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DNA Polymerases, Deoxyribonucleases, and Recombination During Meiosis in *Saccharomyces cerevisiae*

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We utilized strains of *Saccharomyces cerevisiae* that exhibit high efficiency of synchrony of meiosis to examine several aspects of meiosis including sporulation, recombination, DNA synthesis, DNA polymerase I and II, and Mg^{2+}-dependent alkaline DNases. The kinetics of commitment to intragenic recombination and sporulation are similar. The synthesis of DNA, as measured directly with diphenylamine, appears to precede the commitment to recombination. Both DNA polymerase I and II activities and total DNA-synthesizing activity in crude extracts increase two- to threefold before the beginning of meiotic DNA synthesis. Increases of 10- to 20-fold over mitotic levels are found for Mg^{2+}-dependent alkaline DNase activity in crude extracts before and during the commitment to meiotic intragenic recombination. Of particular interest is the comparable increase in a nuclease under the control of the *RAD52* gene; this enzyme has been identified by the use of antibody raised against a similar enzyme from *Neurospora crassa*. Since the *RAD52* gene is essential for meiotic recombination, the nuclease is implicated in the high levels of recombination observed during meiosis. The effects observed in this report are meiosis specific since they are not observed in an αα strain.

During the meiotic stage of development, a large number of biochemical changes can be detected, presumably associated with the events in meiotic recombination. In the case of *Lilium*, DNA metabolic enzymes have been identified during meiosis whose activities are consistent with intermediate steps in recombination (11–13). Among the categories of proteins and enzymes that have been proposed to be involved are nucleases, polymerases, and DNA binding, denaturing, and renaturing proteins (10, 19). Based on observed changes in DNA during meiosis in the limited number of systems available for investigation, some of the enzymes might be expected also to be involved in DNA repair-like processes. Genetic and some biochemical evidence from several systems has, in fact, demonstrated a role for mitotically defined DNA repair systems in normal meiosis (2, 8, 18). Recently, it was demonstrated in *Coprinus* that a β-type DNA polymerase appears specifically during meiosis (27); this enzyme has the characteristics of a repair-like polymerase.

Although there is substantial evidence indicating repair-like activity during meiosis, there have been no studies in which it has been possible to characterize and determine the temporal relationship between recombination, repair, and DNA metabolic enzymes. Such information is absent because either the systems exhibit good meiotic synchrony but are genetically not well defined, or meiotic genetic events can be assessed but biochemical analyses are difficult. Recently, we described strains of yeast with which it is possible to obtain high levels of meiosis (>95%) and good meiotic synchrony (23). By means of the "pull back" or return-to-growth procedure of Sherman and Roman (28), commitment to recombination was followed by plating cells from meiotic to selective vegetative growth medium which would only support the growth of recombinants. Commitment to recombination appeared to correlate with the onset of DNA synthesis (22); however, the precise timing was not determined.

To understand the DNA metabolic events of meiosis, we previously characterized various repair-deficient mutants by their effects on recombination, DNA changes, and DNA repair during meiosis (8). In this article, we further describe the meiotic cycle of the yeast *Saccharomyces cerevisiae* in terms of the following enzymes and activities: DNA polymerases I and II, alkaline single-strand DNases, and a specific single-strand nuclease that is deficient in *rad52* strains (5). The *RAD52* gene is required in meiotic and mitotic recombination (20, 8, 18) and for the repair of double-strand breaks in DNA (24). It has been possible to relate the appearance of these enzymes to (i) the timing of meiotic DNA and RNA synthesis, (ii) sporulation as measured by ascus formation, and (iii) commitment to intragenic (gene conversion) and intergenic recombination. We used this integrated approach to determine the temporal relationship between biochemical and genetic events and to identify those biochemical processes that might account for the high levels of meiotic as compared with mitotic recombination.

MATERIALS AND METHODS

Strains. The data described in this report were obtained with MR48, which was derived from SK-1 (see reference 8). Various well-marked strains were crossed with SK-1, as were the progeny from these crosses. After three or more such crosses, the resulting haploids when intercrossed yielded diploids such as MR48 with the high-efficiency meiotic phenotype of SK-1 (14). Results comparable to those presented in this report were also observed with another SK-1 derivative, g690 (22).

