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Induced recombination in the mitotic cell cycle of the yeast *Saccharomyces cerevisiae*

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SUMMARY

The occurrence of induced recombination in the mitotic cell cycle in yeast has been analysed using conditional cell-cycle mutants held at the restrictive temperature. The strains used were heteroallelic at *gal1* and assaying for functional galactokinase shortly after irradiation (Johnston, 1982) allowed an unambiguous determination of the cell cycle stages in which recombination could occur. Recombination was observed in most strains, including those with the *cdc36* mutation, defective in 'start'; the *cdc4*, *7* and *dbf4* mutations which arrest cells in G1; the *dbf1*, *2* and *cdc6* mutations affecting S phase; *cdc16* and *cdc17* which block cells in G2 and also *cdc14* and *15* which arrest cells in 'late nuclear division'. Recombination can therefore occur within each of the major phases of the yeast cell cycle. This analysis has also revealed that the *cdc8* mutation results in a defect in induced mitotic recombination.

1. INTRODUCTION

Recombination in micro-organisms is commonly detected by formation of colonies on appropriate selective media, so that recombination is actually scored some time after the process has taken place. For certain purposes this is unsatisfactory, such as in determining the occurrence of induced recombination in particular stages of the mitotic cell cycle, when the results may be obscured by subsequent recombination during incubation. Existing data in yeast therefore relate more to the stages of the cell cycle sensitive to induction of recombination, rather than to the occurrence of recombination itself (Holliday, 1965; Esposito, 1968). However, Fabre (1978) has shown that recombination can apparently occur within the G1 phase of the cell cycle by looking at recombination between heteroalleles of *cdc4*, a conditional mutation which arrests cells in G1. Wild-type recombinants which appeared in a population held at the restrictive temperature must have undergone recombination within G1.

We report here a different approach to this question which is applicable to any cell-cycle mutant, even when suitable heteroalleles are not available. Moreover, it avoids any ambiguities arising from scoring recombinants on plates by using strains heteroallelic at *gal1* and detecting recombination by direct assay of the recombinant gene product, namely functional galactokinase (Johnston, 1982). By

this means we have shown that induced heteroallelic recombination can occur within any stage of the mitotic cell cycle in yeast. We have also found that the *cdc8* mutation results in a defect in induced mitotic recombination.

2. MATERIALS AND METHODS

(i) *Media and cultural conditions*

Liquid medium was 1 % Difco yeast extract, 2 % Bacto peptone (YEP) containing 2 % glycerol and 0·2 % galactose (YPGG). Cultures were incubated with vigorous shaking. Solid media contained 2 % agar and consisted of YPD, YEP plus 2 % glucose; EB-gal, YEP containing 2 % galactose and 20 μg/ml ethidium bromide. Incubation was at a permissive temperature of 23 °C or a restrictive temperature of 37 °C.

(ii) *Strains*

The cell cycle, *cdc*, mutants were obtained from the Yeast Genetic Stock Center, Berkeley, and were in an A364A genetic background, *a ade1 ade2 ura1 his7 lys2 tyr1 gal1-1*. The 'start' mutant, SR661-2 *a cdc36-16 trp1 ura1* (Reed, 1980) was provided by Dr Steve Reed, University of California, Santa Barbara. The *dbf* mutants were isolated in this laboratory (Johnston and Thomas, 1982*a*) in D273-11a (α *ade1 his1 trp2*).

For these experiments new *gal1* alleles were isolated in strain D273-11a by ethyl-methane-sulphonate mutagenesis (Johnston, 1977) and tested for recombination with the allele present in A364A. One of these, *gal1-D5*, gave high frequencies of recombination with *gal1-1* and showed very low levels of ultraviolet-light (UV) induced reversion, and was therefore selected for further use.

The *cdc* homozygous diploids were constructed by crossing D273-11a containing *gal1-D5* with strains carrying particular *cdc* mutations and irradiating the diploid cells with UV at 30 J/m^2. Strains homozygous for the *cdc* mutations were then obtained by identifying temperature-sensitive colonies and checking the cells of these to ensure that the appropriate cell cycle morphology was formed at 37 °C.

The *dbf* mutations were first combined with *gal1-1* by crossing with A364A. A suitable spore clone was then crossed with D273-11a *gal1-D5* and a strain homozygous for the *dbf* mutation constructed as described above.

