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The yeast *Saccharomyces cerevisiae* DNA polymerase IV: possible involvement in double strand break DNA repair

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ABSTRACT

We identified and purified a new DNA polymerase (DNA polymerase IV), which is similar to mammalian DNA polymerase β, from *Saccharomyces cerevisiae* and suggested that it is encoded by *YCR14C* (*POLX*) on chromosome III. Here, we provided a direct evidence that the purified DNA polymerase IV is indeed encoded by *POLX*. Strains harboring a *pol4* deletion mutation exhibit neither mitotic growth defect nor a meiosis defect, suggesting that DNA polymerase IV participates in nonessential functions in DNA metabolism. The deletion strains did not exhibit UV- sensitivity. However, they did show weak sensitivity to MMS-treatment and exhibited a hyper-recombination phenotype when intragenic recombination was measured during meiosis. Furthermore, *MATα pol4Δ* segregants had a higher frequency of illegitimate mating with a *MATα* tester strain than that of wild-type cells. These results suggest that DNA polymerase IV participates in a double-strand break repair pathway. A 3.2kb of the *POL4* transcript was weakly expressed in mitotically growing cells. During meiosis, a 2.2 kb *POL4* transcript was greatly induced, while the 3.2 kb transcript stayed at constant levels. This induction was delayed in a *swi4Δ* strain during meiosis, while no effect was observed in a *swi6Δ* strain.

INTRODUCTION

Several nuclear DNA polymerases have been identified and purified from eukaryotic cells (1, 2), prompting questions of why there are so many, and what their roles are. Progress toward answering some of these questions has come from the use of specific inhibitors and the SV40 *in vitro* DNA replication system. Seven cellular factors required for this system (3), including DNA polymerases α and δ (4–6), have also been detected in the yeast *Saccharomyces cerevisiae*, enabling genetic tests of whether the *in vitro* system truly represents *in vivo* chromosomal DNA replication. The pure genetic approach identified a series of cell-division-cycle (*cdc*) mutants, among which were alleles of the genes now known to encode DNA polymerase α and δ catalytic polypeptides (7–10). DNA polymerase ε is the DNA polymerase that was last identified and purified in eukaryotes (1, 2). But, its yeast homolog, DNA polymerase II, was detected enzymatically in yeast cell extracts in early 1970 (11, 12). Its gene, *POL2*, was not found in any genetic screen, nor was its mammalian counterpart identified in the SV40 *in vitro* system. *POL2* was eventually cloned by reverse genetics and has been shown to be required for yeast chromosomal DNA replication (13–15), suggesting that DNA polymerase ε is also required for other eukaryotic chromosomal DNA replication. In vertebrates, another DNA polymerase, DNA polymerase β, has been purified, characterized, and the corresponding gene has been isolated (1, 16). This polymerase is believed to participate in small patch DNA repair and/or DNA recombination in mammalian cells (1). This notion is mainly based on the gap-filling property of DNA polymerase β and inhibitor studies (1). It has been shown that DNA polymerase β is expressed in both proliferating and nonproliferating cells. Expression of the gene is also cell type specific, implicating it in repair synthesis during meiotic recombination (17). Nonetheless, there is no formal genetic evidence for an *in vivo* role of the enzyme. In lower eukaryotes, a low molecular weight DNA polymerase activity similar to DNA polymerase β has not been described (1), although DNA polymerase activities whose molecular weights are larger than that of DNA polymerase β have been previously reported (18–20) and their biochemical properties are similar to those of DNA polymerase β. (It is presently not clear whether these DNA polymerases are derived from either DNA polymerase α, δ or ε, or if they are homologs of *S.cerevisiae* DNA polymerase IV, since immunological studies have not been done.) Recently, we (21) have identified and purified a new DNA polymerase, DNA polymerase IV, from *S.cerevisiae* cell extracts and have shown that its biochemical properties are similar to those of mammalian DNA polymerase β, although the size is considerably larger. We also provided evidence that DNA polymerase IV polypeptide is encoded by the *YCR14C* open reading frame (ORF)

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(or *POLX*) which has been identified on chromosome III (22) and whose translation product has a significant amino acid sequence homology with that of DNA polymerase β from rat and human cells (23). Availability of the gene enables us to test an *in vivo* role of yeast DNA polymerase IV, by construction of DNA polymerase IV-deficient yeast mutants. Here, we describe the characterization of DNA polymerase IV-deficient mutants, expression of the gene during meiosis, and discuss the *in vivo* role(s) of DNA polymerase IV.

