COMMON REGULATION OF RECOMBINATION AT THE *amination-1* AND *histidine-2* LOCI IN *NEUROSPORA CRASSA*

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Summary

Recombination between alleles is reduced by *rec-3*$^+$ at the *am-1* locus and by *rec-x*$^+$ at the *his-2* locus. The two recombination genes are both in the same region of the left arm of linkage group I between *acr-3* and *arg-3*. All known stocks are either *rec-3 rec-x* or *rec-3*$^+$ *rec-x*$^+$. Recombination between *rec-3* and *rec-x* is less than 0·143%, with 95% probability. The evidence suggests that the two *rec* genes are the same, although they act on unrelated loci.

I. INTRODUCTION

It was shown (Catcheside 1966) that allelic recombination between *am-1*^{2} and *am-1*^{6} is greatly reduced by the presence, in either parent or both, of the gene *rec-3*$^+$. The degree of reduction is of the order of 10- to 30-fold and the effect extends to all combinations of *am-1* alleles, 12 in number, so far examined (Smyth 1971). The *rec-3* locus lies in linkage group I between the *mating type* (*mt*) and *arginine-3* loci (Catcheside 1968).

Another gene, *rec-1*$^+$, controls recombination at the *histidine-1* locus. In the course of experiments to determine whether it acts on any other *histidine* locus, a further *rec* gene was found to control allelic recombination at the *histidine-2* locus. A dominant, called *rec-x*$^+$, reducing recombination at the *his-2* locus about sixfold, is present in several wild strains, but not in Emerson a. Moreover, *rec-x* has proved to be linked to *his-2* and a small number of recombinants between the loci of *his-2* and *mt* places it about midway between them (Catcheside and Austin 1969).

This raises the question whether *rec-x* and *rec-3* could be the same gene. There is the parallel between the orders *mt rec-3 arg-3 his-2* and *mt rec-x his-2* and the fact that all stocks known are either *rec-3*$^+$ *rec-x*$^+$ or *rec-3 rec-x*. Until it could be shown that *rec-3* and *rec-x* are different, it was considered more convenient to assign a symbol with a letter instead of a number to the gene which controls recombination at the *his-2* locus. This paper reports more exact mapping of these *rec* loci and unsuccessful attempts to separate them.

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II. Material and Methods

The following mutants have been used in the experiments:

am-1	47305 (*am-1*^{2}) and K314 (*am-1*^{6})	*acr-3*	KH14 (from the Fungal Genetics Stock Centre Nos. 1209 and 1215)
his-2	K584 and K612	*ad-3A*	K118
arg-3	K125	*cot*	C102

Of other loci in the general area, use of *arg-1* and *phen-1* were avoided because the growth supplements, respectively arginine and phenylalanine, also support the growth of *am-1* mutants. The use of ethylacetoacetate for growing *phen-1* mutants was unsuccessful. The locus *ad-5* would be useful, but stocks alleged to contain this mutant appeared to have only *ad-7*. Mutants at the *suc* (*succinate*) locus proved to grow too well on minimal medium to permit their use for selective purposes. The temperature-dependent colonial mutant C102 was incorporated into all stocks to facilitate assay procedures.

All crosses were made in 6-in. tubes containing liquid crossing medium (Westergaard and Mitchell 1947) suitably supplemented and provided with a folded strip of filter paper. Ascospores were harvested in water and, after concentration, were suspended in warm, molten, layer agar in screw-capped bottles and heated in a water-bath for 50 min at 56°C to induce germination. The search for particular kinds of recombinants, especially between *rec-3* and *rec-x*, was done by plating on selective SGF media and testing on plates of SS medium. The basic formula for all media used for vegetative growth is that of Vogel (1964). The SGF media contained, per litre, 5 g sorbose, 0·125 g glucose, and 0·25 g fructose; the SS media contained, per litre, 5 g sorbose and 1 g sucrose; suitable supplements required by mutants were added in the following quantities per litre: 300 mg histidine, 450 mg alanine, 1500 mg glycine, 500 mg arginine, 200 mg citrulline, 10 mg acriflavin, and 100 mg adenine. All plates were incubated at 25°C for 16 hr and then at 34°C for 24–30 hr before counting.

