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A new type of genetic control of gene conversion, from *Ascobolus immersus*

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SUMMARY

A new type of genetic control of gene conversion is described from the Pasadena strains of the fungus *Ascobolus immersus*. It is characterized by *cis/trans* position effects and incomplete dominance. The *P*, *K* and *91* factors segregated from each other like Mendelian alleles and controlled the conversion frequencies and patterns of four nearby, closely-linked *white* ascospore colour mutations, although they did not usually co-convert with these *w* sites. Mutations of different origin responded similarly to the same control factors.

These control factors greatly affected the total conversion frequencies, the relative frequencies of the different detected conversion classes and various other conversion parameters. The detailed results are consistent either with *P*, *K* and *91* affecting both the frequency of hybrid DNA formation and the correction processes for removing mispaired bases, or if they do not affect the correction processes directly, then they must have large effects on the frequency of asymmetrical hybrid DNA formation, which must usually be much more common than symmetric hybrid DNA, and there must be both an inequality in the frequency with which the two homologous chromosomes (in these crosses, + bearing and *w* bearing) invade each other, and in the frequency with which the two strands of each chromatid invade the homologue in asymmetric hybrid DNA formation.

1. INTRODUCTION

One method of studying the mechanisms of gene conversion and crossing-over is to use genetic variants with altered conversion or recombination frequencies. The various systems reviewed by Catcheside (1977) included genes with general effects on crossing-over or conversion, or with effects localized to specific chromosomal regions either remote from or close to the controlling gene.

For genes with localized effects, one type of control is exemplified by the three *rec* loci in *Neurospora crassa* (see Catcheside, 1977, who quotes parallel cases in *Schizophyllum* and yeast). In each case, one allele acts dominantly to depress recombination frequencies in particular unlinked or loosely linked target areas, one dose of the dominant allele being as effective as two. *rec-1* and *rec-3* affected allelic recombination, while *rec-2* primarily affected non-allelic recombination.

A second type of control in *Neurospora* is shown by *cog* genes where in the absence of *rec-2*$^{+}$ the *cog*$^{+}$ allele increases allelic recombination in the adjacent

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his-3 locus about seven-fold compared with *cog* × *cog* crosses. cog^+ is fully dominant for recombination frequencies but with evidence of some *cis/trans* effects when heterozygous: for example, it is the *his-3* allele in the cog^+ chromosome which is preferentially converted (Catcheside, 1977).

A third control type was shown for the *w*-62 locus in Pasadena strains of *Ascobolus immersus* (Emerson & Yu-Sun, 1967), where controlling factors closely linked to the affected site gave much less conversion when heterozygous than when homozygous. The *cv* factors (Girard & Rossignol, 1974) in European strains of the same species acted in a similar but more extreme way, reducing conversion and intragenic crossing-over several hundred fold.

The present work with the Pasadena strains of *A. immersus* has shown the existence of a fourth type of genetic control, characterized by incomplete dominance and clear *cis/trans* position effects for control factors *P*, *K*, *91*, which control the conversion frequencies and patterns for four closely-linked white (*w*) ascospore mutations, *w*-10, *w*-78, *w*-3C1 and *w*-NG1. These three control factors and four *w* mutations are all closely linked to each other but the factors do not usually co-convert with the *w* sites. Certain aspects of the control of conversion at the *w*-10 and *w*-78 sites have been previously studied by Emerson & Yu-Sun, 1967; Lamb & Wickramaratne, 1975, and Wickramaratne & Lamb, 1978.

By using octads with conversion for one of the *w* sites to get recombination between *w* sites and heterozygous control factors, we obtained different combinations of *w* mutations and control factors. Two complete cycles of crosses were made: +,*P* × *w*,*P*; +,*K* × *w*,*K*; +,*P* × *w*,*K*; +,*K* × *w*,*P*; +,*91* × *w*,*91*; +,*P* × *w*,*91*; +,*91* × *w*,*P*; +,*K* × *w*,*91*; +,*91* × *w*,*K*, where *w* was *w*-78 in one cycle, *w*-3C1 in the other. The two different mutations were used to study the effects of these control factors on mutations of different origin and widely different conversion spectrum. *w*-78 is a spontaneous mutation with a conversion spectrum resembling that of base-substitutions in other systems (Leblon, 1972; Yu-Sun, Wickramaratne & Whitehouse, 1977), while *w*-3C1 is ICR 170 induced, with a spectrum typical of frame-shifts in other systems. Using dehisced octads from these + × *w* spore colour marker crosses, both conversion to wild-type and to mutant were studied, which is an advantage over systems that detect only prototrophs in heteroallelic crosses of auxotrophs.