MATERIALS AND METHODS

Bacterial and yeast strains

Escherichia coli DH5α (24) was used for preparation of plasmid DNA. *Saccharomyces cerevisiae* strains used were CB001 (*MATa leu2 trp1 ura3 prb pep4::URA3*) (13), MR966 (*MATα ura3-52 leu2-3, 112 trp1-289 his 1-7*), SLH105 (*MATa lys2 ho::LYS2 ura3 leu2::hisG his4X trp1::hisG*), SLH108 (*MATα lys2 ho::LYS2 ura3 leu2::hisG his4B trp1::hisG*), SLD101 (*MATa/MATα lys2/lys2 ho::LYS2/ho::LYS2 leu2::hisG/leu2::hisG his4X/his4B trp1::hisG/trp1::hisG*), SLH135 (the same as SLH105 except for *pol4Δ::URA3*), SLH139 (the same as SLH108 except for *pol4Δ::URA3*), SLD124 (the same as SLD101 except for *pol4Δ::URA3/pol4Δ::URA3*), SLH145 (the same as SLH108 except for *pms1Δ::LEU2*), SLH149 (the same as SLH105 except for *pms1Δ::LEU2*), SLD129 (SLH145/SLH149), SLH150 (the same as SLH135 except for *pms1Δ::LEU2*), SLH156 (the same as SLH139 except for *pms1Δ::LEU2*), SLD131 (SLH150/SLH156), AMY32 (*MATα rev3° arg4-17 leu2-3, 112 his3-4 trp1 ura3-52*) (25), SLD501 (SLH135/AMY32), SLD109 (the same as SLD101 except for *swi4Δ::LEU2/swi4Δ::LEU2*), SLD113 (the same as SLD101 except for *swi6Δ::TRP1/swi6Δ::TRP1*), AKY102 (*MATa ho::LYS2 lys2 ura3 leu2::hisG ade2::LK his4X-ADE2-his4B*) (26), YHA301 (*MATa ade5-1 leu2-3, 112 ura3-52 trp1-289 pol2-3::LEU2* [YCp *pol2-18*]) (15), YHA302 (same as YHA301, except for [YCp *pol2-9*]) (15), 488 (*MATα trp1 leu2 ura3-52 his1-7 can1 pol1-17*) (27), H17C1A1 (*MATa his7 ura1 cdc17-1*) (from B.Garvik), 336 (*MATa ade1 ade2 ura1 his7 tyr1 lys2 gal1 cdc2-2*) (from B.Garvik) and 346 (*MATa ade1 ade2 ura1 his7 tyr1 lys2 gal1 cdc2-4*) (from B.Garvik).

DNA

To construct *pol4Δ::URA3* allele, The *Xho*I−*Bgl*II DNA fragment containing *POL4* (Fig. 1) was amplified from *S.cerevisiae* CB001 genomic DNA by polymerase chain reaction using appropriate primers, digested with *Xho*I and *Bgl*II and subcloned into pUC118 DNA digested with *Sal*I and *Bam*H1 restriction enzymes. The resultant plasmid (pUC118-POL4) was redigested with *Nsi*I and *Hin*dIII, followed by replacement of the most part of the *POL4* gene (see Fig. 1) with ~1.1kb yeast *URA3* DNA. Yeast *PMS1*, *pms1::LEU2*, *URA3*, and *URA3* DNA were previously described (13, 28). Yeast *swi4::LEU2* and *swi6::TRP1* DNA were obtained from L.H.Johnston.

