TEMPERATURE-SENSITIVE YEAST MUTANTS DEFECTIVE IN MEIOTIC RECOMBINATION AND REPLICATION

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Manuscript received November 10, 1975

ABSTRACT

A system is described for isolating temperature-sensitive mutants of *Saccharomyces cerevisiae* with defects in early meiotic events. We used an otherwise haploid strain disomic ($n+1$) for chromosome *III*, and heteroallelic at the leucine-2 locus. Meiotic development was initiated by exposure of the strain to acetate sporulation medium, and monitored by the appearance of leucine-independent intragenic recombinants. Mutant isolation was based on the recovery of thermally induced defects in recombination. The temperature-sensitive characteristic was included to allow eventual characterizations of the temporal period during meiosis when each gene performs its essential function. Following mutagenesis with either ethyl methane sulfonate or nitrosoguanidine individual clones were tested at 34° and 24° for acetate-induced recombination. Starting with 2700 clones, derived from cells that survived mutagenic treatment, we isolated 48 strains with thermally induced lesions in recombination. In the majority of mutants premeiotic replication occurred normally, or nearly normally, at the restrictive temperature, indicating that the meiotic cycle was initiated and that there was a defect in an event required for intragenic recombination. We also detected mutants where the thermally induced lesion in recombination resulted from temperature-sensitive premeiotic DNA synthesis.

IN the yeast *Saccharomyces cerevisiae*, meiosis is induced by transferring growing cells to nitrogen-free sporulation medium (CROES 1967; ROTH and HALVORSON 1969; TINGLE *et al.* 1973). Under these conditions, vegetative DNA synthesis and cell division cease, but active metabolism including protein and RNA synthesis continues. Within a few hours premeiotic replication begins and a single DNA duplication occurs. Replication is followed by genetic recombination and two reductional divisions which generate four haploid nuclei. Mature ascospores are formed when cell walls develop around the separated nuclei (ROTH 1973). The early events of meiosis in yeast are functionally equivalent to meiosis in other eukaryotes. For this reason, and because of its facile genetic system (MORTIMER and HAWTHORNE 1969), yeast has proven useful to identify gene functions required for meiosis. For example, M. and R. ESPOSITO (1969) devised a system for isolating temperature-sensitive mutants defective in sporulation; temperature-sensitive behavior was valuable for it allowed determination of the temporal period during meiosis when specific genes performed their essential function (ESPOSITO *et al.* 1970). Since sporulation is a terminal event in the meiotic cycle, mutant genes were recovered affecting events spanning the meiotic sequence

from the initiation of DNA synthesis through spore formation itself (ESPOSITO and by the appearance of genetic recombinants (ROTH and FOLEL 1971). Use of a required for early meiotic events, a different mutant selection system was developed which uses a disomic haploid to recover mutants defective in recombination. When exposed to sporulation medium, an appropriately constructed disomic strain initiates meiosis as judged both by successful premeiotic DNA synthesis, and by the appearance of genetic recombinants (ROTH and FOGEL 1971). Use of a disomic strain for mutant isolation eliminates the need for a complete diploid chromosome complement to initiate meiosis, yet allows the use of genetic recombination as a screening device, and also provides for efficient recovery of recessive mutations. In this report we describe the use of this system to isolate a large group of temperature-sensitive mutants with lesions affecting both premeiotic replication and intragenic recombination.

MATERIALS AND METHODS

a. *Strain and media used*: The parent strain used for mutant isolation (Z4521-3C) is disomic $(n+1)$ for chromosome *III*, and has the following genetic structure: *ura3*, *met2*, *ade2*,

$$\frac{+ \quad + \quad \textit{leu2-27} \quad \overset{\textit{a} \quad +}{\frown}}{\textit{his4} \quad \textit{leu2-1} \quad + \quad \underset{\textit{a} \quad \textit{thr4}}{\smile}}$$

