REGULATION OF RECOMBINATION AT THE *his-3* LOCUS IN *NEUROSPORA CRASSA*

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Summary

The frequency of prototrophic recombination between pairs of *his-3* alleles is increased in the absence of the dominant gene *rec-w*$^+$, which is probably the same as *rec*-4$^+$. The locus of *rec-w* is in linkage group V. The degree of increase is determined by genes at a *recognition* locus (*cog*) situated about 1·3 units distally to the *his-3* locus. In the presence of *cog*$^+$, derived from Y8743 which has Lindegren wild stocks as ancestors, the increase is about 30-fold. When *cog*, derived from Emerson a, is present in both parents of a cross the degree of increase is not greater than about fivefold.

The interactions of these genes in their control of recombination at the *his-3* locus limit the possible mechanisms of action. It seems likely that the *cog* locus is the region in which recombination at the *his-3* locus commences and that *rec-w*$^+$ is the gene which controls the recombinase acting at the *cog* locus. Variations of this theory are possible.

Non-allelic recombination in the *his-3 ad-3* segment is increased from about 1·7% to about 4·9% in the presence of *cog*$^+$ in *rec-w* × *rec-w* crosses. This is the first case in which one system controls both allelic and non-allelic recombination.

I. INTRODUCTION

Several *recombination* (*rec*) genes are now known, each of them having specific effects upon allelic recombination at a particular locus. The effects include a reduction in the frequency of recombinants, as measured by numbers of prototrophs, due to the presence in a cross of the dominant *rec*$^+$ gene. Frequently, the distribution of flanking genes, neighbouring the locus, is also altered in the prototrophs.

In the course of testing whether *rec-1*$^+$, so far specific to *his-1*, has any effect on other *histidine* loci, another gene (*rec-w*$^+$) was found to be active at the *his-3* locus (Catcheside and Austin 1969). Three alleles, K504, K26, and K874, whose sites of allelic difference are in that order in the *his-3* locus, were used. It was found that *rec-w*$^+$ has differential effects depending on whether K26 is in the cross. Whereas the ratio of prototroph frequencies in *rec-w* × *rec-w* crosses to those in *rec-w* × *rec-w*$^+$ crosses is of the order of 30 in the presence of K26, the ratio is no more than about 4·5 in its absence. Indeed the prototroph frequencies between adjacent segments are approximately additive in the presence of *rec-w*$^+$, but not at all additive in *rec-w* × *rec-w* crosses.

The crosses also show differences in the distribution of the flanking markers *arg-1* and *ad-3* among the prototrophs. The relative proportions of the two parental combinations of the flanking markers are reversed in *rec-w* × *rec-w* crosses depending on whether K26 is the proximal or the distal allelic difference in the cross. The combination *pd* is in excess in the progeny of K504×K26 crosses, while *PD* is in

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excess in the progeny of K26 × K874 (Table 5). Further, *PD* is in excess in both crosses when they are *rec-w* × *rec-w*$^+$. Thus *rec-w*$^+$ causes a reversal of the proportions of the parental combinations of flankers among prototrophs in K504 × K26 crosses, but not in K26 × K874.

These various effects point to special properties of K26. They could be due to the K26 mutant site itself or to a peculiarity of the *his-3*$^+$ gene from which K26 was derived. K26 was obtained from a stock (Y8743) different from that (Emerson a) which was the source of all other *his-3* mutants used. The properties could, of course, be due to a factor separable from this *his-3* locus. The purpose of the work described below was to determine which of these causes, if any, is responsible for the greater sensitivity to *rec-w* of crosses involving K26.

The *rec-w* locus was originally called *rec-5* (Catcheside 1968) but, because its relationship to *rec-4* (Jha 1967) was uncertain, it was decided to use a letter designation temporarily.

II. Materials and Methods

The original mutant, K26, was obtained from Y8743, a colonial, microconidial mutant described by Barratt and Garnjobst (1949). The progenitors of Y8743 were 1A and 25a, derived by Beadle and Tatum (1945) from the Lindegren A and Lindegren a wild stocks.

To remove the *his-3*$^+$ gene from Y8743, the latter was crossed to K26 *ad-3*; from this cross *his-3*$^+$ progeny were saved and these in turn crossed again to K26 *ad-3*. This gave several fertile strains, mainly Emerson a in genotype and compatible with it in heterocaryons, but with the *his-3*$^+$ gene of Y8743 and, presumably, the neighbouring genetic material also. From these strains, *histidine* mutants were isolated by filtration enrichment after induction by ultraviolet light. Altogether at least 115 histidine mutants were obtained and 46 proved to be *his-3* mutants. These were classified for heterocaryon group by testing with a series of standard *his-3* mutants.

