ACTION OF *rec-3* ON RECOMBINATION NEAR THE *amination-1* LOCUS OF *NEUROSPORA CRASSA*

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Abstract

The *rec-3*$^+$ gene, which strongly reduces allelic recombination in *am-1*, has shown no effect on non-allelic recombination in an 8 map unit interval spanning *am-1*. Allelic recombination in the *gul-1* locus, less than 0·3 map units distal to *am-1*, is also apparently not affected. However, selected recombinants between *am-1* and *gul-1* show differences dependent on *rec-3* constitution. These can be attributed to reduced levels of crossing over and conversion in *am-1* in the presence of *rec-3*$^+$.

This highly localized action of *rec-3*$^+$ can be accounted for if many specific sites exist in this region at which recombination may be initiated. However, a product of *rec-3*$^+$ would recognize only one specific site, closely proximal to *am-1*, and repress the initiation of recombination at this site.

I. INTRODUCTION

The action of recombination (*rec*) genes in *Neurospora crassa* is highly specific. Recombination seems to be affected only in short regions, and these regions are not closely linked to the locus of their master *rec* gene. For example, the *rec-1*$^+$ gene reduces all allelic recombination in the *histidine-1* (*his-1*) locus about 20-fold, but no effect has been detected on non-allelic recombination between markers about 9 map units apart spanning *his-1* (Jessop and Catcheside 1965).

The *rec-3* locus was first described by its effect on allelic recombination at the unlinked *amination-1* (*am-1*) locus (Catcheside 1966). The dominant *rec-3*$^+$ allele reduces recombination between all 13 *am-1* alleles tested by 5- to 25-fold (Catcheside 1968; Smyth 1970). This paper describes experiments to see if *rec-3*$^+$ has effects close to, but outside of the *am-1* locus. High resolution was obtained by following the *gulliver-1* (*gul-1*) locus, less than 0·3 map units distal to *am-1*. This is a recombination frequency similar to that obtainable between alleles. This opportunity to test the interlocus limits of the effect of *rec-3*$^+$ is of interest, since Angel *et al.* (1970) have recently shown that the effect of another *rec* gene, *rec-w*$^+$, extends for at least 1·3 map units in another region, and have further shown the presence of a site of *recognition* (*cog*) in this region.

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II. Mutants and Methods

The following mutants were used:

Locus	Isolation No.	Linkage group	Source of stock
cot-1	C102t	IV	D. G. Catcheside
sp	B132	V	D. G. Catcheside
am-1	19	V	FGSC #1191*
gul-1	G	V	FGSC #817 and #820
	CA1	V	FGSC #1191
his-1	K83	V	D. G. Catcheside

* Fungal Genetics Stock Center Number, Humboldt State College, Arcata, Calif. 95521 U.S.A.

The *colonial temperature-sensitive-1* (*cot-1*) mutant C102t was bred into all stocks to make handling easier. At 25°C, growth is wild type; at 34°C, it is much restricted and branched. The *gulliver-1* (*gul-1*) mutants are only detectable in *cot-1* background; they modify the restricted growth of *cot-1* at high temperatures such that it approaches wild type (Terenzi and Reissig 1967). At the time of this study only one mutant, G, of *gul-1* was available. Another mutant was detected in a stock of $am\text{-}1^{19}$ obtained from the Fungal Genetics Stock Center (#1191). It was judged to be allelic with $gul\text{-}1^{G}$ by its similar phenotype, by a heterocaryon test, and by its frequency of recombination with $gul\text{-}1^{G}$ [Section III (*b*)]. It was given the allele number CA1 and stocks were deposited in the Stock Center (FGSC #1962 and #1963). The morphological mutant, *spray* (*sp*), is readily detectable in a *cot-1*; *gul-1* genotype.

Experimental methods were essentially the same as those described by Smyth (1971).

III. Results

(*a*) *Non-allelic Recombination Close to* am-1

To test if $rec\text{-}3^{+}$ affects recombination immediately adjacent to *am-1*, in which there is a strong effect, four point crosses of the type + + + *his-1* × *sp am-1 gul-1* + were made using both *rec-3* and $rec\text{-}3^{+}$ stocks. The CA1 allele of *gul-1* was used and duplicate crosses were made. Random offspring were collected and classified.

Table 1

Percentage crossing over (with standard errors) among unselected progeny of four-point test crosses in *rec-3* and $rec\text{-}3^{+}$ backgrounds

Cross	Percentage crossing over			Total progeny
	sp — I — *am-1*	*am-1* — II — *gul-1*	*gul-1* — III — *his-1*	
rec-3 × *rec-3*	5·8±0·78	0·00	2·9±0·56	900
rec-3 × $rec\text{-}3^{+}$	4·6±0·63	0·09	3·4±0·54	1100

The values observed in *rec-3* × *rec-3* and *rec-3* × $rec\text{-}3^{+}$ crosses were not significantly different [χ^2 (homogeneity) = 5·4, 5 d.f., $50\% < P < 70\%$]. Table 1 shows percentage crossing over in each case. At this level of analysis, no effect of $rec\text{-}3^{+}$ was detected. Nevertheless an effect in the *sp his-1* region is not excluded.