(III.). Construction and properties of the strain have been described (ROTH and FOGEL 1971). Synthetic complete medium contained per liter of water: yeast nitrogen base (Difco) 6.7 g; glucose 20 g; d-l threonine 60 mg; adenine 20 mg; uracil 20 mg; l-leucine 20 mg; l-histidine 20 mg; l-tryptophan 20 mg; l-methionine 20 mg; l-lysine 40 mg; l-arginine 20 mg; and agar 20 g. Acetate growth medium is supplemented presporulation medium (ROTH and FOGEL 1971) solidified with 2% agar. The remaining media were all prepared as described (ROTH and FOGEL 1971).

b. *Isolation of mutants*: Mutagenesis with ethyl methane sulfonate was previously described (ROTH and FOGEL 1971), for mutagenesis with 1-methyl-1-nitro-nitrosoguanidine (NNMG) cells growing logarithmically in complex medium (30°) were harvested, washed, and exposed to 500 μg/ml of NNMG for 60 min as described by LINGENS and OLTMANNS (1966); survival was 6%.

The methods and rationale used for detection of temperature-sensitive mutants among mutagenized survivors is adapted from a previous procedure (ROTH and FOGEL 1971). After mutagenesis cells were plated onto solidified complex medium to yield about 100 survivors per plate. After 7–10 days' incubation at 24°, samples from individual clones were transferred to master plates of complex medium. For control purposes, each master plate also contained two samples of the untreated parental strain. After two days as 24°, each master was replica-plated to a pair of secondary masters; one was incubated at 24°, the other at 34°. Each secondary master was then used for a parallel series of identical tests at 24° and 34°. After 24 hr the secondary masters were quickly examined to identify temperature-sensitive growth mutants. After 48 hr each master was replica-plated to leucine-free synthetic medium, to acetate growth medium, and to two plates of supplemented acetate sporulation medium. The *leucine-free* plates were exposed to a non-lethal dose of ultraviolet light (200 ergs/mm^2) and incubated for 7 days; U.V.-induced mitotic reversion to prototrophy was used to detect the presence of the *leu-2* heteroalleles, and to rule out the presence of a thermal lesion in the leucine biosynthetic pathway. Acetate growth medium, examined after 24 hr, revealed clones unable to use acetate as a carbon source. The two sporulation plates were used to evaluate the meiotic induction of recombinants as measured by leucine prototroph formation. After 48 hr one sporulation plate was replica-plated directly to leucine-free synthetic medium; at 72 hr this was repeated with the remaining sporulation plate. After 7 days the leucine-free plates were examined for leucine-independent colonies which originated from prototrophic recombinants transferred from the sporulation plates. Control experiments with the

parent strain established that the number of prototrophs on sporulation plates reached a maximum after 48 hr at 34° and after 72 hr at 24°. A large number of mutagenized clones were equally defective in meiotic recombination at both 24° and 34°; these were disregarded. Clones tentatively classified as temperature-sensitive meiotic mutants grew at 24° and 34° on complex and acetate medium, gave a positive heteroallelic response at both temperatures, displayed normal or near-normal levels of meiotic recombinants at 24°, but produced no, or very few, recombinants at 34°. Each tentative mutant was purified on complex medium; numerous separate colonies of each strain were used to prepare a series of new masters. These masters were subjected to a second series of tests at 24° and 34° as described above. In these tests two additional replica plates were included: one was used to assess mating type, the other on synthetic complete medium was used to detect the appearance of unusual nutritional requirements. None of the mutants exhibited a mating type or detectable new nutritional requirements. Each mutant still displaying a thermal defect in meiotic recombination was subjected to more rigorous examination in liquid medium.

c. *Quantitative characterization of mutants*: To initiate meiosis, cells growing logarithmically in acetate presporulation medium at either 24° or 34° were rapidly harvested (at $2–3 \times 10^7$ cells/ml) on sterile membrane filters (no. HAWP; Millipore Filter Corp.), washed with two volumes of water (prewarmed for cultures at 34°), and suspended in 1% potassium acetate sporulation medium (prewarmed for cultures at 34°) at 5×10^7 cells/ml. At intervals samples were withdrawn to monitor increase in mass, which is a measure of acetate utilization (Roth 1970), the formation of leucine prototrophs (Roth and Fogel 1971), and DNA synthesis. Cell concentrations were estimated by absorbance measurements at 600 nm with the Klett colorimeter.