Crosses and the analyses of progeny were made by the methods previously described (Catcheside 1966). The various mutants used are explained in the text.

III. Results

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

Progeny from crosses such as K26 *rec-w*$^+$ × K504 *rec-w* showed that *rec-w* was not linked to *his-3*. Tests showed that Emerson a is *rec-w*. Therefore mutants, other than *his*, derived directly from it were crossed to K26 *rec-w*$^+$ and progeny were examined to detect linkage between *rec-w* and markers in various linkage groups. The results (Table 1) suggest that *rec-w* is in linkage group V, about 32 map units from *ad-7*. The relation of *rec-w* to *rec-4* (Jha 1967) is uncertain, but they appear to be the same gene, since all of 14 stocks analysed are either *rec-4 rec-w* or *rec-4*$^+$ *rec-w*$^+$ with no other combinations known. Jha (1969) has described *rec-6* as also active on *his-3*. The genes *rec-4* and *rec-6* are defined, by their actions, using a different set of *his-3* alleles from those defining *rec-w*. Hence, until *rec-4*, *rec-6*, and *rec-w* are located questions of identity cannot be settled firmly.

(*b*) *Location of Factor for Sensitivity of K26 Crosses*

The data from those progeny, of constitution K26 *rec-w*, arising from the tests used to locate *rec-w*, together with other data in which K26 had been crossed to stocks of Emerson a background, tend to show that the factor for the sensitivity of K26 crosses to allelic recombination is not readily separable from the *his-3* locus.

Among 41 progeny, 35 certainly showed the very high yields of prototrophs, in crosses to K504 *rec-w*, that are characteristic of the presence of the sensitivity factor. A small number (six) of K26 *rec-w* progeny showed rather lower yields (below half of expectation) of prototrophs in these crosses, though still well above what would be expected if the sensitivity factor were absent. Unfortunately, the progeny were discarded before the need to verify the actual yield was realized. Nevertheless, the conclusion must be that the sensitivity factor is fairly closely linked to K26.

The question was examined more explicitly by selecting A *his-3* progeny from a cross (7507) a *arg-1* K26 *cot rec-w* × A K874 *ad-3 cot rec-w*. This experiment concentrated on recombinational events in the *arg-1* to *ad-3* region. Each of the *his-3* progeny was crossed to a K26 *ad-3 cot rec-w* to distinguish the K26 from the K874 progeny. Among 120 progeny, there were 93 A K26 *cot rec-w* arising from recombination between *his-3* and *arg-1* and 27 A K874 *cot rec-w* arising from recombination between *his-3* and *ad-3*. Not all have been analysed, but most of the K874 were identified at first by their ability to complement K480. So far 32 K26 and 19 K874 have been tested by crosses with a K504 *ad-3 cot rec-w*$^+$ and a K504 *ad-3 cot rec-w*.

TABLE 1

TESTS TO DETECT LINKAGE GROUP OF *rec-w*

Linkage Group	Genes	Progeny Examined	Progeny		Conclusion
			Parental	Recombinant	
I	*his-3* (K504, K26, K874)	*his-3*	26	30	No linkage
II	*try-3* (K888)	*try-3*	6	8	No linkage
		try-3$^+$	7	6	
III	*try-1* (K893)	*try-1*	18	15	No linkage
		try-1$^+$	17	15	
IV	*try-4* (K902)	*try-4*$^+$	13	10	No linkage
V	*ad-7* (K77)	*ad-7*$^+$	12	6	Linked
		ad-7	11	4	
VI	*try-2* (K892)	*try-2*$^+$	2	6	No linkage
VII	*nt* (K890)	*nt*$^+$	11	5	Doubtful

The ratio of the prototroph frequencies in the crosses to K504 falls into one of two classes: (1) high, ranging from 15 to 45; and (2) low, ranging from 2·5 to 5. The observations are summarized in Figure 1, where they are plotted on logarithmic scales. All K26 progeny show the high ratio and 14 of the K874 show the low ratio. However, five K874 progeny show the high ratio. All K874 *ad-3* progeny, having no crossover in the *his-3* to *ad-3* segment, show a low ratio.