Construction of yeast strains

Strain SLH135 containing *pol4Δ::URA3* was constructed by transforming strain SLH105 to Ura$^+$ with *Xho*I−*Bgl*II DNA fragment of *pol4Δ::URA3* (Fig. 1). Correct replacement of the *POL4* gene with the *pol4Δ::URA3* gene was confirmed by Southern blot hybridization using chromosomal DNA from the transformants. Similarly, strains containing *pms1Δ::LEU2* mutation was constructed as described (28). To make a *pol4Δ* rev3Δ double mutant, the diploid strain SLD201 was sporulated, the resulted spores were dissected, and *pol4Δ rev3Δ* segregants were identified. At the same time, wild-type, *pol4Δ*, and *rev3Δ* segregants were also used as a control. Similarly, *pol4Δ pol1*, *pol4Δ pol2*, *pol4Δ pol3*, *pol4Δ pol1 pol2*, *pol4Δ pol1 pol3*, and *pol4Δ pol2 pol3* were constructed using SLH135, SLH139, YHA301 (*pol2-9*), YHA302 (*pol2-18*), 488 (*pol1-17*), H17ClA1 (*pol17-1*), 336(*cdc2-2*), and 346 (*cdc2-4*).

Media and general genetic techniques

The media and the standard genetic procedures for the yeast *Saccharomyces cerevisiae* were described (29). Spontaneous mutation frequency of yeast cells was measured as previously described (28). Illegitimate mating of α-cells was measured as described (30).

Transcriptional levels of *POL4*

Steady-state levels of *POL4* transcript were measured by Northern blotting (31). Total RNA was extracted from either mitotically growing cells or cells incubated in meiotic specific medium for various times, and about 20 μg of RNA were fractionated by agarose gel electrophoresis and transferred to Hybond-N nylon membranes (Amersham Corp.) for hybridization. In each experiment, the agarose gel was stained with ethidium bromide to visualize the rRNA and confirm that equal amounts of RNA had been loaded. To make ^{32}P-labeled *POL4* probe, the *DNA* fragment in the open-reading frame of the *POL4* gene (Fig. 1) was nick-translated by *Escherichia coli* DNA polymerase I in the presence of [α-^{32}P]dCTP (> 5,000 Ci/mmol, Amersham Corp.) as described (24).

Other methods

Meiosis-specific double-strand breaks were detected as previously described (31) using *Eco*RV−*Bgl*II DNA fragment in *ARG4* as a probe. After autoradiography, the intensity of bands were quantitated by a Bioimage analyzer BAS2000 (Fuji Film Corp.). Other methods used in this report were also previously described (21, 28, 31).

RESULTS

DNA polymerase IV is encoded by the *YCR14C* ORF (*POLX*)

In the previous study (21), we have shown that a newly identified and purified *S.cerevisiae* DNA polymerase, DNA polymerase IV, is very similar to mammalian DNA polymerase β and suggested that it is encoded by the open reading frame *YCR14C* (*POLX*) that is located on chromosome III (22). To obtain direct proof that DNA polymerase IV is encoded by *POLX*, oligopeptides generated from the purified DNA polymerase IV by endopeptidase Lys-C were separated by reverse phase HPLC and their amino acid sequences were determined by an automated microsequencer. As shown in Table 1, four different amino acid sequences were obtained. These sequences can be found in the ORF of *YCR14C*. Thus, we concluded that DNA polymerase IV is encoded by *YCR14C* (*POLX*). We propose that *YCR14C* (*POLX*) should be renamed as *POL4*.

