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DNA Polymerase I Is Required for Premeiotic DNA Replication and Sporulation but Not for X-Ray Repair in *Saccharomyces cerevisiae*

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We have used a set of seven temperature-sensitive mutants in the DNA polymerase I gene of *Saccharomyces cerevisiae* to investigate the role of DNA polymerase I in various aspects of DNA synthesis in vivo. Previously, we showed that DNA polymerase I is required for mitotic DNA replication. Here we extend our studies to several stages of meiosis and repair of X-ray-induced damage. We find that sporulation is blocked in all of the DNA polymerase temperature-sensitive mutants and that premeiotic DNA replication does not occur. Commitment to meiotic recombination is only 2% of wild-type levels. Thus, DNA polymerase I is essential for these steps. However, repair of X-ray-induced single-strand breaks is not defective in the DNA polymerase temperature-sensitive mutants, and DNA polymerase I is therefore not essential for repair of such lesions. These results suggest that DNA polymerase II or III or both, the two other nuclear yeast DNA polymerases for which roles have not yet been established, carry out repair in the absence of DNA polymerase I, but that DNA polymerase II and III cannot compensate for loss of DNA polymerase I in meiotic replication and recombination. These results do not, however, rule out essential roles for DNA polymerase II or III or both in addition to that for DNA polymerase I.

Eucaryotic cells contain multiple nuclear DNA polymerases: α, β, δ_1, and δ_2 (8, 42). Although these enzymes were first identified and purified many years ago, the precise role of each polymerase in various aspects of DNA metabolism, such as replication, repair, and recombination, is controversial (8). Studies of this question have been limited due to the absence of mutants totally defective in any of the polymerases. *Saccharomyces cerevisiae* contain analogs of both DNA polymerases α and the δ-type enzymes and offer an ideal genetic system in which to explore the cellular functions of these polymerases (8).

A comparison of the metazoan and yeast polymerases is shown in Table 1. It is now clear that DNA polymerase I of *S. cerevisiae* is the counterpart of DNA polymerase α, the conserved subunit structure being the key reason for concluding this (8). DNA polymerase α has been implicated in replication because it is inhibited by aphidicolin, an inhibitor of cellular replication, and because a mouse carcinoma cell line containing a thermolabile DNA polymerase α is also temperature sensitive for DNA replication (16). We have confirmed the requirement for DNA polymerase α in chromosomal replication by cloning the yeast *POL1* gene, showing the gene disruptions are lethal, and deriving conditional lethal mutants which show gross defects in DNA synthesis (5, 22).

Recent evidence suggests that DNA polymerase α may not be the sole polymerase involved in eucaryotic DNA replication, however. At least one form of DNA polymerase δ may also be required. The evidence, while compelling, is indirect in that it relies on the use of differential inhibitors in permeabilized cells and on the requirement for an accessory subunit, rather than the catalytic subunit of DNA polymerase δ, in simian virus 40 DNA replication in vitro (4, 14, 32, 41). One major difference between these δ-type polymerases and DNA polymerase α is the existence of an apparently intrinsic 3′-to-5′ "proofreading" exonuclease in each of the δ-like enzymes. The putative yeast analogs of DNA polymerase δ are DNA polymerases II (9, 43, 44) and III (7), though neither DNA polymerase δ nor DNA polymerase II or III has been well enough characterized yet to warrant a definitive statement on the extent to which the species correspond (7, 13, 25, 42; M. E. Budd and J. L. Campbell, unpublished data). Thus, a critical question in eucaryotic DNA replication today is to what extent each of these polymerases participates in mitotic replication, meiotic replication, repair, and recombination; yeast cells should be useful in answering this question.

Recently, we described a set of seven temperature-sensitive mutants of DNA polymerase I (5). The first set of experiments in the present paper shows that only one of these mutants, *pol1-17*, appears to have the stringency required to test critically the in vivo functions of DNA polymerase I. As reported previously, the *pol1-17* mutants show first-cycle arrest; cells do not divide after shift to the nonpermissive temperature, consistent with a defect in the elongation stage of replication (5). More than 99% of the cells arrest with the dumbbell morphology of a *cdc* mutant with a defect in S phase. In vivo measurements of residual DNA synthesis show a greater defect in this mutant than in any of the other six studied (this work). Finally, biochemical analysis has failed to detect any DNA polymerase I activity during extensive fractionation of extracts of *pol1-17* cells, even when extracts are assayed at the permissive temperature (6). The remainder of the experiments presented below analyze the effect of the *pol1-17* mutations on meiotic DNA replication, meiotic recombination, and repair of damaged DNA. Also, the site of each of the seven mutations within the *POL1* gene is determined by marker rescue, showing that these processes are affected by a conserved region of the DNA polymerase molecule.

MATERIALS AND METHODS

Strains. The genotypes of the strains are listed in Table 2. Strains SS111 and SS111-*pol1*(Ts) have been described previously (5). For the meiotic experiments, *POL1*/*POL1*, *POL1*/*pol1*(Ts), and *pol1*(Ts)/*pol1*(Ts) diploids were con-

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TABLE 1. Eucaryotic DNA polymerases

Polymerase	Template with short single-stranded DNA regions, activated DNA (%)	Template with long single-stranded DNA regions, poly(dA) · oligo(dT) (%)	I_{50} (μg/ml)[a]		M_r (catalytic subunit)	Exonuclease
			Aphidirolin	BuPhdGTP		
Metazoan						
α[b]	100	<20		0.3	180	Cryptic 3′-5′
δ_1[c]	100	100[d]	<25	<70[c]	?	3′-5′
δ_2[c]	<3	100	<25	<70[c]	?	3′-5′
Yeast						
I	100	<20	4[e]	0.3[e]	180	None
II	<5	100	6[e]	2.4[e]	180	3′-5′
III[e,f]	100	8–17	0.6	80	>116	3′-5′, 5′-3′

[a] I_{50}, 50% inhibitory dose.
[b] See Campbell (8) for referenced data.
[c] See Crute et al. (13) for references on δ_1 and δ_2.
[d] In the presence of proliferating-cell nuclear antigen.
[e] Reference 7.
[f] Reference 1.

structed from strains derived from SK-1, a genetic background that allows high-frequency, synchronous sporulation at 36°C. The SK-1-derived haploid strains 488, TC102-D, and g833-1B were obtained from Merle Hoekstra, Scripps Clinic and Research Foundation, La Jolla, Calif. Using the gene replacement technique of Scherer and Davis (35), mutant alleles of *pol1*(Ts) were introduced into both strains 488 and TC102-D. The plasmid used in the gene replacement construction had a 9-kilobase *pol1*(Ts) fragment inserted in the *Bam*HI site of YIp5 (5). The *pol1*(Ts) transductants of TC102-D were then crossed into g833-1D to obtain *MAT***a** *pol1*(Ts) *his1-1* segregants. *MAT***a** *pol1*(Ts) *his1-1* segregants were then crossed into strain 488 to yield the heterozygous diploid X488-2. The isogenic *pol1*(Ts) homozygous diploid was constructed by crossing the same *MAT***a** *pol1*(Ts) *his1-1* segregant into the *pol1-17* transductant of strain 488. These two strains are also heteroallelic at the *his1* locus. *pol1-11*, *pol1-12*, *pol1-13*, *pol1-14*, and *pol1-17* diploid derivatives were all constructed similarly.

spo13-1/spo13-1 POL1/POL1 and *spo13-1/spo13-1 pol1*(Ts)/*pol1*(Ts) strains were constructed by the same gene replacement technique. *pol1*(Ts) mutant alleles were introduced into strain K399-ID (obtained from Rochelle Esposito, University of Chicago, Chicago, Ill.) by transformation with YIp5 plasmids containing mutant polymerase alleles. To construct *MAT***a** *pol1*(Ts) *spo13-1* haploids, the mating type of the *pol1*(Ts) transductants of K399-1D was switched, using plasmid pGAL-HO. K399-1D and the *pol1*(Ts) derivatives were transformed with pGAL-HO (gift of Ira Herskowitz, University of California, San Francisco) which has the *URA3* selectable marker and expresses the HO endonuclease under the control of the *GAL1* promoter (R. Jensen, Ph.D. thesis, University of Oregon, Eugene, 1983). To switch the mating type of the strains, the transformants were grown in 1% yeast extract–2% peptone–2% lactate to a cell density of 3×10^5 per ml. Then galactose was added to a concentration of 2%. Cells were incubated with galactose for 40 min and plated onto YPD medium, and colonies were checked for expression of the *MAT*α allele. The resulting *MAT***a** *POL1 spo13-1* and *MAT***a** *pol1*(Ts) *spo13-1* strains were mated with the respective *MAT*α *POL1 spo13-1* and *MAT*α *pol1*(Ts) *spo13-1* strains to create *POL1* and *pol1*(Ts) isogenic *spo13-1* diploids. The strains are listed in Table 2.