Evidently there is a genetic factor, distal to and close to the *his-3* locus, responsible for the difference in sensitivity to recombination in the absence of *rec-w*$^+$. Transfer of the "high" gene to association with K874 has occurred in five cases. It is relatively closer to K874, for most of the recombinants between it and *ad-3* retain the "low" gene.

The high sensitivity of K26 crosses in the presence of *rec-w* is not a property of the site of change of K26 from normal. Nor is it a property of the *his-3*$^+$ gene

itself, but instead of another locus closely linked to it. Expressed in formal terms, there is a difference between Emerson a and Y8743 in respect of a recognition factor concerned in allelic recombination in the *his-3* locus. It is convenient to refer to this factor as a gene and to give it a symbol; the name *recognition* and the symbol *cog*

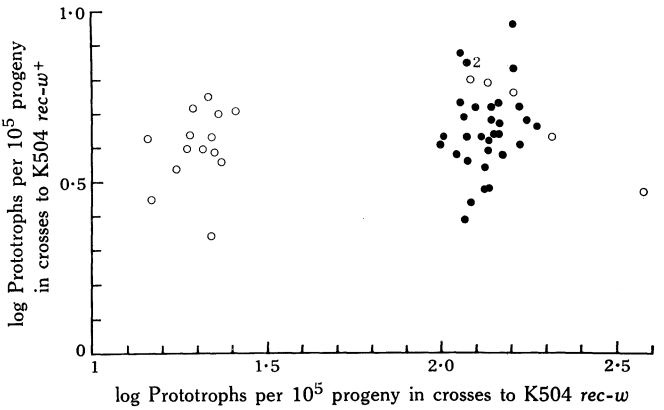

Fig. 1.—Logarithmic plot of the numbers of prototrophic recombinants in crosses, to K504 *rec-w* and *rec-w*$^+$ testers, of progeny of a *arg-1* K26 *cog*$^+$ *rec-w* × A K874 *ad-3 cog rec-w*. ● K26. ○ K874.

seem appropriate. The *recognition* gene in Emerson a and the *his-3* mutants derived from it is *cog*; the gene in Y8743 and the mutants derived from it is *cog*$^+$. The presence of *cog*$^+$ permits a higher frequency of allelic recombination in the *his-3* locus in the absence of *rec-w*$^+$. The possible nature of *cog* and of what it recognizes will be discussed later.

The pooled data from these crosses are summarized in Table 2. They show that *cog*$^+$ is dominant and has the effect of increasing prototrophic recombinants about 6·5 times in the absence of *rec-w*$^+$. It is already known that *rec-w*$^+$ is fully dominant.

TABLE 2

EFFECTS OF *rec-w*$^+$ AND *cog*$^+$ ON FREQUENCIES OF RECOMBINATION BETWEEN *his-3* ALLELES

	K504 *cog rec-w*$^+$	K504 *cog rec-w*	Ratio *rec-w*/*rec-w*$^+$	K26 *cog*$^+$ *rec-w*
K874 *cog*$^+$ *rec-w*	5·1±0·28	167·0±3·4	33·1±1·9	11·5±0·49
K874 *cog rec-w*	4·1±0·15	20·5±0·47	5·0±0·22	14·2±0·34
Ratio *cog*$^+$/*cog*	1·2±0·08	8·1±0·25	6·6±0·48	0·8±0·04
K26 *cog*$^+$ *rec-w*	4·4±0·10	134·0±1·2	30·5±0·72	

It has the effect of decreasing the frequency of prototrophic recombinants by a factor of about five in the absence of *cog*$^+$. Thirdly, *rec-w*$^+$ is completely epistatic to *cog*$^+$. These relationships will be used in the consideration later of possible mechanisms of action.

The analysis is being extended by selecting K26 progeny from a cross of A K26 *cog*$^+$ *ad-3 cot rec-w* × Emerson a in the expectation that some of these progeny will be K26 *cog*, showing low yields of prototrophs in crosses to K504 *rec-w*.

(*c*) *Effects of* rec-w$^+$ *and* cog$^+$ *on Non-allelic Recombination*

The above data show that the *his-3 ad-3* segment is $4{\cdot}35\pm0{\cdot}85$ units in the cross 7507, since *arg-1* to *ad-3* is $19{\cdot}34\pm1{\cdot}89$ in this cross and 27 of the 120 cross-overs in that segment occurred in the *his-3* to *ad-3* part of it. These arguments place the *cog* locus $1{\cdot}14\pm0{\cdot}49$ units distal to K874, measured in the presence of *cog*$^+$.