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MR48: *MATα leu2* + + *tup7* +
MATa leu2 rad52-1 rad1-1 + *ade2-1*

\+ + *his1-1 trp2 lys1-1*
can1 hom3-10 his1-7 + +

Isogenic strains homozygous for mating type were obtained by irradiating MR48 with 4 krad with a ^{125}Cs source and testing the resulting colonies for their ability to mate with *MATa* or *MATα* tester strains. A colony was chosen that had half sectors which mated to opposite mating type strains; presented in this report are results with the cells from a presumptive αα strain derived from this colony.

Growth and recombination. Conditions for growth and sporulation have been described previously (22, 24). In brief, cells were grown at 30°C in SPS (supplemented presporulation medium) containing 1% potassium acetate (KAC), 10 μg of tetracycline per ml, and required amino acids. Cells were grown to a titer of 4×10^7 cells per ml, washed with prewarmed 1% KAC, and suspended in prewarmed sporulation medium (1% KAC, required amino acids, 0.0005% Nonidet P-40). Flasks for sporulation were incubated with vigorous shaking and were no more than one-eighth full to ensure complete aeration. Sporulation was detected with a phase-contrast microscope.

Recombination was monitored by the appearance of histidine prototrophs that arise by gene conversion between *his1-1* and *his1-7* heteroalleles (8). The appearance of colonies resistant to canavanine, which in strains heterozygous for canavanine resistance arise by either recombination or commitment to haploidization, was monitored on synthetic complete medium lacking arginine but containing 60 μg of canavanine per ml. Colonies grown on YEPD (1% yeast extract, 2% peptone, 2% dextrose) were visually inspected for red or red-sectored colonies arising from homozygosis at the *ade2* locus or from commitment to haploidization. Other media and methods have been described previously (8, 26).

Label uptake and DNA measurements. Procedures for labeling newly synthesized nucleic acids during meiosis have been described previously (22). Cells were labeled with [6-^{3}H]uracil (10 mCi/mmol, 1 mCi/ml; New England Nuclear Corp.) at a concentration of 15 μCi/ml. Measurement of incorporation into RNA and DNA has been described previously (22).

Direct DNA measurements were made by a diphenylamine procedure (9, 29) as modified by Craig Giroux (personal communication).

Crude extracts and DNA polymerases. The protocol for making crude extracts from *S. cerevisiae* was essentially the same as described before (15), except that 0.5 M NaCl was used during extraction of proteins. After centrifugation of the cell lysate, supernatants were collected and solid ammonium sulfate was added to 45 to 50% saturation over 20 min at 0 to 4°C. The precipitates were collected by centrifugation at 10,000 rpm for 10 min in a Sorvall SS34 rotor, suspended in 5 ml of buffer A (50 mM Tris-hydrochloride [pH 7.5], 10% glycerol, 10 mM 2-mercaptoethanol, 1 mM EDTA, 1 mM

