Genet. Res., Camb. (1971), **18**, *pp.* 147-151
With 2 text-figures
Printed in Great Britain

Rates of spontaneous mitotic recombination in *Saccharomyces cerevisiae*

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(*Received* 7 *April* 1971)

SUMMARY

The rates of mitotic recombination at the loci *ade3*, *ade5–7*, *ade6* and *ade8* were approximated by the method of 'increasing proportion of variants with growth'. The values (per cell generation) were $3{\cdot}5 \times 10^{-4}$ for *ade8*, $1{\cdot}4 \times 10^{-4}$ for *ade5–7*, $4{\cdot}0 \times 10^{-5}$ for *ade3* and $< 2 \times 10^{-6}$ for *ade6*. The relative frequencies of mitotic recombination in regions between *ade5–7* and the centromere were different from those obtained with radiation-induced recombinants.

1. INTRODUCTION

In the prolonged cultivation of diploid yeast, such as in the commercial production of bakers yeast and in continuous brewing, significant changes in the genetic composition of the culture may occur because of mitotic recombination. The extent of such changes will depend upon (*a*) the rates of mitotic recombination at particular loci which are heterozyous, and (*b*) whether selection operates in favour of or against recombinants. The measurement of rates of spontaneous mitotic recombination, as distinct from frequencies of recombinants, has not been reported, other than a preliminary estimate for *ade8* (Johnston & MacKinnon, 1966). This paper reports the measurement of such rates by a method used for mutation rates in bacteria (Stocker, 1949; Novick & Szilard, 1950).

2. MATERIAL AND METHODS

(i) *Strains*

Two hybrids were constructed from cultures of *S. cerevisiae* originally obtained from Dr R. K. Mortimer, Berkeley, California. Their genotypes are given in Table 1.

(ii) *Media*

Broth was MYPAD (malt extract 0·3%, yeast extract 0·3%, peptone 0·5%, glucose 1%, adenine 40 mg/l.). For plates, the same medium but without the adenine supplement and with addition of 2% agar (MYP) was used. Synthetic complete (SC) and omission media were those of Hawthorne & Mortimer (1960) and actidione agar was SC with the addition of 1·8 mg/l. actidione. Glycerol medium (GLY) was that of Ogur & St John (1956).

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(iii) *Methods*

Both strains form red colonies on MYP medium due to their *ade2* blocks but recombinants homozygous for either *ade3*, *ade5–7*, *ade6* or *ade8* produce adenine-dependent white colonies (Roman, 1956). White colonies, but adenine-independent, are formed by *ade2* revertants. White colonies, smaller in size, are also produced by respiratory-deficient mutants, but these are unable to grow on glycerol medium. The composition of cultures was therefore assayed by plating approximately 1000 cells on MYP medium, with approximately 200 cells per plate, and after 3 days incubation at 30 °C testing white colonies for adenine-dependence and growth on GLY plates.

Table 1. *Genotypes of* Saccharomyces cerevisiae *hybrids*

Hybrid	Genotype*
XT 5	$\frac{a\ ade2}{\alpha\ ade2}\ \frac{ade6\ ade3\ ade8\ try4\ arg4\ his6}{+\ +\ +\ +\ +\ +}$
XT 14	$\frac{a\ ade2}{\alpha\ ade2}\ \frac{act2\ met13\ tyr3\ lys5\ ade5–7}{+\ +\ +\ +\ +}$

* Mitotic linkage has been shown for *ade3–ade6* (Roman, 1956) and *ade8–try4* (Johnston, 1962). Mitotic and meiotic linkage has been shown for the *ade5–7* linkage group (Nakai & Mortimer, 1969).

Cultures were grown in 50 ml flasks containing 10 ml MYPAD medium on an orbital shaker at 30 °C. Initial inocula were from 2-day-old colonies of stock cultures and thereafter 1000 cells were transferred after 24 h growth to fresh medium, i.e. growth was prolonged by serial transfer.