(iii) *Induction of recombination*

Cells were grown in YPGG at 23 °C to approximately 10^7/ml (mid-log phase) and then transferred to 37 °C for 3 h further incubation. They were harvested, washed twice in 0·9 % saline, resuspended at 10^7/ml in saline and irradiated with a UV dose of 125 J/m^2 (unless otherwise stated) using a Hanovia germicidal lamp (254 nm) at a dose rate of 5 J/m^2/s. They were then centrifuged and resuspended at 10^7 cells/ml in fresh YPGG prewarmed to 37 °C and incubated in the dark for 15 h at that temperature before being assayed for galactokinase.

(iv) *Assay for galactokinase*

This has been described in detail previously (Johnston, 1982) and only the salient points will be repeated here. Cells permeabilized with dimethyl-sulphoxide were incubated in an appropriate buffer containing ATP and ^{14}C-labelled galactose. The extent of phosphorylation of the galactose was then determined by spotting the reaction mix on DEAE-cellulose paper which binds phosphorylated compounds. The unreacted reagents were removed by extensive washing of the paper and, after drying, the retained radioactivity was determined in a toluene-based scintillant.

3. RESULTS

(i) *Experimental rationale*

To examine recombination in the mitotic cell cycle, we have used diploids homozygous for *cdc* (Hartwell, 1974) or *dbf* (Johnston & Thomas, 1982*a*, *b*) mutations, both groups of conditional mutations which arrest cells at specific stages of the cell cycle. Each diploid was heteroallelic at the *gal1* locus, which codes for galactokinase, and recombination was detected by assaying for the recombinant gene product, functional galactokinase (Johnston, 1982). All combinations of *gal1* alleles tested give a relatively high background of enzyme (Johnston, 1982), which means that increases in recombination determined by this means appear to be somewhat low when compared to plated recombinants, nevertheless the assay is sufficiently sensitive to detect 5–10 recombinants/10^5 cells. Since the assay measures the phosphorylation of galactose and a fixed number of cells were used in each assay, levels of recombination are expressed as n moles of galactose phosphorylated/10^7 cells/h.

The *GAL1* gene product is inducible and our strains were therefore normally grown in the presence of small amounts of galactose so that they were fully induced for galactokinase at all times, glycerol providing the source of carbon for growth. In practice, mutants were grown to mid-long phase at 23 °C, then transferred to 37 °C for 3 h to allow accumulation at their cell cycle block. To ensure that this had occurred, the cells were examined microscopically to ascertain that they had formed the characteristic terminal cellular morphology associated with their particular arrest point (Hartwell, 1974). In none of the mutants examined did even protracted incubation at 37 °C increase spontaneous recombination above background. Cells were therefore iradiated with UV to stimulate recombination and before assaying were incubated for 15 h to allow completion of recombination and full expression of functional galactokinase (Johnston, 1982). In these experiments this incubation was at 37 °C to ensure that all recombination was confined to the cell cycle stage being examined. This was confirmed by establishing that no increase in cell numbers occurred during the incubation. This also ensured that none of the cells had escaped their cell-cycle block during irradiation, which was at room temperature, as these would divide before again arresting in the cell cycle.

(ii) *Recombination in mutants blocked in the cell cycle*

When irradiated cells were assayed, straight-line plots were obtained, the slopes of which were taken as a measure of recombination (Fig. 1). To determine induced recombination the background present in the unirradiated cells was subtracted from the level of enzyme after irradiation. Thus the functional galactokinase produced due to recombination in the strains carrying *cdc36*, *cdc4*, *dbf1*, *cdc17* and