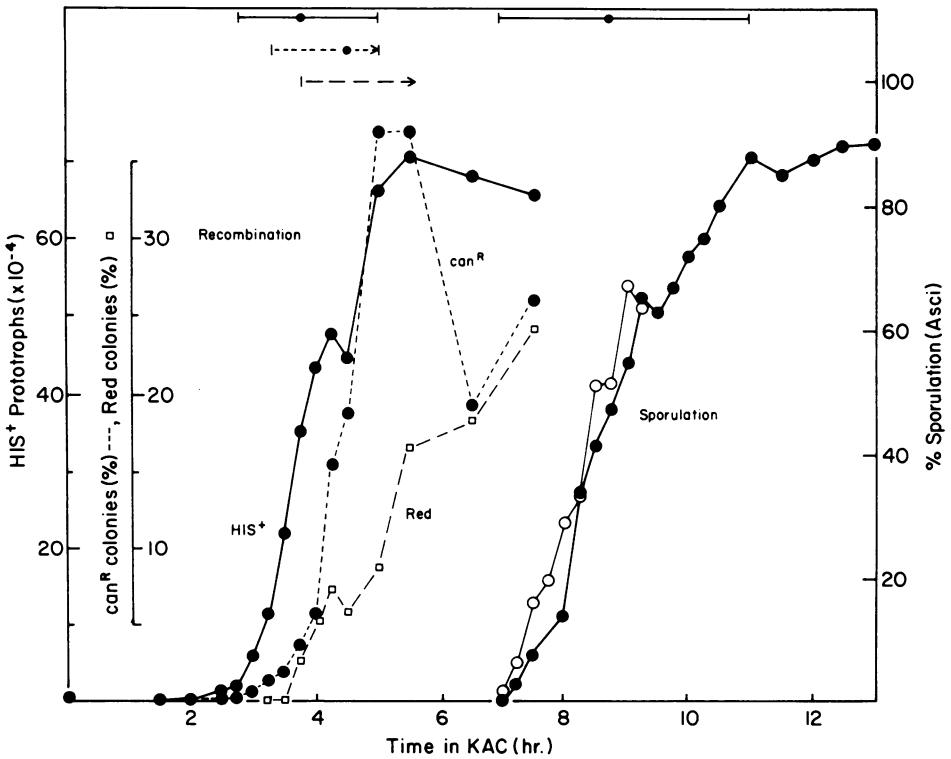

FIG. 1. Sporulation and commitment to recombination. Cells were transferred from SPS to KAC. Commitment to recombination was detected by plating cells to medium lacking histidine (intragenic recombination) and medium containing canavanine for detection of homozygosis for *can^r*. At later times, many of the *can^r* colonies were due to a commitment to haploidization. The red colonies were due to homozygosis for *ade2* or haploidization. Sporulation was detected by the appearance of spherical spores within cells. Shown are the results of experiments on two different days. The horizontal line corresponds to the period over which an increase occurred, and the circle in the line corresponds to the time at which ca. one-half the maximum increase occurred.

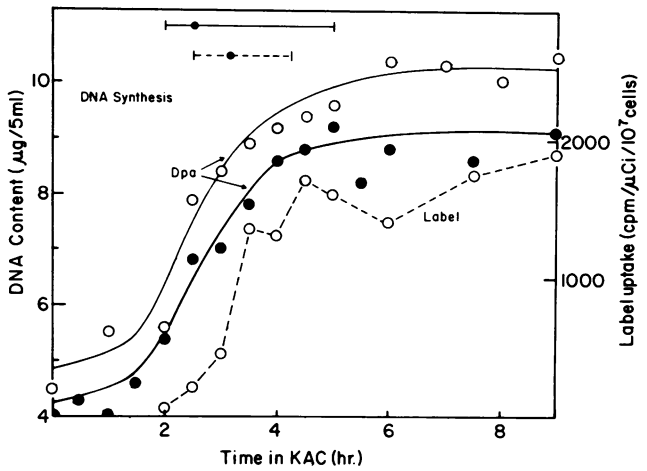

FIG. 2. DNA synthesis in cells undergoing meiosis. Synthesis was measured directly by using a diphenylamine assay or by looking at the radioactivity uptake into alkali-stable, trichloroacetic acid-precipitable material after the addition of [^{3}H]uracil to the cultures. Shown are the results from two separate experiments. The horizontal line corresponds to the period over which an increase occurred, and the circle in the line corresponds to the time at which ca. one-half the maximum increase occurred.

phenylmethylsulfonyl fluoride), and dialyzed against 1 liter of buffer A twice for 4 h at 4°C. The dialyzed crude extract was frozen and stored at −80°C until use.

To obtain DNA polymerases I and II, crude extracts were applied to a DEAE-cellulose column (8 by 10 cm) equilibrated with buffer A. The column was washed with 10 ml of buffer A, and the DNA polymerase activities were eluted by 90 ml of 0 to 0.4 M NaCl linear gradient in buffer A.