Assays for prototrophs were performed by pipetting known volumes of an undiluted suspension of ascospores on agar plates containing suitable selective SGF medium. Samples were also diluted in layer agar and known volumes were pipetted on agar plates containing fully supplemented SGF medium, so that all viable ascospores could grow and form colonies. Where it was necessary to classify the prototrophs further, samples were isolated to plates of SS medium, supplemented with particular growth factors.

Table 1

Data on recombination between the *mating type* locus and other loci

Interval	Progeny Classified		Recombination Value (%)
	Total	Recombinant	
mt acr-3	122	10	8·2±2·5
mt rec-3	287	27	9·4±1·7
mt arg-3	279	42	15·1±2·1
mt his-2	349	63	18·1±2·1
mt ad-3A	238	76	31·8±3·0

III. Results

(*a*) *Location of* rec-3

When *rec-3* was first described (Catcheside 1966), it had been demonstrated that it was linked either to the *am-1 his-1* region in linkage group V or to the *mt* locus in linkage group I. Subsequently, when suitable *rec-3* tester strains had been constructed, it was possible to show linkage, of the order of 10%, to the *mt* locus in the left arm of linkage group I (Table 1).

To determine whether *rec-3* is distal to the *mt* locus or proximal to it, a cross of the consitution A *rec-3*$^+$ *am-1*^{2} *cot* × a *rec-3 ad-3A* was made and the *rec-3* constitution of selected recombinant progeny was determined. Ten a *ad-3A am-1*^{2} *cot* were shown to be *rec-3*, in confirmation of the expected constitution of the a *ad-3A* stock which had been derived directly from Emerson a, itself known to be *rec-3*. Information about the position of the *rec-3* locus was drawn from progeny recombinant between the loci of *mt* and of *ad-3A*. Classification of these progeny gave the following results:

	rec-3	*rec-3*$^+$
A *ad-3A cot am-1*^{2}	**4**	**7**
a + *cot am-1*^{2}	**3**	**2**

The six progeny in bold type are those which show recombination between the *mt* locus and the *rec-3* locus. The proportion of recombinants is similar in the two classes. This is inconsistent with *rec-3* being distal to the *mt* locus and places it between the *mt* and *ad-3A* loci, relatively nearer to the former. The length of the *mt ad-3A* segment is about 32 centimorgans and the distance of *rec-3* from the *mt* locus would be about 12 centimorgans.

Next, progeny from the cross A *rec-3*$^+$ *cot am-1*^{2} × a *rec-3 arg-3* were analysed similarly. The locus *arg-3* was chosen because mutants at this locus can be grown on citrulline, which will not support the growth of *am-1* mutants. Eleven a *arg-3 cot am-1*^{2} progeny were shown to be *rec-3*, confirming the constitution of the K125 stock assumed from its origin directly from Emerson a. The 11 progeny which were recombinant between *mt* and *arg-3*, among the 93 *am-1*^{2} obtained, showed the following results when classified for *rec-3*:

	rec-3	*rec-3*$^+$
A *arg-3 cot am-1*^{2}	**3**	**1**
a + *cot am-1*^{2}	**2**	**5**

A majority of the recombinants between *mt* and *arg-3* are also recombinant between *mt* and *rec-3*, namely the eight in bold type, making the order *mt rec-3 arg-3 ad-3A* quite certain. The *rec-3* locus appears closer to *arg-3* than to *mt*, but the data are too few for certainty.