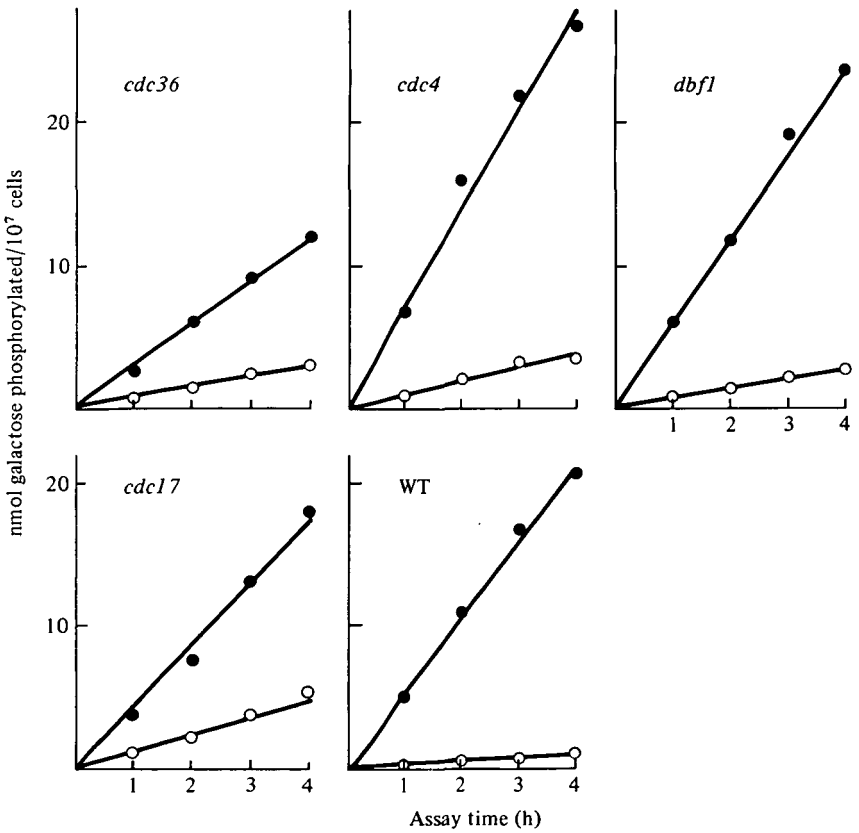

Fig. 1. Assay of functional galactokinase in cells of *cdc36*, *cdc4*, *dbf1*, *cdc17* strains and a wild type after UV-irradiation. The strains were grown to mid-log phase at 23 °C, transferred to 37 °C for 3 h, irradiated and then incubated at 37 °C for 15 h before the cells were permeabilized and assayed. Open symbols indicate the background of galactokinase before irradiation and closed symbols the enzyme produced after irradiation.

the wild type shown in Fig. 1 was 2·2, 6·2, 5·35, 3·2 and 5·3 nmol/10^7 cells/h respectively. It was found that UV-induced reversion in homoallelic diploids of the two *gal1* alleles was only 0·05 nmol/10^7 cells/h for *gal1–1* and was undetectable for *gal1–D5*, so the observed increase in enzyme must be due predominantly to recombination.

As the four mutants mentioned above are defective in each of the major phases

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of the yeast cell cycle, 'start' G1, S and G2, respectively, this suggests that induced recombination can occur over most of the cell cycle. To confirm this, other cell-cycle mutants were examined and the results are summarized in Table 1, together with data on plated recombinants and viability. The viability in all the mutants was in fact very low as a consequence of both the irradiation and the protracted incubation at 37 °C. This should have little effect on recombination, however, as

Table 1. *Recombination in mutants arrested at different points in the cell cycle as determined by galactokinase activity*

Cell cycle stage	Strain	Recombination-galactokinase activity (nmol/10^7 cells/h)	Post-irradiation viability (%)		Plated recombinants per 10^4 viable cells*	
			Immediate	After incubation	Initial	After irradiation
'Start'	*cdc36*	2·24	26	1	4·5	60·0
G1	*cdc4*	6·23	40·8	2·5	1	69·0
	cdc7	3·25	6·3	N.D.[b]	1	77·5
	dbf4	4·1	30·4	1·1	6·5	50·9
S	*cdc6*	4·85	21·2	3·6	4·1	44·4
	cdc8	0	24·8	0·1	12·7	74·8
	dbf1	5·35	50·5	7·8	5·3	50·6
	dbf2	6·33	34·4	6·1	9·1	64·4
G2	*cdc16*	3·13	32·8	3·8	2	60·4
	cdc17	3·2	13·1	1	6·8	73·8
'Late nuclear division'	*cdc14*	2·4	38·5	2·0	1·2	69·5
	cdc15	3·11	44·0	0·8	3·2	60·3
—	Wild type (A364A/D273)	5·15	24·1	65·7	10·8	116·3

* Determined on EB-gal plates. † Not determined.

it can occur in non-viable cells (Johnston, 1982). Indeed this recombination in dead cells probably accounts for the apparent lack of correlation between the viability after the incubation and the amount of enzyme synthesized (Table 1). Recombination was clearly observed in all the mutants, the only exception being strains with the *cdc8* mutation, which blocks cells in S phase.