The *POL4* gene is not essential for yeast mitotic cell growth or meiosis

To investigate an *in vivo* function of DNA polymerase IV, *pol4* deletion mutants were constructed by replacing the *Nsi*I−*Hin*dIII

Table 1. Partial amino acid sequence of DNA polymerase IV polypeptide

Oligopeptide #	Amino acid sequence determined by automated sequencer[1]	Corresponding sequence of *POL4*	(Amino acid number of *POL4*)
1.	DIFQREAGLN(D)(V)(D)	DIFQREAGLNDVD	(67–79)
2.	E(S)EISTDVE(S)(E)(R)	ESEISTDVESER	(138–149)
3.	RXNLLNFE(S)FXVA	RWNLLNFESFCVA	(287–299)
4.	ALRGIDPEXQVELQG	ALRGIDPECQVELQG	(342–356)
5.	XGDIDLLFF	CGDIDLLFF	(365–373)

[1]X in the amino acid sequence represents an unidentified amino acid residue and ( ) indicates an uncertain amino acid residue.

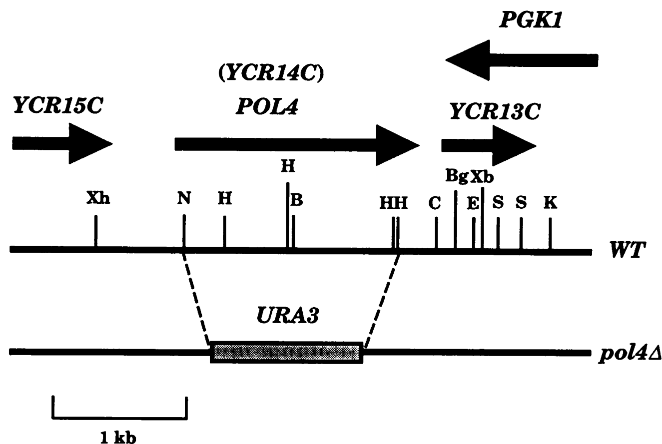

Figure 1. Physical map of the *POL4* region located on chromosome III of *S.cerevisiae* and the construction of *pol4Δ* mutation. An arrow represents the size and direction of open reading frames found in this region, except that only a portion of the *YCR15C* open reading frame is shown. The second line represents the construction of *pol4Δ* mutation by removing the *NsiI–HindIII* fragment followed by replacement with 1.1 kb yeast *URA3* gene indicated by a shaded rectangle. Vertical lines on a horizontal line show representative restriction enzyme sites. Abbreviations used for restriction enzymes: B, *BamHI*; Bg, *BglII*; C, *ClaI*; E, *EcoRI*; H, *HindIII*; K, *KpnI*; N, *NsiI*; S, *SalI*; Xb, *XbaI*; Xh, *XhoI*.

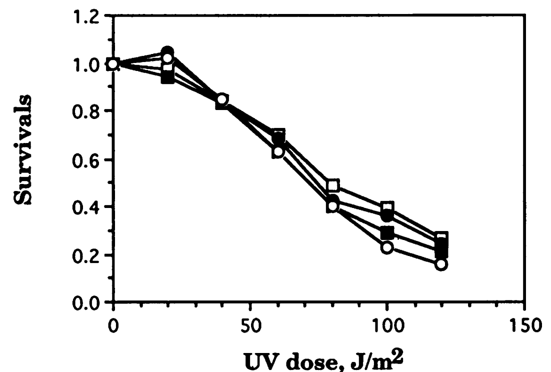

Figure 2. UV-sensitivity of SLH105 (wild type), SLH135 (*pol4Δ*), SLH149 (*pms1Δ*) and SLH150 (*pol4Δ pms1Δ*) strains. Survival of isogenic haploid cells was measured after UV light irradiation by incubating on YPD plates at 30°C for three days. Open and closed circles represent SLH105 and SLH135, respectively. Open and closed square are SLH149 and SLH150, respectively.

fragment in the *POL4* coding region with the yeast *URA3* gene (Fig. 1). One of the wild type genes in a *S.cerevisiae* diploid cell was replaced with the deleted gene by one step replacement method (33). The resultant diploid cells were sporulated, spores were microdissected and germinated. Out of 20 tetrads dissected, 18 tetrads gave four viable spores, 1 tetrad gave 3 viable spores and 1 tetrad gave 2 viable spores. Similar results were obtained when wild-type parental diploids were subjected to tetrad analysis. These results indicate that the gene is not essential for cell growth and therefore, DNA polymerase IV does not play an important role during chromosomal DNA replication in yeast. Homozygous *pol4Δ/pol4Δ* diploid cells sporulated and their spore viability was very similar to that of wild-type diploid cells, indicating that DNA polymerase IV is not required for normal meiosis.