TABLE 2. Strains used in this study

Strain[a]	Genotype
SS111	*MAT***a** *ura3-52 trp1-289 ade2-101 tyr1 gal2 can1*
SS111-pol1-1i	*MAT***a** *pol1-1i ura3-52 trp1-289 ade2-101 tyr1 gal2 can1*
488	*MAT*α *trp1 leu2 ura3-52 his1-7 can1*
TC102-2D	*MAT***a** *leu2 ura3-52 can1*
g833-1D	*MAT*α *leu2 his1-1 trp2 can1*
K399-1D	*MAT*α *ade2 his6 leu2 lys1 aro7 trp1 ura3 spo13-1*
X488-1	*MAT***a**/*MAT*α *POL1/POL1 leu2/leu2/his1-1/his1-7 trp1/TRP1 trp2/TRP2 ura3-52/URA3 can1/can1*
X488-2	*MAT***a**/*MAT*α *POL1/pol1-17 leu2/leu2 his1-1/his1-7 trp1/TRP1 trp2/TRP2 ura3-52/URA3 can1/can1*
X488-11-1	*MAT***a**/*MAT*α *pol1-11/pol1-11 leu2/leu2 his1-1/his1-7 trp1/TRP1 trp2/TRP2 ura3-52/ura3-52 can1/can1*
X488-12-1	*MAT***a**/*MAT*α *pol1-12/pol1-12 leu2/leu2 his1-1/his1-7 trp1/TRP1 trp2/TRP2 ura3-52/ura3-52 can1/can1*
X488-13-1	*MAT***a**/*MAT*α *pol1-13/pol1-13 leu2/leu2 his1-1/his1-7 trp1/TRP1 trp2/TRP2 ura3-52/URA3 can1/can1*
X488-14-1	*MAT***a**/*MAT*α *pol1-14/pol1-14 leu2/leu2 his1-1/his1-7 trp1/TRP1 trp2/TRP2 ura3-52/ura3-52*
X488-17-1	*MAT***a**/*MAT*α *pol1-17/pol1-17 leu2/leu2 his1-1/his1-7 trp1/TRP1 trp2/TRP2 ura3-52/URA3*
X488-17-2	*MAT***a**/*MAT*α *pol1-17/pol1-17 leu2/leu2 his1-1/his1-7 trp1/TRP1 trp2/TRP2 ura3-52/URA3*
XK399-1	*MAT***a**/*MAT*α *ade2/ade2 his6/his6 leu2/leu2 lys1/lys1 aro7/aro7 trp1/trp1 ura3/ura3 spo13-1/spo13-1*
XK399-1i	*MAT***a**/*MAT*α *pol1-1i/pol1-1i ade2/ade2 his6/his6 leu2/leu2 lys1/lys1 aro7/aro7 trp1/trp1 spo13-1/spo13-1*

[a] The sources of the strains not constructed in this study are given in Materials and Methods.

Velocity gradient sedimentation. DNA synthesis was analyzed by a modification of the methods of Resnick et al. (33). Cells were grown overnight in 1% yeast extract–2% peptone–2% glucose and suspended at a concentration of 10^7 cells per ml, and [^{3}H]uracil (25 to 50 μCi/ml) was added to the culture. After a 3-h incubation at 23 or 36°C, cells were harvested, washed with 0.1 M Tris hydrochloride (pH 9.0)–0.01 M EDTA, incubated for 10 min in 2% β-mercaptoethanol–0.1 M Tris hydrochloride (pH 9.0)–0.01 M EDTA, and

then washed with 0.01 M KPO_4–0.01 M EDTA (pH 7.0). Cells were suspended in 0.2 ml of 0.01 M KPO_4–0.01 M EDTA and transferred to nitrocellulose tubes, and 20 μl of a 10-mg/ml concentration of zymolyase and 30 μl of 10% Nonidet P-40 were added. After 10 min, complete lysis was observed. Since lysis and spheroplasting occur simultaneously, all cells are lysed gently and consequently none of the DNA is sheared by experimental handling. After a 10-min incubation at 36°C, a 15 to 30% sucrose gradient containing 0.3 M NaOH, 0.7 M $NaCl_2$, and 0.03 M EDTA was pumped onto the bottom of the tube. Centrifugation was at 12,000 rpm (SW50.1 rotor) for 16 h. Fractions were collected, neutralized with 1 M tris hydrochloride (pH 7.5), and precipitated with acid, and radioactivity was determined. [^{3}H]RNA was degraded to acid-soluble counts during the alkaline centrifugation. Bacteriophage T4 [^{3}H]DNA was used as a molecular weight marker.

The number-average molecular weight (M_n) was calculated with the formula (21)

$$M_n = \frac{\sum C_i}{\sum_i \frac{C_i}{M_i}}$$

where C_i is activity in the ith fraction. M_i is computed by using the relation (20)

$$\frac{M_i}{M_{T4}} = \left(\frac{d_i}{d_{T4}}\right)^{2.63}$$

where d_i is the distance of the ith fraction and d_{T4} is the distance sedimented by T4 DNA. M_{T4} (single stranded) = 6×10^7 (23).

X-ray source and irradiation of cells. The X-ray source was a Machlett OEG 60 tube with a beryllium window operated at 50 kV and 20 mA with unfiltered X rays. The dosimetry was determined by the method of Fricke and Morse (19) and Fregene (18). Cells were grown to 2×10^7 cells per ml in the presence of [^{3}H]uracil, harvested onto nitrocellulose filters, and irradiated with 30 krads. They were immediately placed in YPD medium at 23 and 36°C and incubated for 0 to 6 h. Cells were then harvested and analyzed for extent of damage and repair.

Fluorescence-activated flow cytometry. Total cellular DNA was stained by the Feulgen procedure with acriflavine (11). Cells were harvested and fixed in 70% ethanol. Cells were then suspended in 4 N HCl for 20 min to hydrolyze cellular RNA. The acid hydrolysis removes most histones and depurinates the DNA, thus exposing the aldehyde of the deoxyribose sugar. Then the cells are stained with acriflavine for 20 min. The fluorescence of each cell was measured with a flow cytometer by exciting with 200 mW of laser light at 488 nm and collecting fluorescence signals through a 530-nm high-pass filter. Forward-angle light scatter, which is proportional to cell size, is used to gate for acquisition of fluorescence signals.

Meiosis. Cells were grown in YPD medium to 1.5×10^7 cells per ml and then suspended in presporulation medium [1% potassium acetate buffered with phthalate, pH 5.5, 0.17% yeast nitrogen base without amino acids and $(NH_4)_2SO_4$, 0.5% $(NH_4)_2SO_4$, 0.5% yeast extract, 1% peptone, 1% potassium acetate, pH 5.5] at a cell density of 2×10^6 per ml. Cells were grown for 12 h, washed in H_2O, washed in sporulation medium (1% potassium acetate, supplemented with histidine and leucine at 4 mg/ml), and suspended in sporulation medium at a cell density of 2×10^7 per ml. At 2-h time intervals, samples were fixed in 70% ethanol for analysis of DNA content and sporulation frequency or plated onto histidine-deficient plates for analysis of recombination.

RESULTS

Demonstration that the *pol1-17* mutant is completely deficient in DNA polymerase I activity in vivo. Previous experiments to test the levels of residual DNA synthesis were carried out by placing log-phase *pol1*(Ts) mutants at 36°C and following incorporation of [^{3}H]uracil into the DNA. After 3 h of labeling at 36°C, cells were harvested and incubated overnight in 1 M NaOH to hydrolyze RNA, followed by assaying acid-precipitable [^{3}H]DNA (5). Depending on the mutant allele, the extent of DNA synthesis at 36°C was 25 to 50% that of wild-type. *pol1-17* and *pol1-14* strains appeared to exhibit the greatest defects in DNA synthesis at 36°C. However, the amount of residual synthesis was surprising in view of the fact that five of the seven mutants, *pol1-11*, *pol1-12*, *pol1-13*, *pol1-14*, and *pol1-17*, show first-cycle arrest as dumbbells (5). We wished to clarify whether the observed levels reflected synthesis by other polymerases or problems in the methodology. One problem with quantitating in vivo DNA synthesis in *S. cerevisiae* is that the organism lacks thymidine kinase activity, making it necessary to use uracil rather than thymidine as a radioactive precursor. Uracil labels RNA 20-fold more efficiently than DNA; consequently, if the alkaline hydrolysis of RNA into acid-soluble material is not efficient, aberrantly high levels of DNA synthesis can be recorded. To evaluate the residual synthesis more carefully, products of mitotic DNA synthesis were investigated by neutral (not shown) and alkaline velocity sedimentation.

For analysis of DNA by sucrose gradients, cells were grown to approximately 10^7 cells per ml at 23°C [^{3}H]uracil was added, and cells were grown for 3 h (equivalent to about two generations) at either 23 or 36°C. Cells were converted to spheroplasts on the bottom of centrifuge tubes, followed by the addition of a 15 to 30% alkaline sucrose gradient. Figure 1 illustrates an alkaline sucrose gradient of a *POL1* strain and the isogenic *pol1-17* mutant at 23 and 36°C. The position of T4 DNA (6×10^7 daltons) is indicated by an arrow. A high-molecular-weight chromosomal DNA peak is seen in the gradient of the *POL1* cells at 23°C (Fig. 1A) and 36°C (Fig. 1C) and in the gradient of *pol1-17* cells at 23°C (Fig. 1B). In contrast, no high-molecular-weight chromosomal DNA was observed in the gradient of the *pol1-17* cells labeled at 36°C (Fig. 1D). The calculated M_n for *POL1* DNA at 36°C was 4.1×10^8, and DNA was observed in the gradient up to a molecular weight of about 10^9. These values are consistent with the sedimentation results of Petes and Fangman (29), who observed an M_n of 6.2×10^8 for double-stranded yeast chromosomal DNA.

