

Chromosome Replication During Meiosis: Identification of Gene Functions Required for Premeiotic DNA Synthesis

(yeast)

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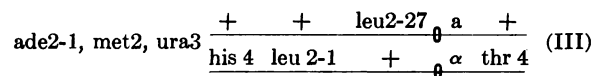
ABSTRACT Recent comparisons of chromosome replication in meiotic and mitotic cells have revealed significant differences in both the rate and pattern of DNA synthesis during the final duplication preceding meiosis. These differences suggested that unique gene functions might be required for premeiotic replication that were not necessary for replication during growth. To provide evidence for such functions, we isolated stage-specific mutants in the yeast *Saccharomyces cerevisiae* which permitted vegetative replication but blocked the round of replication before meiosis. The mutants synthesized carbohydrate, protein, and RNA during the expected interval of premeiotic replication, suggesting that their lesions preferentially affected synthesis of DNA. The mutations blocked meiosis, as judged by a coincident inhibition of intragenic recombination and ascospore formation. The lesions were characterized as recessive nuclear genes, and were designated *mei-1*, *mei-2*, and *mei-3*; complementation indicated that the relevant gene products were not identical.

In eukaryotes major features of chromosomal DNA synthesis are identical in cells undergoing mitosis and meiosis; in both cases replication occurs by a semiconservative mechanism (1, 2) and results in an exact duplication of the genome (3-5). Nevertheless, differences exist in the overall rate and in the spatial and temporal pattern of replication in meiotic and mitotic cells. It has been suggested (6-8) that these differences play a role in determining which of the two characteristic modes of behavior the chromosomes display. Where it has been examined (6, 8-10) the S phase in the last round of replication preceding meiosis is considerably longer than the corresponding S phase of mitosis, and recent autoradiographic data (8) indicate that the slower meiotic rate comes about not by a decrease in the rate of DNA chain growth, but by a reduction in the number of initiation points per genome. In the lily, and in *Trillium*, premeiotic DNA synthesis is altered in such a way that duplication of a small but distinctive portion of the genome is delayed well beyond the normal S period, and only occurs during zygotene of prophase I (11). Differences in the rate and pattern of premeiotic DNA synthesis suggest that unique or modified gene products, distinct from those used during growth, may be required for replication during the sexual phase of the life cycle. One approach to determining whether any new gene products are actually necessary for premeiotic replication involves the isolation of stage-specific mutations that block DNA synthesis preceding meiosis but not mitosis. In this report we describe such mutations isolated using the yeast *Saccharomyces cerevisiae*. The mutants ob-

tained provide additional evidence that distinct biochemical reactions do distinguish the last premeiotic replication from replication during growth.

MATERIALS AND METHODS

Yeast Strains. Mutants M10-2B and M10-6A were isolated from disomic ($n + 1$) strain Z4521-3C. The original disome used to construct Z4521-3C was provided by Dr. G. Fink (13). Construction and properties of Z4521-3C and details of mutant isolation have been described (12). Z4521-3C and both mutants have the following general structure:



Growth, Sporulation, and Analytical Procedures. Media and procedures used for quantitative growth and sporulation experiments reported in Fig. 1 and Table 1 were described (12), as were the methods used to enumerate asci, and to measure DNA synthesis per cell (14). The appearance of leucine prototrophic recombinants served to monitor the initiation of meiosis (15). Intracellular carbohydrate was determined with the anthrone reagent (16). Incorporation of [3 H]leucine and [2 - 14 C]uracil into the macromolecule fraction was determined by standard procedures (17, 18).

Genetic Procedures. Disomic strains heterozygous for *a* and α behaved like heterozygous diploids (19) in that they failed to mate with either *a* or α tester strains. In order to construct diploids and perform crosses, we isolated *a* and α haploid, and *a/a* and α/α disomic segregants from each mutant. These arose spontaneously during mitosis; their recovery and characterization will be fully described (Roth and Fogel, submitted for publication). Identification of those disomic ascospore clones that were simultaneously heterozygous for mating type, and heteroallelic at *leu-2*, as well as the methods used to determine the ability of such clones to undergo meiotic recombination were described (12). All incubations were at 30°.