2. MATERIALS AND METHODS

(i) *Methods*

These were generally as described by Ghikas & Lamb, 1977, and Wickramaratne & Lamb, 1978, with all crosses incubated at 17·5 °C. In some experiments, however, a horse-dung extract agar (devised by S. Helmi) was used for ascospore germination. 60 g of air-dried horse-dung were shaken for one hour in a litre of distilled water. The liquid was filtered through double layers of muslin, then was centrifuged at 17000 rpm for 30 min. The supernatant was filtered through Whatman No. 1 paper before adding 20 g Difco bacto agar per l. and autoclaving at 15 p.s.i for 15 min.

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(ii) *Stocks*

All strains were ultimately derived from the two red-spored wild-type strains, P5 – and K5 +, isolated in Pasadena by Dr Yu-Sun. The original spontaneous ascospore colour mutant strains *w*-10,*K* and *w*-78,*K* were isolated from P5 – × K5 + crosses, while *w*-10,*P* and *w*-78,*P* were isolated from 2 + : 6*w* octads in crosses *w*-10,*K* × +,*P* (i.e. P5 –) and *w*-78,*K* × +,*P* (Emerson & Yu-Sun, 1967). The original P5 – wild-type is presumed to have carried the *P* control factor, with the original K5 + carrying the *K* factor. The *91* control factor was found in strain 91-2R + which was derived in an unrecorded fashion from P5 – and K5 + (Emerson, personal communication). Because some of the older strains, such as K5 +, were sterile or poorly fertile when the present work began, many reisolations had to be made: details and a pedigree diagram of current strains are given by Ghikas, 1978, who also describes the induction of white spore colour mutant *w*-3C1 with the acridine mustard ICR 170. *w*-NG1 was induced with the alkylating agent N-methyl-N′-nitro-N-nitrosoguanidine (NG).

The control factors *P*, *K* and *91* are recognized only by their effects on conversion of these *w* sites, but obtaining various combinations of *w* mutant and control factor was straightforward, especially as these control factors did not generally co-convert with the *w* sites. For example, in the cross +,*P* × *w*-78,*K*, *w*-78,*P* strains could be isolated from two white spores in 2 + : 6*w* octads, where conversion of + to *w* in a *P*-bearing chromatid gives *w*-78,*P*, and +,*K* strains can be reisolated from 6 + : 2*w* octads, where conversion from *w* to + in a *K*-bearing chromatid gives +,*K*. The two +,*K* progeny in the octad are easily distinguished from the four +,*P* progeny by their conversion frequencies in crosses to *w* strains. Using this method, devised by Emerson & Yu-Sun, 1967, all the required strains were obtained. Details and results of tests for phenocopies, false clustering of spores from different octads into groups of eight, mutations and reversion were given by Lamb & Wickramaratne, 1973, and were borne out by further tests in the present work. Virtually all aberrant ratios with apparent conversion to + proved to be genuine convertants, while a very small proportion of those with apparent conversion to *w* were due to new spontaneous mutations to *w*, usually at loci unlinked to *w*-78 or *w*-3C1. Agreement between replicates and repeat crosses for conversion frequencies was usually good: exceptions are noted in Table 1.

