Proc. Natl. Acad. Sci. USA
Vol. 74, No. 9, pp. 3951–3955, September 1977
Genetics

Role of mitotic replication genes in chromosome duplication during meiosis

(yeast/DNA initiation/DNA polymerization)

T. J. ZAMB AND R. ROTH

Biology Department, Illinois Institute of Technology, Chicago, Illinois 60616

Communicated by Adrian M. Srb, June 20, 1977

ABSTRACT In the yeast *Saccharomyces cerevisiae,* DNA synthesis preceding meiosis requires the expression of replication genes used during mitosis. Diploids carrying either a temperature-sensitive lesion in *cdc4* (a gene required for initiation of mitotic DNA synthesis) or a lesion in *cdc8* (a gene controlling mitotic polymerization) completed premeiotic DNA synthesis at a permissive but not at a restrictive temperature. The roles of *cdc4* and *cdc8* were evaluated by characterizing the kinetics of premeiotic DNA synthesis after a shift to a restrictive temperature. In the *cdc8* diploid, DNA synthesis was immediately inhibited, consistent with a role in polymerization. In contrast, *cdc4* exhibited residual DNA synthesis characteristic of an initiation function. The *cdc4* gene function was completed much earlier in the meiotic cycle than the *cdc8*-mediated step.

Meiosis is characterized by a distinctive sequence of nuclear and cytoplasmic events which include recombination, reduction divisions, and gameteogenesis. One of the early events in this sequence is a single round of premeiotic chromosome duplication. As in mitosis, DNA replication is essential for successful completion of the meiotic cycle (1). We have determined whether premeiotic DNA synthesis required the participation of replication genes normally expressed during the mitotic cell cycle and, also, whether required replication genes governed identical functions in both meiotic and mitotic cells. In the yeast *Saccharomyces cerevisiae,* Hartwell (2) identified two groups of genes controlling mitotic DNA synthesis: genes functioning during the G1 interval are required for initiation of replication and genes functioning during the S phase are essential for DNA polymerization. We evaluated premeiotic replication in diploid yeast strains carrying a temperature-sensitive lesion in either a gene (*cdc4*) controlling a mitotic initiation event or a gene (*cdc8*) controlling polymerization. Both gene functions were required for premeiotic replication and controlled identical functions in meiotic and mitotic cells.

In yeast, meiosis is induced by transferring growing cultures to acetate sporulation (starvation) medium. Soon after the transfer to sporulation medium, cell division and mitotic replication cease. A latent period of 4–6 hr, with little or no nuclear replication, precedes the start of premeiotic DNA synthesis. During the latent period, cells are metabolically active and continue to synthesize macromolecules including carbohydrates, protein, and RNA (1). Initiation of DNA synthesis is an early event characteristic of meiotic cells. After replication of the DNA, the cells complete recombination, undergo two meiotic divisions, and initiate sporulation (3). In yeast, sporulation generates asci usually containing four haploid ascospores. Except for the details of spore formation, meiosis in yeast is analogous to meiosis in other eukaryotes.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Strains. *S. cerevisiae* strain A364A is the wild-type parent from which temperature-sensitive mutants ts314 (*cdc4-1*) and ts198 (*cdc8-1*) were derived; these three strains were provided by L. Hartwell (4). Strain X1069-2D was obtained from the stock center at Berkeley. Strain Z1, a thermoresistant control diploid, was derived by crossing A364A and X1069-2D (5); Z1 has the following genotype:

$$\frac{(\text{A364A})}{(\text{X1069-2D})}:\ \frac{+}{his4}\ \frac{+}{leu2}\ \frac{a}{\alpha}\ \frac{+}{thr4}\ \frac{ade1}{ade1}\ \frac{ura1}{ura1}\ \frac{ade2}{+}\ \frac{+}{trp5}\ \frac{+}{met5}\ \frac{his7}{+}\ \frac{lys2}{+}\ \frac{tyr1}{+}\ \frac{gal1}{+}$$

Strain Z198 is homozygous for *cdc8-1;* it was obtained by crossing ts198 and X1069-2D to form a heterozygous diploid and then selecting for a thermosensitive mitotic segregant after a sublethal dose of UV light (6). Strain Z314 is homozygous for *cdc4-1;* it was obtained like Z198, from a diploid formed between ts314 and X1069-2D. Z314 and Z198 were confirmed as homozygous for *cdc4-1* and *cdc8-1,* respectively, by morphological and genetic tests.