The information bearing on this question has been considerably extended by classification of progeny of similar origin kindly provided by Dr. D. E. A. Catcheside. These were derived from a cross designed to map a locus, later found to be *nit-2*, mutants at which interfere with the capacity of *am-1* mutants to adapt to grow on minimal medium. The cross was of the constitution *nit-2* a ++; *cot*; *am-1*^{2}× + A *rec-3* arg-3; *cot*; *am-1*^{2}. The progeny classified for *rec-3* (out of 186) were 31 which were recombinant between *mt* and *arg-3* and 30 which were recombinant between *nit-2* and *mt*. The results, presented as before with recombinants between *mt* and *rec-3* in bold type, are as follows:

	rec-3	*rec-3*$^+$
nit-2 a *arg-3 cot am-1*^{2}	**6**	8
+ A + *cot am-1*^{2}	10	**7**
nit-2 A *arg-3 cot am-1*^{2}	18	**0**
+ a + *cot am-1*^{2}	**0**	11

These data confirm the location of *rec-3* between *mt* and *arg-3*, but suggest a position relatively closer to *mt*.

Pooling of the data from these two experiments shows a map distance of $15 \cdot 1 \pm 2 \cdot 1$ centimorgans between *mt* and *arg-3* (Table 1) with *rec-3* precisely at the mid-point, there being 21 recombinants between *mt* and *rec-3* and 21 between *rec-3* and *arg-3*. The *rec-3* locus is therefore placed, by these experiments, at a distance of $7 \cdot 5 \pm 1 \cdot 6$ centimorgans from *mt* and from *arg-3*. The total number of unselected progeny which have been classified for recombination between *mt* and *rec-3* is 287, the 27 recombinants observed being $9 \cdot 4 \pm 1 \cdot 7\%$. The weighted estimate of the distance *mt rec-3* is $8 \cdot 4 \pm 1 \cdot 2$ centimorgans.

Further refinement of the position of *rec-3* has been achieved by using other loci between *mt* and *arg-3*. The resulting data are considered in Section III(*c*).

(*b*) *Location of* rec-x

When the effect of *rec-x*$^+$ in reducing recombination at the *his-2* locus was discovered, it soon became evident that *rec-x* is closely linked to *mt* and to *his-2*. In crosses of the type a *rec-x his-2* × A *rec-x*$^+$ +, all a *his-2* progeny were *rec-x* while the few A *his-2* were *rec-x* or *rec-x*$^+$ in nearly equal numbers. This means that the locus of *rec-x* is between the *mt* and *his-2* loci.

The *mt* and *his-2* loci are $18 \cdot 1 \pm 2 \cdot 1$ centimorgans apart (Table 1). The distribution of recombination between them in the intervals *mt rec-x* and *rec-x his-2*, derived from the classification of 62 recombinants, is as follows:

Type of Cross	Recombinant Interval	
	mt rec-3	*rec-3 his-2*
a *rec-x his-2* × A *rec-x*$^+$ *his-2*$^+$	24	26
A *rec-x*$^+$ *his-2* × a *rec-x his-2*$^+$	4	8
Totals	28	34

These data place *rec-x* at a position $8 \cdot 2 \pm 1 \cdot 5$ units from *mt* in the direction of *his-2*. This is close to the position of *rec-3*. Since, at this stage, all stocks classified for these genes were either *rec-3 rec-x* or *rec-3*$^+$ *rec-x*$^+$, the evidence suggests that *rec-3* = *rec-x* and *rec-3*$^+$ = *rec-x*$^+$. Closer location of these genes and further tests of their identity were subsequently attempted as joint enterprises.

(*c*) *Experiments to Separate* rec-3 *and* rec-x

It is of course impossible to prove conclusively that *rec-3* and *rec-x* are the same gene. However, if attempts to separate them genetically are unsuccessful it becomes more probable that they are one and the same gene and an upper limit can be set to their distance apart genetically. The method used is to select for recombination between two loci spanning the interval in which *rec-3* and *rec-x* lie and to examine these selected progeny for their *rec-3* and *rec-x* constitutions. The shorter the interval within which recombinants are selected, the more efficient is the test of separability of *rec-3* and *rec-x*.