d. *Analytical procedures*: Samples for measuring DNA synthesis were harvested, stored, and carried through the initial stages of analysis as previously described (Croes 1967). Following lipid extraction, the DNA-containing cell pellets were dried at 60°, and then carried through the remainder of the diphenylamine procedure as usual (Croes 1967). After color development the samples were centrifuged (5000 rpm; 7 min) and absorbance readings were made on the clarified supernatants. Drying samples after lipid extraction allows the volumes required in the usual assay to be substantially reduced, making it possible to measure DNA in samples containing 4×10^8 cells. Measurements of isotope incorporation into DNA and RNA were previously described (Kuenzi and Roth 1974; Hartwell 1971).

RESULTS

Mutants were isolated from an aneuploid parent disomic ($n+1$) for chromosome *III*. The aneuploid was heteroallelic for two non-complementing alleles at the leucine-2 locus; in this way intragenic recombination could be monitored by the formation of leucine-independent (i.e. *leu*$^+$) clones. Since chromosome *III* also contained the mating type locus, the disome was made heterozygous for the mating type alleles **a** and α. The simultaneous presence of **a** and α allowed the otherwise haploid strain to undergo both premeiotic replication and recombination when exposed to acetate sporulation medium.

Selection of the restrictive and permissive temperature

The phenotype used for mutant isolation involved a temperature-dependent deficiency in acetate-induced recombination (gene-conversion) at the *leu-2* locus. We chose 34° as the restrictive temperature and 24° as the permissive temperature. On solid sporulation medium, the wild-type parent (Z4521–3C) grew well and generated comparable levels of leucine prototrophs at both 34° and 24°. Quantitative control experiments, conducted in liquid cultures (Figure 1) established that the kinetics and extent of early meiotic events were similar at the restrictive and permissive temperatures, and corresponded to previous results

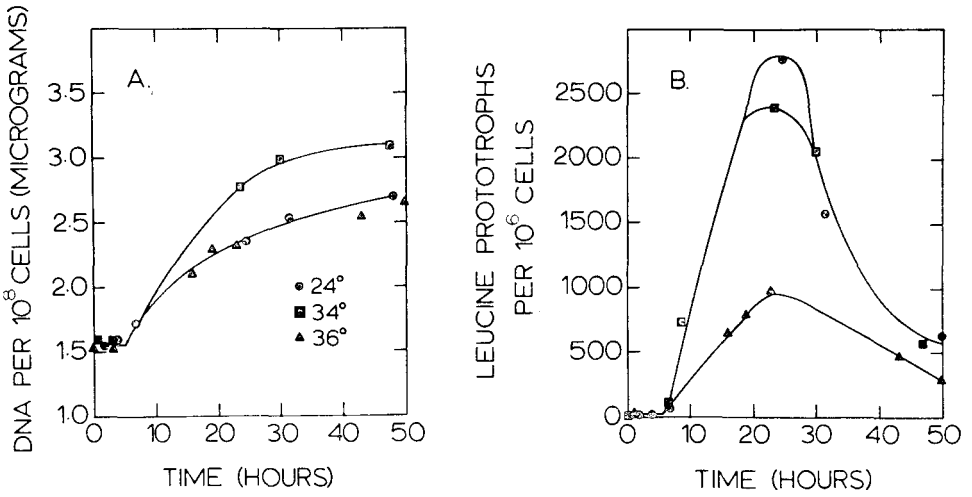

FIGURE 1.—Effects of temperature on premeiotic replication and recombination in a disomic yeast strain.

