensive (in the present study over 250 intervals have been mapped), enough data are obtained to place the vast majority of alleles in a reliable order to establish reasonably accurate distances and to distinguish experimental variation from deviations from the norm produced by biologically significant phenomena.

(ii) *Recombination frequencies.* Over 400 crosses were analysed during this investigation so that the original experimental data are far too extensive for inclusion here. A duplicated table giving full experimental details of each cross performed has been deposited with the editors of this Journal; copies are available from the author. Homoallelic crosses were too infertile for consistent use, but about 78 % of all heteroallelic crosses attempted produced mature fruits.

Table 2. *Recombination frequencies obtained from crosses made between NTG-induced mutants*

	Cluster A													Cluster B														Cluster C															
	469	182	211	463	492	78	15	388	412	421	445	496	505	100	98	83	222	500	167	154	206	70	88	336	168	435	200	183	33	215	121	197	361	60	205	77	32	79	169	65	163	398	
A	3·8	3·2	—	0·3	3·8	—	12·4	—	—	—	—	13·1	27·9	—	—	—	—	53·1	—	—	—	—	—	—	—	—	—	—	35·9	—	—	—	—	110·2	—	—	—	—	—	—	—	—	9
	—	—	26·6	3·8	1·5	0·3	—	—	72·6	—	—	0·9	0·9	2·9	35·0	—	—	30·5	—	16·9	—	—	—	38·0	—	—	—	—	43·6	72·8	—	—	—	59·5	—	—	—	—	—	—	—	—	15
			—	1·7	—	—	—	—	—	—	—	11·8	14·3	—	—	—	—	—	—	13·8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	182
				0·9	1·3	—	—	—	—	—	—	1·9	—	8·2	—	—	22·4	—	—	24·4	—	—	—	42·9	—	—	—	—	—	52·4	—	45·9	—	—	82·1	—	—	—	—	—	—	—	211
				—	0·5	—	—	7·4	3·9	4·7	—	3·6	7·8	—	—	—	13·6	21·6	10·1	—	—	21·3	17·2	17·0	25·3	59·6	39·8	68·0	—	59·0	—	—	58·1	—	—	—	—	51·6	—	61·9	81·5	463
				—	—	1·1	—	—	—	—	—	3·7	5·1	7·6	—	—	—	22·9	—	—	—	—	—	22·9	—	—	—	—	—	45·6	—	—	—	—	—	—	—	—	36·3	—	492
				—	—	—	—	—	—	—	—	1·5	10·8	—	39·1	—	—	—	—	11·6	—	—	26·8	—	—	—	—	—	—	22·1	—	—	—	—	—	—	—	—	—	—	—	78
				0·3	—	—	—	—	—	—	—	13·1	20·9	—	—	—	—	—	—	25·8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	469
										—	—	0	0	—	25·9	—	—	6·1	—	6·2	—	—	30·9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	388
										—	—	0·2	0	—	27·9	—	—	—	—	12·6	—	—	9·7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	412
										—	—	0·4	0	—	19·6	—	—	4·2	—	5·6	—	—	34·6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	421
												0	0	—	15·9	—	—	9·1	—	12·0	—	—	10·7	—	—	—	—	—	—	—	—	29·4	—	—	—	—	—	—	—	—	—	445
													0	2·7	52·1	—	—	10·0	—	—	—	—	—	13·5	—	—	—	—	56·2	27·5	—	—	—	—	—	—	—	—	—	49·2	496
														—	18·3	—	—	15·8	—	—	—	—	—	—	—	—	—	—	60·1	—	—	—	—	—	—	—	—	—	30·6	83·8	505
															48·7	—	—	—	—	—	—	—	—	—	—	—	—	—	30·4	—	—	—	—	—	—	—	—	—	—	—	100
B																1·2	0·9	2·6	0·9	3·7	—	3·9	0·2	6·1	6·0	41·0	—	30·0	16·9	—	—	57·0	—	—	—	—	—	105·6	—	—	17·8	84·3	98
																	—	—	—	2·0	—	—	—	—	—	—	—	—	—	—	—	—	—	22·4	—	27·1	—	47·1	—	—	—	—	83
																		0	1·4	1·9	—	—	—	2·2	—	—	—	7·4	7·7	45·0	—	—	—	—	—	—	—	—	—	—	—	222
																			0·2	2·3	—	5·2	3·5	2·1	4·7	—	12·2	6·2	8·8	—	—	—	—	—	—	—	—	—	—	46·7	500
																				0·9	—	0·9	4·6	0·7	2·3	0·9	—	—	—	—	—	22·3	—	—	—	—	—	—	—	—	—	167
																				—	0	0·8	0·1	—	2·3	—	5·7	3·7	5·9	63·1	42·6	30·3	21·8	—	41·6	—	33·6	46·8	25·4	70·8	21·9	39·0	154
																						—	—	0·9	—	3·1	—	—	—	—	—	20·2	—	—	—	—	—	—	—	—	—	206
																							0	0	—	5·6	—	—	—	—	—	20·1	—	—	—	—	—	—	—	—	—	70
																								0	—	6·8	7·0	—	—	—	—	17·6	—	—	—	—	—	—	57·8	14·9	58·9	88
																									2·7	—	—	—	—	29·5	—	—	—	37·0	—	50·0	—	—	—	—	—	336
																										0·7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	168
																											2·8	3·1	—	—	—	15·6	—	—	—	—	—	—	—	—	—	435
																												—	—	—	—	—	—	—	—	—	—	—	—	—	—	200
																													—	31·9	—	29·5	—	—	—	—	—	—	—	—	—	183
																														—	—	—	—	17·4	—	17·4	—	47·1	—	—	—	—	33
C																															0·7	1·8	—	1·8	1·8	5·2	9·6	5·9	5·8	3·1	21·4	10·5	215
																																5·1	—	—	1·5	—	23·5	—	—	—	—	121
																																	0	0·1	0·2	2·0	3·9	4·4	4·6	18·3	10·9	14·5	197
																																		—	—	—	—	—	—	—	—	—	361
																																			0	2·9	—	3·7	—	—	—	—	60
																																				3·7	—	11·8	—	—	—	—	205
																																					1·1	5·5	3·7	19·2	22·4	25·6	77
																																						1·7	1·6	23·6	36·8	—	32
																																							0	50·2	33·5	41·9	79
																																								43·5	43·3	41·0	169
																																									42·5	29·8	65
																																										45·8	163

The alleles are arranged along the top in their map order. Entries in this, and in similar Tables which follow, are recombination frequencies expressed as wild-types/10^5 viable spores.