3. RESULTS

(i) *The nature and mapping relations of the* P, K *and* 91 *factors*

The different control factors are recognized by their effects on conversion frequencies at any of the four *w* sites, *w*-10, *w*-78, *w*-3C1 and *w*-NG1. *P*, *K* and *91* behaved like Mendelian factors and segregated from each other, giving 1:1 ratios in octads from meiosis and amongst random spores. For example, from the cross +,*P* × +,*91* (+ is wild-type for the *w* site, *P* and *91* are the control factor genotypes), cultures derived from random ascospores were crossed individually to

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strains of *w*-78,*P*, scoring about 1500 octads per cross. Twenty-nine of the 60 progeny tested carried *P*, giving high conversion frequency crosses (about 12%, range – with standard errors – 8·4 ± 0·8 to 14·8 ± 1·0), 30 carried *91*, giving low conversion frequencies (about 4%, range 2·4 ± 0·4 to 5·4 ± 0·6), with just one intermediate value, 6·8 ± 0·6%, which might have been due to chance or some uncontrolled environmental effect. Within each group (high or low conversion frequency strains), the extreme values appeared to be chance variations from their group means, as repeats of the extreme crosses usually gave conversion frequencies nearer to the group mean.

Factors *P* and *K* were present in the originally isolated wild-types P5– and K5+, and we found factor *91* in a strain derived from P5– and K5+, so it might have been present in one of them although it probably arose by recombination or mutation later, so amongst *P*, *K* and *91*, one can not describe any one as being *the* wild-type, nor the others as being mutants.

The four *white* sites with conversion affected by *P*, *K* and *91* all map close to each other. Their recombination frequencies (with standard errors) were: *w*-78,*P* and *w*-NG1,*P*, 0·11 ± 0·23%, *w*-78,*K* and *w*-3C1,*P*, 0·84 ± 0·07% (5·68 ± 0·22% when *w*-78,*P* was used); *w*-78,*K* and *w*-10,*K*, 0·20 ± 0·03% (0·48 ± 0·07% for *w*-78,*P* and *w*-10,*K*; 1·48 ± 0·10% for *w*-78,*P* and *w*-10,*P*). Lack of fertility in some crosses prevented complete ordering of the *w* sites. Recombination between these *w* sites is very largely by conversion (e.g. Emerson & Yu-Sun, 1967, for *w*-10, *w*-78 data), hence the sites are very close in terms of crossover units.

The three control factors all map close to the four *w* sites. In octads with 4+ : 4*w* ratios, one recombinant (*w*-78, *91*) occurred out of 30 tested progeny from the cross +,*91* × *w*-78,*P*, and Wickramaratne & Lamb (1978) found no recombinants in 27 progeny from +,*P* × *w*-10,*K* and +,*P* × *w*-78,*K*. Although *P*, *K* and *91* thus map very close to the four *w* sites, they do not usually co-convert with them. If they did co-convert, then conversion (shown by an aberrant ratio) at a *w* site should be accompanied by conversion at the control factor site, which would not give recombination between the control factors and *w* site. Putting together data of Emerson & Yu-Sun, 1967, Wickramaratne & Lamb, 1978, Ghikas, 1978, and the present work, out of 23 octads with aberrant ratios (6:2, 2:6 and 3:5 were tested – all ratios given as + : *w*) at the *w* site in + × *w*-10 or + × *w*-78 crosses with heterozygous control factors, 22 did have recombination (by conversion only at the *w* site) between the control factors and *w* site, so co-conversion probably only occurred in one octad out of 23. The precise mapping order of the control factors relative to the *w* sites is not yet known, though the control factors are unlikely to be between the *w* sites as they do not usually co-convert with them, though the different *w* mutants often co-convert with each other (data given by Emerson & Yu-Sun, 1967, for *w*-10, *w*-78; present work for *w*-78, *w*-3C1 and *w*-78, *w*-NG1).

(ii) *Specificity of control*

P, *K* and *91* have so far only been shown to control this one nearby series of *white* mutants. Ghikas (1978) crossed 23 other spore colour mutants, unlinked to

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this first series and distributed between various other linkage groups, to strains carrying *P*, *K* or *91*. Although there was evidence for other control factors affecting some of the other mutants, *P*, *K* and *91* seemed to have no clear effects themselves on the unlinked sites. In the present work, similar crosses involving three markers (one white, two granular pigmentation) unlinked to *P*, *K* and *91* also showed no evidence of non-local control of conversion by these factors.