RESULTS

In *S. cerevisiae*, meiosis is induced by transferring growing cells to nitrogen-free sporulation medium (20, 21). After the transfer to sporulation medium, vegetative DNA synthesis

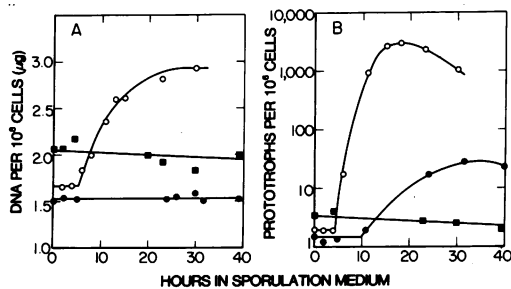


FIG. 1. Premeiotic DNA synthesis and intragenic recombination in disomic yeast strains. At 0 hr, cultures growing logarithmically in acetate presporulation medium were harvested, washed, and suspended in 1% potassium acetate sporulation medium. At intervals thereafter samples were removed for determination of total cellular DNA (A). Initiation of meiosis (B) was followed by monitoring the appearance of leucine prototrophic recombinants (12). (○) Z4521-3C, doubling time in acetate presporulation medium (12) 190 min; (●) M10-2B, doubling time 180 min; (■) M10-6A, doubling time 190 min.

and cell division cease, but active metabolism including the synthesis of macromolecules continues (16, 20, 22). After a lag of 4–5 hr premeiotic replication begins and a single DNA duplication occurs (23). Replication is followed by genetic recombination and two reductive divisions which generate four haploid nuclei. Finally mature ascospores are formed when cell walls develop around the separated nuclei (24). Except for the details of nuclear behavior, yeast follows the general pattern of meiosis established in higher eukaryotes. The organism's simple microbial nature, plus its well-described and versatile genetic system (25), makes it ideal for characterizing gene functions essential for meiosis (26).

Isolation of Mutants. To obtain lesions affecting premeiotic replications, we used an indirect approach beginning with the isolation of mutants unable to initiate intragenic recombination, an early meiotic event close in time to the period of replication (ref. 15 and Fig. 1). We reasoned that such mutants might contain a class defective in premeiotic DNA synthesis. Since our rationale for mutant isolation depended on a test for genetic recombination, it first appeared that we would have to use a diploid strain; yet we realized that a search for mutants involving a diploid could not readily detect recessive lesions. To overcome the ploidy problem we made use of a special haploid containing a single chromosome in the duplex, or diploid, configuration (13). This aneuploid, specifically a disomic ($n + 1$) for chromosome III, was made heteroallelic at the leucine-2 locus; in this way intragenic recombination could be monitored by the formation of leucine-independent clones. Since chromosome III also contained the mating-type locus, the disome was made heterozygous for the mating alleles a and α ; the presence of a and α allowed the otherwise haploid strain to undergo premeiotic replication and recombination when incubated in sporulation medium (12). In essence, the a/α disome was a haploid strain in which recessive mutations affecting early meiotic events could be detected without the necessity of using a diploid. Meiosis, however, was not completed normally in the disome since the final ascospores were imperfectly formed and were inviable.

From the disomic parent (Z4521-3C) we isolated a group of mutants defective in recombination (12). Among these two

strains were detected (M10-2B and M10-6A) which failed to initiate DNA synthesis in sporulation medium; their behavior relative to the wild-type disome is presented in Fig. 1A.

In Z4521-3C, premeiotic replication started 5 hr after the growing cells were resuspended in sporulation medium, and increased the net DNA content from 1.7 to 2.9 μg per 10^8 cells. Under identical conditions the initial DNA contents in the mutants either remained constant (M10-2B) or decreased slightly (M10-6A). The effects of the mutations on other meiotic events were evaluated by monitoring recombination (Fig. 1B) and spore formation. In the wild type, prototrophic recombinants increased from 2 per 10^6 cells at zero time to over 2000 per 10^6 cells by 18 hr. In M10-6A recombination was abolished, while in M10-2B it was decreased to less than 1% of that in Z4521-3C. Sporulation, which reached 15% in the wild type, was not detectable in either mutant. The absence of recombination and sporulation indicated that meiotic events subsequent to replication had also been blocked by the mutations.