Table 3. *Recombination frequencies obtained from crosses made between spontaneous mutants and NTG-induced mutants*

Spontaneous allele	NTG-induced-allele									
	9	505	98	500	154	336	435	215	60	197
2	—	—	129·5	—	18·3	14·3	—	46·0	—	40·5
3	—	22·5	—	—	3·9	0·4	—	—	—	23·4
4	—	68·4	61·3	36·1	8·0	19·3	—	—	—	35·9
7	—	15·2	—	—	33·8	—	—	—	—	71·6
10	—	49·5	—	—	14·9	—	—	0	—	23·2
11	—	26·4	—	—	15·9	—	—	—	17·9	4·2
18	—	11·6	—	—	1·5	0·1	—	—	—	11·5
24	—	25·8	—	—	46·4	—	—	—	—	66·4
26	—	0	22·6	17·5	3·5	5·3	—	—	—	34·1
29	8·0	14·5	—	—	29·0	—	—	—	—	67·8
30	—	—	12·5	—	4·8	2·7	1·5	—	—	—
31	—	27·6	14·1	—	13·2	0·9	—	—	—	23·1
32	—	19·9	23·8	—	8·7	6·1	—	—	—	35·3

In each cross the spontaneous mutant was used as a parent with A_6B_6 mating type. Note that no wild-type recombinants were found among a total of $2{\cdot}36 \times 10^6$ progeny tested from a cross made between S29/6,6 and S24/40,40.

Basidiospore viabilities averaged $47{\cdot}0 \pm 18{\cdot}8\,\%$ and a mean of $1{\cdot}49 \pm 0{\cdot}88 \times 10^6$ viable progeny were tested from each cross. Over the first 300 crosses performed there was no correlation between the recombination frequency and either spore viability or the number of spores tested (correlation coefficients were 0·005 and 0·014 respectively; the 99% critical value for the correlation coefficient of a sample of this size is approximately 0·15).

Results of the crosses performed are summarized in the form of recombination frequencies in Tables 2 and 3. The recombination frequencies were calculated relative to the number of viable spores tested. Throughout the text of this paper distances between sites in the allele map will be given in 'units'; it should be understood that one unit = one wild-type recombinant per 10^5 viable progeny.

(iii) *The allele map.* The map of this cistron which can be drawn using the recombination frequencies of Tables 2 and 3 is shown, to approximate scale, in Fig. 1. The most obvious feature of the map is that the majority of the alleles are clustered into three distinct regions which are identified as clusters A, B and C. Cluster A is arbitrarily placed on the left. If this clustering of sites truly reflects some sort of differentiation within the cistron then it has considerable interest in terms of the correlation of genetic structure with protein function. However, we must first discount a number of other plausible explanations of allele clustering.

(*a*) The set of mutants which have been mapped may not be a representative sample of the mutants available. However, these mutants were chosen entirely at random, and though they represent only 13 % of the total number of *ftr* mutants in the collection the number mapped is still sufficiently large to make this simple explanation unsatisfactory.

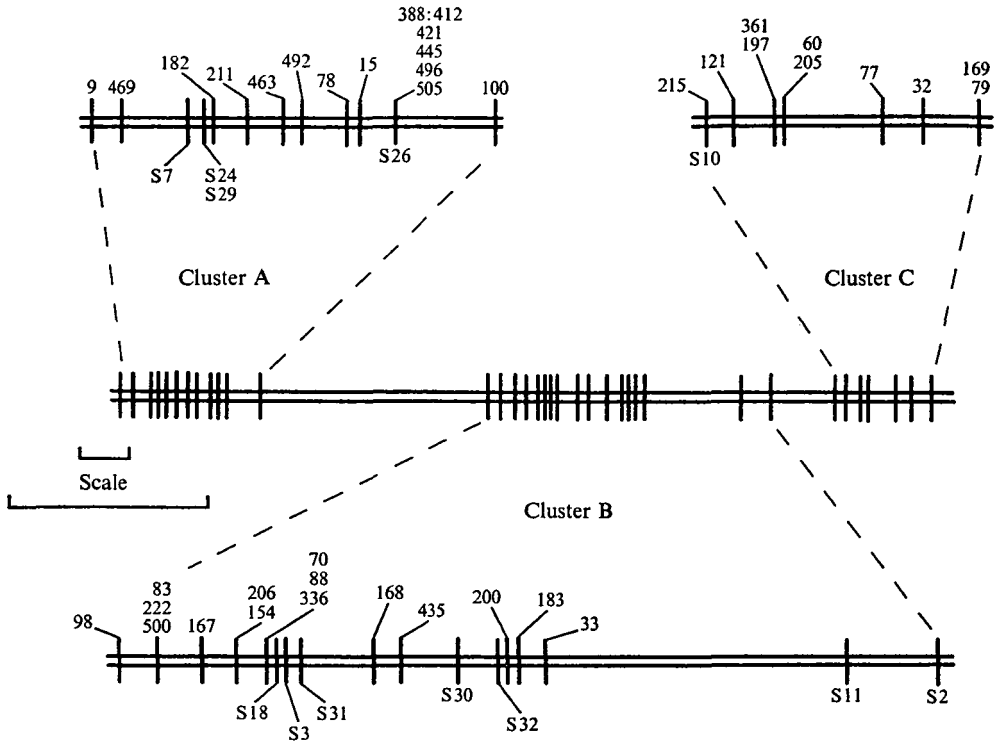

Fig. 1. Allele map of the *ftr* cistron of *Coprinus lagopus*. The map of the whole cistron is drawn to scale in the centre and the clusters are shown to expanded scale above and below the full map. Isolation numbers of NTG-induced mutants are indicated above the 'chromosome' without prefix and those of spontaneous mutants, prefixed with the letter S, below. The scale bars each correspond to a length of 5 units (1 unit = 1 wild-type recombinant/10^5 progeny) at the two scales used in the figure. Note that alleles S 2 and S 11 are included in the drawing of cluster B for graphical convenience only. It is considered that they represent mutational sites situated approximately midway between clusters B and C.