The low-molecular-weight DNA seen in all of the gradients, and most visible in the *pol1-17* gradient, is most likely due to mitochondrial DNA synthesis. It has an M_n of about 2×10^7, which approximates the molecular weight of single-stranded mitochondrial DNA, about 3.1×10^7 or 0.45 times the molecular weight of T4 DNA (23). Furthermore, the peak represents about 5% of the total DNA synthesis observed in SS111 *POL1* at 36°C, consistent with the amount of mitochondrial DNA synthesis observed by Petes and Fangman (29).

To verify the conclusions derived from the data of Fig. 1, the *pol1-17* allele was introduced into strain 167 *tmp1*

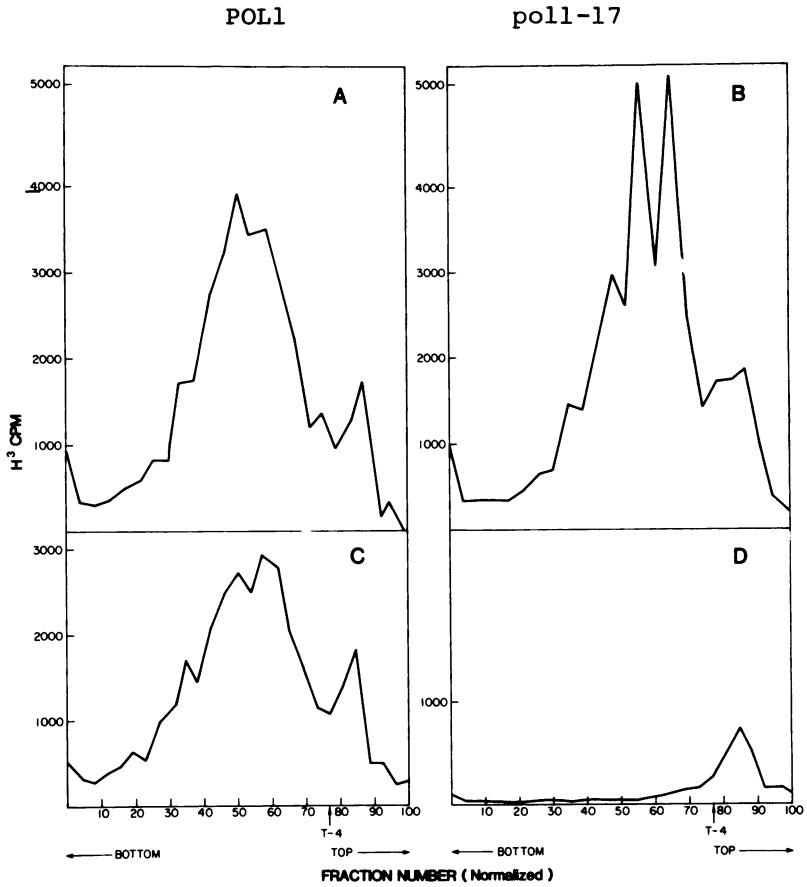

FIG. 1. Alkaline sucrose gradient analysis of residual DNA synthesis in *POL1* and *poll-17* cells. Methods are described in Materials and Methods. The arrow points to the sedimentation position of the T4 phage DNA. *POL1* (A) or *poll-17* (B) cells were incubated with [^{3}H]uracil (25 μCi/ml) at 23°C for 3 h and harvested. (C) *POL1* cells were incubated with [^{3}H]uracil (25 μCi/ml) at 36°C for 3 h and harvested. (D) *poll-17* cells were incubated with [^{3}H]uracil (50 μCi/ml) at 36°C for 3 h and harvested.

tut1:pJM81TK$^+$. Plasmid pJM81 carries the herpes simplex virus thymidine kinase gene, and in the presence of the *tmp1* and *tut1* mutations, expression of the thymidine kinase gene allows direct labeling of DNA with [^{3}H]thymidine (37). An alkaline sucrose gradient of DNA synthesized in strain *poll-17 tmp1 tut1*:pJM81 during a 3-h [^{3}H]thymidine-labeling period at 36°C produced a pattern identical to that of Fig. 1D, in which <10% of the label sedimented as chromosomal length DNA. A neutral sucrose gradient (data not shown) of *poll-17 tmp1 tut1*:pJM81 strains labeled with [^{3}H]thymidine at 36°C also showed 91% of the label at the top of the gradient. This further suggested that the peak at the top of the alkaline sucrose gradient of *poll-17* cells grown at 36°C (Fig. 1D) represents largely mitochondrial DNA synthesis.

In summary, inactivation of DNA polymerase I in this mutant immediately blocked chromosomal DNA synthesis, showing that DNA polymerase I is necessary for the elongation stage of replication in yeast cells. Furthermore, the very low level of residual synthesis suggested that, if DNA polymerase II or III plays a role in elongation, this role is not independent of DNA polymerase I. Finally, the severe defect in this mutant suggested that it is suitable for critically evaluating the role of polymerase I in other aspects of DNA metabolism.

Analysis of DNA synthesis by flow cytometry. An alternative method of determining DNA synthesis that does not involve radioactive pulse-labeling is flow cytometry. For flow cytometry, cultures were grown to logarithmic phase, harvested, suspended in fresh YPD medium, placed at 36°C for a specific time period, harvested, and fixed in 70% ethanol. The cells were subjected to acid hydrolysis, which removes RNA and depurinates DNA. Total cellular DNA was stained by the Feulgen procedure with acriflavine, resulting in single-cell fluorescence which is proportional to DNA content per cell (12). The distribution of single-cell fluorescence was measured by flow cytometry. In wild-type proliferating cells, two peaks of fluorescence, corresponding to unreplicated (haploid, 1 N) and replicated (diploid, 2 N) cells, are seen (Fig. 2A). The 1 N peak contains cells in G1; the region between the peaks contains the cells in S phase, and the 2 N peak represents G2 + M cells. When the temperature is shifted to 36°C in a DNA replication mutant, the G2 + M cells that have completed DNA replication should divide and join the 1 N population. If the mutant is deficient in initiation of DNA replication, the G1 population should not enter S phase. In an elongation mutant, cells in S phase at the shiftup should arrest there. Thus, the G1 peak should increase and there should be a second peak or shoulder of cells in S phase. Ideally, for initiation and elongation mutants, one would expect a pattern that shows only the 1 N peak, with a small percentage of the cells remaining in S phase for elongation mutants.

Flow cytometric results divided the mutants into three separate groups (Fig. 2). First, a 36°C, mutant *poll-17* shows

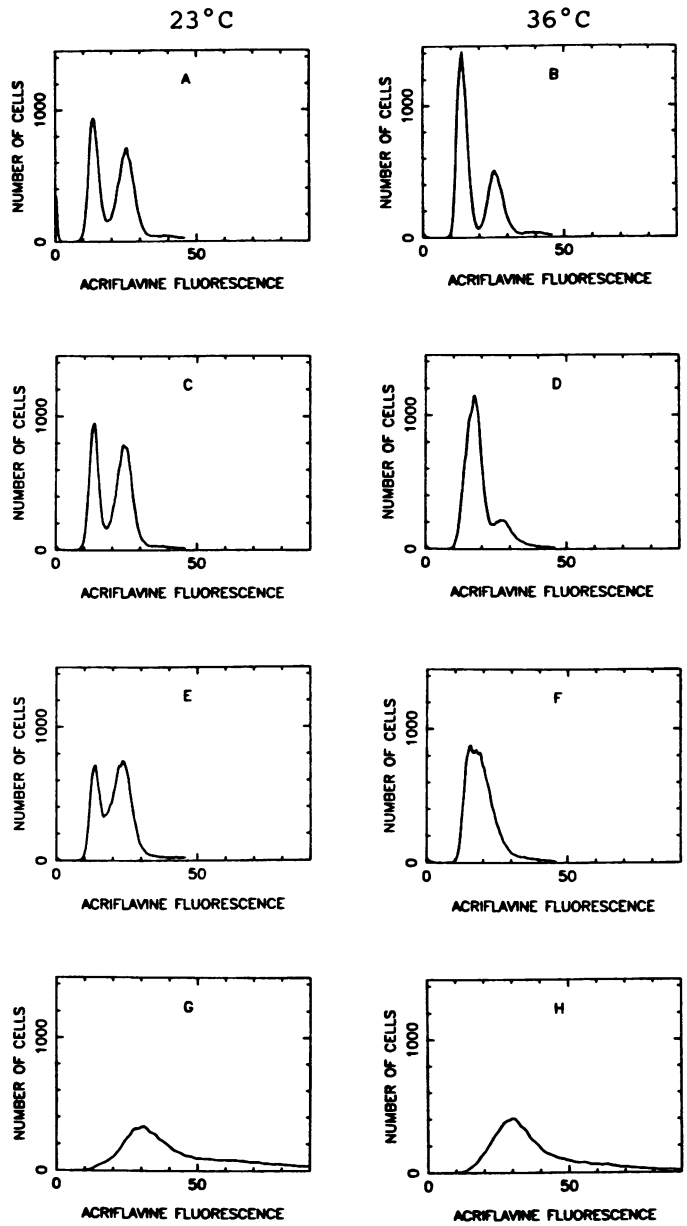

FIG. 2. Flow cytometry analysis of *POL1* and *pol1*(Ts) mutants during vegetative growth. Flow cytometry analysis of fixed cells is described in Materials and Methods. The vertical axis labels the number of cells, and the horizontal axis labels the amount of acriflavine fluorescence at 530 nm. (A) *POL1* cells were grown at 23°C and then fixed in 70% ethanol. (B) *POL1* cells were grown at 23°C and then at 36°C for 3 h followed by fixing in 70% ethanol. (C) *pol1-17* cells were grown at 23°C and then fixed. (D) *pol1-17* cells were grown at 23°C, incubated at 36°C for 3 h, and then fixed. (E) *pol1-11* cells were grown at 23°C and then fixed. (F) *pol1-11* cells were grown at 23°C, incubated at 36°C for 3 h, and then fixed. (G) *pol1-14* cells were grown at 23°C and then fixed. (H) *pol1-14* cells were grown at 23°C, incubated at 36°C for 3 h, and then fixed.

a pattern expected for an elongation mutant (Fig. 2C and D). The fluorescence of the 1 N peak increases slightly, and the peak remains symmetrical, with an increase in fluorescence due to cells stuck in S or to mitochondrial DNA synthesis. The mutant does not reenter G2 phase, since there is no reappearance of the G2 peak in the histograms. The small reproducible shoulder at the G2 position may indicate that some cells with nearly completely replicated chromosomes cannot divide at the nonpermissive temperature.