Cultures growing at the permissive temperature (23.5°) were rapidly brought to the restrictive temperature (36.5°) and, at various times after the shift, were examined by phase-contrast microscopy. Z314 and Z198 exhibited the terminal phenotype characteristic of *cdc4-1* and *cdc8-1* tester strains at 36.5° (4). Z314 and Z198 were sporulated at the permissive temperature, and meiotic spore clones were checked for *cdc4-1* or *cdc8-1* by complementation with appropriate tester strains (5). With Z198, spore viability was 48% but all 120 segregants were temperature-sensitive; the spores from a four-spored ascus all contained *cdc8-1.* With Z314, spore viability was 51% but all 74 spore clones were temperature-sensitive; four spores from one ascus contained *cdc4-1.*

Growth and Sporulation Conditions. Strains were maintained on a complex medium (5) further supplemented as follows (in mg/liter): adenine, 20; *l*-histidine, 20; *l*-methionine, 20; *l*-arginine, 40; *l*-phenylalanine, 40; *l*-tryptophan, 40; *l*-tryosine, 100; *d,l*-isoleucine, 120; *l*-leucine, 120; *l*-lysine, 120; uracil, 200; *d,l*-valine, 300; *d,l*-threonine, 300. For physiological experiments, cells were grown in acetate presporulation medium (7) supplemented with amino acids and bases as described above; at 23.5° the doubling time of Z1, Z314, and Z198 was 4 hr ± 10 min. Meiosis was initiated as described (8) except that cells were harvested from presporulation medium at a density of 3 to 4 × 10^7 per ml and resuspended in sporulation medium at 3.0 × 10^7 cells per ml.

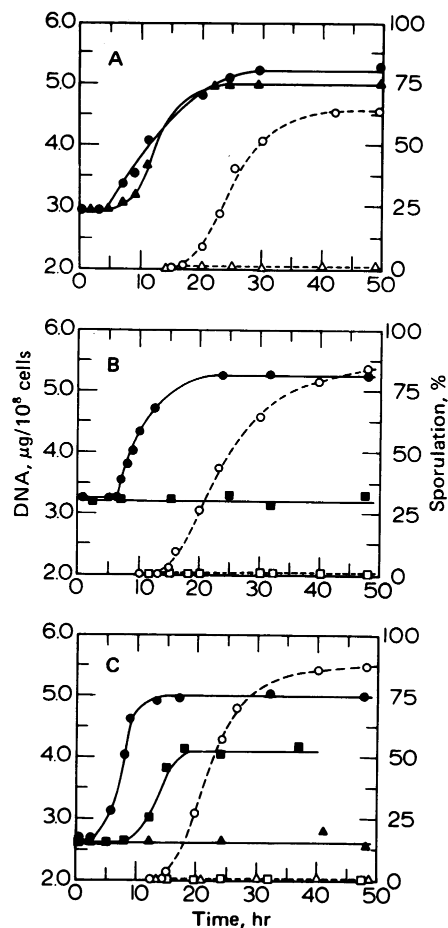

FIG. 1. Premeiotic replication and sporulation in diploid yeast strains. At zero time, cultures growing logarithmically in presporulation medium were harvested, washed, and resuspended in sporulation medium; subcultures were immediately established, and continuously incubated at 23.5° and at 34.5° or 36.5°. At intervals, samples were removed for measurement of: (*i*) absorbance at 600 nm, which is a measure of mass increase (10); (*ii*) total cellular DNA by a diphenylamine assay; and (*iii*) the percentage sporulation by phase-contrast microscopy. Solid symbols and lines present DNA data; open symbols and broken lines depict sporulation. (*A*) Strain Z1 (wild-type control). ● and ○, DNA and sporulation, respectively, at 23.5°; ▲ and △, DNA and sporulation at 36.5°. Increases in mass between 0 and 24 hr were: at 23.5°, 2.6-fold; at 34.5°, 2.5-fold; at 36.5°, 2.6-fold. (*B*) Strain Z198 (homozygous for the *cdc8-1* mutation). ● and ○, DNA and sporulation at 23.5°; ■ and □, DNA and sporulation at 34.5°. Mass increases, 0 to 24 hr: at 23.5°, 2.5-fold; at 34.5°, 2.5-fold; at 36.5°, 2.3-fold. (*C*) Strain Z314 (homozygous for the *cdc4-1* mutation). ● and ○, DNA and sporulation at 23.5°; ■ and □, DNA and sporulation at 34.5°; ▲ and △, DNA and sporulation at 36.5°. Mass increases, 0 to 24 hr: at 23.5°, 2.4-fold; at 34.5°, 2.4-fold; at 36.5°, 2.3-fold.