So far, satisfactory data have been obtained using *acr-3* and *arg-3* as the boundary loci of the segment in which *rec-3* and *rec-x* lie. Two crosses, as follows, have been used:

10154 a *acr-3*^{r} *rec-3 arg-3* +; *cot*; *am-1*^{2} × A + *rec-x*$^+$ + *his-2*; *cot*; +

10155 a *acr-3*^{r} *rec-3*$^+$ *arg-3* +; *cot*; *am-1*^{2} × A + *rec-x* + *his-2*; *cot*; +

Progeny of the constitution *acr-3*^{r} *his-2* *am-1*^{2} were selected from each of them by plating on SGF + acriflavin + histidine + alanine and saving those which require both histidine and alanine for growth as well as being resistant to acriflavin. These progeny, of constitution a *acr-3*^{r} *his-2*; *cot*; *am-1*^{2}, were classified for *rec-3* versus *rec-3*$^+$ and *rec-x* versus *rec-x*$^+$ by analysing crosses made to suitable testers.

The frequency of recombination between the *acr-3* and *arg-3* loci was determined by selective plating and counting one (*acr-3*^{r} *arg-3*$^+$) of the two kinds of recombinant in this segment. The data, which are consistent for four different crosses of different parentage, show 1367 recombinants of this class in a population estimated to be 117,910. This shows the interval to be $2 \cdot 32 \pm 0 \cdot 07$ centimorgans.

So far, 47 recombinants between *acr-3* and *arg-3* have been classified for *rec-3* and *rec-x* with the following results, those recombinant in the *acr-3* to *rec-3* and *rec-x* interval being in bold type:

Cross	*rec-3 rec-x*	*rec-3*$^+$ *rec-x*$^+$
10154	7	**2**
10155	**13**	27

The difference in frequency of *am-1*$^+$ prototrophs in tests of *rec-3* and *rec-3*$^+$ progeny is great enough to allow an unambiguous distinction. The highest frequency of *am-1*$^+$ prototrophs in tests of *rec-3*$^+$ progeny was $2 \cdot 2 \pm 0 \cdot 6$ per 10^5 and the lowest frequency in tests of *rec-3* progeny was $17 \cdot 4 \pm 2 \cdot 1$ per 10^5. The distinctness of the two classes is illustrated graphically in Figure 1(*a*), in which the logarithms of the prototroph frequencies at the *am-1* and *his-2* loci are plotted against one another. The distinction between *rec-x* and *rec-x*$^+$ is less clear. The *rec-3*$^+$ progeny ranged, in the crosses made to the 7560 tester, from $2 \cdot 4 \pm 1 \cdot 1$ to $12 \cdot 9 \pm 3 \cdot 6$ per 10^5 in *his-2*$^+$ prototroph frequency. The *rec-3* progeny ranged from $8 \cdot 6 \pm 1 \cdot 8$ to $72 \cdot 0 \pm 18 \cdot 7$ per 10^5. The ranges overlapped and there was no distinct separation into two frequency classes. Hence the classification of some progeny in the border zone definitely as *rec-x* or *rec-x*$^+$ could not at first be made with confidence. Nevertheless, no *rec-3* progeny were clearly *rec-x*$^+$ and no *rec-3*$^+$ progeny were clearly *rec-x*.

Some of the uncertainty arose from rather poor fertility of some of the test crosses, but some probably arose from genetic modifiers of the action of *rec-x* and *rec-x*$^+$. It was decided to retest all progeny with observed values ranging from $6 \cdot 3$ to $18 \cdot 4$ per 10^5 (Table 2, column 2) with a number of additional *rec-x* tester strains. The next smaller and larger values shown by progeny not retested were $5 \cdot 5 \pm 0 \cdot 8$ and $22 \cdot 3 \pm 2 \cdot 3$. Altogether, 11 progeny, 10 being recombinants between *acr-3* and *arg-3*, were re-examined with the results summarized in Table 2. Collectively, the more extensive data allow an unambiguous classification into *rec-x* and *rec-x*$^+$ classes. Those progeny which are *rec-3* now show higher frequencies of *his-2*$^+$ progeny and those which are *rec-3*$^+$ show lower or unchanged frequencies. The classification is illustrated in Figure 1(*b*), in which the logarithms of the *am-1*$^+$ and *his-2*$^+$ prototroph frequencies are used as coordinates, the 11 which were in the boundary zone, between the solid horizontal lines in Figure 1(*a*), being identified by distinct symbols.