Two further experiments were done to search for effects using higher resolution.

(*b*) *Allelic Recombination in* gul-1

Since *gul-1* is so close to *am-1*, only one recombinant being obtained in 2000 unselected progeny (Table 1), allelic recombination in *gul-1* may also be affected by *rec-3*$^+$. To test this, *gul-1* alleles G and CA1 were intercrossed in *rec-3* and in *rec-3*$^+$ backgrounds. Relatively few progeny could be obtained due to the infertility of crosses between *gul-1* mutants and the lack of a method for selecting recombinants.

Table 2 shows the frequency of *gul-1*$^+$ progeny obtained. The presumption that the *gul*$^+$ progeny were recombinants, rather than revertants or contaminants, was strengthened by the lack of such progeny in control crosses homozygous for G or CA1. The *gul-1*$^+$ genotype of offspring of *gul*$^+$ phenotype was confirmed by crossing to *gul-1*$^+$ testers. No *gul-1* progeny segregated.

TABLE 2

FREQUENCY (WITH 95% CONFIDENCE LIMITS) OF *gul-1*$^+$ RECOMBINANTS FROM CROSSES BETWEEN *gul-1* ALLELES G AND CA1 IN *rec-3* AND IN *rec-3*$^+$ BACKGROUNDS

Constitution of cross		Progeny		
gul-1	*rec-3*	*gul-1*$^+$	Total	$10^{-5}\times$frequency
CA1×G	3×3	10	8,571	117 (56–215)
CA1×G	3×3$^+$	8	11,497	70 (30–137)
G×G	3×3$^+$	0	10,366	0 (0–29)
CA1×CA1	3×3	0	10,462	0 (0–29)

The frequency of *gul-1*$^+$ recombinants is not significantly different between *rec-3* and *rec-3*$^+$ crosses. Using overlap of 95% confidence limits in Stevens' table (Stevens 1942), it is possible to calculate that 3·4 or more times *gul-1*$^+$ progeny would need to have been scored in the *rec-3* cross than in the *rec-3*$^+$ cross for a significant difference to have been shown. Thus, if *rec-3*$^+$ does affect *gul-1* recombination, the effect is less than that in the *am-1* locus where the smallest reduction shown so far is fivefold.

(*c*) *Recombination between* am-1 *and* gul-1

The single recombinant between *am-1* and *gul-1* [Section III(*a*)] was sufficient to order the two loci, but statistically insufficient to show any *rec-3*$^+$ effect. To obtain a larger sample, recombinants between these two loci were selected. Progeny of the same four crosses described in Section III(*a*) were germinated at the restrictive temperature (34°C) on medium lacking a supplement for the *am-1* mutant. Under these conditions only *am-1*$^+$ *gul-1* recombinants grow. The *gul-1*$^+$ progeny germinate, but growth is slight owing to the presence of *cot-1*, carried by all parents.

Table 3 shows the frequency of such recombinants, and their constitution at the unselected flanking loci *sp* and *his-1*. In this case, *rec-3*$^+$ crosses show a significantly lower recombinant frequency. This may be due to *rec-3*$^+$ or to other factors present in the stocks. Evidence that it is due to *rec-3*$^+$ comes from an examination of the flanking markers.

In neither case do the results fit those expected if recombinants arose from crossing over alone (Table 3). Both *rec-3* and *rec-3*$^+$ results show an excess of apparently multiple cross-overs, which can be interpreted to result from "negative interference". However, if conversion is also considered a significant source of recombinant progeny, multiple cross-overs need not be invoked. The *sp* + and + *his-1* classes would then arise from conversion of *am-1* to *am-1*$^+$ and of *gul-1*$^+$ to *gul-1* respectively. The minor class, *sp his-1*, could arise from either of these conversions plus a single independent cross-over in the non-adjacent region outside region II. Thus, 3% of the *am-1* → + converts would fall into the minor class by independent crossing over in region III and 5% of the + → *gul-1* converts by cross-overs in region I. The observed numbers of *sp his-1* recombinants are close to these expectations.

TABLE 3

FREQUENCY AND DISTRIBUTION OF FLANKING MARKERS AMONG *am-1*$^+$ *gul-1* PROGENY SELECTED FROM CROSSES OF THE TYPE ++ +*his-1*×*sp am-1 gul-1*+ USING *rec-3* AND *rec-3*$^+$ GENOTYPES

Cross	Frequency of *am-1*$^+$ *gul-1* recombinants ± S.E.	Flanking Markers				
		++	*sp*+	+*his*	*sp his*	Total
rec-3×*rec-3*	0·143%±0·011%	632	172	34	16	854
		74·0%	20·1%	4·0%	1·9%	
rec-3×*rec-3*$^+$	0·064%±0·004%	206	15	18	0	239
		86·2%	6·3%	7·5%	0·0%	
Origin of recombinants:						
(1) Crossing over in regions:		II	I,II	II,III	I,II,III	
Expected frequency*:		91·9%	4·9%	3·0%	0·2%	
(2) Crossing over or conversion:		II	*am* → +	+ → *gul*	—	

*Using pooled map distances from Table 1 and assuming no interference. Regions are those shown in Table 1.