Analytical Procedures. DNA synthesis was monitored as described (8, 9) except the samples contained 1.5×10^8 cells. Protein synthesis was estimated by the incorporation of [^{14}C]-leucine (New England Nuclear Corp.) into a fraction precipitated with hot trichloroacetic acid (1).

RESULTS

At the permissive temperature (23.5°), Z1, Z314, and Z198 completed premeiotic replication and meiosis in a similar fashion (Fig. 1). Before evaluating the effects of restrictive temperatures on the mutants, we determined thermal limits for meiotic events in the wild type (Fig. 2). Z1 grown in presporulation medium at 23.5° was washed and resuspended in

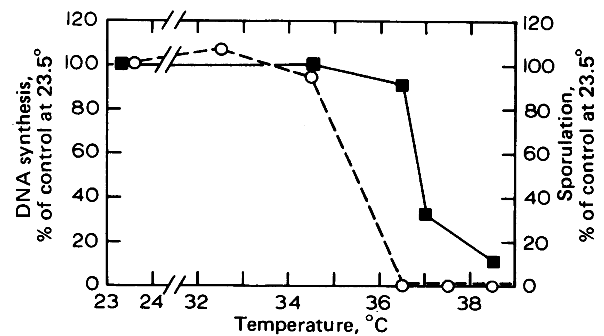

FIG. 2. Effects of temperature on premeiotic replication and sporulation in strain Z1. At zero time, independent meiotic cultures were established at temperatures between 23.5° and 38.5°. DNA synthesis and sporulation were determined in each culture from 0 to 50 hr as described in Fig 1. The amounts of DNA synthesis and sporulation at 23.5° (control) (cf. Fig 1*A*) were taken as 100% and the values for each of the other temperatures were calculated as a percentage of the control. ■, DNA synthesis; ○, sporulation.

sporulation medium; at zero time, subcultures were established at temperatures between 23.5° and 38.5°. Periodically, samples from each subculture were taken for measurement of DNA synthesis and sporulation. Premeiotic replication was essentially the same at 23° and 36.5°; DNA synthesis was inhibited by about 70% at 37° and by about 90% at 38.5°. Next, cultures of Z314 and Z198 were washed, resuspended in sporulation medium, and subcultured at either 34.5° (Z198) or at 34.5° and 36.5° (Z314) (Fig. 1 *B* and *C*). Samples were taken to monitor DNA synthesis. In Z198 (Fig. 1*B*), replication was fully inhibited at 34.5°, which was chosen as the restrictive temperature. In Z314 (Fig. 1*C*), replication was partially inhibited at 34.5° but fully inhibited at 36.5°; 36.5° was used as the restrictive

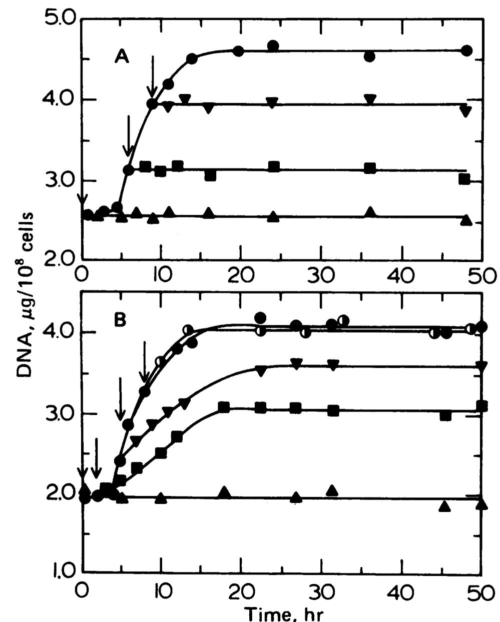

FIG. 3. Effects of a restrictive temperature on premeiotic DNA synthesis in strains Z198 and Z314. Meiotic cultures were established at 23.5°. At zero time and at intervals, subcultures were rapidly brought to a restrictive temperature for the remainder of the experiment; one culture (control) remained at 23.5°. DNA synthesis was monitored in each culture. (*A*) Strain Z198. ●, Control; ▲, subculture shifted to 34.5° at 0 hr; ■, subculture shifted at 6 hr; ▼, subculture shifted at 9 hr. (*B*) Strain Z314. ●, Control; ▲, subculture shifted to 36.5° at 0 hr; ■, subculture shifted at 2 hr; ▼, subculture shifted at 5 hr; ◑, subculture shifted at 8 hr.