(iii) *The* cdc8 *mutation results in defective recombination*

Cells with the *cdc8* mutation failed to show any recombination as measured by increased synthesis of galactokinase (Table 1). This could be due either to the mutant being blocked at a particular stage of the cell cycle preventing recombination, or to a direct involvement of the gene product in recombination. The latter interpretation is supported by the occurrence of recombination in other mutants blocked in S (Table 1); and in addition, when cells were irradiated without the 3 h preincubation at 37 °C but were placed at 37 °C immediately aftewards, they failed

to recombine (Fig. 2A). This suggests the cells do not have to be at their cell-cycle block for the defect in recombination to be apparent.

The viability of the mutant after irradiation and incubation is very low (Table 1), which could account for the lack of recombination. However in an experiment using a dose of only 60 J/m² the viability was 79 % after irradiation and 1 % after

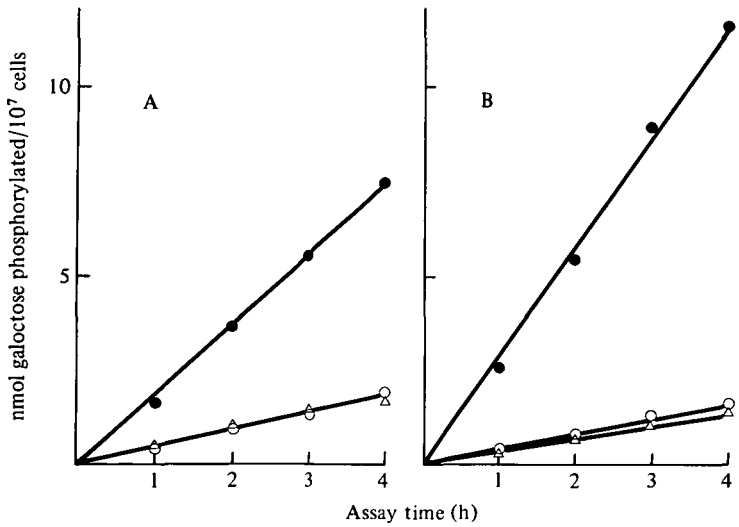

Fig. 2. Production of functional galactokinase by a *cdc8* strain after UV-irradiation. Cells were grown to mid-log phase at 23 °C, irradiated and transferred to 37 °C for 15 h before permeabilization and assay. (A) 125 J/m². (B) 60 J/m². ○——○, Background galactokinase; △——△, enzyme produced at 37 °C; ●——●, enzyme produced at 23 °C.

the incubation. This is comparable to several of the mutants in Table 1 which recombined successfully, including *dbf4*, which produced 80 % of the wild-type level of enzyme, yet again *cdc8* mutants showed no recombination (Fig. 2B). Furthermore *GAL1* cells of *cdc8* mutants grown with glycerol as sole carbon source and induced after irradiation by addition of galactose were able to synthesize new galactokinase at 37 °C, even 3 h after the irradiation (Fig. 3), indicating that the intracellular machinery was functional. Incidentally, when we performed this control with cells of a *cdc9* mutant defective in DNA ligase (Johnston & Nasmyth, 1978), after only 25 J/m² irradiation damage to the cells was such that very little galactokinase was induced at 37 °C, invalidating this particular approach with *cdc9* mutants.

Finally, Fabre & Roman (1979) developed a method for assessing the effect on recombination of a gene containing a conditional lethal mutation by means of conventional plating experiments. This employs heteroalleles within the gene of interest, both alleles being temperature-sensitive, and recombination is detected by the appearance of colonies at the restrictive temperature. We have performed these experiments with *cdc8* to confirm that it confers a recombination defect. A *cdc8–141/cdc8–172* heteroallelic diploid was irradiated with a low dose of UV having no effect on viability of the mutant (Fig. 4, inset) and the cells were then