At 0 hr, logarithmically growing cultures of strain Z4521-3C were harvested, washed, and resuspended in acetate sporulation medium to initiate meiosis. At intervals samples were removed to determine total cellular DNA (Part A). Recombination was followed (Part B) by monitoring the appearance of leucine prototrophic recombinants. (●) culture grown and incubated in sporulation medium at 24°; (■) culture grown and incubated at 34°; (▲) culture grown and incubated at 36°.

obtained at the optimal temperature of 30° (ROTH and FOGEL 1971). At both 24° and 34° premeiotic DNA synthesis began 6 to 8 hrs after cells were suspended in sporulation medium and resulted in an approximate doubling of the cellular DNA content. At 24° leucine prototrophs increased from less than 20 per 10^6 cells at zero time to over 2700 per 10^6 cells at 24 hrs; at 34° recombinants rose from 2 per 10^6 cells to 2400 per 10^6 by 24 hrs. At temperatures above 34° premeiotic replication occurred, but later stages of meiosis were severely inhibited. For example, at 36° DNA synthesis was apparently normal, while recombination was reduced to 30% of that at 24° (Figure 1).

Isolation of mutants

Mutagenesis was accomplished with ethyl methane sulfonate (EMS) and with 1-methyl-1-nitro-nitrosoguanidine (NNMG). We examinted 2700 clones derived from cells that survived mutagenic treatment (1,212 from EMS; 1,488 from NNMG) for thermally induced defects in meiotic recombination. These tests were conducted by replica plating, and thus were semi-quantitative in nature. Of the 2700 clones examined, 262 grew at both temperatures but exhibited reduced recombination at 34° compared with simultaneous identical tests run at 24°. Each presumptive mutant was purified by cloning, and re-examined in a second series of tests on solid medium. With these tests 82 of the original 262 presumptive mutants were eliminated—most because they either grew very poorly at 34° or were "leaky" (that is, displayed significant levels of recombina-

TABLE 1

Quantitative characterization of recombination-deficient (rec⁻) *mutants at 34°**

Total number of mutant strains examined	Did not grow, or grew poorly†	Deficient in acetate utilization‡	"Leaky" i.e., >15%§ *leu*+ recombinants	*rec*⁻, i.e., <15% recombination
164	32	23	57	52

* Cells of each strain were grown, and meiosis initiated, as described in MATERIALS AND METHODS. Samples were withdrawn at 24, 48 and 72 hr to monitor increases in mass and the number of leucine prototrophic (*leu*+) recombinants.

† In Z4521–3C the doubling time was 3 h; strains with doubling times longer than 6 hr were considered defective.

‡ In the wild type (Z4521–3C), mass increased between 125% and 150% (FOGEL and ROTH 1974) strains displaying less than 50% increase in mass were considered defective.

§ In Z4521–3C, leucine prototrophs reached a maximum of 2400 per 10^6 cell plated at 24 hr and then declined (c.f. Figure 1). In some mutants the maximal frequency of *leu*+ cells was attained after 48 hr; the highest frequency reached by a mutant, of either 24 hr, or 48 hr, was compared to the level attained by Z4521–3C at 24 hr.

tion at 34°). Other strains were discarded because their defect at 34°, although severe, was not significantly improved in tests run at 24°. After the above tests, 164 presumptive mutants remained; each was now quantitatively examined for its ability to generate recombinants during incubation in liquid sporulation medium at 34° (Table 1). Based on preliminary studies we expected that many presumptive mutants which appeared useful in semi-quantitative tests would be found unsuitable after a more rigorous quantitative examination. As anticipated, 112 of the original 164 presumptive mutants were eliminated from further consideration for various reasons, as described in Table 1; 52 presumptive mutants remained, and were now examined in additional quantitative tests run simultaneously at 24° and 34° (Table 2). These tests were designed to confirm and quantify the temperature-sensitive *rec*⁻ phenotype. Of the 52 mutants, 48 displayed a thermosensitive response, as evidenced by a preferential reduction in recombination at 34° compared to 24°. As shown (Table 2), many dramatic temperature-sensitive *rec*⁻ strains were isolated. In addition many mutants which were extremely defective at 34° also displayed moderate to rather severe defects in recombination at 24°. Detailed kinetics of recombination, at both the permissive and restrictive temperature, are shown for four representative mutants in Figure 2.