For the *w*-10, *w*-78, *w*-3C1 and *w*-NG1 sites, *P*, *K* and *91* appear to be the only major genetic controls in our crosses that segregate and affect their conversion frequencies and patterns. For example, where crosses were homozygous for their control factors, e.g. +,*P* × *w*-78,*P*, in octads with 4+ : 4*w* ratios (i.e. with no detected conversion at the *w* site, although some might be 'correction' 4:4s or aberrant 4:4s), only 1 out of 27 octads had any progeny with significantly altered conversion properties compared with the parental strains.

(iii) *The effects of* P, K *and* 91 *on conversion at the* w *sites*

The main results for the effects of different combinations of *P*, *K* and *91* on conversion at the *w*-78 and *w*-3C1 sites are given in Table 1. For brevity, only results for narrower ratio classes (4:4, 6:2, 2:6, 5:3, 3:5) are given because the wider ratio classes (8:0, 0:8, 7:1, 1:7) were much rarer and their omission has little effect. Comparing the total narrower ratio conversion frequencies (NRCFs) in crosses with different control factor combinations, it is clear that these two white mutation sites, *w*-78 and *w*-3C1, responded in generally the same way to the control factors, even though the mutations are probably of different kinds, *w*-78 probably being a base-substitution and *w*-3C1 probably a frame-shift. For example, both showed about 11·5% total conversion in *P* × *P* crosses, about 1·9% in *K* × *K*, and more conversion in *P* × *K* and *K* × *P* than in *K* × *K* or *91* × *91* crosses.

Although the total conversion frequencies in comparable crosses for the two mutations were thus generally – though not uniformly – comparable, these total NRCFs were made up in quite different ways for the two mutants. Thus the 11·4% total conversion for *w*-78 in *P* × *P* crosses had 6:2 octads as its main component (59·5%), with small quantities of 2:6, 5:3 and 3:5 octads (Table 1), while the corresponding 11·8% total conversion for *w*-3C1 was mainly due to 2:6 octads (71·8%) and had extremely few of the postmeiotic segregation classes, 5:3 and 3:5. As well as these two complete cycles of crosses for *w*-78 and *w*-3C1, we have results for incomplete cycles involving *w*-10 and *w*-NG1: all four sites respond in similar ways to the control factors. For *w*-10, see Wickramaratne & Lamb, 1978, where 'HCF' strains of *w*-10 and *w*-78 carry *P*, as do P5 – and 1WT3 + : 'LCF' *w* strains carry *K*; 91-2R + carries 91.

If total narrower ratio conversion frequencies are considered for sets of crosses homozygous and heterozygous for particular pairs of control factors, the general result is incomplete dominance between the control factors, with the low-conversion-causing factors *K* and *91* being incompletely dominant to the high-conversion-causing *P*. For example, in the set of crosses for *P* and *K* with *w*-78, the homozygous crosses +, *P* × *w*,*P* and +,*K* × *w*,*K* gave 11·4% and 1·95% NRCFs

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Table 1. *Conversion data from* + × w *crosses with different combinations of control factors* P, K *and* 91, *where* w *is* (*i*) w-*78 or* (*ii*) w-*3C1*