The second class contains mutants *pol1-11*, *pol1-12*, and *pol1-13* (Fig. 2E and F). The data from *pol1-11* show an initial reduction in the G2 peak, followed by gradual rightward spreading of the G1 peak, presumably indicating residual DNA synthesis. There was no reappearance of the G2 peak, indicating little conversion to 2 N chromosomes. These mutants are probably leaky, although they may be more temperature sensitive for assembly into a replication complex or holoenzyme than for elongation. This class of mutant may behave analogously to a mouse DNA polymerase α temperature-sensitive mutant (16).

The third class of mutant is *pol1-14*. This mutant showed an unexpected pattern of DNA fluorescence (Fig. 2G and H). Radioactive pulse-labeling had previously shown that, at both 23 and 36°C, the *pol1-14* mutants are defective in DNA

TABLE 3. Meiosis of *pol1*(Ts)

Genotype	Strain	% Sporulation	
		30°C	35°C
POL1/POL1	X488-1	61	50
pol1-11/pol1-11	X488-11-1	10	<0.1
pol1-12/pol1-12	X488-12-1	12	<0.1
pol1-13/pol1-13	X488-13-1	49	0.1
pol1-14/pol1-14	X488-14-1	5	<0.1
pol1-17/pol1-17	X488-17-1	12	<0.1
POL1	XK399-1	26	5
pol1-11 spo13	XK399-11	5	<0.1
pol1-12 spo13	XK399-12	<0.1	<0.1
pol1-13 spo13	XK399-13	10	<0.1
pol1-14 spo13	XK399-14	<0.1	<0.1
pol1-17 spo13	XK399-17	44	<0.1

synthesis compared to wild type (5). Nevertheless, flow cytometry indicates that there is a higher DNA content in these originally haploid isolates than in wild-type cells. At the permissive temperature, *pol1-14* cells contained approximately 2 N chromosomes as represented by fluorescence. It is possible that these mutants have either undergone endoreduplication or nondisjunction to increase their ploidy, an interesting phenotype, but not one previously predicted for a DNA polymerasae mutant. The fluorescence distribution also suggests the presence of cells with DNA content of >2 N. There does not appear to be any change in the DNA histograms after a shift to the nonpermissive temperature, which correlates with the observation by in vivo labeling that DNA replication is severely defective in this mutant at 23°C (5).

Meiosis: sporulation and DNA synthesis. Studies of *cdc* mutants show that meiotic and mitotic DNA replication share some functions but not others. For instance, *CDC8* (thymidylate kinase) is required for both types of replication. *CDC7* function, however, is required for mitotic replication but not for premeiotic DNA replication, despite the fact that *CDC7* is required for meiotic recombination (17). It was therefore of interest to extend our studies of the role of DNA polymerase I in mitosis and to ask whether POL1 was required for any stage of meiosis. Since some steps in sporulation are inherently temperature sensitive in many yeast strains, the *pol1-11*, *pol1-12*, *pol1-13*, *pol1-14*, and *pol1-17* alleles were introduced into a strain derived from SK-1, using the gene replacement technique of Scherer and Davis (35) as described in Materials and Methods. The SK-1 background allows for high-frequency, synchronous sporulation at temperatures up to 36°C. Care was taken that all strains used in the meiotic DNA replication and recombination studies were isogenic.

First, the ability of the *pol1*(Ts) mutants to sporulate was tested at 23 and 36°C. The diploids were grown on YPD media, replica plated onto presporulation media, and then replica plated onto sporulation media after 16 h. After 24 h, *POL1* diploids and *pol1*(Ts) diploids were examined for asci. The data are shown in Table 3. None of the five *pol1*(Ts) strains tested showed any visible asci at 35°C except X488-13-1. Strain X488-13 showed about 0.1% asci relative to wild type.

pol1-17 diploids were chosen for further experiments on premeiotic DNA replication and recombination. Log-phase cells were harvested and suspended in presporulation medium, grown for 12 h, and then transferred to sporulation medium as described in Materials and Methods. After 24 h at 23°C, both X488-2 *POL1* and X488-17-2 *pol1-17* had sporulated with a frequency of 95%. At 36°C, X488-2 *POL1* sporulated with a frequency of 50%, while X488-17-2 *pol1-17* sporulated with a frequency of <0.1%.

Analysis of premeiotic DNA synthesis. Previous studies of premeiotic DNA synthesis in yeasts have used radioactive labeling or fluorescence measurements of DNA content. They have been difficult because cells become impermeable to precursors during meiosis and because extensive RNA degradation presumably changes the uracil pool sizes. Flow cytometry has not been used to study yeast meiosis previously, and it seemed a method that might overcome some of the difficulties encountered in the past. In our studies, change in DNA content during meiosis was first assayed by measuring acriflavine fluorescence with a flow cytometer. Strains X488-2 *POL1* and X488-17-2 *pol1-17* exhibited little difference in their fluorescence pattern at 0 h (Fig. 3A and B). After 24 h in sporulation media, however, the fluorescence patterns of X488-2 *POL1* cells at 23 and 36°C and X488-17-2 *pol1-17* cells at 23°C changed dramatically (Fig. 3C, D, and E). After sporulation most of the cells fluoresce with an intensity about 50- to 100-fold greater than that of mitotic cells. Interestingly, however, when X488-17-2 *pol1-17* was sporulated at 36°C, only a small fraction of cells moved into the high fluorescence peak (Fig. 3F). To test whether the appearance of the high fluorescence peak requires DNA replication, strains X488-2 *POL1* and X488-17-2 *pol1-17* were sporulated at 23°C in the presence of hydroxyurea, which has been shown in several studies to be an inhibitor of elongation during premeiotic DNA synthesis (36, 39). Figure 3G and H illustrates that the appearance of the high fluorescence peak is significantly reduced in the presence of hydroxyurea. Thus, the appearance of the high fluorescence peak correlates with meiotic DNA replication. That *pol1-17* displayed a phenotype at the restrictive temperature that was very similar to the phenotype of cells blocked in DNA synthesis by hydroxyurea suggests that meiotic DNA synthesis is severely defective in *pol1-17* strains at 36°C.

To verify the latter conclusion, DNA content was determined during meiosis by the diaminobenzoic acid procedure of Hopper and Hall (21a). The third column of Table 4 shows the amount of DNA synthesis at 24 h relative to time in $POL1^+$/*pol1-17* and *pol1-17/pol1-17* homozygous diploids. The $POL1^+$ strain carries out premeiotic synthesis at both 23 and 36°C, while the *pol1-17* mutant carries out premeiotic DNA synthesis at 23°C but not at 36°C. The result shows directly that DNA polymerase I is required for premeiotic DNA replication.