Initiation of meiosis in recombination-deficient mutants

Mutants defective in prototroph formation could fail to initiate the entire meiotic sequence rather than have a specific defect in an event required for recombination. To examine this possibility each *rec*⁻ strain listed in Table 2 was tested for premeiotic DNA synthesis at 34°; replication is an early event specific to the meiotic sequence (ROTH and LUSNAK 1970). The majority of strains (38) exhibited normal or nearly normal levels of pre-meiotic replication at 34°. The kinetics of DNA synthesis of two representative strains (M37–25 and M41–13) are shown in Figure 3. We considered replication strong evidence that these

TABLE 2

*Meiotic intragenic recombination at 24° and 34° in 52 presumptive temperature-sensitive, recombination-deficient mutants**

Strain	% recombination at 24°	% recombination at 34°	Strain	% recombination at 24°	% recombination at 34°
Z4521-3C (wild type)	100%	100%	M38-12	30%	7.5%
M41-13	98	0.17	M37-20	30	0
M44-29	89	0.31	M40-21	25	5.1
M40-22	89	0.76	M39-21	25	0.76
M35-27	84	12	M43-4	25	1.8
M42-8	83	0.80	M44-9	22	4.1
M40-15	77	0.73	M43-23	20	6.5
M43-28	77	2.7	M39-13	19	6.1
M38-27	74	7.8	M39-28	18	1.3
M44-25	74	5.0	M40-7	17	3.0
M39-19	67	3.0	M36-21	16	2.0
M37-11	65	15	M41-4	15	1.9
M43-11	67	7.0	M43-21	15	0.15
M44-30	65	6.4	M40-18	13	2.4
M44-4	58	11	M36-4	13	2.0
M37-25	51	2.5	M36-12	11	0.83
M35-29	51	0.42	M38-13	8.5	0
M40-16	47	5.0	M41-3	7.1	0
M41-7	42	0	M37-22	7.1	2.6
M37-13	41	6.8	M39-22	6.7	0
M41-26	39	2.0	M35-26	6.5	10
M42-12	35	5.8	M43-25	3.8	0.80
M39-17	35	6.3	M37-23	3.5	0
M44-20	33	5.5	M35-23	1.0	0
M41-5	33	7.1	M39-8	0.10	0
M44-13	32	11	M38-8	0	0
M43-14	31	2.1			

* Duplicate cultures of each strain were grown and inoculated into sporulation medium as described in MATERIALS AND METHODS. One culture was grown and continuously incubated at 24°; the other was grown and continuously incubated at 34°. Samples were taken at 24, 48 and 72 hr to measure the frequency of leucine prototrophs. In Z4521-3C, at 24°, *leu*+ recombinants reached a maximum of 2800 per 10^6 cells plated; at 34° the maximum was 2400. In some mutants the kinetics of recombinant formation varied from those shown for Z4521-3C in Figure 1, (e.g., see Figure 4 strain M40-7). The maximal frequency of recombinants reached in each mutant, at any time, is presented as a percentage of the maximum attained by Z4521-3C at the corresponding temperature.

strains initiated meiosis at the restrictive temperature, but were deficient in some later event(s) required for successful gene conversion.

Defective replication in recombination-deficient mutants

Ten mutants exhibited significantly reduced levels of replication at the restrictive temperature (Table 3); DNA synthesis varied from none in strain M40-7 to