Molecular Specificity of the Mutations. The ability of the mutants to metabolize the carbon source (acetate) in sporulation medium was estimated by monitoring intracellular carbohydrate synthesis; during sporulation carbohydrate formation accounts for at least 67% of the total increase in cellular mass (16). In wild type and both mutants, intracellular carbohydrate rose from 0.2 mg per 10^8 cells, upon inoculation into sporulation medium, to over 1.2 mg per 10^8 cells by 30 hr; in all strains 50% or more of this increase occurred during the expected interval of replication. From these results it appears that the mutants are not deficient in overall acetate metabolism.

We estimated protein and RNA synthesis by following [³H]-leucine and [^{2-¹⁴C}]uracil incorporation into the macromolecular fraction (Fig. 2) (17, 18, 26). Under the nongrowing conditions that obtain in sporulation medium the observed rates of precursor incorporation may not accurately reflect the true internal rates of protein and RNA synthesis (27); nevertheless,

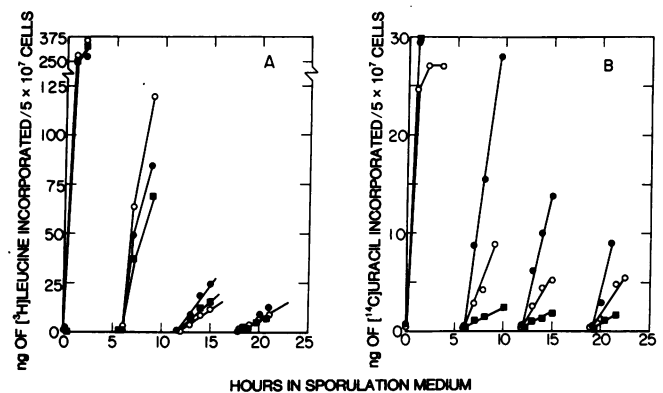


FIG. 2. Incorporation of [³H]leucine and [^{2-¹⁴C}]uracil by disomic yeast strains during incubation in sporulation medium. At 0 hr, cultures were inoculated into sporulation medium as described in Fig. 1. At intervals samples were subcultured into warmed flasks containing either (A) [³H]leucine (4.5×10^4 cpm/ μg ; $5 \mu\text{g}/\text{ml}$) or (B) [^{2-¹⁴C}]uracil (1.0×10^5 cpm/ μg ; $5 \mu\text{g}/\text{ml}$). Immediately upon subculturing, and at 1-hr intervals thereafter duplicate 1-ml aliquots were withdrawn. Incorporation into the macromolecular fraction was determined by standard procedures (17, 18). (○) Z4521-3C; (●) M10-2B; (■) M10-6A.

TABLE 1. Premeiotic DNA synthesis, intragenic recombination, and sporulation in diploid yeast strains

Strain*	Genotype†	DNA synthesis‡			Recombination§ (leucine prototrophs formed per 10 ⁶ cells)	Sporulation (48 hr)
		Initial	Final ($\mu\text{g}/10^8$ cells)	Increase (%)		
[1] 4579 (control)	Wild type	2.6	4.4	72	4570	57%
[2] D-M10-2B	$\frac{mei-1}{mei-1}$	2.8	2.5	0	41	0
[3] D-M10-6A	$\frac{mei-2 \quad mei-3}{mei-2' \quad mei-3}$	2.7	2.5	0	0	0
[4] D- $\frac{M10-2B}{M10-6A}$	$\frac{mei-1}{+} \quad + \quad +$ $\quad \quad \quad + \quad mei-2' \quad mei-3$	3.0	4.9	63	5330	62%

* Strains 1, 2, and 3 were obtained by hybridizing a and α haploid segregants derived from Z4521-3C, M10-2B, and M10-6A, respectively. 4579 is a wild-type diploid.