UV- and MMS-sensitivities of *pol4Δ* mutants

To further investigate an *in vivo* function of DNA polymerase IV, UV-, γ-ray and MMS-sensitivities of *pol4Δ* mutants were measured. As shown in Fig. 2, no difference in UV-sensitivity was detected between wild-type and *pol4Δ* mutant cells. Furthermore, introduction of either *pms1Δ* (one of mismatch correction deficient mutations) or mutagenic repair deficient mutation, *rev3Δ*, did not change the sensitivity. However, it was still possible that the function of DNA polymerase IV can be substituted by other DNA polymerases, such as DNA polymerases I(α), II(ε) and/or III(δ). To test this possibility, we constructed various double and triple mutants between *pol4Δ* and other DNA polymerase mutants *pol1-17*, *cdc17-1*, *cdc2-2*, *-4*, *pol2-9* and *-18*. None of these multiple mutant cells exhibited increased UV-sensitivity of *pol4Δ* mutation (data not shown). Even single-mutants of *pol1-17*, *pol2-9*, *pol2-18*, *cdc2-2*, and *cdc2-4* exhibited approximately the same UV-sensitivity as wild-type cells (data not shown).

pol4Δ mutant cells exhibited a weak, but significant MMS-sensitivity (Fig. 2). This sensitivity was almost the same as *rev3Δ* mutant cells. MMS-sensitivity of the double mutant *pol4Δ rev3Δ* cells seems additive (Fig. 3A). However, MMS-sensitivity of *pol4Δ* was repressed by the *pms1Δ* mutation, since *pol4Δ pms1Δ* double mutants behaved like wild-type cells (Fig. 3B). MMS-sensitivity of the *pol4Δ* mutant cells was completely reversed to that of wild-type cells by a single-copy plasmid DNA containing the *POL4* gene (Fig. 4), indicating that the MMS sensitivity is associated with *pol4Δ* mutation. *pol4Δ* mutant cells also showed similar weak sensitivity to γ-ray as to MMS-treatment (data not shown).

UV-induced intragenic recombination in *pol4Δ* mutant cells

To detect any effect of *pol4Δ* mutation on recombination, we measured UV-induced intragenic recombination between *his4X* and *his4B*. As shown in Fig. 5, no significant difference of intragenic recombination could be observed between wild type