Control factors in: +	; *w*	Narrower ratio segregation classes (+ : *w*) 4:4	6:2	2:6	5:3	3:5	Total octads	Total narrower ratio conversion frequency, % ± S.E.	Narrower ratio conversion classes 6:2	2:6	5:3	3:5
		Frequency, % of total octads							Relative frequency, %			
					(i) + × *w*-78							
P	P	88·46	6·78	2·91	0·78	0·91	37,051	11·38 ± 0·16	59·5	25·6	6·9	8·0
K	K	98·04	0·94	0·11	0·73	0·17	6,567	1·95* ± 0·17	48·4	5·5	37·5	8·6
91	91	99·12	0·53	0·21	0·07	0·06	28,599	0·87* ± 0·05	60·6	24·1	8·4	6·8
P	K	96·69	1·42	0·91	0·37	0·57	19,040	3·27 ± 0·13	43·3	27·8	11·4	17·5
K	P	96·12	2·81	0·29	0·59	0·17	41,164	3·85* ± 0·09†	72·9	7·6	15·2	4·4
P	91	97·53	0·92	1·15	0·12	0·27	27,376	2·38 ± 0·09	37·2	46·7	5·0	11·0
91	P	95·10	3·35	0·68	0·47	0·25	25,532	4·75* ± 0·13	70·6	14·3	9·9	5·2
K	91	98·45	0·77	0·44	0·19	0·12	28,544	1·52 ± 0·07	50·8	28·7	12·4	8·0
91	K	98·55	0·67	0·21	0·16	0·33	12,795	1·38 ± 0·10	48·9	15·3	11·9	23·9
					(ii) + × *w*-3C1							
P	P	88·15	3·31	8·43	0·00	0·01	17,211	11·75 ± 0·25	28·1	71·8	0·0	0·1
K	K	98·10	0·99	0·84	0·02	0·04	30,060	1·89* ± 0·08†	52·4	44·8	0·9	1·9
91	91	98·60	0·36	1·00	0·04	0·01	41,800	1·40* ± 0·06†	25·8	71·1	2·6	0·5
P	K	96·78	1·92	1·22	0·04	0·02	45,717	3·20 ± 0·08	59·9	38·0	1·4	0·6
K	P	97·42	1·19	1·30	0·05	0·04	17,966	2·57* ± 0·12	46·1	50·4	1·9	1·5
P	91	99·34	0·29	0·32	0·03	0·02	27,264	0·66 ± 0·05	44·4	47·8	4·4	3·3
91	P	96·71	1·68	1·27	0·19	0·02	5,134	3·16* ± 0·24	53·1	40·1	6·2	0·6
K	91	98·64	0·58	0·73	0·03	0·00	8,600	1·35 ± 0·12	43·1	54·3	2·6	0·0
91	K	99·19	0·28	0·48	0·02	0·03	12,493	0·81* ± 0·08	34·7	59·4	2·0	4·0

* Difference between vertically adjacent values for conversion frequency significant at the 1 % level in 2 × 2 χ^2 tests.

† Replicates or repeat crosses heterogeneous at the 5 % level. All experiments included at least two repeat experiments, each of at least two replicates.

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respectively, while the heterozygous crosses $+,P \times w,K$ and $+,K \times w,P$ gave 3·27 % and 3·85 % respectively, which are significantly different at $P = 0{\cdot}01$ from both homozygous cross values, but closer to the $K \times K$ than to the $P \times P$ value. The incomplete dominance holds for all w-78 sets of crosses (for P and K, P and *91*, and K and *91*) and for most but not all w-3C1 sets, where one value ($+,K \times w$, *91*) is not significantly different from the lower homozygous cross value, and two values ($+,P \times w$,*91* and $+$,*91* $\times w,K$) are lower than the lowest homozygous value.

In addition to incomplete dominance, the results in Table 1 show clear *cis/trans* position effects of the control factors on conversion of the w sites. That is, in a $+ \times w$ cross with heterozygous controlling factors, such as P in one parent, K in

Table 2. *Some conversion parameters* from* + × w *crosses with different combinations of control factors* P, K *and* 91, *where* w *is* (*i*) w-*78 or* (*ii*) w-*3C1*

Control factors in:		Direction of conversion ratio				Efficiency of correction ratio	
		a	*b*	*c*	*d*	*e*	*f*
+	*w*	6:2 / 2:6	5:3 / 3:5	2(6:2)+5:3 / 2(2:6)+3:5	(6:2)(3:5) / (2:6)(5:3)	2(6:2+2:6)+5:3+3:5 / 5:3+3:5	6:2+2:6 / 5:3+3:5
				(i) + × *w*-78			
P	P	2·3	0·9	2·1	2·7	12·5	5·7
K	K	8·9	4·4	6·9	2·0	3·3	1·2
91	91	2·5	1·2	2·4	2·0	12·4	5·6
P	K	1·6	0·7	1·3	2·4	6·0	2·5
K	P	9·6	3·5	8·3	2·8	9·2	4·1
P	91	0·8	0·5	0·8	1·7	11·6	5·2
91	P	4·9	1·9	4·5	2·6	12·2	5·6
K	91	1·8	1·5	1·7	1·1	8·8	3·9
91	K	3·2	0·5	2·0	6·4	4·6	1·8
				(ii) + × *w*-3C1			
P	P	0·4	0·0†	0·4	†	2349†	1010†
K	K	1·2	0·5	1·2	2·6	62·0	34
91	91	0·4	5·0	0·4	0·1†	55·4	32
P	K	1·6	2·2	1·6	0·7	106	49
K	P	0·9	1·3	0·9	0·7	56·3	28
P	91	0·9	1·3	0·9	0·7	25·4	12
91	P	1·3	10·0	1·4	0·1†	29·1	13
K	91	0·8	†	0·8	0·0†	88·3	38†
91	K	0·6	0·5	0·6	1·1	31·4	16