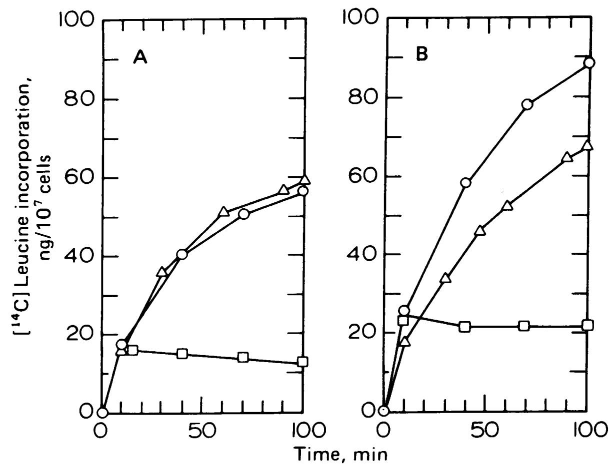

FIG. 4. Effects of a restrictive temperature and cycloheximide on protein synthesis in Z198 and Z314. Meiotic cultures were established at 23.5°. During replication (at 7 hr for Z198; at 5 hr for Z314), sufficient [^{14}C]leucine was added to each culture to give a final concentration of 2.2μg/ml (specific activity, 2×10^5 cpm/μg). Immediately thereafter, each culture was divided into three portions: one portion (○) remained at 23° (control); one portion (△) was brought to a restrictive temperature; and the third portion (□) was incubated at 23.5° for 10 min and then cycloheximide was added to a final concentration of 100 μg/ml. Incorporation of [^{14}C]leucine was monitored for 100 min. (*A*) Strain Z198, restrictive temperature 34.5°. (*B*) Strain Z314, restrictive temperature 36.5°.

temperature. Thermal inhibition of DNA synthesis in Z198 and Z314 indicated that *cdc4* and *cdc8* gene functions were necessary for premeiotic replication.

Molecular Specificity of *cdc* Lesions during Meiosis. At their restrictive temperatures, both Z198 and Z314 showed typical increases in cell mass, suggesting normal rates of acetate metabolism (see legend, Fig. 1). In both mutant strains, the restrictive temperature had little or no effect on protein synthesis as monitored by incorporation of [^{14}C]leucine (see Fig. 4). These data suggest that mutations *cdc4-1* and *cdc8-1* affect primarily DNA synthesis.

Physiological Roles of *cdc4* and *cdc8* in Premeiotic Replication. Hartwell's conclusion that *cdc4* and *cdc8* controlled DNA initiation and polymerization, respectively, relied in part on the effects of a restrictive temperature on the kinetics of ongoing replication and on the time during the cell cycle when each gene completed its essential function (4). Experiments performed with meiotic cultures of Z198 and Z314 established that *cdc4* and *cdc8* performed similar physiological functions during meiosis and mitosis.

A meiotic culture of Z198 was established at 23.5°; at zero time and at intervals (6 and 9 hr) subcultures were shifted to 34.5° and DNA synthesis was monitored (Fig. 3*A*). The shift at zero time, prior to the start of replication, blocked all DNA accumulation. The shifts during replication itself immediately stopped any further increase in DNA accumulation. Data from other thermal shifts throughout the DNA synthetic period all were consistent with an immediate thermal inhibition of replication (see Fig. 6). Rapid inhibition of ongoing DNA synthesis is characteristic of a defect in a polymerization function (4, 11).