It may therefore be concluded with confidence that none of the recombinants between *acr-3* and *arg-3* are recombinants between *rec-3* and *rec-x*. If it is assumed that *rec-3* and *rec-x* are two separate genes, the absence of recombinants sets an upper

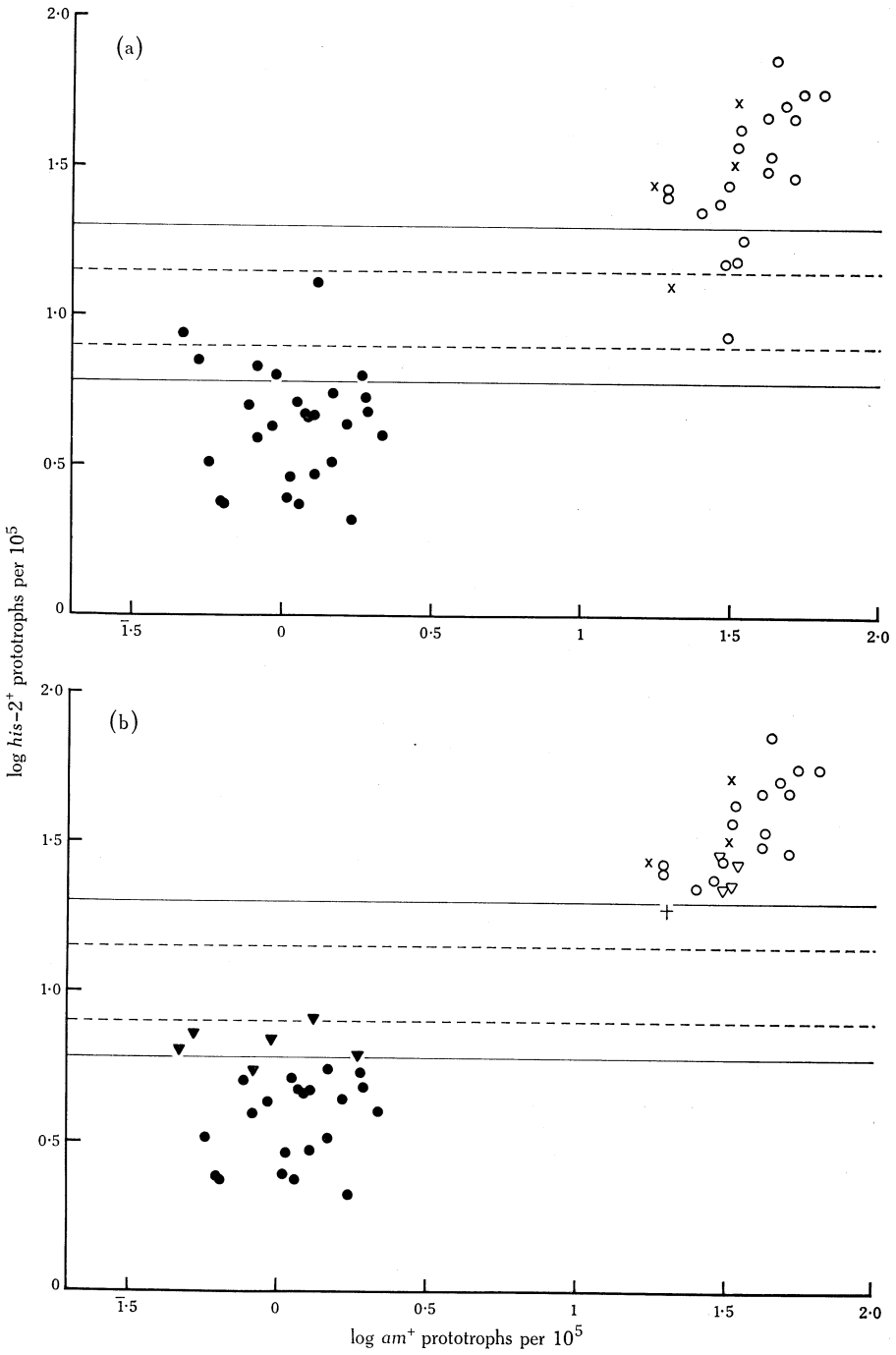

Fig. 1.—Diagrams to show absence of recombination between *rec-3* and *rec-x*, suggesting they are identical genes. Progeny of constitution a *acr-3*^{r} *his-2 am-1*^{2} (shown by circles) from crosses of the type a *acr-3*^{r} *rec-3*$^+$ *rec-x*$^+$ *arg-3* +; *am-1*^{2} × A *acr-3*^{s} *rec-3 rec-x* + *his-2*; +, are classified

limit to their possible separation. If the chance of separating them is p and selection of recombinants is made in the interval *acr-3 arg-3*, length d, between which loci *rec-3* and *rec-x* lie, the chance of finding no recombinants among n progeny analysed is $(1-p/d)^n$. The solution of $(1-p/d)^n = 0{\cdot}05$ gives the upper limit of p with 95% probability as 0·143%, which is equivalent to 143 recombinants per 10^5 progeny.

TABLE 2

PROTOTROPHS PER 10^5 ASCOSPORES OBTAINED IN CROSSES OF CERTAIN PROGENY TO SEVERAL *his-2 rec-x* TESTER STRAINS

K584 Tester Strains				Mean	K579 Tester Strain 7567
7560	11121	11122	11124		
			rec-3$^+$		
6·3±1·1	7·8±1·5		7·1±0·9	7·0±0·6	
6·4±1·8	7·3±2·2		5·9±1·0	6·2±0·8	1·9±0·4
6·7±2·2	3·1±0·9		6·2±0·8	5·5±0·6	1·3±0·3
7·2±1·0	5·4±0·9	8·7±1·3	8·7±1·8	7·2±0·6	1·6±0·4
8·7±1·6	3·2±0·7	7·0±1·1	10·6±2·2	6·5±0·6	
12·9±3·6	9·3±1·6	6·2±1·1		8·0±0·9	1·5±0·3
			rec-3		
8·6±1·8	35·8±3·5	25·5±2·3	17·2±2·5	22·3±1·3	10·0±0·9
12·7±1·6	13·4±4·3	26·2±2·3		19·2±1·3	
15·1±4·0	24·2±6·5		24·3±2·2	22·8±1·9	8·7±0·9
15·1±3·2	43·9±6·1		29·1±1·9	28·6±1·7	13·1±1·3
18·4±2·4	36·7±5·7	30·2±2·3		27·0±1·6	16·0±1·6