* See Wickramaratne & Lamb, 1978, for details of theory, assumptions etc.

a–d Direction of conversion ratio (conversion to +/conversion to *w*): *a*, value for detected conversion classes with meiotic segregation; *b*, value for detected conversion classes with postmeiotic segregation; *c*, overall value for dual hybrid chromatid models; *d*, overall value for single hybrid chromatid models.

e–f Efficiency of correction ratio (detected correction events/detected non-corrections): *e*, dual hybrid chromatid models; *f*, single hybrid chromatid models.

a–f Fuller versions of these formulae (Wickramaratne & Lamb, 1978) include aberrant 4:4s and correction 4:4s, but both these classes were indistinguishable from normal 4:4s in these experiments.

† Value unreliable because of small numbers observed for at least one figure used in this calculation.

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the other, different conversion results were obtained according to whether a given factor (say *P*) is in *cis* to +, e.g. +,*P* × *w*,*K* or in *trans* to +, e.g. +,*K* × *w*,*P*. This showed as either a significant difference in NRCFs between the two heterozygous crosses of a set, e.g. between +,*P* × *w*-78,*91* and +,*91* × *w*-78,*P*, and/or a clear difference in relative frequencies for the narrower ratio conversion classes 6:2, 2:6, 5:3, 3:5. The latter differences occurred even when total conversion frequency differences between two heterozygous *cis/trans* crosses were less marked, e.g. +,*K* × *w*-78,*91* and +,*91* × *w*-78,*K*.

Table 2 shows various conversion parameters estimated for these cycles of crosses, using the formulae of Wickramaratne & Lamb, 1978. Factors *P*, *K* and *91*, and the nature of the mutation involved, all affected the direction of conversion ratio (*a*, *b*, *c*, *d* give different estimates for relative frequencies of conversion to + and to *w*) and the efficiency of correction ratio (*e*, *f*), both with formulae for dual hybrid chromatid and single hybrid chromatid models of recombination. For example, *w*-78 crosses usually, but not always, had a higher direction of conversion ratio than *w*-3C1 crosses, while *w*-3C1 crosses had a higher efficiency of detected correction than did *w*-78 crosses, with various combinations of *P*, *K* and *91* clearly affecting the various parameters for each mutant.

4. DISCUSSION

In the present work, both conversion to wild-type and to mutant were studied for the *w* sites. If the data in Table 1 are used to calculate the percentage of spores with conversion to wild-type, to make these results more comparable to others where selective systems using only prototroph frequencies were used, one still gets incomplete dominance and *cis/trans* effects. The *P*, *K* and *91* system of conversion control in *Ascobolus immersus* is thus genuinely different from previously reported systems (references in Catcheside, 1977) such as the *rec/cog/con* type in *Neurospora*, *Schizophyllum* and *Schizosaccharomyces*, or systems where heterozygosity for control factors depresses conversion below the level of both homozygous arrangements, as with the *w*-62 controls and *cv* factors in *Ascobolus*.