DNA synthesis in vitro. DNA polymerase activity in crude extract and DEAE-celluose fractions was assayed in the reaction mixture (0.1 ml) containing 35 mM Tris-hydrochloride (pH 8.0), 10 mM $MgCl_2$, 2 mM dithiothreitol, 50 μM 4× dNTPs ([α-^{32}P]dTTP was used as a radioactive nucleotide; specific activity was ca. 200 cpm/pmol), 100 μg of bovine serum albumin per ml, 10% glycerol, 5 μg of activated calf thymus DNA (4), and the extract. After incubation of the reaction mixture at 30°C for 30 min, 2 ml of 5% trichloroacetic acid–1% sodium PP; was added and acid-insoluble radio-

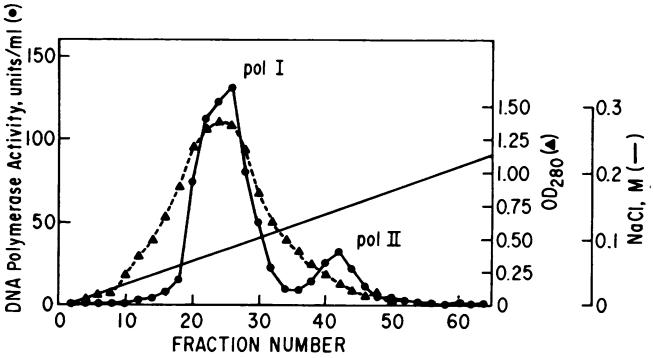

FIG. 3. *S. cerevisiae* polymerases I and II are separated by DEAE-cellulose column chromatography. The DNA polymerase activities were assayed in mitotic cells as described in the text, and the optical density at 280 nm (OD_{280}) corresponding mainly to protein was measured.

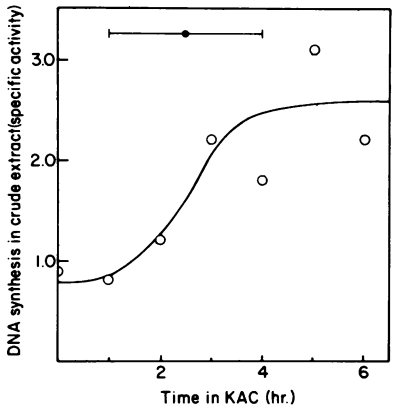

FIG. 4. Total DNA-synthesizing activity in crude extract during meiosis. Total DNA synthesis activities in crude extracts were measured as described in the text.

active materials were collected on a GF/C glass filter as described previously (1).

DNases. For obtaining crude extracts for DNase assays, cells were suspended in 10 mM Tris-hydrochloride–1 mM EDTA (pH 7.5) containing 1 mM phenylmethylsulfonyl fluoride and 1 μM pepstatin "A" (protease inhibitors). The cell suspensions were then disrupted with a French pressure cell (18,000 lb/in^2), and the resulting extracts were centrifuged at 4,100 × *g* for 20 min to remove cell debris.

DNase activities were examined by the procedure described by Mills and Fraser (16), except that ^{3}H-labeled heat-denatured *Escherichia coli* single-stranded or native DNA was used as substrate. The amount of DNA present in each assay mixture was 12 μg. One unit of activity was defined as the amount of DNase activity that converted 1 μg of DNA to acid-soluble form in 30 min.

For measurement of the presence of nuclease activity that showed cross-reactivity with a rabbit antiserum raised against a *Neurospora crassa* nuclease (6, 7), the antiserum was added to the *S. cerevisiae* crude extracts and the mixture was incubated overnight at 4°C. Protein A-Sepharose 4B (Pharmacia Fine Chemicals) was added and the

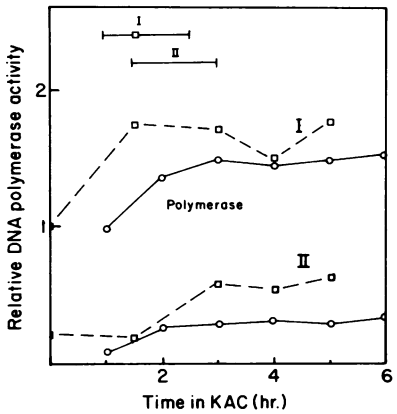

FIG. 5. Polymerase I and II activities from cells at various stages of meiosis. Polymerases were separated on DEAE-cellulose columns, and activities were measured as described in the text. Shown are results from two separate experiments. The horizontal line corresponds to the period over which an increase occurred, and the circle in the line corresponds to the time at which ca. one-half the maximum increase occurred.