The position of the *rec-3* (*rec-x*) locus is 0·71±0·15 units proximal to *acr-3* and 1·62±0·15 units distal to *arg-3*. In these crosses the interval *rec-3 arg-3* is a quarter or a fifth that in the data discussed in Section III(*a*). Evidently, there is a factor in these crosses reducing non-allelic recombination, analogous to the effect of *rec-2*$^+$ (Smith 1966) on the *pyr-3 his-5* region in linkage group IV. Presumably it has been brought in by the *acr-3* stock, but nothing is known about its location.

IV. DISCUSSION

The evidence provided by these experiments strengthens the view that *rec-x* is the same gene as *rec-3* and that *rec-x*$^+$ is the same as *rec-3*$^+$. If *rec-x* and *rec-3* were different they are, with 95% probability, less than 0·143 centimorgans apart. This value is within the range of frequencies of prototrophic recombinants between different allelic mutants. However, the precise values do not signify equivalent

for *rec-3* versus *rec-3*$^+$ and *rec-x* versus *rec-x*$^+$. The *rec-3* alleles are assessed by the frequency of *am-1*$^+$ prototrophs in test crosses, the *rec-x* alleles by the frequency of *his-2*$^+$ prototrophs, each plotted on a logarithmic scale. Four A *rec-3 rec-x his-2 am-1*^{2} are also plotted (crosses). (*a*) Initial classification, showing a number of progeny between the horizontal lines which could not be definitely assessed as *rec-x* or *rec-x*$^+$. Solid circles, *rec-3*$^+$; open circles, *rec-3*. (*b*) Final classification after additional tests for *rec-x* or *rec-x*$^+$ constitution of all progeny between the horizontal lines in (*a*). These are now shown as solid or open triangles, replacing solid or open circles, and a dagger replacing a cross.