Meiotic recombination. The effect of the *pol1-17* allele on meiotic recombination was studied with an interrupted meiosis experiment (38). The "isogenic" strains X488-2 *POL1* and X488-17-2 *pol1-17* contain *his1-1/his1-7* heteroalleles, allowing one to follow meiotic gene conversion by removing cells from sporulation medium, plating onto histidine-deficient medium, and assaying for HIS^+ prototrophs. After incubating these *POL1* and *pol1-17* diploids for 12 h at 23°C in sporulation medium, the frequency of HIS^+ prototrophs increased about 200-fold in both strains. Sporulating the *POL1* strain at 36°C also resulted in a 200-fold increase in HIS^+ prototrophs after 24 h. However, after sporulating the *pol1-17* strain at 36°C for 24 h, the number of HIS^+ prototrophs increased by only fourfold (Table 5). Consequently, meiotic recombination is severely defective in the *pol1-17* mutant. The residual meiotic gene conversion occurring in

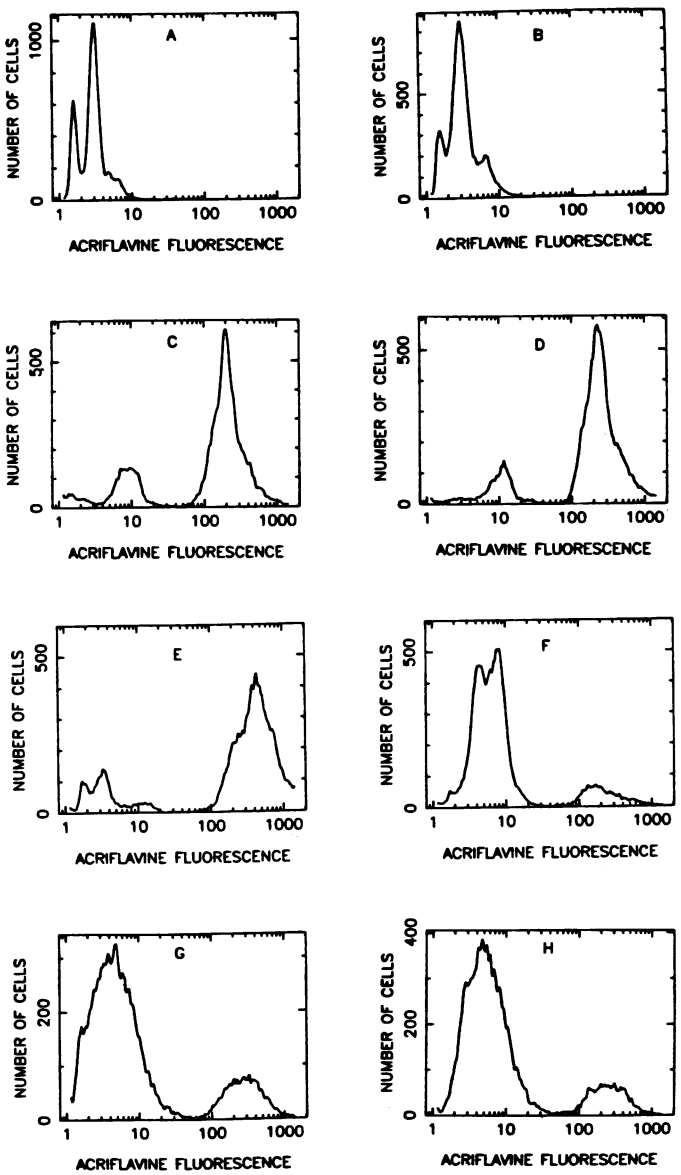

FIG. 3. Flow cytometry analysis of DNA content of *POL1* and *pol1-17* diploids during meiosis. In all samples, log-phase cells were harvested and grown in presporulation medium for 12 h at 23°C. The horizontal axis labels the intensity of acriflavine fluorescence (logarithmic scale), and the vertical axis measures cell number. (A) *POL1* and (B) *pol1-17* cells, fixed immediately after transfer to sporulation medium. (C) *POL1* and (D) *pol1-17* cells, incubated in sporulation medium at 23°C for 24 h. (E) *POL1* and (F) *pol1-17* cells, incubated in sporulation medium at 36°C for 24 h. (G) *POL1* and (H) *pol1-17* cells, incubated in sporulation medium with hydroxyurea (100 mM) at 23°C for 24 h. The *POL1* strain was X488-2 and the *pol1-17* strain was X488-17-2.

TABLE 4. Meiotic DNA replication in *POL1/pol1-17* and *pol1-17/pol1-17* strains

Strain	Temp (°C)	DNA synthesis[a]
X488-2 (*POL1*)	23	1.79
X488-2 (*POL1*)	36	1.72
X488-17-2 (*pol1-17*)	23	1.58
X488-17-2 (*pol1-17*)	36	1.08

[a] Expressed as DNA content at 24 h relative to that at 0 h, the time at which cells were placed in sporulation medium. Samples were measured in duplicate.

the *pol1-17* diploid is probably a result of slight leakiness of the mutant at 36°C.

One further experiment was carried out to see whether the requirement for DNA polymerase I could be bypassed in meiosis. The *spo13-1* mutation blocks the first meiotic reductional division, leading to the formation of two diploid spores after the second meiotic division. Thus, haploid *spo13-1* cells which express both **a** and α mating-type information are capable of sporulation and production of two haploid spores. Diploid *spo13-1* strains were constructed by the technique of Scherer and Davis (35) by gene replacement of mutant alleles of *pol1* into a *spo13-1* strain as described in Materials and Methods. Diploids were sporulated on plates at 35°C (Table 3). The conclusion is that the *pol1* mutants

TABLE 5. Recombination at 36°C of *Pol1/pol1-17* and *pol1-17/pol1-17* diploids

Time in sporulation medium (h)	*POL1/pol1-17* HIS$^+$ (10^5)	*pol1-17/pol1-17* HIS$^+$ (10^5)
0	2.9	9
3	2.4	8.8
6	37	8
12	500	16
24	600	40

remain defective in sporulation in the *spo13-1* background. Thus, in the absence of the first reductional division, there is still a requirement for DNA polymerase I in meiosis.

In summary, this set of experiments show that, as in the mitotic cell division cycle, neither DNA polymerase II nor DNA polymerase III can compensate for the absence of DNA polymerase I in meiotic gene conversion or completion of meiosis.

Role of DNA polymerase I in repair. Originally, experiments showing that aphidicolin inhibited repair of X-ray-induced single-strand breaks in mammalian cells were interpreted as showing that DNA polymerase α was the DNA repair polymerase. However, aphidicolin also inhibits DNA polymerase δ, and recent studies that used other approaches have suggested that DNA polymerase δ, and perhaps β, are involved in repair. To help resolve the question of which polymerase(s) carries out repair, we have studied the ability of the *pol1-17* mutant to repair X-ray-induced damage. Since the *POL1* gene is essential, survival at the nonpermissive temperature could not be used to assess sensitivity of the *pol1-17* mutant to radiation. Instead, actual physical repair of breaks had to be assessed. To establish conditions for introducing X-ray damage, irradiated cells were first analyzed qualitatively by orthogonal field gel electrophoresis. The strains used were isogenic *POL1* and *pol1-17* diploids. Cells were grown to log phase, harvested onto nitrocellulose filters, and irradiated with 30 krads. They were subsequently incubated in YPD medium at 36°C for 3 or 6 h. The unirradiated cells gave rise to bands corresponding to the individual chromosomes. Irradiation led to a smear of low-molecular-weight DNA. Incubation following irradiation restored the original pattern, although considerable smearing remaining after a 6-h incubation. Surprisingly, repair appeared to occur to the same extent in both *POL1* and *pol1-17* (data not shown).

To confirm and quantitate this, repair was analyzed by alkaline velocity sedimentation. Alkaline sucrose gradients measure primarily single-strand break repair because the ratio of X-ray-induced single-strand breaks to double-strand breaks is approximately 10:1 (3, 10, 34). Cells were grown for 17 h in the presence of [^{3}H]uracil to label the DNA and irradiated as in the experiments just described. They were then incubated for 0, 1, or 2 h to allow repair, harvested, converted to spheroplasts, and then lysed on the bottom of a nitrocellulose tube. A 15 to 30% alkaline sucrose gradient was then pumped into the tube. The sedimentation properties of the DNA are illustrated in Fig. 4. The 30-krad X-ray dose reduced the chromosomal DNA of the *POL1* strain

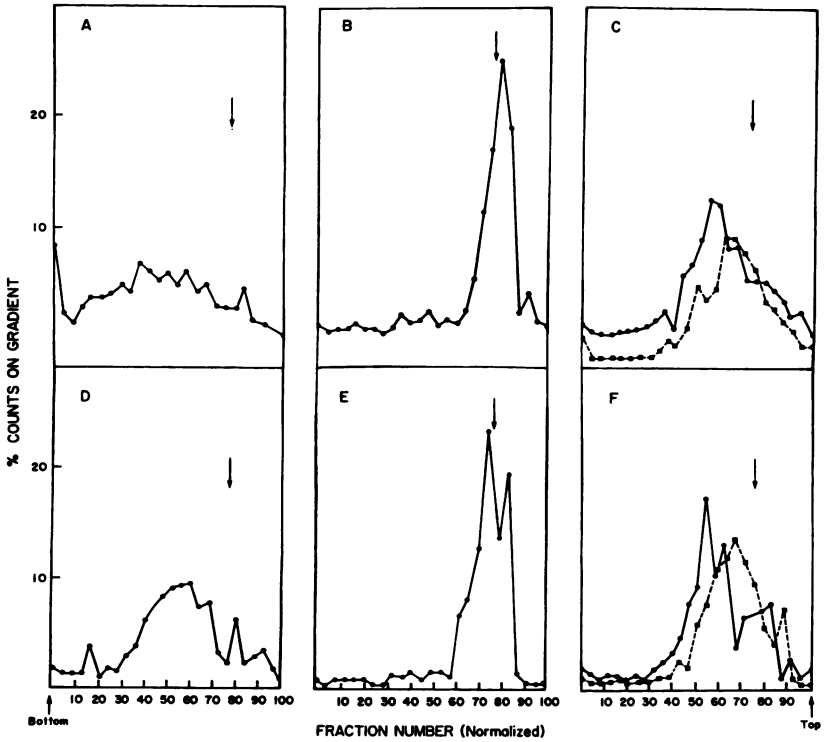

FIG. 4. Alkaline sucrose gradient analysis of X-ray-induced single-strand break repair in SS111 *POL1* and SS111 *pol1-17* strains. The experimental details are described in Materials and Methods. Cells were labeled with [^{3}H]uracil (10 μCi/ml) for 16 h and then harvested. The arrow points to the sedimentation position of the T4 DNA. (A) Untreated SS111 cells. (B) SS111 cells irradiated with 30 krads. (C) SS111 cells irradiated with 30 krads and then incubated at 36°C for 1 (▮) or for 2 (●) h. (D) Untreated SS111 *pol1-17* cells. (E) SS111 *pol1-17* cells irradiated with 30 krads. (F) SS111 *pol1-17* cells irradiated with 30 krads and then incubated at 36°C for 1 (▮) or for 2 (●) h.