(*b*) The set of mutants which have been isolated may themselves be an unrepresentative sample because of some mutational peculiarity. The main foundation of the map is provided by recombination frequencies obtained from crosses made between NTG-induced mutants. The common mutagenic origin might lead to the suggestion that the clusters result from some particular and localized response to the mutagen. Any such suggestion is negatived by the experience with spontaneous mutants which were included specifically to test this possibility. The spontaneous mutants were subjected to a slightly simplified testing regime, in particular fewer viable spores than usual were analysed, so that individual spontaneous alleles are positioned in the map with less precision than the NTG-induced alleles. But the fact that 10 of the 12 spontaneous mutants mapped fall into the clusters originally defined by NTG mutants establishes categorically that clustering was not due to any mutational specificity.

The only plausible explanation which remains is that clustering results from

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some functional differentiation within the cistron; i.e. that the clustered regions in the allele map correspond to functionally differentiated regions of the polypeptide and that the selection conditions used (all alleles were selected for resistance to deGlc) in some way favoured mutants in the clusters in preference to any which might have occurred in the 'silent' regions between the existing clusters. It is here assumed that the regions between the clusters of the *ftr* cistron are relatively empty regions which are genuinely of unusual length. That is, that the allele map is a reasonably direct representation of the physical arrangement of the mutant sites in the DNA. In contrast, Holliday (1964) has interpreted inter-cluster intervals as map-expanded regions which are not of unusual length but which are bounded by sites which show extreme marker effect enhancement of recombination frequencies; while Whitehouse & Hastings (1965) assumed that clustering of sites in allele maps reflected variation in the mapping unit rather than variation in the physical separation of sites. Neither of these interpretations can be supported here. Both would create the need for a further explanation of the experimental observation that the majority of crosses between alleles belonging to different clusters yielded high recombination frequencies while the majority of those made between members of the same cluster yielded low recombination frequencies, despite the fact that the same strains and alleles were used in each type of cross. It is abundantly clear that a high recombination frequency across an inter-cluster interval is a characteristic of all sites in a cluster and not just the sites which are immediately adjacent to that interval. Taking the stand that the best arrangement is that which satisfies the greatest majority of the results, a reliable map can be constructed which can then be used for the interpretation of inconsistent recombination frequencies. As will be shown in detail below, this approach allows the specific recognition of marker effect sites even though there is a general additivity of map distances over the entire length of the map.

(iv) *Functional differentiation.* Some support for the idea that the clusters represent functionally differentiated regions of the gene arises from the fact that a comparison of the physiological characteristics of the mapped alleles indicates that the clusters are functionally differentiated both within themselves and from one another. At the present time the only measures of function which are available in sufficient number to make such a comparison worth while are based on the rates and extents of mycelial growth made on various liquid and solid media. There are obvious difficulties in interpreting such measurements. However, if we restrict ourselves to generalities it does seem reasonable to assume that the rate of growth of an *ftr* mutant (which, remember, has a lesion in sugar transport) on a medium containing an inhibitory sugar analogue is a measure, however indirect, of the efficiency with which that analogue is transported across the cell membrane; and similarly that the dry weight of mycelium formed on a fructose medium is a measure of the efficiency of fructose transport. Using these assumptions qualitative comparisons can be made for a variety of alleles between their positions in the allele map and their abilities to transport the three sugars deGlc, sorbose and fructose (Fig. 2). A feature of this display is that the curves drawn for cluster A are

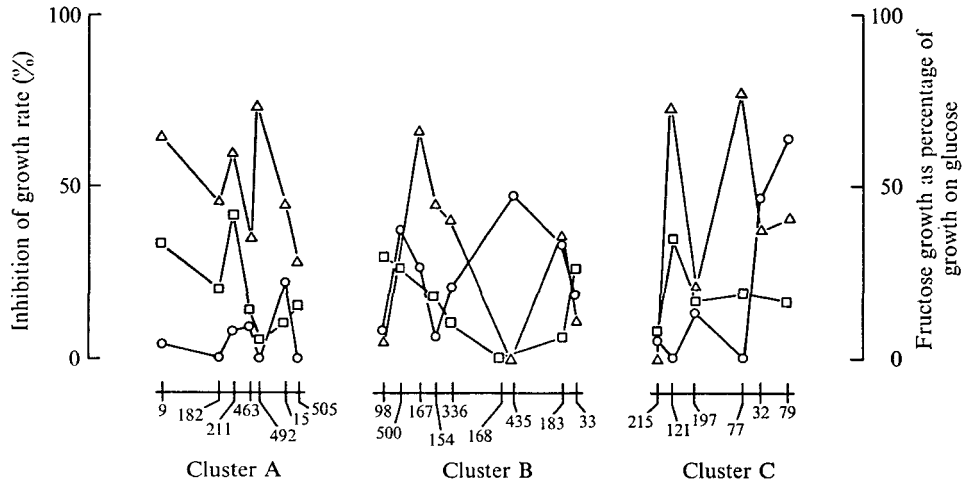

Fig. 2. Comparison of the physiological characters of some alleles with their positions in the allele map. The physiological characters plotted are: percentage inhibition of growth rate on medium containing 4 mm acetate + 1 mM deGlc (△) or 5 mM sorbose (○), and dry weight of mycelium formed on 5 mM fructose expressed as a percentage of that made on 5 mM glucose (□).

strikingly similar, but those drawn using alleles from clusters B and C show some remarkably consistent differences. Specifically, it seems that alleles placed centrally or just right of centre in cluster B were able to transport sorbose quite well while being far less able to transport fructose or deGlc. Conversely, alleles placed in the centre of cluster C had a greater ability to transport deGlc than either fructose or sorbose. Since the mutants have been positively identified as having some lesion in sugar transport it is tempting to conclude that the cistron specifies a permease molecule and that the clusters correspond to regions of the polypeptide which act as substrate binding sites. If this is true then we might further conclude from Fig. 2 that cluster A corresponds to a site which is equally important to the permease binding of all three sugars, that cluster B is representative of a site which is relatively less important to overall binding with the substrate sorbose, and cluster C relatively less important to binding with substrate deGlc. These cannot be considered as definitive statements because of the simple and indirect nature of the experiments. Nevertheless it is clear that a more comprehensive quantitative study would be very interesting indeed.

