NOTES AND COMMENTS

FINE CONTROL OF GENETIC RECOMBINATION IN YEAST

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Received 5.vii.70

1. Introduction

The fine and coarse controls of genetic recombination differ by the following criteria (Simchen and Stamberg, 1969*a*). (*a*) The latter has an all-or-none effect on recombination while the former alters its frequency only. (*b*) The coarse control affects the whole genome while the fine control shows specificity to short chromosomal segments and presumably involves recognition between the controlling genes and the controlled segments. (*c*) Variation in the fine control of recombination is commonly found as naturally occurring differences between various strains, for instance in Neurospora (Catcheside, 1968) and Schizophyllum (Stamberg, 1968; Simchen and Stamberg, 1969*b*); on the other hand, the coarse control can usually be demonstrated to occur from the behaviour of rare mutants, for instance in *E. coli* (Clark, 1967) and barley (Riley and Miller, 1966). An implication of these three differences is that the fine control has an evolutionary role of adjusting the frequencies of recombination independently in different regions of the genome, thus producing new genotypes at carefully controlled rates. The coarse control provides the essential machinery for the occurrence of crossing-over and variants in which any element of this machinery fails are usually selected against. The following experiments demonstrate the occurrence of a fine control of recombination in the yeast *Saccharomyces cerevisiae.*

2. Material and methods

Two pink heploid strains, *X1266-1C a* hi_4^- le_2^- ur_1^- me_{10}^- ad_2^-, obtained from Dr R. K. Mortimer (Donner Laboratory, Berkeley), and α ad_{2-1}^-, obtained from Dr J. H. Croft (Genetics Dept., University of Birmingham), were mated on YEPD plates (1 per cent. yeast extract, 2 per cent. peptone, 2 per cent. dextrose, 2 per cent. agar), and formed an almost white diploid which was isolated by streaking on YMIN (4 per cent. dextrose, 0·2 per cent. NH_4Cl, 0·05 per cent. $CaCl_2$, 0·15 per cent. KH_2PO_4, trace-elements and vitamins, 1·5 per cent. agar). The diploid, designated *Dip 8*, was grown in liquid presporulation medium (5 per cent. dextrose, 2·3 per cent. nutrient broth, 1 per cent. yeast extract) overnight, and then in liquid sporulation medium (0·4 per cent. anhydrous sodium acetate) for a week, both periods in a shaking waterbath. The suspension of diploid cells, intact tetrads and ascospores was spread on YEPD plates and incubated for a few days at 30° C. Haploid colonies were recognised by their pink colour, while the parental diploid was almost white (Mortimer's ad_2^- and its allele ad_{2-1}^- complement in this diploid) and intact tetrads gave sectoring colonies. Haploid progeny were transferred on to YEPD plates in 5×5 matrices, 25 colonies per plate,

and were afterwards tested by replica-plating on differential media (YMIN + supplements: 20 mg./l. histidine, uracil, methionine and adenine, and 40 mg./l. leucine). Mating-type determination was carried out by replica-plating each of the 5 × 5 matrices on a lawn of replicated *a* ad_1^- and on another lawn of replicated α ad_1^- (obtained from J. H. Croft), both on YEPD plates. These plates were incubated at 30° C. for three days and afterwards replicated on to YMIN plates. Diploid colonies developed after one day of incubation, thus indicating the mating types of the original colonies. Replica plating was performed with sterile velvet pads.

3. Results and discussion

Two adjacent segments on chromosome III (Hawthorne and Mortimer, 1968) were examined for frequency of recombination throughout these experiments, namely $hi_4 \sim le_2$ and $le_2 \sim \alpha$, with the centromere included in the

Table 1

Frequencies of recombinants for the $hi_4 \sim le_2$ *chromosomal segment. The diploids were produced by mating ten different siblings to one common strain* α ad_1^-.

Diploid	Per cent. recombinants	Sample size
Dip 8-1	11·6	216
Dip 8-2	6·0	218
Dip 8-3	0·4	240
Dip 8-4	3·0	221
Dip 8-5	7·5	186
Dip 8-6	3·2	252
Dip 8-7	2·2	274
Dip 8-8	7·3	303
Dip 8-9	2·5	236
Dip 8-10	8·3	217

latter. The following frequencies of recombinants were obtained from *Dip 8*: $hi_4 \sim le_2$ 16·9 per cent. and $le_2 \sim \alpha$ 30·3 per cent. (see also table 2). In this cross, as well as in the crosses that follow, the two alleles at each of the three loci under examination were recovered at approximately equal frequencies. There was no apparent evidence for differential viability affecting any particular genotype.

Ten haploid progeny were picked for further study, all of which were of the genotype *a* hi_4^- le_2^- ad_2^- and contained the wild-type alleles of ur_1, and me_{10}. These were designated *Hap 8-1* to *Hap 8-10* and were all mated to α ad_1^-. The diploids, *Dip 8-1* to *Dip 8-10*, were sporulated and haploid progeny were isolated from each of them as described above for *Dip 8*. Only recombination frequencies in the $hi_4 \sim le_2$ segment were examined. Table 1 contains these frequencies which vary over a wide range and clearly show heterogeneity (contingency $\chi^2_{(9)} = 50{\cdot}45$, $P < 0{\cdot}001$). The heterogeneity can be eliminated by dividing the data into two groups, namely *Dip 8-1*, *8-2*, *8-5*, *8-8* and *8-10* as the high-recombination group (within group contingency $\chi^2_{(4)} = 5{\cdot}23$, P = 0·30 ~ 0·20) and *Dip 8-3*, *8-4*, *8-6*, *8-7* and *8-9* as the low-recombination group (contingency $\chi^2_{(4)} = 5{\cdot}74$, P = 0·30 ~ 0·20). The pooled percentage of recombinants for the " high " group is $8{\cdot}07 \pm 0{\cdot}81$ and the pooled percentage for the " low " group is $2{\cdot}21 \pm 0{\cdot}44$. Clearly, at least one locus with two alleles which have different effects on recombination in $hi_4 \sim le_2$, is

segregating among the haploid progeny of *Dip 8*; thus *X1266-1C* and $\alpha\ ad^{-}_{2-1}$ (the two parents) differ in this locus, which does not seem to be linked to the affected region.