from an M_n of 4.7×10^8 to 3.5×10^7, resulting in about 11 breaks per chromosome or an efficiency of 7.8×10^{-10} breaks per krad per dalton. After 1 h at 36°C, the chromosomal peak shifted to a higher molecular weight corresponding to M_n of 1.1×10^8, and after 2 h at 36°C a further increase in the molecular weight was observed (M_n, 1.7×10^8). After 2 h, about 1.8 breaks per chromosome remained. Thus, about 80% of the single-strand breaks are repaired during the incubation. A calculation of M_n for the control peak (no X rays) of *pol1-17* is about 3.3×10^8, which is somewhat less than that for the corresponding *POL*$^+$ strain. The 30-krad dose reduced the M_n to approximately 5.5×10^7, causing five breaks per chromosome and yielding an efficiency of 5.1×10^{-10} breaks per krad per dalton. A 1-h incubation at 36°C resulted in a significant increase in the chromosomal size corresponding to an M_n of 1.4×10^8. After 2 h, a further increase in the chromosomal size occurred with a calculated M_n of 1.6×10^8. Since about one break per chromosome remained compared with the unirradiated control, about 80% of the original X-ray-induced single-strand breaks were repaired. There does not appear to be any significant difference in repair between *POL1* and *pol1-17* strains at 36°C.

Although survival could not be measured at the nonpermissive temperature, growth does occur at the "semipermissive" temperature of 30°C, allowing us to assess survival after radiation. Mutants in *cdc8*, for instance, show decreased susceptibility to UV-induced mutagenesis at a semipermissive temperature (31). Isogenic diploid strains were used for X-ray survival studies of *POL1* and *pol1-17* at 30°C. Strains SS111 *POL1* and SS111 *pol1-17* were diploidized by transforming each strain with a plasmid carrying the HO gene under *GAL* control. HO endonuclease was induced by addition of galactose followed by mating. After irradiation, cells were incubated at 30°C, a semirestrictive temperature for *pol1-17* cells. There was no significant difference in survival between *POL1* and *pol1-17* diploids after a dose of 36 krads (20% for *POL1* versus 19% for *pol1-17*). (It is also worth noting that the *pol1-17* mutant survived the double-strand break introduced by HO endonuclease during diploid formation.)

The UV survival of isogenic *POL1* and *pol1-17* haploids was also determined. After UV irradiation at 1.5 J/m^2, cells were incubated at 30°C for 24 h followed by incubation at 23°C. The survival of SS111 *POL1* was 5.4% and that of the *pol1-17* strain, SS111 *pol1-17*, was 4.0%, again not significantly different from that for wild type.

The above results, in particular those carried out at 36°C, suggest that the *pol1-17* mutation does not result in a repair deficiency. Perhaps DNA polymerase II or III or both either fulfill the repair function normally in yeast cells or compensate for loss of polymerase I. A trivial explanation of our results is, of course, that so little DNA synthesis is needed for repair of the lesions we have studied that the mutant is leaky enough to provide function.

Marker rescue. Since the seven *pol1*(Ts) mutants appear to differ on the physiological level (5) (Fig. 2), we attempted to determine differences on the molecular level. Marker rescue of the *pol1*(Ts) mutations was carried out with cloned fragments of the *POL1* gene. Five restriction fragments spanning the *POL1* gene (see Fig. 5) were cloned into the polylinker of plasmid pSEY8, a yeast vector containing 2μm sequences, and the URA3 selectable marker (gift of Scott Emr, California Institute of Technology, Pasadena). The resulting plasmids were designated pSEY8A-E. Each of the *pol1*(Ts) mutants was transformed with the pSEY8A-E set of plasmids, and transformants were selected by growth on uracil-deficient plates. Transformants were replica plated onto YPD media, and the replicas were placed at 36°C. Appearance of papillation indicated recombination and hence marker rescue (Fig. 5). No papillation occurred in any *pol1*(Ts) mutant when cells were transformed with the parental vector pSEY8. Only one fragment, in fact, pSEY8-C, carrying the internal *Eco*RI-*Xho*I fragment of *POL1*, allowed papillation, suggesting that all of the mutations were located in this portion of the gene. Furthermore, the finding that the insert in plasmid pSEY8-D, which overlaps the insert in pSEY8-C, does not allow papillation in any mutant placed all of the *pol1*(Ts) mutations in the 1,096-base-pair *Eco*RI-*Hin*dIII fragment of *POL1* (see reference 30 for coordinates).

The *Eco*RI-*Xho*I (fragment C) region was further subdivided and subcloned as shown in Fig. 5, and marker rescue was carried out as above. The *pol1-17* mutation was mapped to the *Hpa*I-*Hin*dIII fragment. The *pol1-13* and *pol1-14* mutations were mapped to the *Cla*I-*Eco*RI fragment. The *pol1-15* mutation was mapped to the *Eco*RI-*Sal*I fragment. The fine-structure mapping of the *pol1-11* and *pol1-12* mutations were more ambiguous than that of the *pol1-13*, *pol1-14*, *pol1-15*, and *pol1-17* mutations. Both plasmids pSEY8-*Cla*I-*Hin*dIII and pSEY8-*Cla*I-*Eco*RI, which carry restriction fragments that are adjacent but nonoverlapping in the *POL1* gene, rescued the *pol1-11* and *pol1-12* strains. *pol1-11* strains that were rescued with the *Eco*RI-*Cla*I fragment, however, grew into normal sized colonies, while strains rescued with the *Cla*I-*Hin*dIII fragment gave rise to very small colonies. It thus appears that the temperature-sensitive phenotype in the *pol1-12* and *pol1-11* mutants results from changes in both the *Eco*RI-*Cla*I and *Cla*I-*Hin*dIII regions of the *POL1* gene. In the *pol1-11* strain, mutations in the *Eco*RI-*Cla*I region contribute predominantly to the temperature-sensitive phenotype. In summary, the fine-structure map reveals that the 376-base-pair *Eco*RI-*Cla*I region appears to be the most susceptible target for creating *pol1*(Ts) mutants. However, the tightest mutation at 36°C, *pol1-17*, lies outside the *Eco*RI-*Cla*I fragment. This region coincides with the proposed catalytic core of the polymerase as determined by sequence comparisons among yeast DNA polymerase I, human DNA polymerase α, and the herpes simplex virus DNA polymerases (45). *pol1-11*, *pol1-13*, *pol1-14*, and *pol1-15* fall in region II, while *pol1-17* falls in region III (45).

DISCUSSION

This study addresses the question of whether DNA polymerase I, the yeast analog of metazoan DNA polymerase α, is required in all cellular processes requiring DNA synthesis such as meiotic DNA synthesis and repair of damaged DNA. The absence of a requirement for DNA polymerase I would suggest a role for DNA polymerase II or III independent of DNA polymerase I. This was examined by using a previously isolated DNA polymerase I mutant, *pol1-17*, which appears to be totally deficient in DNA polymerase I activity at 36°C both in vitro and in vivo (5, 6). The sucrose gradients of Fig. 1 show that the amount of chromosomal DNA synthesized in *pol1-17* cells at 36°C is about 1 to 2% that of *POL1* cells at 36°C. Therefore, DNA polymerase I is required for the elongation stage of replication in *S. cerevisiae* and neither DNA polymerase II nor III can compensate for the absence of DNA polymerase I in elongation. Although DNA polymerases II and III cannot continue synthesis when DNA polymerase I is inactivated in the *pol1-17* mutant, studies with inhibitors of DNA polymerases α and δ in mammalian cells suggest that DNA polymerase δ can func-