(v) *Additivity of distances in the allele map.* Additivity of intervals in the map is very good indeed, and this holds over the entire length of the map. It has been frequently observed in similar studies involving other fungi that additivity is lost over large allele to allele distances. Typically, large intervals have been found to have recombination frequencies which greatly exceed the sum of those of their smaller constituent intervals. Holliday (1964) called this phenomenon map expansion and introduced the map expansion plot in which the recombination frequency for each interval is compared with its map distance, the latter being computed by

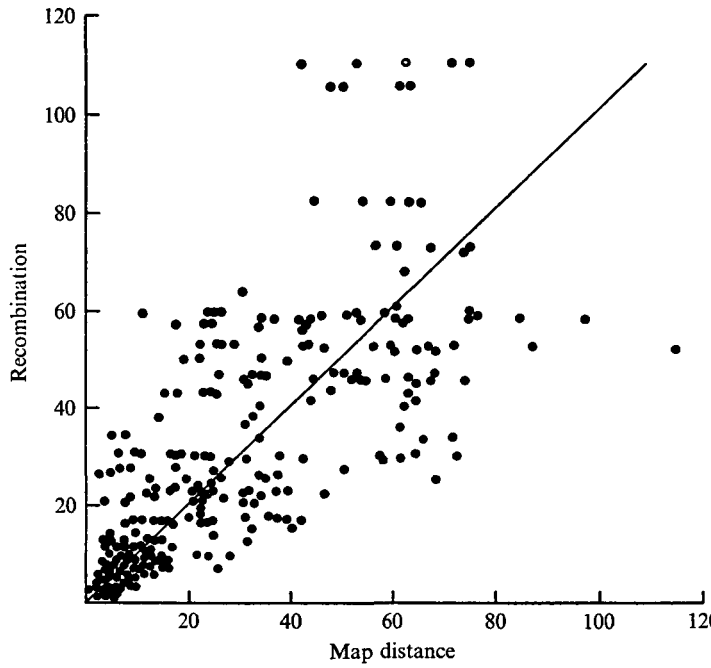

Fig. 3. Map expansion plot for the data obtained with the *ftr* cistron. Recombination frequencies are compared with map distances computed by summing the recombination frequencies of smaller intervals. For simplicity the plot contains comparisons against map distances computed only from trios of alleles. The line of slope 1 is drawn for reference.

summing the recombination frequencies of the smaller intervals of which the major one is composed. With strict additivity such a plot should indicate a line with slope 1; but in practice map expansion occurs and the plot produces a curve with a steadily increasing positive slope. The map expansion plot for the *ftr* cistron is shown in Fig. 3. It is clear that though there is a good deal of scatter of the points there is no forceful indication that anything other than a 1 : 1 relationship holds. In other words map expansion is absent. It is interesting to note that a quite detailed allele map of the *ur*-1 locus of *Coprinus radiatus* (Gans & Masson, 1969) also lacks map expansion.

The basic characteristic of the *ftr* cistron, therefore, is that the intervals between alleles are additive. There were, however, a number of significant deviations from this general rule. Non-additive recombination frequencies fell into three classes. There were those which were clearly due to experimental variation. For example, a cross between the NTG alleles 463 and 183 yielded a recombination frequency of 68·0. The interval between these two alleles spans 15 sites (considering only NTG mutants) and the experimental data for these smaller intervals can be combined (to calculate map distance) in 162 different ways. Map distances calculated in this way varied from 13·1 (519 % map expansion) to 62·7 (7·8 % map expansion) and had a mean of 32·2 (211 % map expansion). Conversely the recombination frequency between 463 and the site closest to 183 – site 200 which is only 0·3 units to the left

Table 4. *Recombination patterns of marker-effect alleles of cluster A which show enhancement of recombination frequency*

Marker effect allele	Reference allele			
	492	505	500	'60'
9	3·8	27·9	53·1	110·2
469	—	20·9	—	—
182	—	14·3	—	—
S7	—	15·2	—	71·6
S24	—	25·8	—	66·4
S29	—	14·5	—	67·8
15	1·5	0·9	30·5	59·5
463	0·5	7·8	21·6	58·1

Alleles 15 and 463 are included as examples of non-marker effect sites, they are respectively 3·7 and 1·7 units to the right of allele 182. Entries in the column headed '60' include crosses against other alleles which map at site 60.

of 183 – was 39·8. Data from other crosses involving sites 463 or 183 did not show any consistent trend towards unusually high recombination frequencies so it must be that the 463–183 result was caused by some random error rather than by any biologically significant phenomenon. Similar considerations can be applied to other crosses which yielded recombination frequencies which were not only inconsistent with the allele map but were also inconsistent with the pattern of results obtained from other crosses involving the same parents. In this way random errors can be distinguished from inconsistencies which had an overall trend suggestive of a biologically significant effect.

Two types of trend were evident in the inconsistent recombination frequencies recorded in this investigation. Of the 56 alleles included in the crossing programme, 4 (3 NTG-induced mutants – 65, 163 and 398; and 1 spontaneous mutant, S 4) each gave a set of recombination frequencies which could not be fitted into any consistent pattern. Such alleles cannot be mapped but the reasons for this behaviour are not at all clear. The recombination frequencies obtained with these alleles were almost always unusually high. Although it is noticeable that allele 163 has shown an unusually high frequency of reversion (Table 1) no definite suggestion can be put forward to explain this situation because of the lack of relevant information. It could well be that these alleles are representatives of that class of mutant site, evident for example in the *mtr* cistron of *Neurospora* (Stadler & Kariya, 1969) and the *lac* operon of *Escherichia coli* (Norkin, 1970), which has such a disturbing effect on recombination that their recombination frequencies are of no use in map construction.

In contrast to the type of allele just discussed there were a number of alleles in the fine structure map which showed sufficient consistency in their recombination frequencies with closely linked sites for them to be positioned unambiguously, and yet gave unusually high recombination frequencies in crosses against more

distantly placed alleles. Alleles showing this pattern of recombination are concluded to be marker effect alleles and are discussed in detail below.