To test variation in $hi_4 \sim le_2$ and $le_2 \sim \alpha$ simultaneously, three of the ten haploids were each mated to both $\alpha\ ad^{-}_{2-1}$ and $\alpha\ ad^{-}_{2-R8}$ (the latter also obtained from J. H. Croft) and the six diploids were isolated, sporulated and progeny-tested as described above. These diploids were *Dip 8-1-1* and *Dip 8-1-R8* which were derived from *Hap 8-1* mated with $\alpha\ ad^{-}_{2-1}$ and $\alpha\ ad^{-}_{2-R8}$ respectively, *Dip 8-4-1* and *Dip 8-4-R8* which were derived from *Hap 8-4*, and *Dip 8-6-1* and *Dip 8-6-R8* which were derived from *Hap 8-6*. Frequencies of recombinants are given in table 2, from which the following conclusions can be drawn.

TABLE 2

Frequencies of recombinants for two adjacent chromosomal segments, $hi_4 \sim le_2$ *and* $le_2 \sim \alpha$, *in the original diploid and six derived diploids. For the derivation of the diploids, see text*

Diploid	Per cent. recombinants $hi_4 \sim le_2$	Per cent. recombinants $le_2 \sim \alpha$	Sample size
Dip 8	16·9	30·3	320
Dip 8-1-1	11·6	28·4	190
Dip 8-1-R8	9·2	22·1	195
Dip 8-4-1	4·6	46·4	151
Dip 8-4-R8	3·3	39·2	153
Dip 8-6-1	15·7	32·5	166
Dip 8-6-R8	16·8	32·1	196

(1) $\alpha\ ad^{-}_{2-1}$ and $\alpha\ ad^{-}_{2-R8}$ are very similar in their effects on recombination in the two regions studied. Pooled frequencies of recombinants can therefore be calculated for each pair of diploids. (2) There is genotypic control of recombination in $hi_4 \sim le_2$ ($\chi^2_{(2)} = 30{\cdot}48$, $P < 0{\cdot}001$) and of recombination in $le_2 \sim \alpha$ ($\chi^{2}_{(2)} = 21{\cdot}75$, $P < 0{\cdot}001$). For each segment at least two controlling loci must be postulated because three distinct levels were obtained. (3) The recombination frequencies in the two regions are affected differently by the genotypic differences, probably due to at least one locus affecting each segment independently of the other segment. (4) *Hap 8-6* is very similar to the parental haploid *X1266-1C* with respect to genes affecting recombination in $hi_4 \sim le_2$ and $le_2 \sim \alpha$ because both haploid strains gave the same recombination values when mated to $\alpha\ ad^{-}_{2-1}$.

The possibility that factors other than fine-control genes result in the variation observed can be ruled out by a reasoning similar to that given in detail by Stamberg (1968). Thus these experiments demonstrate the occurrence of fine control of recombination in *S. cerevisiae* as specified by the three criteria presented in the beginning of this report.

4. SUMMARY

1. Frequency of meiotic recombination in the $hi_4 \sim le_2$ region of *Saccharomyces cerevisiae* varies considerably among sibling strains and is under the control of at least one locus elsewhere in the genome.

2. The adjacent region of $le_2 \sim \alpha$ is affected differently by genotypic differences thus suggesting that specific and independent genes control each region.

Acknowledgment.—We thank Miss Rachel Ronen for technical assistance.

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THE FIFTH CHROMOSOME HISTOCOMPATIBILITY TYPES OF MOUSE STRAINS Hg/Hu AND C3Hf/A

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Received 18.viii.70

1. Introduction

THERE is some indication, in the case of both rats and mice, that maternal-foetal incompatibility may lead to the preferential survival of offspring having histocompatibility antigens different from those of their mother (Hull, 1969; Palm, 1970). Since there is evidence that maternal-foetal " autoincompatibility " of this type is associated with differences between offspring and their mother at the *H-3/H-13* chromosome V region (Hull, 1969), which also includes the *a* locus (Snell *et al.*, 1967), it was decided to type the two strains CeHf/HeHa and Hg/Hu to see if they showed chromosome V histocompatibility differences: these two strains gave rise to the stock in which incompatibility between female parent and offspring of the same genotype at the $+/a^t$ locus was originally seen (Hull, 1964).

2. Experimental material and methods of interpretation of results

Reciprocal F_1 tests were used to type Hg/Hu* and C3Hf with strains B10LP and C57BL/Sn, a pair of coisogenic resistant strains bred by Dr Snell, with skin grafts as the test transplants. A piece of skin 1·5 cm. in diameter was taken from donor animals of the same sex as the recipient and grafted on to the right flank. The graft was attached by five or six stitches, dusted with antibiotic powder and covered with a plaster of paris bandage. After

* It is almost certain that strain Hg/Hu is the result of a cross between two strains both of C57BL derivation (K.P. Hummel, personal communication.)