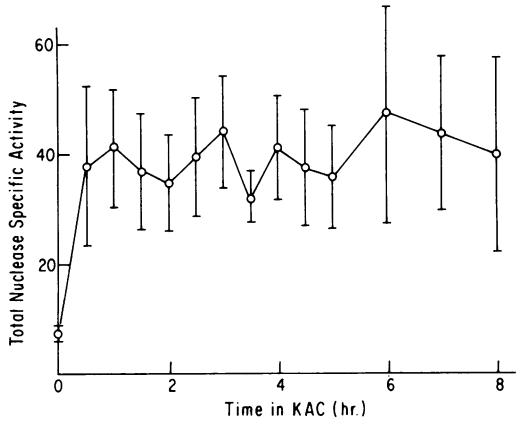

FIG. 6. Total Mg^{2+}-dependent alkaline DNase activity in extracts of meiotic cells. Shown is the total Mg^{2+}-dependent nuclease activity at pH 8.0. Nuclease activity is expressed as the amount of DNA rendered perchloric acid soluble per milligram of cellular protein added. One unit of nuclease activity is the amount of activity necessary to render 1 μg of *E. coli* DNA acid soluble in 30 min.

mixture was incubated on ice for 30 min; it was then centrifuged in a Beckman microfuge for 5 min at maximum speed, and the supernatant was assayed for nuclease activity as described above.

RESULTS

Cells can be switched from logarithmic growth in SPS to meiotic development by collecting and suspending them in 1% KAC plus the required amino acids. The meiotic products, asci, began to appear by ca. 7 h (t = 7.0) after cells were introduced into KAC, and by t = 9 h over 80% of the cells had sporulated (Fig. 1). Typically, we found that ca. 95% of the cells were capable of sporulating by t = 24 h. Since this and related strains (23) are efficient in sporulation and relatively synchronous, it is expected that the timing of genetic and biochemical events and the relative increases can be studied accurately. Following the procedure of Sherman and Roman (28), and as we have reported earlier (22), cells were transferred back to selective medium for monitoring of commitment to intragenic recombination when cells were plated to selective medium. Such commitment began at ca. t = 2.5 h. During the subsequent 3 h, the level of commitment to recombination increased by a factor of 100. In these and related experiments (22), we have found that commitments to the appearance of both *can*^{r} colonies and red (*ade2*) colonies follow the commitment to intragenic recombination. These colonies can arise by haploidization or, alternatively, by a commitment to reciprocal recombination and, therefore, homozygosity in diploid cells. Since these markers are not tightly linked to their respective centromeres, it is expected that a significant number of colonies at early times would exhibit *can*^{r} or *ade2* (red) phenotypes or both if there were coincident commitments to homozygosity along with *HIS1* intragenic recombination.

We have found the kinetics of commitment to intragenic recombination and the appearance of asci to be highly reproducible from culture to culture. Results shown in Fig. 1 are for one culture; essentially the same results are found from parallel cultures derived from different single-colony isolates when experiments are done on the same or different days (data not shown). Thus, we used these parameters to verify that the kinetics of meiosis between different experiments were the same and to verify that there were no differences between different flasks in the same experiment.

As shown previously and in Fig. 2, the commitment to recombination follows the meiotic round of DNA synthesis. The DNA was measured directly by using diphenylamine assays. Label uptake of ^3H into DNA from [^3H]uracil added at t = 1.5 was also measured. There appeared to be small increase in DNA when the cells were transferred to the meiotic medium, which presumably was due to the completion of mitotic DNA synthesis. Between 1.5 and 2 h, the meiotic round of synthesis began, and the amount of DNA in the culture nearly doubled in the following 2 to 2.5 h. No such increase was found in an isolate homozygous for the mating type locus (αα). Since the growth cycle for this strain was 2 h, these results suggest that there was little additional asynchrony introduced into the culture. It appears that for the loci examined in this strain, commitment to recombination follows the initiation of DNA synthesis by ca. 0.5 to 1 h if comparisons are made on the basis of the time at which a one-half maximum increase in total DNA is detected.