(vi) *Marker effect.* It was noted earlier that the map expansion plot for the data recorded here shows a considerable scatter of points. A large number of the most divergent points were found to be contributed by crosses involving allele 9. Crosses in which one of the parents carried this allele generally yielded unusually high recombination frequencies. Similar patterns of results were obtained from crosses involving the NTG alleles 182 and 469 and the spontaneous mutants S 7, S 24 and S 29. These effects are illustrated in Table 4. There is no doubt that the alleles listed in Table 4 can be unambiguously mapped in a group at the extreme left-hand end of the cistron. Equally there is no doubt that the alleles individually expressed a marker effect which acted to increase the apparent frequency of recombination. So it can be concluded that this marker effect was not allele-specific but was instead a character of the entire region of the cistron in which these alleles were found. The region which can generate marker-effect alleles in this area of the cistron is quite clearly of limited extent. The boundary seems to be in the vicinity of site 211 for this allele had, on the whole, an unexceptional pattern of recombination frequencies. It is clear that marker effect was only seen when the interval between the alleles involved in a cross exceeded a certain minimum distance. The minimum seems to have been about 5–7 units. All of the alleles showed marker effect in crosses against members of the hotspot at site 505, but expression of the effect was variable, with alleles proximal to the latter site. Once the minimum distance was exceeded, though, it is evident that the effect extended over the whole length of the cistron (a distance of over 60 units); there was no upper limit to the size of interval which would allow expression of marker effect. There were some indications that the marker effect activity of allele 9 was responsive to the identity of the background genome. Inflated recombination frequencies were more often observed when this allele was used as a parent with $A_{40}B_{40}$ mating type, implying that the background attendant on this mating specificity emphasized the particular activity of the mutant. Expression of marker effect was not restricted to the $A_{40}B_{40}$ mating type though, and alleles 182, 469, S 7, S 24 and S 29 were exclusively used with their original A_6B_6 mating type and yet still exhibited specific enhancement of recombination frequencies. It might be imagined that this can be taken to imply that some sort of response to recombination control genes were taking place. That is, that the $A_{40}B_{40}$ background contained factors similar to the *rec* genes which have been identified in *Neurospora* (Catcheside, 1968). Two points argue against this idea. Firstly, *rec* genes are fully recessive and would not be expected to have expression in the crosses performed in this study; secondly, *rec* genes affect all allele pairs indiscriminately whereas there is a selective effect operating here. It is much more probable that the background effect resulted from some quite unspecific action, possibly but not necessarily expressed at the chromosome level, on metabolic stages which themselves influenced recombination. It may not be irrelevant to point out that the ZBw601/40,40 wild-type is far more vigorous and fertile than the BC9/6,6 wild-type and may therefore possess a far more 'efficient'

genetic background. Whatever the cause of the background effect it can be interpreted as indicating that allele 9 is at or near the left-hand boundary of the marker effect region of cluster A. This interpretation is made on the grounds that allele 9 marker effect is exhibited most strongly in a permissive background whilst alleles like 182 and 469 exhibit marker effect irrespective of genetic background. Allele 9, of course, is currently the most leftward site in the cistron and may coincide with the leftward terminus of the gene.

Allele 98 is situated at the extreme left-hand end of cluster B, and this allele also exhibited a marker effect which tended to enhance recombination frequencies. Examination of Table 2 will show that, like the marker effect region of cluster A, allele 98 enhanced recombination frequencies in crosses with other alleles providing these latter were about 7 units distant from 98; and that the effect had no upper limit of distance but extended over the whole cistron. In contrast to the cluster A situation, however, there was no evidence that sites closely linked to allele 98 shared its marker effect activities. In this case, then, the effect was site-specific. Neither did the effect show any mating type dependence in this case, but reference to Table 3 will reveal a different, but equally interesting, property of marker effect. This is that the effect was grossly emphasized in crosses against spontaneous mutants. This may have been due to the different natures of NTG-induced and spontaneous mutations. Malling & de Serres (1970) showed that NTG caused point mutations in *Neurospora* which were probably AT → GC transitions. If it can be assumed that this is true for *Coprinus* and, further, that spontaneous mutants could often be frameshift mutations rather than transitions then a possible explanation emerges. Marker effect must be exerted at the molecular level during recombination. It could therefore be that marker effect is further emphasized when a marker effect site is crossed with a second mutant which has a grossly disturbed coding pattern. An alternative explanation would be that the spontaneous mutants used were of the type which have an enhanced frequency of reversion at meiosis (Magni & Puglisi, 1966; Magni, von Borstel & Steinberg, 1966), but if this were so then one would have to add the constraint that enhanced meiotic reversion was not a character of the allele but of the particular pair of alleles under consideration since it does not seem to have grossly affected crosses against alleles other than 98. On the whole, the first suggestion is preferred, but as there is no data directly bearing on this point it is probably best to simply state that the expression of allele specific marker effect can be influenced by the nature of the mutation at the second site involved in a cross.

By far the most remarkable instance of marker effect revealed by this analysis was that which characterized the seven alleles (NTG mutants 388, 412, 421, 445, 496 and 505 and spontaneous mutant S 26) which map together in a hotspot in cluster A. This site was first recognized with allele 505 and so the whole group will be referred to as 'the site 505 hotspot'. These alleles were unique in that they showed consistently *reduced* recombination frequencies in certain crosses (Table 5). No explanation based on chromosomal abnormalities is satisfactory. The data of Table 5 show no consistency in this direction and the same strains which yielded

Table 5. *Patterns of recombination observed with marker effect alleles of cluster A which show reduction of recombination frequency*

Marker effect allele	Reference allele					
	463	98	500	154	88	336
505	7·8	18·3	15·8	—	—	—
388	7·4	25·9	6·1	6·2	30·9	—
412	3·9	27·9	—	12·6	9·7	—
421	4·7	19·6	4·2	5·6	34·6	—
445	—	15·9	9·1	12·0	10·7	—
496	3·6	52·1	10·0	—	—	13·5
S26	—	22·6	17·5	3·5	—	5·3
15	3·8	35·0	30·5	16·9	—	38·0
492	0·5	—	22·9	—	—	22·9

Alleles 15 and 492 are included for comparison, they are respectively 0·9 and 2·4 units to the left of the site 505 hotspot.

the recombination frequencies illustrated in that Table were used in other crosses whose results led to the unambiguous ordering of cluster B and the relative ordering of clusters A and B. It follows, therefore, that this must have a genuine site-specific marker effect of the site 505 hotspot members. Three interesting characteristics of this inhibition of apparent recombination are evident from Table 5. First, the effect was of limited extent; it seems to have lessened in severity the greater the interval between the site 505 allele and the second mutant in the cross. Specifically it appears to die out at about the centre of cluster B. Secondly, the effect was unidirectional, crosses between site 505 and other members of cluster A were not affected. Thirdly, crosses against site 98 yielded approximately 'correct' recombination frequencies. To illustrate: the weighted mean of all crosses (not including sites 505 or 98) made between clusters A and B can be used to set the probable maximum size of the interval between those clusters; this works out at 28·2 units. The weighted mean of all crosses made between site 98 and members of the site 505 hotspot is 24·2. Site 98 has already been identified as one which shows marker effect enhancement of recombination frequencies. This behaviour in crosses against site 505 leads to the conclusions that (*a*) site 98 marker effect extends in both directions from that site (see also the higher than expected recombination frequency with allele 15), and (*b*) site 98 *enhancement* of recombination frequency was equalled and cancelled by site 505 *reduction* of recombination frequency.