† The overall structure of the homozygous mutant diploids is presented below. The genotypes shown in the table refer to the meiotic (mei) lesion(s) present in each strain. These diploids were identical to 4579 except for the mei lesions indicated.

$$\frac{\text{ade2-1} \quad \text{met-2} \quad \text{ura-3} \quad \text{mei}}{\text{ade2-1} \quad \text{met-2} \quad \text{ura-3} \quad \text{mei}} \quad \frac{+ \quad + \quad \text{leu2-27} \quad a \quad +}{\text{his-4} \quad \text{leu2-1} \quad + \quad \alpha \quad \text{thr-4}}$$

Strain 4, used to test complementation, was derived by hybridizing the a segregant from M10-2B and the α segregant from M10-6A.

‡ The initial value was an average of determinations at 0 and 2 hrs; the final value was an average of determinations at 24 and 36 hr.

§ The number of leucine prototrophs (per 10⁶ cells) present at zero time was subtracted from the frequency at 24 hr to obtain the number generated during meiosis.

isotope incorporation did allow a comparison of mutants with wild type. The pattern of [³H]leucine incorporation (Fig. 2A) was essentially the same in the mutants and wild type; incorporation was most rapid during the first few hours of incubation in sporulation medium and then decreased. It appears that the mutants are not deficient in protein synthesis.

[²⁻¹⁴C]Uracil incorporation is shown in Fig. 2B. In Z4521-3C, uracil incorporation was most rapid between 0 and 1 hr, then quickly fell to a lower rate for the remainder of the experiment. In M10-6A, incorporation between 0 and 1 hr was comparable to that of wild type; afterwards incorporation was consistently lower (30–35%). In M10-2B, incorporation was again initially similar to that of the control; at later times however, this mutant incorporated uracil faster than the control. In a control experiment (not shown) we demonstrated that [¹⁴C]uracil incorporated by the mutants between 12 and 15 hr was rapidly hydrolyzed by alkali. This observation indicated that the uracil was predominantly labeling RNA. We conclude that both mutants synthesized RNA under sporulation conditions, but that M10-6A may be partially defective in this process.

Effects of the Mutations in Diploids. The absence of premeiotic replication observed in M10-2B and M10-6A did not depend on some peculiarity of disomic structure. Diploids homozygous for the mutations originally present in M10-2B and M10-6A also failed to initiate premeiotic DNA synthesis; in addition they showed little or no recombination and no sporulation (Table 1). In a wild-type diploid derived from Z4521-3C replication, recombination, and sporulation occurred normally (12).

Recessiveness, Segregation, and Complementation of the Mutations. The behavior of the mutations in crosses was

examined using recombination-deficiency as a phenotypic marker. We assumed that the recombination-deficient behavior was a pleiotropic consequence of the primary lesion blocking replication. Genetic analysis revealed a single mutant gene in M10-2B; it was designated $mei-1$. In M10-6A we tentatively identified two separable mutant genes designated $mei-2$ and $mei-3$. The mei notation was chosen to indicate the overall inhibitory effects of the lesions on meiosis.

A disomic α/α segregant obtained from each mutant was crossed to a wild-type a/a disome to form a tetrasomic ($2n + 2$) diploid heterozygous for the relevant mutant gene (Table 2). A cross between a wild-type α/α disome and the a/a parent served as a control (Table 2). The control strain and both heterozygous diploids sporulated well, and exhibited a high frequency of recombination at $leu-2$. This observation established that the mutations were recessive. This fact was confirmed independently by the complementation test reported in Table 1.