The length of the *his-3* to *ad-3* segment has generally been reported as about 1 unit. The results of measurements using stocks of K874 *cog rec-w* and K874 *cog*$^+$ *rec-w* each crossed to *ad-3 cog rec-w* and *ad-3 cog rec-w*$^+$, bred from suitable sources, are summarized in Table 3. The prototrophic recombinants were counted after selective plating, the total population being estimated from the numbers of colonies growing on fully supplemented plates grown from suitably diluted suspensions. It is clear that there is a large effect due to the presence of *cog*$^+$ in *rec-w* × *rec-w* crosses.

TABLE 3

EFFECT OF *cog*$^+$ AND *rec-w*$^+$ ON RECOMBINATION BETWEEN *his-3* (K874) AND *ad-3* (K118)

Recombinants (%) in crosses between stocks with the constitutions in the column heads and left-hand column of the table

	his-3 cog rec-w	*his-3 cog*$^+$ *rec-w*
ad-3 cog rec-w	$1{\cdot}66\pm0{\cdot}05$	$4{\cdot}93\pm0{\cdot}19$
ad-3 cog rec-w$^+$	$1{\cdot}59\pm0{\cdot}05$	$1{\cdot}76\pm0{\cdot}06$

Recombination between *his-3* and *ad-3* is more than three times smaller in the absence of *cog*$^+$ or the presence of *rec-w*$^+$. This is the first system with an effect simultaneously upon the frequency of allelic recombination at a locus and upon crossing over between that locus and a neighbouring locus.

The question whether the presence of *cog*$^+$ affects the whole of the *his-3 ad-3* segment cannot be answered certainly with the data available. In the presence of *cog*$^+$ the length is $4{\cdot}93\pm0{\cdot}19$ units, the *his-3 cog* section being $1{\cdot}3\pm0{\cdot}5$ units, leaving $3{\cdot}63\pm0{\cdot}52$ units for the *cog ad-3* region. In the absence of *cog*$^+$ or the presence of *rec-w*$^+$, the *his-3 ad-3* segment is $1{\cdot}7$ units. In the latter case, the minimum value for the *cog ad-3* section is $0{\cdot}4$ and the maximum increase in it due to *cog*$^+$ in *rec-w* × *rec-w* is about nine times. The minimum increase is about twice assuming that the *his-3 cog* section is virtually zero in the absence of *cog*$^+$. It seems most likely that there are effects in both the *his-3 cog* and *cog ad-3* regions, but further extensive experiments will be needed to establish this and show whether they are equal or unequal.

(*d*) *Sensitivity of New* his-3 *Mutants from Y8743 Source*

All of these mutants, when crossed to K26 *rec-w* and *rec-w*$^+$, show the characteristic large difference in yield of prototrophs in the two kinds of cross (Table 4). A preliminary map is shown in Figure 2.

With a few special exceptions, large yields of prototrophs occur in *rec-w* × *rec-w* crosses of each of the new mutants to K504 and K874. Generally, the ratio of prototrophs in *rec-w* × *rec-w* to those in *rec-w* × *rec-w*$^+$ is as large as in the case of crosses involving K26. All of the mutants, except TM429, show this effect. Clearly, all of them carry *cog*$^+$. The clear conclusion that the high sensitivity to *rec-w* resides in a gene separate from *his-3* itself was reached before the experiments reported in Section III(*b*) were done.

TABLE 4

EFFECT OF *rec-w* AND *rec-w*$^+$ ON CROSSES OF *his-3* MUTANTS, DERIVED FROM Y8743 SOURCES, TO K26, K504, AND K874 MUTANTS

Data given are frequencies of prototrophs per 10^5 ascospores

		TM428	TM429	TM504	TM521	TM522
K26	*rec-w*	20·5±1·3	23·0±3·1	173·0±6·3	0·7±0·12	109·0±9·9
	rec-w$^+$	0·7±0·14	0·8±0·37	4·8±0·3		
	Ratio	29·7±6·2	28·0±13·0	36·2±2·6		
K504	*rec-w*	60·4±1·4	11·1±0·5	141·0±7·0	122·0±7·0	36·0±1·9
	rec-w$^+$	4·6±0·8	10·9±1·4	7·3±0·4	6·9±0·7	1·2±0·2
	Ratio	12·6±2·1	1·02±0·14	19·3±1·5	17·8±1·9	30·7±5·1
K874	*rec-w*	52·7±1·5	0·19±0·04	41·8±1·04	29·6±1·3	102·0±3·4
	rec-w$^+$	2·1±0·18	0·21±0·05	1·6±0·14	1·1±0·1	5·5±0·3
	Ratio	25·7±2·3	0·9±0·3	25·8±2·3	27·6±2·9	18·4±1·3