The various conclusions about marker effects on recombination which can be drawn from these experimental data can be summarized as follows: (*a*) marker effect may act to enhance or reduce the frequency of recombination; (*b*) enhancement is about equal to and is effectively opposite to reduction of recombination frequency; (*c*) there was a certain minimum distance between alleles which had to be exceeded before marker effect enhancement was observed, but there was no upper limit, i.e. additivity was not restored even though intervals closely approaching the total length of the map were represented in the data; (*d*) marker effect

enhancement may appear to be unidirectionally polarized when the site is terminal or subterminal in the cistron, but centrally placed marker effect sites exert their effect in both directions; (*e*) marker effect reduction of recombination frequency did show unidirectional polarization, though obviously this need not necessarily always be the case; (*f*) expression of marker-effect enhancement was influenced both by the background genome and by the nature of the mutation at the second site in a heteroallelic cross. It is also worth noting that of the three marker-effect situations recognized, one involved a relatively lengthy region of the cistron while another involved a mutational hotspot. This is taken to imply that marker effect activity of a site is related to other peculiarities of the DNA; in the first case a particular coding differentiation related to function and in the second a particular coding pattern which is unusual also in its response to mutagens.

(vii) *The interpretation of marker effect and map expansion.* The data for the *ftr* cistron certainly do not satisfy the predictions of the marker-induced excision-repair model of map expansion suggested by Fincham & Holliday (1970). This model was based on the assumption that map expansion and recombination itself were initiated by some sort of marker induction effect at the excision-repair stage. It seems likely that this basic assumption is wrong. Map expansion is certainly not an inevitable observation. The overall impression given by the *ftr* data is that recombination does, in general, occur at random and that marker effects are imposed sophistications rather than necessary prerequisites of the process.

The apparent regularity with which map expansion has been observed in Ascomycetes may mean that the process of recombination differs significantly from that which operates in Basidiomycetes. This seems unlikely. It is noticeable that in the *ftr* cistron marker effects are correlated both with mutational hotspots and with particular short regions of the gene. If this is generally the case then the regular observations of map expansion in Ascomycetes may be an artifact. If a marker-effect site is one of a number of similar sites in a small region of the gene, or is a single site which is also a mutational hotspot, then that site will be represented unusually frequently in any randomly isolated set of mutants. If an allele map is constructed from very few alleles then it is not inconceivable that non-marker effect sites will not be represented at all. Many of the allele maps which show 'classic' map expansion do indeed contain very few sites (see Fincham & Holliday, 1970). This is not intended to suggest that map expansion is itself an artifact for the interpretation of map expansion as an expression of marker effect is favoured in this discussion. Rather, it is suggested that organized map expansion of the sort which lends itself to formalization as a typical map expansion plot is most often an artifactual result of inadequate data. These interpretations cannot be easily reconciled with current models of recombination by hybrid DNA formation. Although specific adjustments could be suggested both current models have a serious fault which has received scant attention.

(viii) *Recombination through the formation of hybrid DNA.* Current theories of recombination via the formation of regions of hybrid DNA envisage hybrid formation to be a consequence of initial breakage of DNA strands (Whitehouse, 1963,

1966; Whitehouse & Hastings, 1965; Holliday, 1964, 1968). There are considerable difficulties with this premise. Unless restrictions are placed on the formation and/or positioning of the initial strand breaks one can rapidly reach the uncomfortable conclusion that strand breakage can occur at any time between any pair of adjacent bases. The conditions which are usually applied to prevent the argument reaching this position are that breakage results from the activity of specific enzymes and/or occurs only at fixed points.

The polaron model of recombination (Whitehouse & Hastings, 1965) seeks to restrict initial strand breaks (the opening points) to the end or ends of a gene or sequence of genes, with the possibility that the opening point coincides with the operator of a operonic structure (Whitehouse, 1966). Such an extreme restriction in the position of the point of breakage leads to predictions of site distributions in allelle maps which are not supported by available information. Mutant sites close to the point where DNA hybridity is most often initiated would more often show recombination than would sites distant from the opening point. A composite allele map would thus show a clustering of apparently unusually closely linked sites in the region where hybrid DNA was least frequently formed. Clustered allele maps are common but they usually have more than one cluster, e.g. the *me*-2 and *mtr* loci of *Neurospora* (Murray, 1963; Stadler & Kariya, 1969) and the *ftr* cistron of *Coprinus* illustrated above. This situation is not compatible with the polaron model unless further restrictions are imposed on the mean lengths of hybrid DNA regions (Whitehouse & Hastings, 1965).

The concept that there exist 'linkers' between genes at which breakage of strands occurred was a common feature of the original versions of both current models. The Holliday model has moved away from this original conception however. An argument is developed for coding sequences which are genetic discontinuities only in that they are substrate sequences for a recombinase enzyme (Holliday, 1968). These recombinator sequences are considered to be relatively uncommon and thus often to be outside the gene in which recombination events are being studied. They are also suggested to have meaning in the DNA-to-protein sense so that they could occur within genes rather than be restricted to positions between genes. These ideas are quite plausible, but as will be discussed below they do create additional mechanical problems for the Holliday model.

(ix) *Unwinding of the DNA helix.* Any model of recombination which postulates the formation of hybrid DNA must take into account the unwinding of established helices which is necessary before the new hybrid helices can be constructed. Conceptually, models of recombination bring the participating chromatids into such close proximity that molecular exchanges become possible. It follows that unwinding of the helix must also be assumed to take place within a very confined space. Levinthal & Crane (1956) showed that the energy required for unwinding is small, but they pointed out that unwinding from the end of a DNA molecule involves the danger of entanglement of loose ends and that considerable viscous drag would be experienced by the free ends as they rotated through the suspending fluid. Recombination models postulating initial strand breakage must face the same

dangers during unwinding of one (or both) of the free ends of the broken molecule. Both current models postulate breaks and unwinding in one strand of each of two non-sister chromatids (each chromatid being assumed to consist of one DNA double helix). Thus unwinding of a free end is taking place in the same regions of two closely appressed molecules and entanglement is a real possibility. However, the dangers are increased by particular features of each model. The Whitehouse model requires that the strands, after unwinding, remain free for a length of time sufficient for the synthesis of duplicates of the unwound segments (using the unbroken but now unpaired complementary strands as templates). On completion of this synthesis the newly formed strands themselves unwind from their templates and when this is accomplished there are four free ends and six unpaired segments of DNA in the same region. As indicated above, the later version of the Holliday model abandons the idea of linkers between genes in favour of recombinator sequences scattered along the chromosome. If, as is suggested, there are few recombinator sequences then in the majority of cases recombination would require the unwinding of a considerable length of DNA. In this situation the possibilities for entanglement, folding and aberrant pairing are obviously great.