Upon completion of meiosis and sporulation in a tetrasomic diploid, each of the four ascospores will be disomic. In many of the asci two of the spores, and in some asci all four spores, were simultaneously heterozygous for a and α and heteroallelic at $leu-2$. These heterozygous and heteroallelic spores are potentially able to undergo meiosis and thus generate leucine recombinants; however, actual recombinant formation in any spore clone is governed by the presence or absence of the mei lesion(s), which was simultaneously segregating in the cross (Table 2). In the control hybrid where no mei lesions were segregating we expected all $\frac{a \quad \text{leu2-1} \quad +}{\alpha \quad + \quad \text{leu2-27}}$ spore clones to exhibit a high frequency of recombination. If the mei lesions were single genes we expected among asci with four potentially meiotic spores only 2 plus:2 minus segregants (minus signifies the recombination-deficient pheno-

TABLE 2. Tetrad analysis of the meiotic (*mei*) mutations from M10-2B and M10-6A

Crosses*	Genotype† of hybrid	Segregation of recombination-deficient phenotypes†														
		Segregation of <i>ura-3</i>			Segregation of <i>met-2</i>			A. Asci with two competent spores				B. Asci with four competent spores				
		+: -	+: -	+: -	+: -	+: -	+: -	Total	2+	1+:	2-	Total	4+:	3+:	2+:	1+:
3:1	2:2	1:3	3:1	2:2	1:3			1+:	1-	2-		0-	1-	2-	3-	
[1] (control)																
Z4521-88A(α/α) × Z4521-96C(a/a)	Wild type	0	40	0	-	-	-	18	18	0	0	6	6	0	0	0
[2] M10-2B(α/α) × Z4521-96C	<i>mei-1</i> +	0	38	0	0	38	0	11§	1	9	1	8	0	1	7	0
[3] M10-6A(α/α) × Z4521-96C	<i>mei-2</i> , <i>mei-3</i> +, +	0	34	0	0	33	1	13	¶			6	¶			

* Z4521-96C had the following structure:

	{	+	+	leu2-27	0	a	+	ade2-1	+	+	+	
	}	+	leu2-1	+	0	a	+					

Z4521-88A was:

	{	+	+	leu2-27	0	α	thr4	ade2-1	ura-3	+	+	
	}	his4	leu2-1	+	0	α	thr4					

The α/α segregants derived from M10-2B and M10-6A had the following overall structure:

	{	+	+	leu2-27	0	α	thr4	ade2-1	ura-3	met2	<i>mei</i>	
	}	his4	leu2-1	+	0	α	thr4					

† *mei* represents the appropriate mutational lesion(s). The sporulation frequencies of the hybrids obtained in crosses 1, 2, and 3 were 75, 56, and 60%, respectively; in each hybrid ascospore viability was greater than 90%.

‡ 0-25 leucine-independent colonies per test was considered a mutant (-) phenotype; >125 leucine-independent colonies per test was considered a wild-type (+) response.

§ Fit to a 1:2:1 ratio confirmed by χ^2 ; ($\chi^2 = 4.5$).

¶ As described in the text, a majority of the ascospore clones from this hybrid displayed an intermediate response yielding 40-80 leucine-independent colonies per test.

type; plus, the high-frequency recombination response). Among asci containing two potentially meiotic spores we expected a 1:2:1 ratio of ascus types with 2 plus, 1 plus and 1 minus, and 2 minus responses. Table 2 reports the data from the relevant crosses. As predicted, all potentially meiotic spores from the control hybrid generated a high frequency of leucine prototrophs. In the M10-2B hybrid, heterozygous for *mei-1*, the pattern of wild-type and recombination-deficient phenotypes was consistent with a single gene defect except for a single ascus with a 3 plus:1 minus segregation. About half of the potentially meiotic spore clones from the M10-6A hybrid could not be unequivocally scored as either + or -, but exhibited an intermediate response. Pooling the data from 19 asci revealed 19 plus spores, 30 intermediate spores, and 13 minus spores; this closely fits a 1:2:1 ratio ($\chi^2 = 1.23$) expected if two unlinked genes, each with a partial effect, must cooperate to fully inhibit recombination. The conclusion, however, that M10-6A contained two mutant genes (*mei-2* and *mei-3*) is only tentative; to firmly establish this point would require reconstruction of the strain by combining the isolated single components.