TM429 is unlike the other new mutants in that the *his-3* mutation is associated with a structural change with one break apparently in the locus itself. Study of the proportions of different classes of asci with different numbers of defective spores (d) shows that there is an interchange, presumably between linkage group I and another one, not yet identified. Observed were 185 asci with 8+ : 0d, 194 with 0+ : 8d, 146 with 4+ : 4d, 56 with 6+ : 2d, and 46 with 2+ : 6d. The relatively low proportion of 4+ : 4d asci shows that the points of interchange are relatively close to the centromeres. A cross of *arg-1* and *ad-3* stocks of TM429 shows that these genes are unlinked in these stocks and therefore that the break is between them. Observed were 37 *arg-1*, 36 *ad-3*, 40 ++, and 31 *arg-1 ad-3* progeny.

In crosses of TM429 to K504 and K874, *rec-w*$^+$ has little or no effect in reducing the yield of prototrophs. No prototrophs arising from any of these crosses carry TM429, as judged by the absence of asci with defective spores in test crosses. Further, as the data in Table 5 show, the combinations of flanking markers found in the prototrophs are virtually restricted to one parental and one recombinant class, the parental class having the flankers introduced by the parent other than TM429. The results suggest that TM429 cannot itself be converted back to normal, perhaps because the *his-3* mutation in it is the result of the fracture of the original *his-3*$^+$ gene.

Further, the low frequencies of recombination in the presence of *rec-w* in these crosses suggest that the *cog*$^+$ region has been separated from the rest of the *his-3* gene, so that the initiation of recombination from this region is blocked from spreading to the rest of the gene.

(*e*) *Effect of* cog$^+$ *and* rec-w$^+$ *on the Distribution of Flanking Genes*

The genes *arg-1* (K166) and *ad-3* (K118) have been introduced into stocks of many *his-3* mutants and used, respectively, as markers of the proximal and distal regions neighbouring the *his-3* locus. Information about the disposition of these flanking markers amongst the prototrophs is summarized in Table 5. Data for a selection of alleles with sites of difference distributed through the locus are shown.

TABLE 5

EXAMPLES TO SHOW THE EFFECTS OF *rec-w*$^+$ AND *cog*$^+$ ON THE DISTRIBUTION OF FLANKING MARKERS AMONG *his-3* PROTOTROPHS

The *arg-1 his-3* segment is about 14 units; the *his-3 ad-3* segment is 1·7 units in the presence of *rec-w*$^+$ and in *cog rec-w* × *cog rec-w* crosses, but 4·9 units in *rec-w* × *rec-w* crosses in the presence of *cog*$^+$. The *his-3* allele with the proximal difference from normal is given first in each cross; its flankers are *P* and *D*, the distal allele having *p* and *d*

his-3 Alleles Crossed		*rec-w* × *rec-w*				*rec-w* × *rec-w*$^+$			
		PD	*pd*	*pD*	*Pd*	*PD*	*pd*	*pD*	*Pd*
cog	× *cog*								
K504	K874	249	230	310	163	178	97	183	126
K504	K480	142	114	146	71	60	33	79	38
K874	K480	189	123	211	173	138	80	174	100
cog	× *cog*$^+$								
K504	K26	142	377	350	151	142	49	207	50
K504	TM428	66	195	175	60	11	5	22	4
K504	TM502	93	198	256	62	116	51	211	28
K874	TM502	95	152	280	72	57	18	136	14
cog$^+$	× *cog*								
TM522	K874	93	50	171	45	93	44	128	34
TM522	K458	150	73	219	78	17	8	24	15
TM428	K874	259	80	357	96	48	17	77	16
K26	K874	194	80	204	70	39	20	70	13
cog$^+$	× *cog*$^+$								
TM428	K26	55	100	85	61	3	9	15	7
TM428	TM504	41	77	69	50	75	132	120	54
K26	TM502	76	170	126	106	48	47	42	40
cog	× *cog*$^+$								
K504	TM429	197	7	446	5	19	0	52	0
cog$^+$	× *cog*$^+$								
K26	TM429	20	4	11	2	4	0	1	0
TM429	TM504	19	166	111	47	0	8	6	0

The data are grouped according to the *cog* and *rec-w* constitutions of the parents. There are some clear differences between the groups, but the data in each group are

not always homogeneous. It is not known to what extent the heterogeneity is due to uncontrollable variation, perhaps genetic in character, and to the different positions in the *his-3* locus of the differences between the mutant alleles.