Thermal shifts with Z314, performed in an identical fashion, gave results characteristic of a lesion in DNA initiation (Fig. 3*B*). A thermosensitive defect in initiation exhibits extensive residual DNA synthesis after a thermal shift; presumably, cells that have completed the thermosensitive initiation event at the permissive

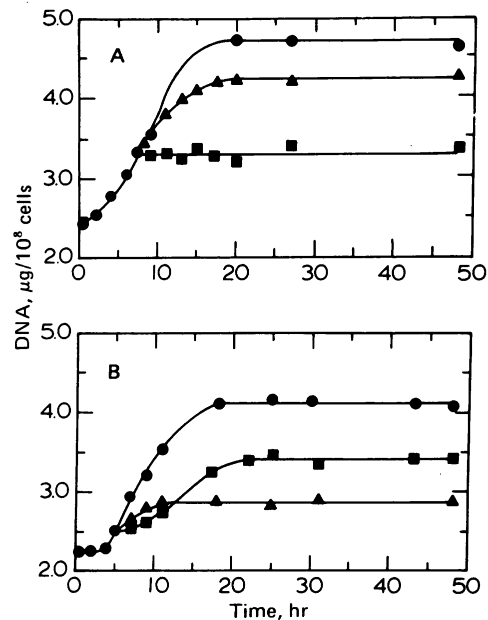

FIG. 5. Comparative effects of cycloheximide and restrictive temperature on DNA synthesis in strains Z198 and Z314. At zero time, meiotic cultures were established at 23.5°. During replication (at 7 hr for Z198; at 5 hr for Z314) the cultures were divided into three portions: one portion (●) remained at 23.5° as a control; the second portion (■) was brought to a restrictive temperature; the third portion (▲) was kept at 23° but received cycloheximide to a final concentration of 100 μg/ml. DNA synthesis was monitored in each culture. (*A*) Strain Z198, restrictive temperature 34.5°. (*B*) Strain Z314, restrictive temperature 36.5°.

temperature can finish polymerization at the restrictive temperature (4, 11).

Effects of Cycloheximide on Meiotic Replication. In yeast, protein synthesis is required for DNA initiation but not for continued polymerization (12). Thus, an inhibitor of protein synthesis, such as cycloheximide, can be used to distinguish the two types of functions. In control experiments with meiotic cultures of Z198 and Z314 we confirmed that cycloheximide at 100 μg/ml effectively blocked [^{14}C]leucine incorporation into protein (Fig. 4) (13). Next, meiotic cultures of Z198 and Z314 were established at the permissive temperature. During replication (at 5 hr for Z314, at 7 hr for Z198), one portion of each culture was shifted to the restrictive temperature, a second portion was exposed to cycloheximide at 23.5°, and the remainder was kept as a control. DNA synthesis was monitored in each subculture. In Z198, the thermal shift blocked replication immediately (Fig. 5*A*). In contrast, inhibition of protein synthesis allowed substantial residual DNA synthesis. The results imply that the rapid thermal inhibition in Z198 represented inhibition of polymerization rather than an initiation defect in a highly asynchronous population. In Z314, residual DNA synthesis was observed both with cycloheximide at 23.5° and after the thermal shift (Fig. 5*B*). These results are consistent with the idea that both treatments affect initiation. In Z314, there was more residual synthesis after the thermal shift than after cycloheximide treatment. This may be accounted for by assuming that an initiation event requiring protein synthesis occurs after the *cdc4*-mediated step; consequently at 5 hr, more cells in the population have completed the *cdc4* step than a later cycloheximide-sensitive step.

Timing of *cdc4* and *cdc8* Gene Functions during Meiosis. The *cdc4* gene function occurs during the G1 phase of mitosis whereas *cdc8* functions during S phase (2). Thermal shift ex-

periments established the same relative temporal order for these gene functions during meiosis. Our experiments attempted to estimate the time when each gene function was completed in asynchronous meiotic populations. Cultures incubated at the permissive temperature were periodically shifted to the restrictive temperature and the amount of subsequent DNA synthesis (if any) was compared to a control kept continuously at 23.5°. In order to combine data from different strains, involving numerous independent experiments, we had to correct for variability in the latent period prior to replication, for variations in the overall length of the DNA synthesis period in the population, and for differences in the DNA content per cell. To accomplish this, we adopted the following conventions: first, the total increase in DNA in the control was taken as 100% and the DNA increase in any sample was expressed as a percentage of this value; next, the period from zero time to the estimated completion of DNA synthesis in the population was set equal to 1.0 and termed the "G1 + S" interval and the time when each DNA sample was taken from a culture was expressed as a fraction of the total G1 + S interval. With these conventions, various DNA control experiments involving Z1, Z198, and Z314 were replotted on a single graph (Fig. 6*A*). The data formed a smooth continuous curve, indicating a common latent period from 0 to 0.25 and a synthesis period extending from 0.25 to 1.0. The latent period probably represents the minimal G1 period in the population because cells with longer G1 periods are concealed by the start of DNA accumulation in precocious cells. The relative orders of the *cdc4* and *cdc8* events are compared in Fig. 6*B*. These data evaluate the timing for completion of the temperature-sensitive event at the permissive temperature. In Z198, completion of the temperature-sensitive event coincided with the completion of replication itself, as expected of a polymerization function. In contrast, completion of the temperature-sensitive function in Z314 occurred much earlier (at 0.38 on time scale), consistent with a role in DNA initiation. Comparison of the times for half-completion of the steps gives approximately 0.19 for *cdc4* and 0.47 for *cdc8*.