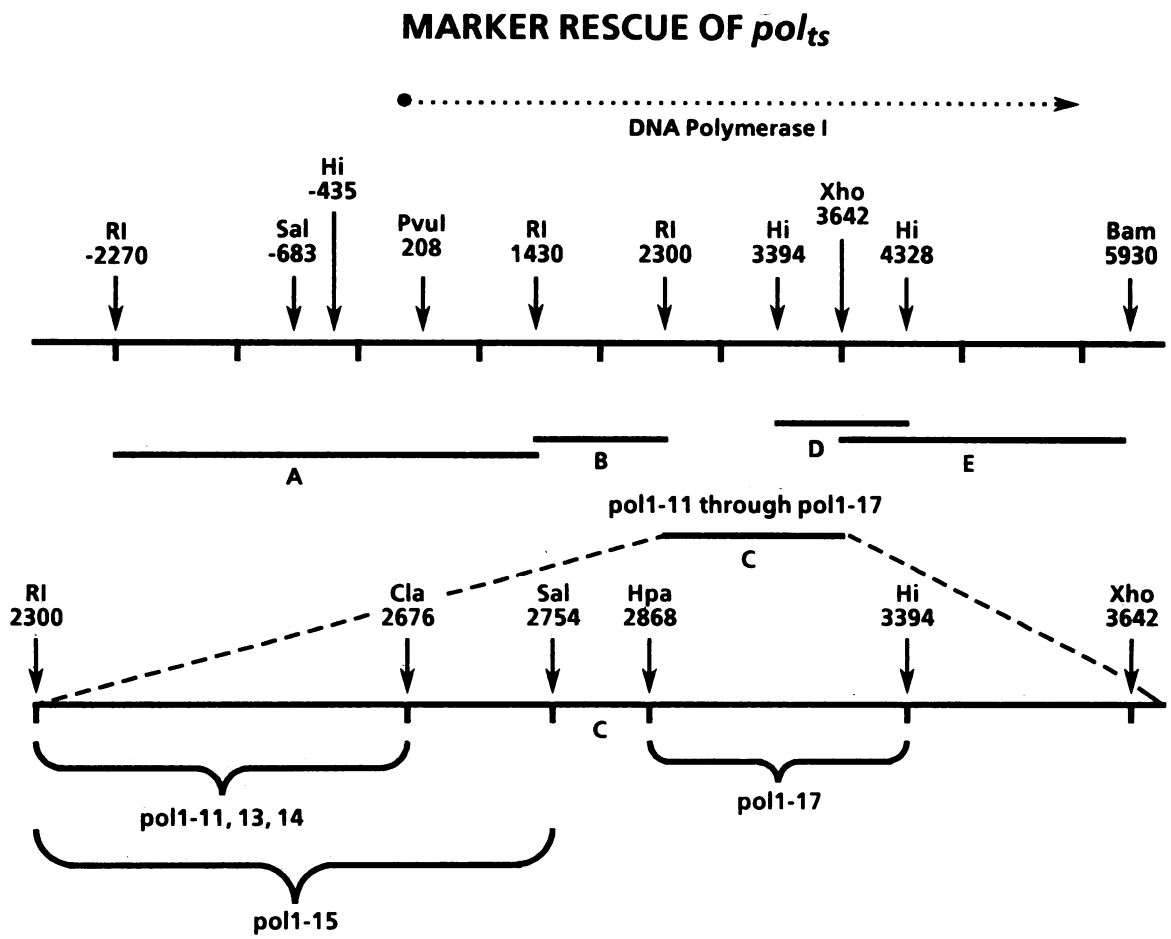

FIG. 5. Restriction map of plasmid pPOL1-1 and illustration of the restriction fragments of pPOL1-1 which were cloned into vector pSEY8. The *pol1*(Ts) mutants were transformed with the resulting plasmids for the marker rescue experiment. Only the 1.4-kilobase *Eco*RI-*Xho*I-C fragment recombined with the *pol1*(Ts) mutants to give a POL^+ phenotype. The C fragment was further subcloned into pSEY8 as described in the text, and marker rescue was carried out with plasmids with overlapping inserts. The fragments that marker rescued each *pol1*(Ts) mutant allele are shown. The dotted arrow is the coding sequence for polymerase I. Note that *pol1-15* has only been mapped to the *Eco*RI-*Sal*I fragment as described in the text.

tion independently of DNA polymerase α (14, 15, 27). The difference in amount of residual synthesis observed in *pol1* mutants and in the presence of DNA polymerase α inhibitors can best be explained by the fact that the inhibitors functionally inactivate DNA polymerase α, leaving the polypeptide intact. In the temperature-sensitive mutants we describe, on the other hand, thermal inactivation of the DNA polymerase I (α) polypeptide apparently leads to inactivation of the whole replication complex and neither polymerase II nor III can carry out measurable synthesis under these conditions.

It is important to emphasize that we do not wish to imply that the absence of DNA synthesis in the *pol1-17* mutant suggests that DNA polymerase α is the sole polymerase involved in yeast chromosomal replication. In fact, we would like to directly test the hypothesis, based on data implicated DNA polymerase δ in mammalian cell replication, that DNA polymerase II or III or both do participate. Our results are consistent with this idea if we propose that the nature of the *pol1-17* mutation leads to disruption of the replication fork, or at least to disruption of essential interactions between the two polymerases. The requirement for two polymerases at the replication fork is best rationalized in the "trombone" model, which suggests that interaction of two DNA polymerase molecules is required for orderly propagation of both the leading and the lagging strands (40). However, it is also possible that one polymerase is involved in initiation and another in elongation.

DNA polymerase I mutants that do not affect elongation would be important to isolate. Eki et al. (16), using a mouse carcinoma cell line with temperature-sensitive DNA polymerase α activity, found that elongation of preexisting replicons proceeding normally at the restrictive temperature, but that the frequency of replicon initiation decreased, suggesting a defect in assembly of replication complexes. Similar mutants have been obtained in yeast cells. They do not arrest in the first cell cycle after transfer to the restrictive temperature but, rather, synchronously in the second cell cycle (26). Since marker rescue presented in this paper shows that all of our mutations fall in the catalytic portion of the DNA polymerase I protein, other types of mutants should be sought by directing mutagenesis to the N- or C-terminal portion of the protein (26, 45).

The data of Table 4 show that DNA polymerase I is required for meiotic DNA synthesis. Thus, DNA polymerase II or III or both cannot carry out meiotic DNA replica-

tion in the absence of DNA polymerase I. When meiotic DNA replication was analyzed by using acriflavine fluorescence of DNA, we unexpectedly observed that fluorescence increased about 100-fold after sporulation. Nevertheless, the acriflavine fluorescence change appears to be dependent on premeiotic DNA replication because it is significantly inhibited in wild-type cells by hydroxyurea, an inhibitor of DNA replication. The appearance of the highly fluorescent peak is also significantly inhibited when *pol1-17* diploids are incubated in sporulation medium at 36°C. The acriflavine fluorescence data are consistent with the diaminobenzoic acid fluorescence data, showing that DNA polymerase I is required for meiotic DNA replication. Furthermore, the data of Table 5 show that DNA polymerase I is required for meiotic gene conversion. Thus, DNA polymerase I is required for sporulation and meiotic gene conversion, in addition to replication, and neither DNA polymerase II nor III can compensate.

Since flow cytometric studies of meiosis in yeasts have not been reported previously and since the stoichiometric relationship between acriflavine fluorescence and DNA content is apparently not maintained through meiosis, the experiment was repeated with a different DNA dye, olivomycin. Olivomycin, when complexed with magnesium ions, preferentially binds to G-C base pairs by a nonintercalating, noncovalent mechanism (12). Ethanol-fixed cells can be stained directly with olivomycin, and the sample can be measured while still in the dye solution (24). Mitotically dividing cells stained with olivomycin yielded DNA content histograms identical to those obtained with the acriflavine staining procedure. However, when strain X488-2 was sporulated at 23°C, the mean single-cell fluorescence decreased by about half, and the fluorescence distribution consisted of two peaks: a sharp peak at a fluorescence half as intense as the 2 N mitotic peak, and a broad distribution spanning fluorescence intensities between 2 N and 4 N. When X488-2 was sporulated at 36°C, the mean single-cell fluorescence approximately doubled, but the distribution was very broad, spanning intensities from 2 N to 8 N. Thus, neither acriflavine nor olivomycin fluorescence maintain a constant stoichiometric relationship with DNA content during sporulation, perhaps due to changes in chromatin structure. Further analysis of this interesting possibility will require a separate study.

Another important and surprising finding of our studies was the ability of the *pol1-17* mutant to carry out repair of X-ray-induced single-strand breaks. In spite of the severe replication defect in the *pol1-17* mutant and apparent tightness of the mutation, we were not able to show a defect in the mutation in repair of X-ray-induced damage at any temperature. Thus, DNA polymerase I seems to be dispensable for repair of strand breaks and presumably DNA polymerase II or III or both play the role of the repair polymerase. This is in keeping with recent drug inhibition studies in mammalian cells. Dresler and Kimbro (15) found that UV-induced repair synthesis is permeable human fibroblasts was more sensitive to ddTTP than was isolated DNA polymerase α. They also observed that repair synthesis in permeable fibroblasts was more resistant to N^2-(*p-n*-butylphenyl)-2′-deoxyguanosine 5′-triphosphate than isolated DNA polymerase α. Their results are consistent with a role for DNA polymerase δ in repair. Most recently, Nishida et al. (28) demonstrated that addition of DNA polymerase δ to depleted permeabilized cells restores the ability to carry out repair of UV-induced damage. Our results are also consistent with a yeast analog of DNA polymerase δ playing a role in repair, and it will be interesting to investigate mutants affecting these polymerases as they become available.