3. RESULTS

The percentage of white colonies, all of which were adenine-dependent and glycerol-positive, in cultures between 4 and 14 days is shown in Fig. 1. Each point is the average of six flasks. The results prior to this time are not included as the sampling variation of cultures with $< 1\%$ recombinants (< 10 recombinants transferred) is high. All of a large sample of white colonies were presumed to be diploid because of their ability to sporulate.

These points (Fig. 1) are best fitted by a slightly concave curve which becomes more pronounced after 14 days, so that recombinants comprise 25% of the population after 18 days. This result shows that recombinants have a selective advantage, for if their growth rate were equal to that of cells of original genotype, a convex curve should be obtained because of the decreasing proportion of heterozygotes available for recombination.

These data, however, also show a good approximation to linearity and the calculated 'line of regression' is shown in Fig. 1. The slope of this line is $3{\cdot}9 \times 10^{-4}$ recombinational events per cell generation, since strain XT 5 completed 19 generations of exponential growth each 24 h. This is therefore the estimated rate for recombination at the three loci *ade3*, *ade6* and *ade8*. To obtain estimates for indi-

vidual rates at these loci, white colonies were first tested for tryptophan dependence. The results (Fig. 2) show that an average of 67% of these colonies are tryptophan-dependent, although this proportion increases slowly with time, as would be expected if the relative frequency of tryptophan-dependent recombinants is > 50%

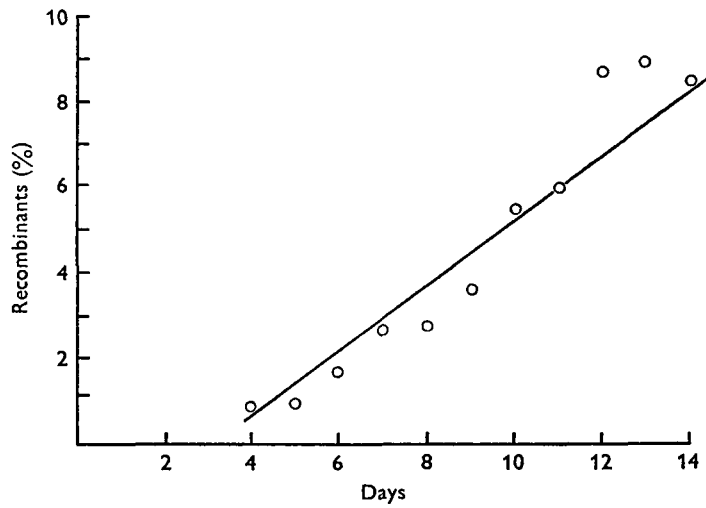

Fig. 1. Overall percentage of adenine-dependent white colonies of hybrid XT5 grown by daily serial transfers (19 generations/day).

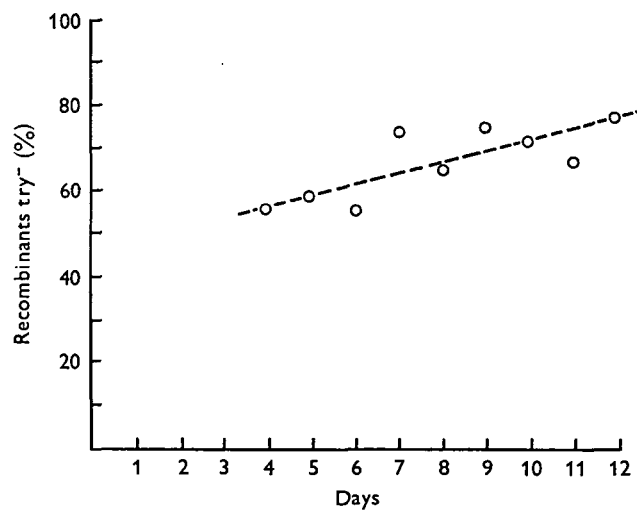

Fig. 2. Percentage of all adenine-dependent white colonies of strain XT5 which are also tryptophan-dependent.