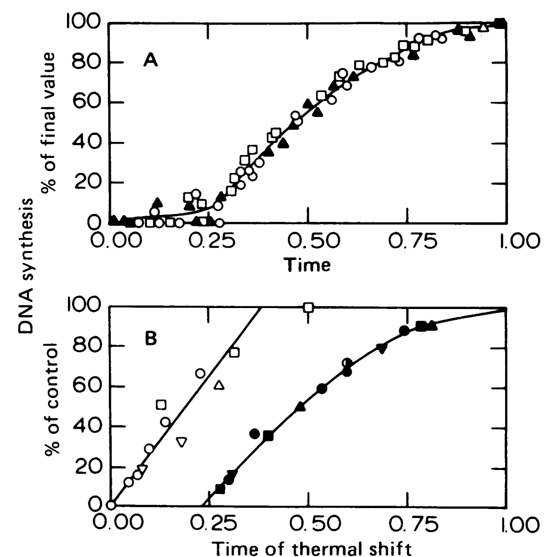

FIG. 6. Timing of *cdc4* and *cdc8* gene functions during meiosis. Time scale is shown as fraction of total G1 + S interval. (*A*) Method for normalizing the amount and timing of meiotic DNA synthesis in different strains or in multiple experiments with a single strain. The data are from four separate experiments at 23.5° with each of three strains from Figs. 1, 3, and 5: □, strain Z1; ○, strain Z198; ▲, strain Z314. (*B*) Data from thermal shift experiments with Z198 and Z314 were normalized (expressed as % of control at 23.5°) and used to determine when the functions specified by *cdc4* and *cdc8* were completed at the permissive temperature. Data from Z198 (six experiments) are presented as solid symbols and include experiments shown in Figs. 1*B*, 3*A*, and 5*A*. Data from Z314 (five experiments) are presented as open symbols and include experiments shown in Figs. 1*C*, 3*B*, and 5*B*. DNA was measured at 48 hr.

DISCUSSION

Genetic studies of development often attempt to identify gene functions required for a specific developmental event or sequence. Usually this involves the isolation and characterization of mutants affecting the process in question, and generally such mutants are stage-specific in that their effects are limited to a unique portion of the life cycle. Less frequently used is an analysis that identifies a physiological role in development for gene functions expressed at other stages of the life cycle. The sporulation cycle in yeast offered a convenient opportunity to explore the role of mitotic gene functions in meiotic development. Gene functions required for specific events during the cell division cycle are well known (2) and the sporulation cycle is convenient to analyze. In an earlier study, Simchen (6) took advantage of these facts to demonstrate that defects in gene functions necessary for mitosis did in fact affect meiosis and sporulation. To define more precisely the roles of mitotic gene functions in development, we examined premeiotic replication in strains carrying mutations in genes controlling well-characterized mitotic replication events. Replication was especially amenable to analysis because appropriate experiments provided information about the actual physiological functions of a gene.

In yeast, Hartwell (4, 11) showed that mitotic DNA synthesis required at least five gene functions: three genes (*cdc4*, *-7*, *-28*) controlled DNA initiation events, and two genes (*cdc8*, *-21*) governed polymerization. Our experiments show that expression of functions specified by *cdc4* and *cdc8* also was essential for replication during meiosis, in which the gene apparently controlled physiological functions identical to those performed in mitotic cells.