These findings on synchrony of various events suggest that it is possible in this system to relate enzymatic changes in the population specifically to various landmarks of meiosis. Total DNA synthesis activity in crude extracts and the DNA polymerase I and II activities have been assayed in meiotic cells throughout the periods of meiotic DNA synthesis and heightened genetic activity. The DNA polymerase activity in crude extract of mitotic cells can be readily separated on DEAE-cellulose into polymerase I and II activities (4; Fig. 3). In agreement with previous reports, the polymerase II activity in mitotic cells (t = 0) is ca. one-fifth that of polymerase I. Because of the small amount of polymerase II activity, observations must be considered as being somewhat qualitative rather than quantitative. No other polymerase activities were observed under these conditions of growth in the SPS medium or during meiosis. Total DNA-synthesizing activity in crude extracts (Fig. 4) began to increase at ca. t = 1.5 h to a final level two times greater than that in mitotic cells. Much of this activity was due to polymerase I. An increase in polymerase I activity of 50 to 75% was detected by t = 2.0 h, and the activity remained constant over the rest of the meiotic period examined (Fig. 5). The polymerase II activity also increased, although the increase occurred slightly later and remained approximately constant during the rest of meiosis. There were no apparent

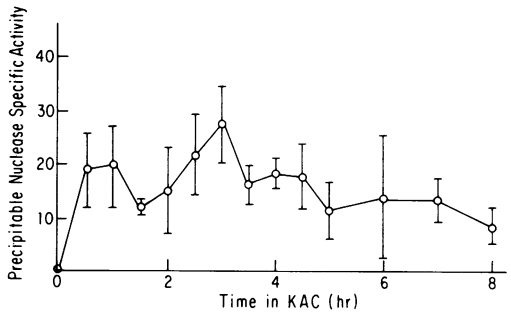

FIG. 7. Antibody-precipitable Mg^{2+}-dependent alkaline DNase activity in extracts of meiotic cells. Crude extracts were incubated with antiserum against a *N. crassa* single-strand endo-exonuclease (7). Shown is the activity in the crude extract that is precipitated by the antibody plus protein A-Sepharose 4B. Nuclease activity is expressed as the amount of DNA rendered perchloric acid soluble per milligram of cellular protein added.

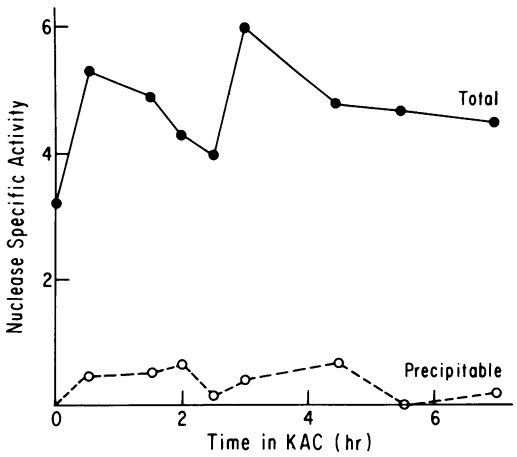

FIG. 8. Total alkaline nuclease activity and antibody-precipitable nuclease activity in extracts of an αα strain. The αα strain was grown to a titer of 4×10^7 per ml in SPS and suspended in KAC. No increase in histidine prototrophs is seen after 7 h, and no ascospores are detected after 24 h (data not shown).

changes in either polymerase since the positions of elution remained constant and no new polymerase activities were detected. The increase in polymerase activity is specific to cells undergoing meiosis since αα diploids exhibit no such increase (data not shown).

DNases would be expected to have an important role in the events of meiotic recombination and possibly in processing DNA during replication. We have centered our studies on two groups of nucleases in crude extracts that were active under alkaline (pH 8) conditions: Mg^{2+} independent and Mg^{2+} dependent. Under the present conditions of late logarithmic growth in SPS, we found very low activities of Mg^{2+}-independent or -dependent alkaline nuclease activity in crude extracts. During meiosis, no Mg^{2+}-independent nuclease activity was detected. On the other hand, Mg^{2+}-dependent nuclease activity increased considerably in cells transferred to KAC and remained high throughout meiosis (Fig. 6).