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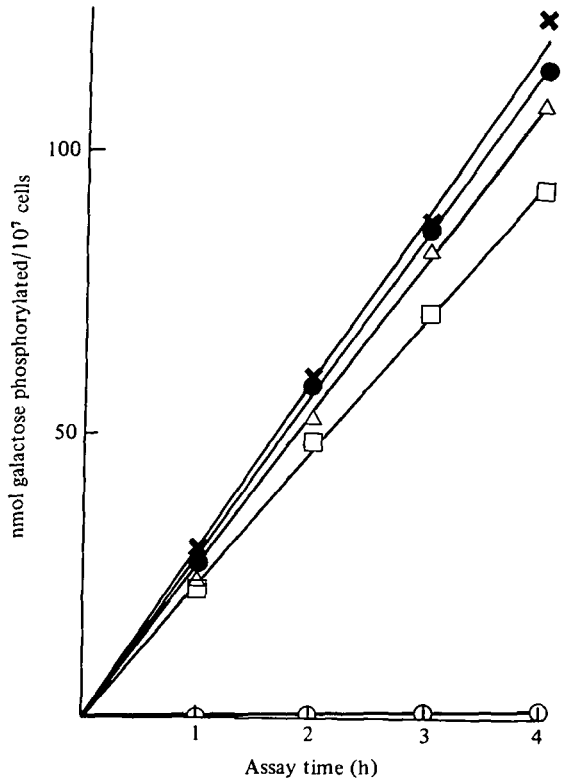

Fig. 3. Induction of galactokinase by galactose in a *cdc8 GAL1* strain. A *GAL1* recombinant of the *cdc8 gal1* diploid was obtained and was grown in YEP containing 2% glycerol to mid-log phase at 23 °C when 1% galactose was added to part of the culture and incubation continued at 23 °C. The remaining cells were irradiated with 60 J/m² and divided into 3 aliquots. The first aliquot was resuspended in YEP containing 2% glycerol and 1% galactose and was incubated at 23 °C; the second aliquot was resuspended in the same medium but was incubated at 37 °C; and the third aliquot was resuspended in the same medium without galactose, was incubated at 37 °C, and galactose to 1% final concentration was added 3 h later. The cells were harvested after a total of 12 h incubation, permeabilized and assayed for galactokinase. ○——○, Background galactokinase; ●——●, unirradiated incubated at 23 °C; □——□, irradiated incubated at 23 °C; ×——×, irradiated incubated at 37 °C; △——△, irradiated incubated at 37 °C, induced after 3 h incubation.

incubated at 23 °C and transferred to 37 °C at intervals. Only those cells recombining to produce wild-type *CDC8* gene were able to form colonies at the high temperature and as expected for a temperature-sensitive protein involved in recombination, the longer the incubation at 23 °C the greater the number of colonies at 37 °C (Fig. 4).

4. DISCUSSION

UV-induced reversion of the *gal1* alleles used in these experiments was very low and the increase in detectable galactokinase after irradiation must have been due to recombination. We believe this recombination was confined to the particular

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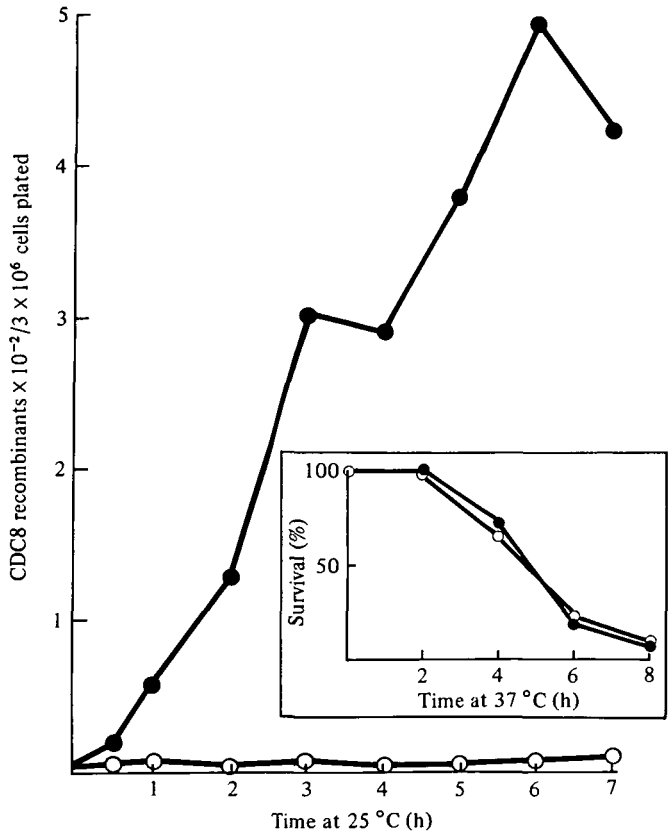