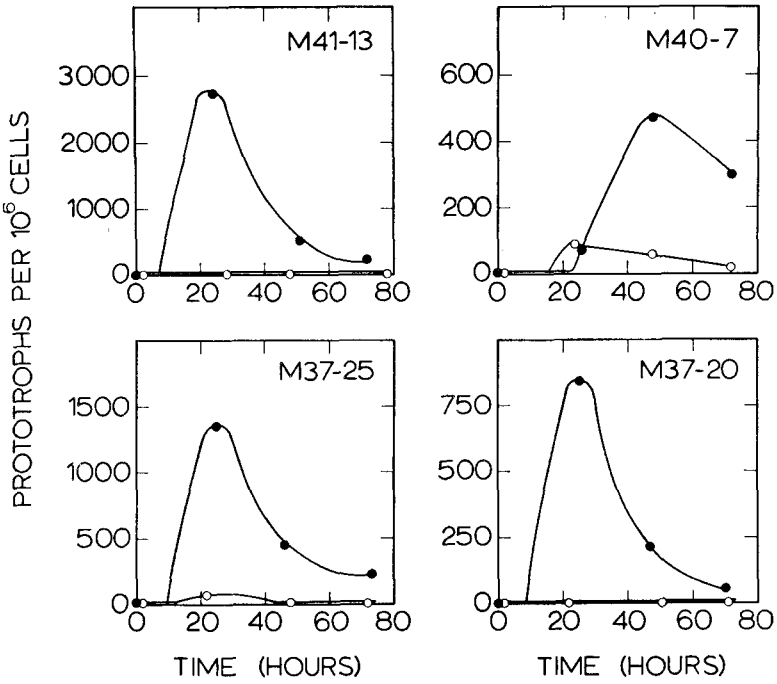

FIGURE 2.—Kinetics of recombination in temperature-sensitive recombination-deficient mutants.

At 0 hr, duplicate cultures of each mutant were harvested from growth medium, washed, and resuspended in sporulation medium to initiate meiosis. One culture was grown and continuously incubated in sporulation medium at 24° (●); the other culture was grown and incubated at 34° (○). Recombination was followed by monitoring the appearance of leucine prototrophic recombinants. Note that the scale for recombination frequency is somewhat different for each mutant.

about ⅓ of normal in strain M38–13. Since replication is a normal prerequisite for gene conversion (SILVA-LOPEZ, ZAMB and ROTH 1975), we considered it likely that the defects in replication accounted for the failure in recombination in these mutants. If this were true we anticipated that DNA synthesis would also be temperature-sensitive. To test this, replication was measured at 24° in each of the 10 mutants (Table 3). In five of the mutants DNA synthesis was nearly normal, or at least significantly better at 24° compared to 34°, and thus behaved in the expected temperature-sensitive fashion. DNA synthesis in two mutants of this type (M37–20, M40–7) are shown in Figure 4. In the other five mutants replication was defective at both the permissive and restrictive temperature. Consistent with this, a comparison of Tables 2 and 3 reveals that in these five mutants recombination was also severely defective at both temperatures.

Molecular specificity of the defects in replication

^{14}C-uracil incorporation was used to estimate meiotic RNA synthesis in the five mutants with temperature-sensitive defects in replication (Table 4). In the

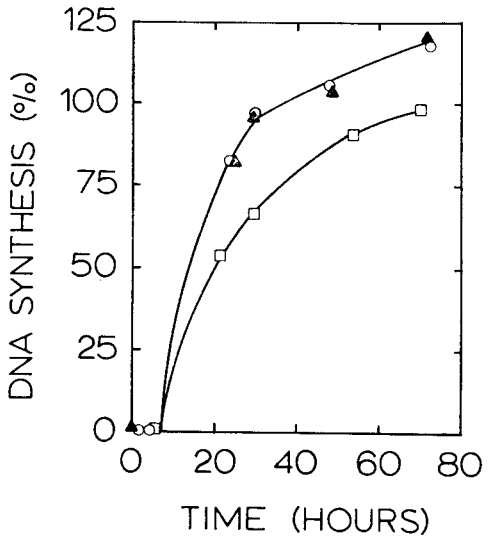

FIGURE 3.—Initiation of meiosis in temperature-sensitive recombination-deficient mutants.