Although unwinding of the double helix is a necessary prerequisite of hybrid DNA formation, strand breakage is not a necessary prerequisite of unwinding. Fong (1964) has shown that the conditions for unwinding are far more favourable if it is assumed that unwinding is initiated *within* the *intact* double helix. In this analysis it was assumed that the process started by the severance of a few hydrogen bonds at the midpoint of the molecule. The argument was then developed to show that once the bonds are released the intact segments of the molecule are free to rotate essentially as rigid rods in order to take up the unwinding of the interstitial regions. Even a completely randomized rotation process was shown to contribute to extremely rapid unwinding. No covalent bonds are broken in the process, viscous drag is minimized and the dangers of gross entanglement are greatly reduced. Cross & Lieb (1967) proposed a model of prophage detachment which involved unwinding of the helix before strand breakage. As we have seen, there is considerable theoretical support for such a model. Application of this concept to eukaryote recombination leads to the hypothesis which is detailed below and illustrated in Fig. 4.

(x) *A model of recombination which does not require initial strand breakage.* The chromatid is assumed to consist of a single DNA double helix and during meiosis homologous chromatids lie side by side in close molecular proximity (Fig. 4*a*). At random points within one chromatid hydrogen bonding between a few bases fails and the released bases swing out of the helix (Fig. 4*b*). Strand separation by interstitial unwinding is thus initiated in this chromatid and as it proceeds a similar series of events is triggered in the homologous (non-sister) chromatid, possibly as the result of interference with pairing relations by the freed bases of the adjacent molecule. Unwinding will continue until the wound parts of the molecule are no longer free to rotate (Fig. 4*c*). Extensive overwinding of a double helix is not admissible because of the already close atomic packing. However, even if the ends

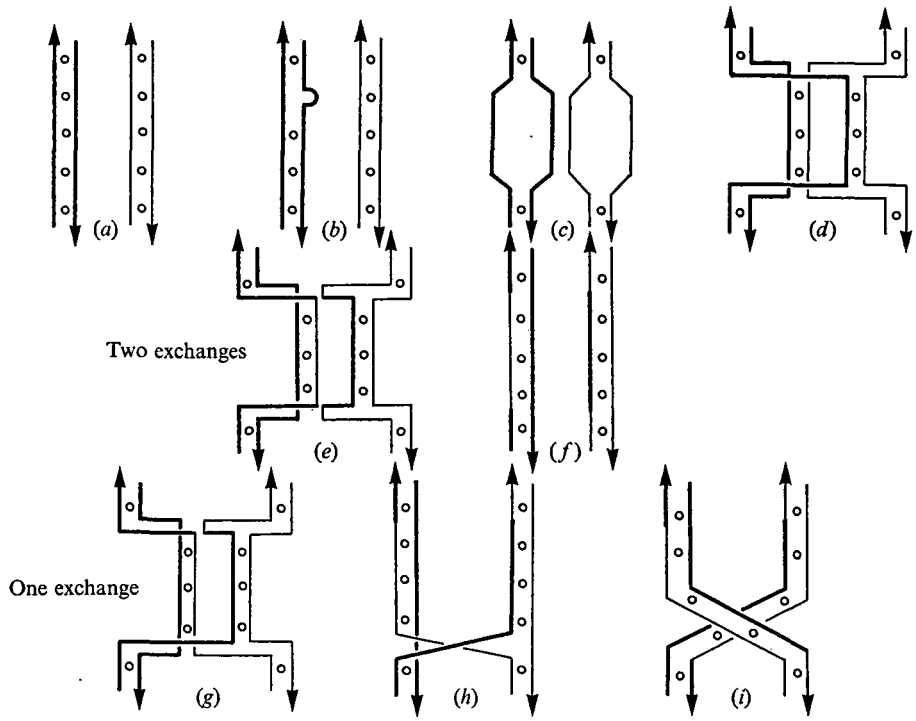

Fig. 4. Recombination through interstitial unwinding. The chromatid is assumed to consist of a single DNA double helix and only the two non-sister chromatids actually involved in recombination are figured. Hydrogen bonding is indicated by open circles and chemical polarity of the strands by arrow heads. (*a*) represents the initial configuration of the molecules; in (*b*) hydrogen bonding has failed between a few bases of the 'heavy' helix and those bases have swung out of the helix; (*c*) unwinding proceeds in this molecule and is induced in the neighbouring 'light' helix; (*d*) shows the configuration attained when the strands pair illegitimately; in (*e*) two breaks have occurred, one at each end of the illegitimately paired region, giving hybridity but no crossover (*f*); in (*g*) a single exchange has taken place, forming a half-chiasma (*h*) which is completed by breakage and exchange between the two strands not so far involved (*i*).

of the molecule are fixed, the extent of any one unwinding event may be so small that the tendency to overwind is acceptable. Alternatively, the rotation may be taken up in the form of molecular deformation as relational or supercoiling. In either case a limitation on rotation would be expected, and when this point is reached rewinding will tend to occur in order to release the potential energy stored by the unwinding process.

The amount of unwinding which occurs before rewinding commences will depend on the extent of rotation available to the wound parts of the molecule. This will in turn depend on a number of conditions: the amount of overwind which is admissible; the position within the molecule of the unwinding region; the amount of relational and/or supercoiling already existing; the number of unwinding events taking place. One would therefore expect that the length of DNA unwound would be normally distributed about a certain mean value and that the mean value will differ

between different chromosomes and even between different regions of the same chromosome.