Fig. 4. Allelic recombination within the *cdc8* gene as a function of time at 23 °C before transfer to 37 °C. Mid-log phase cells of *cdc8–141/cdc8–182* heteroallelic diploid were spread on plates, irradiated with 1·5 J/m² and placed at 23 °C. At intervals plates were transferred to 37 °C for a further 3 days incubation before scoring recombinants. ○——○, Unirradiated; ●——●, irradiated. Inset: survival of the *cdc8* heteroallele at 37 °C without irradiation (●——●) and after irradiation with 1·5 J/m² (○——○).

cell-cycle stage under examination for two interrelated reasons. First, there was no increase in cell numbers during the post-irradiation incubation at 37 °C, so few, if any, of the cells have escaped the cell-cycle block. Secondly, the levels of UV-induced enzyme were high, ranging from 43 % to 120 % of the wild type and it is unlikely that this amount of recombination could be the result of a small, undetectable, number of cells escaping their block and recombining subsequently. Assuming that production of galactokinase indicates completion of recombination the technique used suggests that mutants blocked in all the major phases of the cell cycle, namely 'start', G1, S and G2/mitosis, are capable of recombining.

Only diploids homozygous for *cdc8* failed to recombine; however, three other mutants blocked in the S phase gave high levels of recombination, suggesting that the defect in *cdc8* strains may be a specific consequence of the mutational lesion. In support of this, cells irradiated before accumulation at their cell-cycle block and then immediately transferred to 37 °C did not recombine. Moreover, controls

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suggested that the *cdc8* defect was unlikely to be due to an inability to produce new enzyme after irradiation, or simply to a loss of viability, since non-viable cells are able to recombine (Johnston, 1982). The *CDC8* gene product is believed to be a single-stranded DNA binding protein (Arendes, Kim & Sugino, 1983) and activities of this sort could well have a role in recombination, indeed an *E. coli* mutant defective in a similar protein is deficient in recombination (Glassberg, Meyer & Kornberg, 1979). Thus the recombination defect caused by *cdc8* is probably due to a direct involvement of the gene product in recombination rather than being a secondary consequence of its cell cycle block.

Previous studies relating to recombination in the cell cycle have involved the use of synchronized cells treated with recombinogens at various points in the cell cycle (Holliday, 1965; Esposito, 1968; Davies, Tippins & Parry, 1978). This has provided information on stages of the cycle sensitive to induction of recombination, rather than demonstrating the actual occurrence of recombination in different parts of the cell cycle. The only existing experiments to provide clear data on this point are those of Fabre (1978), who showed, using *cdc4*, that recombination could occur within G1, in agreement with our results.

In experiments using cell-cycle mutants the various phases of the cycle are prolonged, and the amount of recombination observed may not accurately reflect the extent to which it would occur in a normal cell cycle. In fact, existing evidence suggests that spontaneous recombination is initiated in G1 (Esposito, 1978), although sister chromatid exchange obviously must occur in S or G2. We observed most recombination in *cdc4* strains, blocked in G1, and in *dbf1* and *dbf2* strains, both blocked in S phase. However, precise quantitation in our experiments was difficult. The numbers of plated recombinants obtained varied somewhat from mutant to mutant, and there was also considerable variation in viability of the mutants after irradiation and incubation at 37 °C, although loss of viability in itself does not prevent recombination (Johnston, 1982).

The results imply that homologous chromosomes are able to pair at any stage of the cell cycle. This may be a consequence of centromere attachment, as suggested for *Schizosaccharomyces pombe* by the more frequent occurrence of spontaneous mitotic gene conversion in centromere-linked genes than in unlinked genes (Minet, Grossenbacher-Grunder & Thuriaux, 1980). *Gal1* is in fact loosely linked to its centromere so possibly the recombination we observed is a reflection of this. Alternatively it could reflect a more complete pairing of homologous chromosomes. Our results provide no information on the nature of this association but simply demonstrate that it can occur at least over much of the mitotic cell cycle in yeast.

We have attempted to extend these observations to the meiotic cycle but have been unable to detect an increase in galactokinase at any stage of meiosis. Even a strain constitutive for galactokinase production and able to sporulate in the presence of inducing levels of galactose, showed no increase in enzyme. The expected increase in gal^+ plated recombinants occurred, however, suggesting that synthesis of galactokinase may be repressed in meiosis. Possibly the use of a different locus is required or a different technique for detecting recombination.

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