At 0 hr, logarithmically growing cultures of each strain were harvested, washed, and resuspended in sporulation medium. The cultures were grown and continuously incubated in sporulation medium at 34°. At intervals samples were removed to determine cellular DNA synthesis; the net increase in DNA content between samples taken at later times and those taken at t=0 hr was converted to a percentage of the DNA present at t=0 hr, (○) strain Z4521-3C; (▲) strain M41-13; (□) strain M37-25.

TABLE 3

Premeiotic DNA synthesis in 12 thermosensitive rec⁻ *mutants**

	Strain	Increase in DNA content at 34°	Increase in DNA content at 24°
Wild type:	Z4521-3C	120%	73%
group "a":	M40-7	0	22
	M41-3	3	36
	M43-21	10	62
	M37-20	17	137
	M40-16	33	49
group "b":	M39-8	10	10
	M35-23	12	8
	M43-25	19	17
	M36-12	33	21
	M38-13	38	19

* Meiotic cultures of each strain were established at 24° and 34° as described in Table 2. For every culture the start of incubation in sporulation was considered zero time. At interval (t=0, 24, 48, and 72 hr) duplicate samples were removed and analyzed for DNA. The net increase in DNA content between samples taken at t=0 hr and t=72 hr was converted to a percentage of the DNA present at t=0.

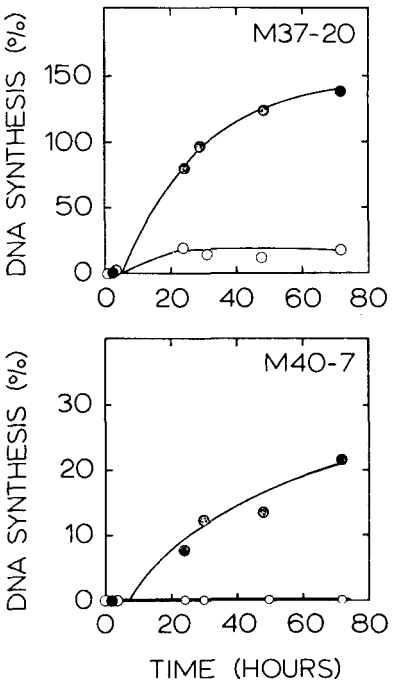

FIGURE 4.—Defective premeiotic replication in temperature-sensitive recombination-deficient mutants.

At 0 hr, duplicate cultures of each mutant were harvested, washed, and suspended in sporulation medium. One culture was grown and continuously incubated in sporulation medium at 24° (●); the other culture was grown and incubated at 34° (○). DNA synthesis was determined as described in legend to Figure 3.

TABLE 4

*RNA synthesis in temperature-sensitive mutants defective in premeiotic replication**

Strain	RNA synthesis at 34° (ng of uracil incorporated per hour)	RNA synthesis at 24° (ng of uracil incorporated per hour)
wild type		
Z4521-3C	0.78	0.73
M40-7	0.98	0.74
M41-3	0.80	0.84
M43-21	1.33	1.06
M37-20	1.19	0.50
M40-16	2.34	1.95

* Duplicate meiotic cultures of each strain were established at 24° and 34° as described in Table 2. Each culture was exposed to ^{14}C-uracil as previously described (KUENZI and ROTH 1974). At intervals duplicate samples were removed, precipitated with ice-cold trichloroacetic acid and processed to determine incorporation into the RNA fraction (HARTWELL 1971). RNA synthesis is expressed as the average hourly rate of uracil incorporation (per 2.5×10^7 cells) over the interval t=3 hr to t=24 hr.

TABLE 5

*Premeiotic DNA synthesis, measured by isotope incorporation, in replication-deficient mutants**

Strain	DNA synthesis at 34° (picomoles of pyrimidine incorporated)	DNA synthesis at 24° (picomoles of pyrimidine incorporated)
Wild type:		
Z4521-3C	26.4	22.9
M40-7	5.72	9.03
M41-3	3.04	10.2
M43-21	9.13	28.8
M37-20	6.52	22.5
M40-16	30.6	61.0
M39-8	2.94	2.50