It might be argued that DNA duplication associated with meiosis is actually regulated as in a typical mitotic cell cycle. Numerous lines of evidence, both in higher eukaryotes and in yeast, argue against this interpretation. First, it has been established in various higher eukaryotes that premeiotic replication is distinct in both its temporal and spatial patterns from that in mitotic cell (1, 14). In yeast, where the pattern of meiotic replication has not yet been examined, it is known that premeiotic replication is governed by a set of unique, stage-specific genes, typical of most developmental sequences (1, 4, 15, 16). In a previous study (17), it was shown that mitotic replication occurred in diploids either homozygous or heterozygous for the two alleles of the mating-type locus, but that replication during meiosis only occurred in the heterozygous strains. This result suggested initially that meiotic replication had genetic controls distinct from those for mitosis. Subsequently, additional stage-specific gene functions required for premeiotic replications were discovered (9, 15, 16). Previous studies and those reported here lead to the conclusion that meiotic replication requires the participation of two distinguishable types of genes: those that function during mitosis and meiosis, like *cdc4* and *-8*, and others that act in a strictly stage-specific fashion. Roles for two types of genes can be envisioned based on characteristics of premeiotic replication known from higher cells. For example, because mitotic and meiotic DNA synthesis use identical deoxynucleotides and share a semiconservative mode of replication, they might be expected

to use some common enzymes for precursor synthesis or for DNA chain elongation. In contrast, a stage-specific function might determine a unique initiation pattern restricted to meiotic cells (14) and perhaps required for efficient chromosome pairing or recombination. Biochemical characterizations may help assign more precise roles to the various gene functions.

This work was supported by Grant GM 19,330 and a Research Career Development Award to R.R. (1 K04 GM 70,274) from the National Institutes of Health.

1. Roth, R. (1973) "Replication during meiosis: Identification of gene functions required for premeiotic DNA synthesis," *Proc. Natl. Acad. Sci. USA* **70**, 3087–3091.
2. Hartwell, L. H. (1974) "*Saccharomyces cerevisiae* cell cycle," *Bacteriol. Rev.* **38**, 164–198.
3. Tingle, M. A., Singh Klar, A. J., Henry, S. A. & Halvorson, H. O. (1973) "Ascospore formation in yeast," in *Microbial Differentiation* (Symposium of the Society for General Microbiology), (Cambridge University Press, Cambridge, England), No. 23, pp. 209–243.
4. Hartwell, L. H. (1971) "Genetic control of the cell division cycle in yeast II. Genes controlling DNA replication and its initiation," *J. Mol. Biol* **59**, 183–194.
5. Mortimer, R. K. & Hawthorne, D. C. (1969) "Yeast Genetics," in *The Yeasts*, eds. Rose A. H. & Harrison, J. S. (Academic Press, New York), Vol. 1, pp. 385–460.
6. Simchen, G. (1974) "Are mitotic functions required in meiosis?," *Genetics* **76**, 745–753.
7. Roth, R. & Halvorson, H. O. (1969) "Sporulation of yeast harvested during logarithmic growth," *J. Bacteriol.* **98**, 831–832.
8. Küenzi, M. T. & Roth, R. (1974) "Timing of mitochondrial DNA synthesis during meiosis in *Saccharomyces cerevisiae*," *Exp. Cell Res.* **85**, 377–382.
9. Roth, R. (1976) "Temperature-sensitive yeast mutants defective in meiotic recombination and replication," *Genetics* **83**, 675–686.
10. Roth, R. (1970) "Carbohydrate accumulation during the sporulation of yeast," *J. Bacteriol.* **101**, 53–57.
11. Hartwell, L. H. (1973) "Three additional genes required for deoxyribonucleic acid synthesis in *Saccharomyces cerevisiae*," *J. Bacteriol.* **115**, 966–974.
12. Hereford, L. M. & Hartwell, L. H. (1973) "Role of protein synthesis in the replication of yeast DNA," *Nature New Biol.* **244**, 129–131.
13. Magee, P. T. & Hopper, A. K. (1974) "Protein synthesis in relation to sporulation and meiosis in yeast," *J. Bacteriol.* **119**, 952–960.
14. Callan, H. G. (1972) "Replication of DNA in the chromosomes of eukaryotes," *Proc. R. Soc. London Ser. B.* **181**, 19–41.
15. Esposito, M. S. & Esposito, R. E. (1974) "Genes controlling meiosis and spore formation in yeast," *Genetics* **78**, 215–225.
16. Simchen, G., Piñon, R. & Salts, Y. (1972) "Premeiotic DNA synthesis, readiness and commitment," *Exp. Cell Res.* **75**, 207–218.
17. Roth, R. & Lusnak, K. (1970) "DNA synthesis during yeast sporulation: Genetic control of an early developmental event." *Science* **168**, 493–494.