The initial work on *P* and *K* was by Emerson & Yu-Sun, 1967, with a complete cycle for *w*-10 and an incomplete one for *w*-78: most of our corresponding results are similar. They concluded, however, that *P* gave completely dominant enhancement of conversion when in *cis* to *w* but not in *trans*. This conclusion hinges on their +,*K* × *w*-10,*P* high conversion result which is quite different from any of their other results, with 3:5 octads as the most frequent conversion class. They state that crosses to +,*K* were poor, with few counts made. Our *w*-78,*P* results, in crosses to three separately isolated +,*K* strains and with large samples, consistently gave fairly low conversion frequencies, as did +,*K* × *w*-3C1,*P*. In view of the consistency of our results on the incomplete dominance of *K* over *P*, and of our different kinds of *cis/trans* effects from those of Emerson & Yu-Sun, 1967, their results for the crucial cross +,*K* × *w*-10,*P* seem inexplicable, possibly a freak result from rather infertile crosses.

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Of the control factors, *P* tends to give high conversion frequencies and is easily distinguished from *K* and *91*. Although *K* and *91* both give low conversion frequencies, they differ in their extent of conversion-suppression when homozygous and in giving differences in relative frequencies of various conversion classes (Table 1). *K* and *91* also often had quite different effects on various conversion parameters (Table 2). The heterozygous *K*, *91* crosses gave interesting 'negative evidence' on relations between these two factors. If *K* and *91* were non-allelic recessive mutations causing low conversion frequencies, they should complement in *trans* to each other, to give high conversion frequencies, but that did not occur. Neither did they have additive or synergistic effects, as might have occurred in crosses containing *K* and *91* if they depress recombination in different ways. Crosses heterozygous for a pair of control factors, e.g. $+,P \times w,K$ are the *trans* part of a classical *cis*/*trans* test for allelism between control factors, but there are several obstacles to completing this test. They include incomplete dominance, lack of a suitable *cis* test, and the fact that one cannot say what a 'wild-type' phenotype for conversion frequency at the *w* sites would be because in the two original wild-types, one contained *P*, one contained *K*, and *91* may have been present in one of them.

Detailed analyses and molecular interpretations of the action of *P*, *K* and *91* are still in progress (Helmi & Lamb, in preparation) but some conclusions relevant to recombination models can already be drawn. On models postulating the symmetric formation of hybrid DNA in two homologous non-sister chromatids, as on the Holliday (1964) and Whitehouse & Hastings (1965) models, changes in just the frequency of hybrid DNA formation at a site should cause all narrower ratio conversion class frequencies to change in parallel (unless amounts of correction enzymes become limiting). In the present results (Table 1), however, these classes did not change in parallel and showed big differences in their relative frequencies in crosses with different combinations of *P*, *K* and *91*. These data cannot therefore be explained solely in terms of changes in frequency of symmetric hybrid DNA, so either effects on asymmetric hybrid DNA formation and/or on the correction of mispaired (or non-paired) bases must have been involved.

Certain changes in relative frequencies of the different conversion classes and the *cis*/*trans* effects could be explained in terms of single hybrid chromatid models (references in Wickramaratne & Lamb, 1978), or of models involving both symmetric and asymmetric hybrid DNA, such as that of Meselson & Radding, 1975. For example, a difference in relative frequencies of conversion classes in the crosses $+,P \times w$-78,*K* and $+,K \times w$-78,*P* could arise if *P* increased the frequency with which the chromatid carrying it was invaded by the homologous non-sister chromatid in asymmetric hybrid DNA formation. In the former cross, this would increase the invasion of + by *w*, increasing the 2:6 and 3:5 classes, while in the latter cross there would be increased invasion of *w* by +, increasing 6:2 and 5:3 conversion classes.

The formulae of Wickramaratne & Lamb, 1978, can be used to obtain comparative (but not absolute) estimates of the efficiency of correction of mispairs and the

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direction of conversion ratio (conversions to + / conversions to *w*) on both dual and single hybrid chromatid models. Large changes in these parameters occurred (Table 2) for the *w* sites with different combinations of control factors. There are therefore several possibilities for the action of *P*, *K* and *91* in changing total conversion frequencies and relative frequencies of conversion classes: (i) the action is on hybrid DNA formation alone; (ii) the action is on the correction of base mispairs (or non-pairs) alone; (iii) both hybrid DNA formation and correction are involved.