The Mg^{2+}-dependent nucleases can be separated further into two categories: (i) activity precipitated by an antibody (precipitable nuclease) raised against a single-strand DNase (6, 7) from *N. crassa* (Fig. 7), and (ii) activity that is not precipitated. The former activity has been identified as being deficient in *rad52* mutant strains of *S. cerevisiae* both mitotically and meiotically (5; manuscript in preparation). The level of precipitable nuclease also rose quickly upon switching to KAC; the highest level of activity occurred at ca. 3 h (Fig. 7). The level of precipitable nuclease activity declined slightly after 3 h, but substantial activity was detectable throughout meiosis (Fig. 7). No increase in either total nuclease or antibody-precipitable nuclease was seen when an isogenic αα strain was switched from SPS to KAC (Fig. 8). The αα strain shows no commitment to intragenic recombination, no increase in DNA polymerase I or II, and no sporulation (data not shown). This is consonant with the requirement for heterozygosity at the mating type locus for meiosis to occur (25).

DISCUSSION

The meiotic cycle of *S. cerevisiae* is characterized by high levels of genetic recombination. Assuming a total genetic map length of 5,000 centimorgans (17), there are ca. 100 recombinational events per meiotic cell. This is much more than occurs spontaneously in mitotic cells, although DNA-damaging agents can raise the levels considerably (21). Several enzymatic steps presumably must take place to bring about these recombinational events. As discussed elsewhere (10), chromosomal pairing, DNA interactions, exchange, synthesis, and possibly DNA degradation and resolution of recombinant structures are required for recombination. Although it has been possible to examine DNA biochemical processes during meiosis in some systems, it has not been possible to specifically relate biochemical changes to genetic events. In this report, we have been able to determine the temporal relationships between various biochemical activities, recombination, and meiotic development. Figure 9 relates these events and activities to the time of incubation in meiotic medium. The apparent order of events is an increase in DNA polymerases I and II, DNA synthesis, commitment to recombination, and sporulation. The total Mg^{2+}-dependent alkaline nucleases increase substantially within 0.5 h after cells are placed in KAC and are maintained at high levels.

Because of the high levels of recombination, we expected that some related enzymes might appear in unusually high amounts as compared with mitotic cells. However, this need not be so since there may be sufficient activity from available enzymes to bring about the recombinational events in the time involved. In this case, all that might be needed is a triggering event such as pairing. There was no evidence for unique DNA polymerases, unusually large increases of polymerase I or II, or DNA-synthesizing ability in extracts during the various stages of meiosis. Their increases corresponded in time to the meiotic round of DNA synthesis and commitment to recombination; however, their roles in these

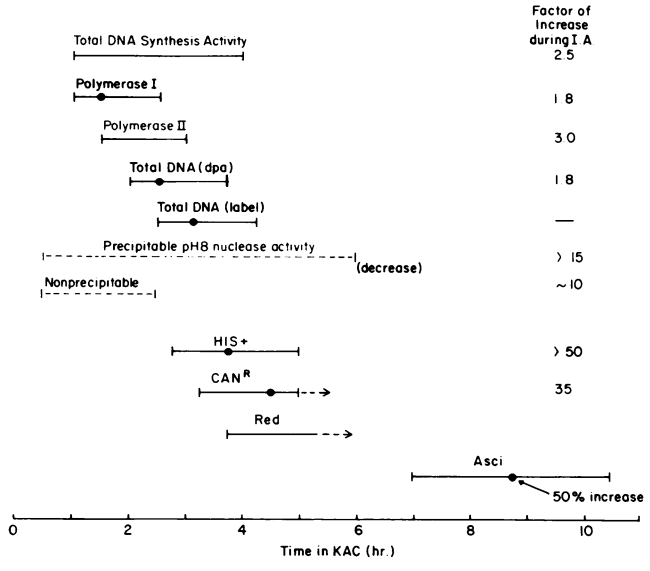

FIG. 9. Timing of events during meiosis. This figure summarizes the results from Fig. 1 through 7. The horizontal lines show the time during which increases in the endpoint were detected. The circles correspond to the time at which approximately half the maximum increase was observed. The numbers to the right represent the factor of increase observed during meiosis. Because the mitotic levels were so low for the nucleases, the factor of increase was not accurately determined. There was an initial increase in *can*^{r} and red colonies that was presumably due to commitment to recombination; the increase at later times was associated with haploidization.