Despite the heterogeneity of the data, the genes cog^+ and $rec\text{-}w^+$ have distinctive effects upon the frequencies of the four classes of flankers amongst the $his\text{-}3^+$ prototrophs. Broadly, the effects indicate a preferential participation of the cog^+ chromosome in generating prototrophs:

(1) *pD* is considerably larger than *Pd*, except in $cog^+ \times cog^+$. Of course this majority of *pD* is the criterion upon which the order of the differences between the *his-3* alleles is based or, rather, the basis upon which *PD* and *pd* are identified. Despite the lack of definite evidence in the $cog^+ \times cog^+$ data, the order in the fine structure map (Fig. 2) is quite consistent. The difference between *pD* and *Pd* is generally greater in crosses which contain $rec\text{-}w^+$.

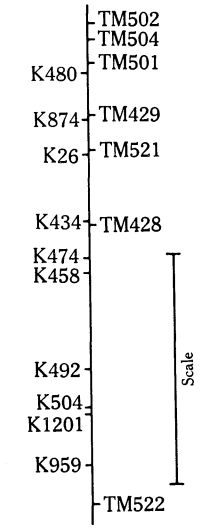

Fig. 2.—Map of *his-3* locus, showing the order of the sites of difference of several mutant alleles. The spacing is approximate, with the scale on the right representing roughly 120 prototrophs per 10^5 progeny in cog^+ *rec-w* crosses, 20 per 10^5 in *cog rec-w* crosses, and 4 per 10^5 in $rec\text{-}w^+$ crosses.

(2) The parental classes are unequal, except in *cog* × *cog* crosses which do not contain $rec\text{-}w^+$. One class is generally about twice the size of the other.

(3) In $cog \times cog^+$ crosses, which are *rec-w* × *rec-w*, the majority parental class is always that which is similar to the cog^+ parent. When the cog^+ parent's site of difference is distal the majority parent is *pd*, when proximal the majority parent is *PD*.

(4) In *rec-w* × $rec\text{-}w^+$ crosses the majority parental class is always *PD*. Thus, in $cog \times cog^+$ crosses in which the cog^+ allele has a distal site of difference, there is a switch in the majority parental class of flankers from *pd* to *PD*.

(5) There is a residual effect of cog^+ in the presence of $rec\text{-}w^+$ in $cog \times cog^+$ crosses; the recombinant classes are more unequal than in *cog* × *cog* and $cog^+ \times cog^+$.

When TM429 is in a cross, the behaviour indicates strongly that it does not itself produce any prototrophs. When it is crossed to a *his-3* allele carrying *cog*, the predominant parental class amongst prototrophs is the one carrying the flankers which accompanied that allele. Indeed the minority parental class (equivalent to the TM429 parent) and the minority recombinant class are both very small. Similar features are seen in $cog^+ \times cog^+$ crosses involving TM429, though the minority classes are larger.

IV. Discussion

Possible theories of the mode of action of the *rec* genes and the *cog* genes, assuming that the latter also occur generally, are restricted by the dominance relationships. Dominance can be taken to indicate the presence of an activity, absent or reduced in the recessive. The dominant rec^+ genes always reduce recombination specifically and they are epistatic to cog^+. In the absence of rec^+, the dominant cog^+ genes increase recombination. A second general feature of *rec* genes is that they are usually not linked to the loci at which they exert control. If linked they are not close to the target loci, from which they are separated by other loci insensitive to them. On the other hand, the only known *cog* gene is very close to the locus it affects and the cog^+ allele shows a principal action on the *his-3* allele to which it is attached and on the segment of chromosome in which it lies.