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LITERATURE CITED

1. **Bauer, G. A., H. M. Heller, and P. M. J. Burgers.** 1988. DNA polymerase III from *Saccharomyces cerevisiae*. J. Biol. Chem. **263**:917–924.
2. **Boeke, J. D., J. Truehart, G. Natsoulis, and G. Fink.** 1986. 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. Methods Enzymol. **154**:164–175.
3. **Bonura, T., C. D. Town, K. C. Smith, and H. S. Kaplan.** 1975. The influence of oxygen on the yield of DNA double-strand breaks in X-irradiated *Escherichia coli* K-12. Radiat. Res. **63**:567–577.
4. **Bravo, R., R. Frank, P. A. Blundell, and H. MacDonald-Bravo.** 1987. Cyclin/PCNA is the auxiliary protein of DNA polymerase-δ. Nature (London) **326**:515–517.
5. **Budd, M., and J. L. Campbell.** 1987. Temperature sensitive mutants of yeast DNA polymerase I. Proc. Natl. Acad. Sci. USA **84**:2838–2842.
6. **Budd, M., C. Gordon, K. Sitney, K. Sweder, and J. L. Campbell.** 1988. Yeast DNA polymerases and ARS binding proteins, p. 347–357. *In* Cancer cells: eukaryotic DNA replication, vol. 6. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **Burgers, P. M. J., and G. A. Bauer.** 1988. DNA polymerase III from *Saccharomyces cerevisiae*. J. Biol. Chem. **263**:925–930.
8. **Campbell, J. L.** 1986. Eukaryotic DNA replication. Annu. Rev. Biochem. **55**:733–771.
9. **Chang, L. M. S.** 1977. DNA polymerases from baker's yeast. J. Biol. Chem. **252**:1873–1880.
10. **Christensen, R. C., C. A. Tobias, and W. A. Taylor.** 1972. Heavy-ion-induced single- and double-strand breaks in ΦX-174 replicative form DNA. Int. J. Radiat. Biol. **22**:457–477.
11. **Crissman, H. A., P. F. Mullaney, and J. A. Steinkamp.** 1975. Methods and applications of flow systems for analysis and sorting of mammalian cells. Methods Cell Biol. **9**:179–246.
12. **Crissman, H. A., and J. A. Steinkamp.** 1987. Multivariate cell analysis: techniques for correlated measurements of DNA and other cellular constituents, p. 179. *In* J. W. Gray and Z. Darzynkiewics (ed.), Techniques in cell cycle analysis. Humana Press, Clifton, N.J.
13. **Crute, J. J., A. F. Wahl, and R. A. Bambara.** 1986. Purification and characterization of two new high molecular weight forms of DNA polymerase δ. Biochemistry **25**:26–36.
14. **Dresler, S. L., and M. G. Frattini.** 1986. DNA replication and UV-induced repair synthesis in human fibroblasts are much less sensitive than DNA polymerase α to inhibition by butylphenyldeoxyguanosine triphosphate. Nucleic Acids Res. **14**:7093–7102.
15. **Dresler, S. L., and K.-S. Kimbro.** 1987. 2′,3′-Dideoxythymidine 5′-triphosphate inhibition of DNA replication and ultraviolet-induced DNA repair synthesis in human cells, evidence for involvement of DNA polymerase δ. Biochemistry **24**:2664–2668.
16. **Eki, J., Y. Murakami, T. Enomoto, F. Hanaoka, and M. Yamada.** 1986. Characterization of DNA replication at a restrictive temperature in a mouse DNA temperature sensitive mutant ts FT20 strain, containing heat-labile DNA polymerase α activity. J. Biol. Chem. **261**:8888–8893.
17. **Esposito, R. E., and S. Klapholz.** 1982. Meiosis and ascospore development, p. 211–287. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces cerevisiae*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Fregene, A. U.** 1967. Calibration of the ferrous sulfate dosimeter

by ionometric and calorimetric methods for radiations of a wide range of energy. Radiat. Res. **31**:256–272.

19. **Fricke, H., and S. Morse.** 1927. The chemical action of roentgen rays on dilute ferrosulfate solutions as a measure of dose. Am. J. Roentgenol. **18**:426–430.
20. **Friefelder, D.** 1970. Molecular weights of coli phages and coli phage DNA. J. Mol. Biol. **54**:567–577.
21. **Green, M. H. L., A. S. C. Medcalf, C. F. Arlett, S. A. Hancourt, and A. R. Lehmann.** 1974. DNA strand breakage caused dichlorous, methyl methane sulfonate and iodoacetamide in *E. coli* and cultural Chinese hamster cells. Mutat. Res. **24**:365–378.

21a. **Hopper, A. K., and B. D. Hall.** 1975. Mating type and sporulation in yeast. I. Mutations which alter mating type control over sporulation. Genetics **80**:41–59.

22. **Johnson, L. M., M. Synder, L. M. S. Chang, R. W. Davis, and J. L. Campbell.** 1985. Isolation of the gene encoding yeast DNA polymerase I. Cell **43**:369–377.
23. **Kornberg, A.** 1980. DNA replication. W. H. Freeman Co., San Francisco.
24. **Kuchenbecker, D., and G. Braun.** 1985. Rapid yeast DNA staining method for flow cytometry. J. Basic Microbiol. **25**:509–512.
25. **Lee, M. Y. W. T., and N. L. Toomey.** 1987. Human placental DNA polymerase δ: identification of a 170-kilodalton polypeptide by activity staining and immunoblotting. Biochemistry **26**:1076–1085.
26. **Lucchini, G., C. Mazza, E. Scacheri, and P. Plevani.** 1988. Genetic mapping of the *Saccharomyces cerevisiae* DNA polymerase I gene and characterization of a *pol1* temperature-sensitive mutant altered in DNA primase-polymerase complex stability. Mol. Gen. Genet. **212**:459–465.
27. **Miller, M. R., R. G. Ulrich, T. S.-F. Wang, and D. Korn.** 1985. Monoclonal antibodies against human DNA polymerase α inhibit DNA replication in permeabilized human cells. J. Biol. Chem. **260**:134–138.
28. **Nishida, C., P. Reinhard, and S. Linn.** 1988. DNA repair synthesis in human fibroblasts requires DNA polymerase δ. J. Biol. Chem. **263**:501–510.
29. **Petes, T. D., and W. L. Fangman.** 1972. Sedimentation properties of yeast chromosomal DNA. Proc. Natl. Acad. Sci. USA **69**:1188–1191.
30. **Pizzagalli, A., P. Valsasnini, P. Plevani, and G. Lucchini.** 1988. DNA polymerase I gene of *Saccharomyces cerevisiae*: nucleotide sequence, mapping of a temperature-sensitive mutation, and protein homology with other DNA polymerases. Proc. Natl. Acad. Sci. USA **85**:3772–3776.
31. **Prakash, L. D., D. Hinkle, and S. Prakash.** 1979. Decreased UV mutagenesis in *cdc8*, a DNA replication mutant of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **172**:249–258.
32. **Prelich, G., M. Kostura, D. R. Marshak, M. B. Mathews, and B. Stillman.** 1987. The cell-cycle regulated proliferating cell nuclear antigen is required for SV40 DNA replication in vitro. Nature (London) **326**:471–475.
33. **Resnick, M. A., J. N. Kasimos, J. C. Game, R. J. Braun, and R. M. Roth.** 1981. Changes in DNA during meiosis in a repair-deficient mutant (rad 52) of yeast. Science **212**:543–545.
34. **Roots, R., and K. C. Smith.** 1974. Induced single-strand breaks in mammalian cells. Int. J. Radiat. Biol. **26**:467–480.
35. **Scherer, S., and R. W. Davis.** 1979. Replacement of chromosome segments with altered DNA sequences constructed *in vitro*. Proc. Natl. Acad. Sci. USA **76**:4951–4955.
36. **Schild, D., and B. Byers.** 1978. Meiotic effects of DNA-defective cell division cycle mutations of *Saccharomyces cerevisiae*. Chromosoma **70**:109–130.
37. **Sclafani, R. A., and W. L. Fangman.** 1986. Thymidine utilization by TUT mutants and facile cloning of mutant alleles by plasmid conversion in *S. cerevisiae*. Genetics **114**:753–767.
38. **Sherman, F., and H. Roman.** 1963. Evidence for two types of allele recombination in yeast. Genetics **48**:255.
39. **Simchen, G., D. Idar, and Y. Kassir.** 1976. Recombination and hydroxyurea inhibitor of DNA synthesis in yeast meiosis. Mol. Gen. Genet. **144**:21–23.
40. **Sinha, N. K., C. F. Morris, and B. M. Alberts.** 1980. Efficient *in vitro* replication of double-stranded DNA templates by a purified T4 bacteriophage replication system. J. Biol. Chem. **255**:4290–4303.
41. **Tan, C.-K., C. Castillo, A. G. So, and K. M. Downey.** 1986. An auxiliary protein for DNA polymerase-δ from fetal calf thymus. J. Biol. Chem. **261**:12310–12316.
42. **Wahl, A. F., J. J. Crute, R. D. Sabatino, J. B. Bodner, R. L. Marraccino, L. W. Harwell, E. M. Lord, and R. A. Bambara.** 1986. Properties of two forms of DNA polymerase δ from calf thymus. Biochemistry **25**:7821–7827.
43. **Wintersberger, E.** 1974. Deoxyribonucleic acid polymerases from yeast. Further purification and characterization of DNA dependent DNA polymerases A and B. Eur. J. Biochem. **50**:41–47.
44. **Wintersberger, U., and E. Wintersberger.** 1970. Studies on deoxyribonucleic acid polymerases from yeast. I. Partial purification and properties of two DNA polymerases from mitochondria-free cell extracts. Eur. J. Biochem. **13**:11–19.
45. **Wong, S. W., A. F. Wahl, P.-M. Yuan, N. Arai, B. E. Pearson, K. Arai, D. Korn, M. W. Hunkapiller, and T. S.-F. Wang.** 1988. Human DNA polymerase α gene expression is cell proliferation dependent and its primary structure is similar to both prokaryotic and eukaryotic replicative DNA polymerases. EMBO J. **7**:37–47.