and there is no selection against this genotype. Tryptophan-dependence identifies these colonies as *ade8 try4* recombinants. Approximately 30% of *ade8* recombinants do not show coincident mitotic segregation for the *try4* locus, however (Johnston & MacKinnon, 1966), and thus tryptophan-independent white colonies may be either

https://doi.org/10.1017/S0016672300012544 Published online by Cambridge University Press

ade8 or *ade3* or *ade6* recombinants. To identify which, a sample of 60 tryptophan-independent white colonies, selected from different cultures, were subjected to tetrad analysis and segregants were tested for complementation with 'tester' strains of both mating types carrying the *ade3*, *ade6* and *ade8* mutations respectively. Of these, 40 colonies were homozygous for *ade8*, 20 for *ade3*, and none for *ade6*. None were homozygous for both *ade3* and *ade8*. Thus, within experimental limits, the proportion of all recombinants which are *ade8* homozygotes is 90% and the remaining 10% are *ade3* recombinants. The individual rates of mitotic recombination (per cell per generation) are therefore $3{\cdot}5 \pm 0{\cdot}7 \times 10^{-4}$ for *ade8* and $4{\cdot}0 \pm 0{\cdot}8 \times 10^{-5}$ for *ade3* (95% confidence limits are shown). Since none of the 60 colonies tested by tetrad analysis were *ade6* homozygotes, the rate of mitotic recombination for *ade6* can be estimated as $< 2 \times 10^{-6}$ per cell generation. In this small sample, no mitotic linkage is shown by *ade3* and *ade6*.

With strain XT14, reasonable linearity of similar data was apparent only between 4 and 9 days. Thereafter, the increase in percentage of white colonies curved more steeply and recombinants comprised 7·5% of the population after 12 days. Clearly *ade5–7* recombinants have more of a selective advantage over 'red' cells than do corresponding *ade8* and *ade3* recombinants. The rate of mitotic recombination of *ade5–7*, equal to the slope of the line of regression through six points, was therefore more approximate, its value estimated as $1{\cdot}4 \pm 0{\cdot}8 \times 10^{-4}$ per cell generation. The results of testing *ade5–7* recombinants for linked markers were surprising. Only 10% showed coincident recombination and all of these were recombinants for *lys5*, *tyr3*, *met13* and *act2*. The relative frequencies of spontaneous mitotic recombination in this linkage group are therefore considerably different from those observed when segregation is induced by X-rays and u.v. light (Nakai & Mortimer, 1969).

4. DISCUSSION

These results show the relatively high rates of mitotic recombination for non-centromere-linked genes (*ade3*, *ade5–7*, *ade8*). Similar rates for other non-centromere-linked genes might be expected to lead to significant population changes in (heterozygous) yeast cultures within relatively short times during prolonged growth. Clearly, there is a need to study the rate of increase of recombinants when no selection applies. The population kinetics will then also depend upon the mechanism of spontaneous mitotic recombination. Different end-results should be obtained depending on whether the recombinational event is (*a*) reciprocal, by mitotic crossing over, or (*b*) non-reciprocal, by mitotic conversion. Although different mechanisms have been proposed for radiation- and chemical-induced mitotic segregation in yeast (Wilkie & Lewis, 1963; Hurst & Fogel, 1964; Zimmermann, Schwaier & Laer, 1966), the process appears to be most often reciprocal (James & Lee-Whiting, 1955; Johnston, 1962; Nakai & Mortimer, 1969). Similar results of spontaneous mitotic segregants, however, have shown that a high proportion of events are non-reciprocal (Thornton & Johnston, 1970; Johnston, unpublished data). No attempt was made to investigate the mechanism of mitotic recombination in the experiments reported

here. However, the high rates obtained make it unlikely that (forward) gene mutation was responsible in those cases where there was no recombination for linked markers.

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