The rec^+ gene is presumed to specify a product which reduces recombination. The specificity of *rec* genes implies recognition loci at which their products exert their functions. These could be at or near the loci where recombination is controlled or elsewhere. In any case, it is necessary to assume that there are recognition loci associated with those at which recombination is detected, though no assumption is made about what they recognize. Naturally, all such recognition is mutual, between the recognition locus and the product of a gene able to act at the recognition locus. Two kinds of effect are likely. One is an action upon the recognition locus, the other an association to prevent some other product acting upon it.

The product of $rec\text{-}w^+$ could be a regulator specific to *his-3*, where recombination is occurring, and with *cog* as its target [Fig. 3(*a*)]. It would act to prevent access of a recombinase, such as endonuclease causing an initial nick, or frustrating the initial activity of such an enzyme. If all rec^+ genes had this function a very large number may occur. Indeed, there might be an apparent infinity. There must be some limit, even if the number of *rec* genes is large. Limitation to their number implies a corresponding limit to the number of target loci, each of which could be present several times in different parts of the genome. With this kind of mechanism, if there were only a single recombinase initiating recombination at all loci, it is difficult to reconcile the relative lack of specificity of the recombinase with the high degree of specificity shown by *recognition* loci in their discrimination between the products of different rec^+ genes. Moreover, the cog^+ and *cog* variants near the *his-3* locus are distinguished by the recombinase, but not by $rec\text{-}w^+$. If *cog* and cog^+ were the target of the product of $rec\text{-}w^+$, a differential response to the presence of $rec\text{-}w^+$ would have been expected rather than to its absence.

Alternatively, the product of $rec\text{-}w^+$ could be a regulator specifically controlling the recombinase which initiates recombination at the *cog* locus [Fig. 3(*b*)]. Since there

are several different *rec* loci, each with a distinct specificity, there should be several loci (*comb*) responsible for recombinases. Moreover, the product of each *comb* locus appears specific to one locus or more probably to a limited number of loci. Again, there must be a *recognition* locus related to each locus at which recombination shows control. In this case, the *recognition* locus is the target of the recombinase. On this interpretation, there should be a limited number of *rec* genes, each in control of one of an equally limited number of recombinases. Any one of the latter would be capable of recognizing a particular sequence of nucleotides in a DNA molecule, or any of a group of similar sequences though with different efficiencies, and of causing initiation of recombination. In this case, *cog* variants at a given locus would be expected to respond differently to a given recombinase, in the absence of the *rec*$^+$. This conforms to the relationships exhibited by the *cog*$^+$ and *cog* variants at the *his-3* locus with respect to *rec-w*$^+$ and *rec-w*.

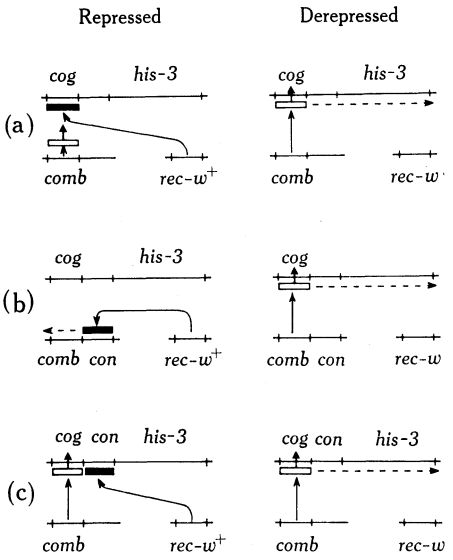

Fig. 3.—Diagrams to illustrate theories of the mechanism of control of recombination by *rec-w* genes; *cog* = recognition; *rec* = recombination; *comb* = recombinase; *con* = control. (*a*) Product of *rec-w*$^+$ interacts with *cog* to prevent access of recombinase; (*b*) product of *rec-w*$^+$ interacts with an operator site (*con*) at *comb* to prevent production of recombinase; (*c*) product of *rec-w*$^+$ interacts with a control site (*con*) to interfere with the spread of the effect of the recombinase acting primarily at a promoter locus *cog*.