The characteristic 2:2 segregation pattern for the *uracil-3* and *methionine-2* genes (Table 2) demonstrated that meiosis apparently occurred in normal fashion in both heterozygous diploids.

Genetic complementation was tested by construction of a diploid heterozygous for the mutations and analysis of its meiotic behavior (Table 1). This hybrid exhibited normal

premeiotic replication; in addition recombination and sporulation were restored. Full complementation indicated that the defective gene products specified by *mei-1*, *-2*, and *-3* were not identical.

DISCUSSION

In this report we characterized two yeast mutants that failed to initiate premeiotic DNA synthesis but could replicate normally during growth. The mutants were used to identify a series of recessive nuclear genes (designated *mei-1*, *-2*, and *-3*) which affect different gene products. Carbohydrate, protein, and RNA synthesis occurred in the mutants during the expected interval of premeiotic replication, suggesting that the lesions preferentially inhibited synthesis of DNA. The mutations had striking inhibitory effects of meiosis. In M10-6A (containing *mei-2* and *-3*) meiotic intragenic recombination was completely arrested, while in M10-2B (*mei-1*) it was reduced to less than 1% of that of the control (in both mutants, UV-induced mitotic intragenic recombination at the *leu-2* locus was at, or near, control levels); both mutants also failed to sporulate. It is likely that the overall inhibitory effects on meiosis were a direct consequence of the defect in replication which somehow prevented continuation of meiotic development.

In addition to the genes described here, premeiotic replication in yeast is controlled by the mating-type locus (14). DNA synthesis under sporulation conditions occurs only in strains heterozygous for the *a* and α alleles of this locus, while strains homozygous for either allele behave just like the mutants in

that they undergo neither replication nor meiosis. The *mei* mutations, however, cannot be explained as defects in mating type since strains carrying these mutations can exist in fully functional *a* and α states. Recently a dominant mutation that inhibits premeiotic replication has been described (28).

Successful replication, whether it precedes mitosis or meiosis, requires three distinct functional groups of gene-controlled reactions (29). These include: (i) reactions necessary for deoxynucleotide formation, (ii) polymerization reactions, and (iii) reactions that initiate polymerization. In trying to propose functions for mutant genes that only affect replication during meiosis, we considered two possibilities. The first possibility is that the lesions affect functions (i.e., enzymes) common to replication during mitosis and meiosis, but that conditions inside the meiotic cell (e.g., pH, nucleotide levels, etc.) are sufficiently different from those during mitosis that the defect is only expressed during meiosis. The second possibility is that one or more of the three major replication functions might require different reactions during meiosis from those used during growth. For example, it has been proposed (28) that deoxynucleotides used for premeiotic replication are supplied by a pathway different from that used during growth. Clearly, a defect in a reaction required only to supply deoxynucleotides during meiosis could easily account for a stage-specific affect on replication. Mutant M10-6A, where inhibition of replication was accompanied by a simultaneous reduction in the apparent rate of RNA synthesis (Fig. 2B), may well be an example of a defect in meiotic nucleotide metabolism.

Other possible gene-controlled replication functions that might require new components during meiosis are polymerization and initiation. At present, there is no evidence to support the existence of new or modified polymerizing enzymes for premeiotic replication. On the other hand, Callan (8) recently proposed, after comparing autoradiograms of replicating chromosomes from meiotic and mitotic tissues of the newt *Triturus*, that the control of initiation points along the chromosome was different during meiosis and mitosis. Conceivably new patterns of initiation may require new types of gene-specific initiator molecules (18). A lesion in the synthesis or function of such an initiator would be expected to act in a strictly stage-specific fashion.

While the actual roles in DNA synthesis controlled by the stage-specific genes identified here remain to be determined, their existence does tend to support the growing evidence that the last round of replication preceding meiosis differs in significant ways from replication during growth. In attempting to identify the molecular mechanisms responsible for these differences, mutants, available in yeast, offer advantages for analysis that do not exist in more complex eukaryotes.

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