* The cultures used to measure incorporation into DNA are those described in Table 4. At intervals duplicate samples were removed to determine ^{14}C-incorporation into the alkali-stable, trichloroacetic acid precipitable fraction (HARTWELL 1971). DNA synthesis is expressed as total picomoles of ^{14}C-pyrimidine (derived from ^{14}C-uracil) incorporated by 5×10^7 cells between t=3 hr and t=48 hr.

parent strain uracil incorporation occurred at an equivalent rate at both 24° and 34°; the five mutants behaved in a similar fashion, although the absolute rates of incorporation varied from strain to strain. These results indicated that the thermal defects in replication did not result from a non-specific blockage of nucleic acid synthesis at the restrictive temperature.

In the experiments used to monitor RNA synthesis it was also possible to simultaneously measure incorporation of ^{14}C-uracil-derived pyrimidine into DNA (Table 5). At 24° and 34° the parental strain incorporated equivalent amounts of ^{14}C-pyrimidine into the DNA fraction; in contrast each presumptive mutant incorporated much less pyrimidine at 34° than at 24°. In mutant M39–8, previously identified (Table 3) as being defective in DNA synthesis at 24° and 34°, incorporation was depressed by an equal amount at both temperatures. The incorporation studies independently confirmed the thermo-sensitive nature of DNA synthesis in these five mutants.

DISCUSSION

In this report we used a disomic yeast strain to isolate mutants with thermally induced lesions in meiotic intragenic recombination. The presence or absence of premeiotic DNA synthesis at the restrictive temperature was used to divide the mutants into two broad categories. The majority of mutants carried out DNA synthesis at 34°; we considered successful replication as evidence that these mutants initiated meiosis but were defective in an essential function required for intragenic recombination. Some of the problems involved in identifying the basis for the defects in recombination were discussed previously (FOGEL and ROTH 1974). A smaller group of mutants were defective in replication at the restrictive temperature but displayed normal, or more normal, levels of replication at the permissive temperature. In these mutants we considered the thermally induced,

recombination-deficient phenotype a consequence of a thermal lesion blocking replication. This conclusion is supported by studies with the DNA inhibitor hydroxyurea which established that replication was essential for meiotic gene conversion (SILVA-LOPEZ, ZAMB and ROTH 1975). Temperature-sensitive mutants defective in premeiotic replication have also been isolated by ESPOSITO and ESPOSITO (1974).

Previously (ROTH and FOGEL 1971) a smaller group of *rec*⁻ mutants were isolated using the disomic strain. However, unlike the mutants described here, these mutants were not conditional; that is, their meiotic defects were absolute. Five of these mutants were chosen for further analysis; two were defective in replication, while three completed replication but were blocked at a later step (ROTH 1973; FOGEL and ROTH 1974). By appropriate genetic techniques, the mutations originally present in the non-mating, heterozygous **a**/α, configuration were obtained in **a** and α haploids capable of hybridization. This allowed genetic analysis and the construction and evaluation of true diploids homozygous for each mutant gene. Tetrad analysis and complementation studies revealed that the mutations behaved as simple, independent recessive genes. Diploids, homozygous for each mutation, displayed identical meiotic defects exhibited by the original disome; this indicated that mutations isolated using an aneuploid do not depend on some peculiarity of disomic structure but represent lesions in essential meiotic functions. These preliminary studies, conducted with non-conditional mutants, showed that a disome was indeed useful for probing the genetic control of early meiotic events.

The availability of thermal lesions in premeiotic replication and recombination offers advantages for analysis of gene-function not present with non-conditional mutants. For example, thermal sensitivity can be used to determine the temporal period during the meiotic cycle when each gene performs its essential function (ESPOSITO *et al.* 1970). In the case of replication genes such temporal information offers important clues to the type of replication reaction controlled by the relevant genes (HARTWELL 1971). Current efforts are directed at using such techniques to characterize genes identified here.

This work was supported by a grant (GM 19330) and a Research Career Development Award (1 KO4 GM 70274) from the National Institutes of Health.

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Corresponding Editor: R. E. ESPOSITO