processes are not established. The increase of ca. 1.5- to 3-fold in specific activity may simply correspond to the increase in DNA content during this time. Although polymerase II has repair-type activities associated with it (4), the role of either polymerase in mitotically growing cells has not been determined. These results are in marked contrast to those obtained with the basidiomycete *Coprinus* (27). In the vegetative phase of this organism, nearly all of the polymerase activity is accounted for by polymerase A, whereas in meiotic S phase, polymerase A and B are nearly equal and at pachytene, the polymerase B accounts for over 95% of the activity. It is not obvious why the two organisms should differ in their two polymerases with regard to meiosis. Since DNA synthesis and recombination are closely related temporally in *S. cerevisiae*, it may utilize available enzymes, whereas in *Coprinus* there is a significant delay between synthesis and recombination. Another possibility is that the polymerase B of *Coprinus* is required for recombinational functions that polymerase I and II of *S. cerevisiae* can accomplish jointly.

Although large variations in the DNA polymerases were not observed in these studies, the pattern of alkaline DNase activities changes considerably between mitotically growing and meiotically developing cells. Although Mg^{2+}-independent nucleases account for as much as 30% of the alkaline nuclease activity in logarithmically growing cells in a growth medium containing a fermentable carbon source (1% yeast extract, 2% peptone, 2% dextrose), no activity is detected in cells growing in SPS that contains acetate rather than dextrose or in cells incubating in meiotic medium (data not shown). These results suggest that this category of nucleases does not play an active role in meiotic processes.

Contrary to these results, the Mg^{2+}-dependent alkaline nuclease activity increases considerably during meiosis, suggesting important functions for at least some of the nucleases in this category. Of special interest is the precipitable nuclease activity controlled by the *RAD52* gene, particularly since this gene is essential during meiosis. Meiotic recombination is abolished in *rad52* mutants, and cells die as they enter into the meiotic cycle. Evidence to date suggests that *RAD52* functions during recombinational events (M. A. Resnich, T. Chow, J. Nitiss, and J. Game, Cold Spring Harbor Symp. Quant. Biol., in press). In *S. cerevisiae* as in most eucaryotic organisms, chromosome pairing is mediated through synaptonemal complex structures (3). Although the initiating events in recombination are not well characterized, we have demonstrated (23) that single-strand interruptions accumulate in the *rad52* strains during or shortly after premeiotic DNA synthesis. These are thought to be related to the recombination process, possibly as sites of initiation that are not resolved in the *rad52* mutants. We had proposed that the nuclease activity under the control of the *RAD52* gene might be involved in processing these single-strand interruptions so that its absence in *rad52* mutants resulted in accumulation of the interruptions (5; Resnick et al., in press). It is not surprising, therefore, that this nuclease activity increases considerably during the meiotic cycle in RAD^+ strains.

The antibody-precipitable nuclease activity contributes as much as 50% to the large increase in alkaline DNase activity during meiosis beginning before the time of DNA synthesis and extending into the period of commitment to recombination. Although the function of this enzyme activity in recombination and repair is not known, the large factor of increase further supports its role in meiotic events. The change in media by itself is not sufficient to cause an increase in the nuclease, since an isogenic $\alpha\alpha$ strain exhibits no change when switched to KAC. Thus, we suggest that the increase is due to the meiotic process. The substantial increase in activity of other Mg^{2+}-dependent alkaline nucleases (nonprecipitable) at comparable times suggests a role for these nucleases in meiosis as well. It is reasonable to expect that more than one DNase may be involved in the process of meiotic recombination.

Investigations are under way to determine the specific role of the precipitable nuclease in meiosis and to identify the other alkaline DNases that increase during meiosis. The system we have described here will enable us to characterize other biochemical activities that are necessary for meiotic recombination.

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