The results can now be interpreted by claiming that *rec-3*$^+$ (1) reduces crossing over in region II (presumably often occurring within *am-1* itself since the length of *am-1* is of the same order as this region), and (2) reduces conversion of *am-1* to *am-1*$^+$. If this were the case, the relative number in the *sp* + class would fall and the number in the + *his-1* class increase, as is observed. Also, the observed reduction in total frequency of recombinants is expected.

This explanation is consistent with that previously used to account for the effect of *rec-3*$^+$ on allelic recombination in *am-1* (Smyth 1971). Hence it is possible to account for the apparent effects of *rec-3*$^+$ on recombination between *am-1* and *gul-1* solely in terms of its already-known effects on recombination within *am-1*.

IV. DISCUSSION

Recombination between distant loci almost always results from crossing over, whereas allelic recombination frequently occurs by conversion, often with associated crossing over. Data supporting this have long been known in fungi (Holliday 1968), and conversion has also recently been demonstrated in *Drosophila* (Smith *et al.* 1970).

If detection of conversion requires close markers, rather than being a unique characteristic of allelic recombination, then conversion should also contribute to recombinants between close but non-allelic markers. Stadler and Towe (1968) showed this for recombination between the close *cys-1* and *cys-2* loci of *Neurospora*. Murray's (1970) data for the *me-7* and *me-9* loci of *Neurospora* are also in strong support.

The present results also can be readily explained if conversion provides some recombinants between non-allelic markers at *am-1* and *gul-1*. An apparent effect of *rec-3*$^+$ on recombination between them can then be interpreted simply as an effect of *rec-3*$^+$ on conversion of the *am-1*^{19} mutant to wild type and of crossing over in *am-1* itself.

In spite of a detailed search, I have been unable to show any effect of *rec-3*$^+$ on recombination close to, but outside of, *am-1*. No effects were shown by crosses using markers 5 units proximal, 0·3 units distal, and 3 units distal of *am-1*. It is possible that studies using other markers more closely proximal to *am-1* would be successful in detecting effects outside of *am-1*. Even so, any hypothesis concerning *rec-3*$^+$ action must account for this very localized effect in the *am-1* region.

The *rec-3* locus has also been tested for effects on allelic recombination in 10 loci apart from *am-1*. No effect has been found in *gul-1* (this paper), *his-1* (Catcheside 1966), *inos* (Catcheside 1968) and *his-6* (Catcheside and Austin 1971), all on the same linkage group arm as *am-1*, and none in the unlinked loci *his-3*, *his-5*, *his-7* (Catcheside and Austin 1969), *try-1* (Catcheside and Austin 1971) and *nit-2* (D.E.A. Catcheside 1970). However, Catcheside and Austin (1971) have discovered that *his-2* (unlinked to *am-1*) is also sensitive to *rec-3*$^+$. Thus two loci out of 11 tested so far are affected.

The action of *rec-3* can be interpreted in the light of the discoveries made by Angel *et al.* (1970) on an analogous *rec* gene *rec-w*, which affects allelic recombination in the unlinked *his-3* locus. The de-repressed level of recombination in *his-3* depends on the genotype at a *recognition* (*cog*) locus, closely distal to *his-3*. Much higher levels are obtained in the presence of a dominant promotor of recombination, *cog*$^+$. Furthermore, map distances closely distal to *his-3* are larger in the presence of *cog*$^+$.

We can propose that recombination in the 8 unit region spanning *am-1* is initiated at a large but limited number of *recognition* sites. Only one site closely proximal to *am-1* would be recognized by a product of *rec-3*$^+$, and the initiation of recombination repressed. Since little if any effect of *rec-3*$^+$ has been shown proximal to *am-1*, it is possible all stocks used carry the recessive *cog* allele at this recognition site. Recombination would occur in the *am-1* locus by the extension of hybrid DNA into it from this recognition site. The present results indicate that hybrid DNA does not extend much beyond *am-1* and certainly not the 0·3 map units into *gul-1*. The *rec-3*$^+$ product would block this recombination specifically (and, incidentally, also that at a site with identical specificity near *his-2*). The residual recombination in *am-1* of different polarity (Smyth 1971) might result from hybrid DNA extending into *am-1* from an insensitive point distal (Smyth 1971) or more proximal (Whitehouse 1972) to *am-1*.

Just how the *rec-3*$^+$ gene product might repress recombination in the *am-1* locus is unknown, but discovery of variants of the proposed recognition sites (Angel *et al.* 1970) and closer study of the region proximal to *am-1* will help delimit possibilities.

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VI. References

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