The changes in relative class frequencies disprove hypothesis (i) for models with only symmetric hybrid DNA formation, as previously discussed, and would disprove it for models with asymmetric hybrid DNA formation if both types of chromatid (+ bearing and *w* bearing in these crosses) were invaded by the other with equal frequency, irrespective of *P*, *K* or *91* combinations present, or if there were no strand preference within chromatids during invasion. The following example shows how with asymmetric hybrid DNA formation, strand preference within chromatids and unequal chromatid invasion frequencies could change relative class frequencies and various conversion and correction parameters, even with no direct effects of *P*, *K* and *91* on correction. Suppose that at the point of mutation each + bearing chromatid carries an *A.T* base pair in its two strands and each *w* bearing chromatid carries a corresponding *C.G* pair, and that the *A* bearing single polynucleotide strand invades the homologue more often than does the *T* bearing strand, and the *C* bearing strand invades more often than the *G* bearing strand. Then if a single strand from the + bearing chromatid invades the *w* bearing chromatid more often than a single strand from *w* invades +, there will be a preponderance of *A.G* mispairs formed, but if with another combination of control factors *P*, *K* and *91*, a single strand from the *w* chromatid invades the + chromatid more often than a single strand from + invades *w*, there will be a preponderance of *C.T* mispairs. As *A.G* and *C.T* mispairs may well have different efficiencies and direction (to + or to *w*) of correction, the different combinations of control factors could – by acting solely on asymmetric hybrid DNA formation, with no direct action themselves on correction – thus affect correction parameters and relative class frequencies. If there is no strand preference within chromatids for invasion, then even if + bearing and *w* bearing chromatids invade each other with different frequencies, the two kinds of mispair, *A.G* and *C.T* in this example, will be formed with equal frequencies, and changes in relative conversion class frequencies could not be explained by changes in hybrid DNA formation alone, even on asymmetric hybrid DNA models.

Hypothesis (ii), that the controlling factors only affect correction but not hybrid DNA formation, is unlikely because of the very large changes in total observed conversion frequencies in different crosses. As aberrant 4:4s and correction 4:4s were indistinguishable from normal 4:4s in most of these experiments, the same hybrid DNA frequency could in theory give different frequencies of detected conversion (6:2, 2:6, 5:3, 3:5 and wider ratio classes) if the action of *P*, *K* and *91* on correction processes altered the relative frequencies of aberrant and correction

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4:4s compared with detected conversion classes. The work of Ghikas & Lamb, 1977, disproves that major changes in total conversion frequency were due to changes in aberrant 4:4 frequencies, because aberrant 4:4s had the same low frequency, $0{\cdot}15 \pm 0{\cdot}02\,\%$, in both a high conversion frequency cross (+ ,*P* × *w*-78,*P*, NRCF = 11·4 %) and a low conversion frequency cross (+ ,*P* × *w*-78,*K*, NRCF = 3·3 %). A rise in correction efficiency could increase detected conversion classes by changing potential undetected aberrant 4:4s into detected classes such as 5:3 or 2:6, or reduce detected conversion classes by changing potential 5:3s and 3:5s into undetected correction 4:4s, but a detailed examination of Tables 1 and 2 will not support such explanations for large changes in NRCF. For example, Table 2 shows that a high conversion cross, +,*P* × *w*-78,*P*, NRCF = 11·4 %, had a very similar efficiency of correction ratio to a very low conversion cross, +,*91* × *w*-78,*91*, NRCF = 0·9 %.

Hypothesis (iii), that *P*, *K* and *91* affect both hybrid DNA formation and correction, is consistent with the present data. There are therefore two acceptable explanations for the actions of these controlling factors on conversion frequencies and patterns of the nearby *white* sites. Either they affect both hybrid DNA formation (which in this case could be symmetric or asymmetric) and correction, or they affect a predominantly asymmetric hybrid DNA formation, with + and *w* bearing chromatids being able to invade each other with different frequencies and with different single strands within each chromatid having different invasion frequencies, and with no direct effects of *P*, *K* and *91* on correction processes. Further work and analysis to distinguish these alternatives are in progress.

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