physical lengths, since *rec* and *cog* genes between them may cause more than 30-fold differences in frequencies of recombination between any given pair of allelic differences.

The lowest values of recombination encountered, indicating closeness of differences, are less than 0·001%. To reduce the minimum possible separation of *rec-3* and *rec-x* to this level would require the analysis of more than 7000 recombinants between *acr-3* and *arg-3*. It does not seem worth while, and it is hardly feasible, to undertake an analysis of such magnitude, which would require several years to accomplish.

Accepting that *rec-x*$^+$ is the same gene as *rec-3*$^+$, it specifically controls the frequency of recombination at the *am-1* and *his-2* loci, but not at any of the following according to our data, mostly unpublished: *his-1* (Catcheside 1966), *his-3*, *his-5*, *his-6*, *his-7*, *inos*, and *try-1*. Other evidence confirms the lack of effect on *his-5* (Smith 1969) and adds *nit-2* as another insensitive locus (Catcheside 1970). Thus, the degree of specificity is towards 2 loci out of 10 tested. While the presently available information shows that there are several to many *rec* loci, it does not suggest that there is a separate *rec* locus for each locus at which recombination occurs. Rather it appears that each *rec*$^+$ gene has effects upon a number of loci scattered through the genome. Circumstantial evidence, which we have, suggests that the genes *rec-1*, *rec-2*, *rec-3*, *rec-4*, *rec-x*, *rec-w*, and *rec-z* may represent no more than three distinct loci, with two alleles known at each. Experiments to determine the number of independent loci are under way.

Specificity of response, in which some parts of the genome, but not all, are the apparent targets of a given *rec*$^+$ gene, imply the existence of a recognition gene in the target area, just as the specific action of *rec*$^+$ implies that it has a specific region at which it is recognized and where it has its primary action. Recently, Angel, Austin, and Catcheside (1970) have discussed the possible functional relations between these two kinds of recognition gene and *rec*$^+$. One possibility is that the target of *rec*$^+$ is the recognition gene in the region whose recombination is controlled. Another possibility is that the target of *rec*$^+$ is the operator of a gene concerned with the specification of a recombinase active on some parts of the genome but not on others. These two hypotheses, each subject to variations, imply respectively that the specificity of action of *rec*$^+$ may be direct or indirect. Recombination is a complex process comprising, after pairing of the homologous chromosomes which are already replicated, such events as breakage of DNA chains by endonucleases, erosion of broken chains by exonucleases, repair of eroded chains by new synthesis using existing chains as templates, and closure of breaks by ligases. The most highly specific of these events might well be the initial breakage by an endonuclease at a special place. Recognition of the latter would depend upon a sequence of nucleotide pairs, equivalent to a recognition gene, which might occur at a number of different places in the genome. This gene might also be the target of the product of *rec*$^+$. Alternatively, the gene that specifies the specific endonuclease may be controlled, its regulator being the product of *rec*$^+$. The simultaneous control of recombination at the *am-1* and *his-2* loci would be consistent with either interpretation though we prefer the latter. The different degrees of sensitivity may be only apparent, due to the different levels of residual recombination being produced by distinct systems.

Means of proceeding further are far from simple. It would be important (1) to be able to identify the gene responsible for the presumed endonuclease, (2) to identify its operator, (3) to find further loci at which recombination appears to be controlled by *rec-3*$^+$, and (4) to identify *cog* genes at the *am-1* and *his-2* loci similar to those near the *his-3* locus (Angel, Austin, and Catcheside 1970).

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