Once rewinding starts there are two possible outcomes. Within the unwound parts of the two homologous chromatids there now exist four single-stranded DNA molecules, each of which is capable of pairing with one of two others. Legitimate pairing relationships may be re-established, so that rewinding will restore the chromatids to their original configurations. On the other hand, illegitimate pairing, between the '+' strand of one chromaid and the '−' strand of the other, is equally likely (Fig. 4*d*). In this configuration complete rewinding is impossible and there will inevitably be regions of stress at each end of the illegitimately paired segments where the strands exchange their legitimate for their illegitimate partners. This stress will be caused by the attempted release of the potential energy for rewinding stored in the intact parts of the molecules. It can be relieved by the exchange of covalent links between strands of the same polarity since this will immediately legitimize an illegitimate pairing relationship. If two such exchanges occur the situation shown in Fig. 4(*e*,*f*) will result; each chromatid having a region of hybrid DNA but with no crossover resulting. If only one exchange occurs, rewinding over the hybrid segment will lead to the situation shown in Fig. 4*h*, and a second break in the strands not so far involved in exchange of covalent links will form both hybridity and a crossover (Fig. 4*i*). Strand breaks will be enzymically repaired to reconstitute intact molecules, and immediately legitimate pairing relationships are established rewinding of the molecules can proceed normally.

(xi) *Implications of the model.* The sequence of events postulated in this model is (*a*) strand separation, (*b*) illegitimate pairing and (*c*) strand breakage. The fundamental difference between this and other current models of recombination is thus that here strand breakage is assumed to occur after unwinding and after the formation of hybrid regions of DNA. Events subsequent to the formation of hybrid DNA need not necessarily differ from those postulated in other models, although some specific differences are suggested later.

The proposed model has the virtue of simplicity. It appears to make use of the natural abilities of the molecules involved and this makes it attractive from the evolutionary point of view. The evolutionary development of organized genetic information must have carried with it the almost inevitable need to exchange that information. The model proposed here, making use as it does of what is essentially an error in the arrangement of DNA, could be envisaged even in a very primitive cellular system. Apart from the repair of strand breaks enzymic processes are not absolute requirements. The model would not be affected, however, by the introduction of such processes. For example, a specific 'unwinding enzyme' could be suggested, although it could, perhaps more reasonably, be considered instead that DNA molecules are subject to a continuous flux of unwinding and rewinding of very short randomly located regions in response to fluctuations in the cellular environment. On the other hand strand breakage is an obvious candidate for enzymic attention. The potential for rewinding may not be sufficiently great to cause spontaneous breakage of the covalent bond. However, the tangled strands at

each end of the illegitimately paired regions could well be recognized as points of error by DNA repair systems. It would then be unnecessary to postulate either linkers or recombinator sequences to account for breakage in the same place in two physically different molecules.

The most cogent reason for postulating fixed opening points in the DNA molecule is that this simple suggestion provides an elegant explanation for the polarization in recombination which has been frequently observed. If initial strand breakage is, as here, to be dismissed then some other explanation of polarization must be offered. To suggest that there may be sites or regions in a DNA molecule which are more than usually liable to commence unwinding seems no more unlikely than to suggest that there are sites at which breakage occurs with unusual frequency. Hence to explain polarization with the model proposed here simply requires that the fixed (or preferred) opening points of the Whitehouse and Holliday models become fixed (or preferred) unwinding points. However, the position with regard to polarization is now extremely complex (see comments and references in Paszewski, 1970). It could well be that polarization can best be explained as some sort of site-specific effect so that it is less important to provide a general explanation which requires the imposition of a fundamental restriction on the proposed model.

In the context of the data presented for the *ftr* cistron the proposed model has a number of advantages. In particular, marker effect can be interpreted as having a secondary rather than a primary role in recombination. Randomized unwinding events offer a means of explaining a general equivalence between recombination frequencies and the relative distances between alleles, particularly if it is assumed that the amount of DNA unwound in any one event is small compared with the length of the gene. However, it is relatively easy to foresee situations, resulting from peculiar coding sequences, which could impose some preference on this randomized process and thus lead to marker effect. Norkin (1970) has in fact demonstrated that the pattern of marker effect is altered by specific base changes either at a mutant site or even at some distance from the mutant site. A reasonable interpretation seems to be that a marker effect site is one at which mutation has so changed the coding sequence that unwinding of the helix is more (or less) likely than normal in its vicinity. This means that marker effects on recombination depend not only on the coding pattern at the mutant site but also on the coding pattern of the normal gene. One could envisage genes which have normal coding patterns in which the most usual mutations can never produce sequences which have a disturbing effect on the basic process of recombination. Such genes would have almost perfectly additive allele maps and an example might be the *ur*-1 locus of *Coprinus radiatus* (Gans & Masson, 1969). At the other extreme there would be genes whose coding pattern was such that more often than not mutation produced a marker effect sequence. In this case any relationship between recombination frequency and distance may be entirely lost so that allele maps would be difficult or impossible to construct; an example might be the *mtr* locus of *Neurospora* (Stadler & Kariya, 1969). One would expect that the majority of genes would lie between these extremes, showing reasonably additive allele maps but with some

significant departures from the general trend caused by the occasional marker effect sites or regions; e.g. the *Coprinus ftr* cistron described above and the *ad*-8 locus of *Saccharomyces cervisiae* (Esposito, 1968).

This discussion assumes, of course, that marker effect is exercised at an initial stage of recombination, specifically helix unwinding, a stage which does not actually produce recombinant chromatids but which establishes the molecular configuration which is necessary if these are to be formed. This is contrary to Fincham & Holliday's (1970) interpretation of map expansion being due to marker-induced excision-repair. The difference in emphasis results mainly from the relative importance which is attached to the phenomenon of map expansion. It is here shown that marker effects can result in map contraction and that, consequently, map expansion is not an inevitable observation. The possibility remains that effects which operate at the excision-repair stage may also disturb the postulated general correspondence between recombination frequency and distance. Such effects can be accommodated if the further assumption indicated earlier is made; that is, that the primary error recognized by the excision-repair system involved in recombination is not the mismatching of bases but the disorganized and tangled strands at either end of the illegitimately paired segments of DNA. This would mean that for the most part excision-repair would be induced by the tangled regions and that the system would proceed with DNA excision and replacement independently of the character of the mutational alterations present in the hybrid region. Exceptions to this general rule would be those situations where a mutation has produced a coding pattern which does significantly inhibit or assist the repair process and these would yield another class of marker effect site.

The proposed model primarily rests on a series of assumptions which are, unfortunately, extremely difficult to test. The contrasts between initial unwinding and initial breakage essentially depend on points of interpretation and strength of argument, there being no obvious means of experimentally deciding between the two except at the ultimate molecular level. On the other hand the model would gain considerable support if it could be shown that enzymes which might be implicated in recombination are able to recognize and respond to conformational alterations in paired DNA molecules in addition to specific chemical (i.e. base mismatch) alterations.

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