The discovery of variants of a *recognition* locus associated with *his-3* has allowed the demonstration of several properties of the system of recombination which acts at this locus. The variants *cog*$^+$ and *cog* do not respond differently towards *rec-w*$^+$, suggesting that they do not react with the product of *rec-w*$^+$ itself. Secondly, if the more sensitive recognition gene (*cog*$^+$) is removed from the rest of the *his-3* locus, as in TM429, recombination is no longer sensitive to the *rec-w* and *rec-w*$^+$ difference in crosses to *cog* strains. The frequencies in *rec-w* × *rec-w* crosses are reduced to the base level characteristic of *rec-w* × *rec-w*$^+$ crosses. This shows that *cog* is a region in which recombination starts and that *his-3* itself does not contain sequences of nucleotides appreciably sensitive to enzymes which initiate recombination. Thirdly, *cog* and *cog*$^+$ appear to respond differently to the same recombinase, suggesting that the latter may attack different sequences of nucleotides with different efficiencies.

The effect of variation at the *cog* and *rec-w* loci predictable on different theories of their action may be compared with the observations. If the *cog* locus were the

target of the product of *rec-w*$^+$, one would expect a difference between the *cog* and *cog*$^+$ genes in respect of their ability to recognize or retain the *rec*$^+$ product. Observation shows that *cog* and *cog*$^+$ strains have recombination equally repressed in the presence of *rec-w*$^+$. This suggests that the target of the product of *rec-w*$^+$ is not the *cog* locus or, at least, not that part of the *cog* locus which responds differently to recombination in the absence of *rec-w*$^+$. Thus the available evidence is more readily compatible with there being two distinct *recognition* loci, one (*con*) for the product of *rec-w*$^+$ and the other (*cog*) for the presumed recombinase. The *recognition* locus for the recombinase is regarded as being the *cog* locus, while that for the *rec-w*$^+$ product could be a regulatory locus (*con*) near the recombinase gene (*comb*) or near the locus exhibiting recombination.

The second of these possibilities arises in the third theory, illustrated in Figure 3(*c*). This is essentially a modification of the first theory formed by introducing elements of the second one into it. In it the recognition site *con* is near the *his-3* locus and has the function of preventing the spread of the effect of the recombinase when the product of *rec-w*$^+$ is bound to *con*. This theory would probably require a large number of *rec* genes, but need not require more than a single recombinase if all variants of *cog*, wherever they are, could react to it with different efficiences. This third theory requires a larger number of regulatory genes than does the second one. In the former case, every locus at which recombination is controlled requires a *cog* and a *con* gene. In the latter case the number of *con* loci would be equal to the number of *comb* loci, in turn equal to the number of *rec* loci.

Test of the validity of the theories outlined, the second [Fig. 3(*b*)] being regarded as the more likely, requires the discovery of the gene (and therefore variant alleles) for the hypothetical recombinase, variants of *con*, and also other loci under the "control" of *rec-w*$^+$.

The distinct effect of *cog*$^+$ on recombination between *his-3* and *ad-3*, provided that *rec-w*$^+$ is absent, cannot be fully reconciled with the theories outlined. The evidence nevertheless makes it highly probable that the *cog* locus is a region in which recombination is initiated. A puzzling feature is that *rec-w* has no apparent effect on recombination between *his-3* and *ad-3* in *cog* $\times$ *cog* crosses. It could be argued that the effect is so small that it is lost in the uncontrolled variation. If the magnitude of the increments to *his-3*/*ad-3* recombination were proportional to the increments to allelic recombination in the *his-3* locus, one would expect the increment in *rec-w cog* $\times$ *rec-w cog* to be one-eighth of the increment in *rec-w cog* $\times$ *rec-w cog*$^+$. The latter is about 3·2 units, so the former should be 0·4 units. A value of 2·1% should be readily distinguishable, in the experiments performed, from the base value of 1·7%. One must conclude that *rec-w* has no effect on recombination between *his-3* and *ad-3* in *cog* $\times$ *cog* crosses. It seems that *cog*$^+$ has special properties in this respect. Before speculating further, it is necessary to determine whether *cog*$^+$ acts differently upon the *his-3 cog* and *cog ad-3* segments and whether *cog*$^+$ is fully dominant in its effects upon allelic and non-allelic recombination.

There is an obvious analogy between *rec-w*$^+$ and *rec-2*$^+$ (Smith 1966, 1968) in their control of non-allelic recombination. Several questions are raised. Is there a gene, between *pyr-3* and *his-5*, with properties like those of *cog*$^+$? Is allelic recombination at the *pyr-3* locus controlled by *rec-2*$^+$? It is known that the substitution of

rec-2 for *rec-2*$^+$ has no effect upon allelic recombination at the *his-5* locus. Thirdly, are *rec-w* and *rec-2* the same gene? They appear to be located in the same region of linkage group V.

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