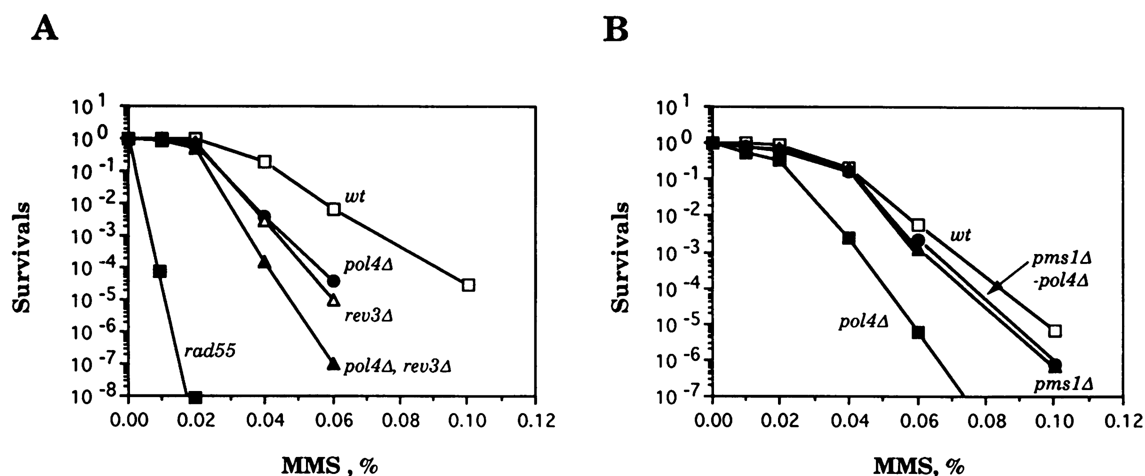

Figure 3. MMS-sensitivity of *rad55*, *pol4*Δ, *rev3*Δ, *pms1*Δ, *pol4*Δ, *pms1*Δ. and *pol4*Δ *rev3*Δ mutant cells. (**A**) Isogenic strains of wild-type cells, *pol4*Δ, *rev3*Δ, and *pol4*Δ *rev3*Δ mutant cells, as well as *rad55*, were grown to 1×10^7 cells/ml in YPD medium, plated on YPD plates containing indicated amounts of MMS, incubated at 30°C for three days, and colonies were counted. (**B**) MMS-sensitivity of isogenic strains of wild-type cells, *pol4*Δ, *pms1*Δ, and *pol4*Δ *pms1*Δ mutant cells were measured as (A).

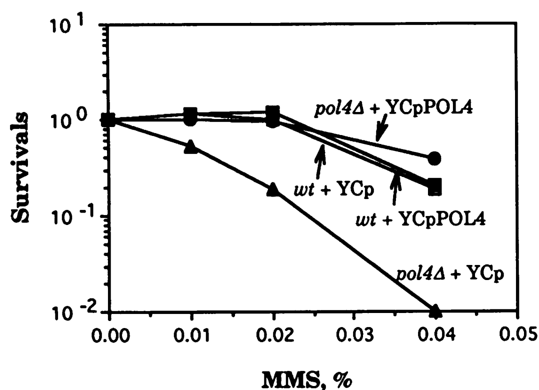

Figure 4. MMS-sensitivity of *pol4*Δ mutant cells is complemented by the *POL4* gene. SLH105 (wild-type) strain containing either vector YCplac111 (46) or YCplac111POL4 plasmid and SLH135 strain (*pol4*Δ) containing either vector YCplac111 or YCplac111POL4 plasmid were grown to 1×10^7 cells/ml in SD complete medium without leucine, plated on YPD containing the indicated concentrations of MMS, incubated at 30°C for three days and colonies were counted.

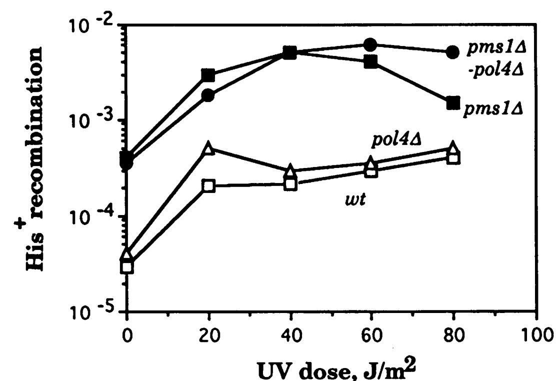

Figure 5. UV-induced intragenic recombination. Diploid strains SLD101 (wild-type), SLD124 (*pol4*Δ/*pol4*Δ), SLD129 (*pms1*Δ/*pms1*Δ), and SLD131 (*pol4*Δ/*pol4*Δ *pms1*Δ/*pms1*Δ) were treated by the indicated amount of UV-light, plated on both SD complete plates without histidine and YPD plates, incubated at 30°C for 3 days, and His^+ recombinants were counted. -□-, SLD101; -Δ-, SLD124; -■-, SLD129; -●-SLD131.

and *pol4*Δ mutant cells after UV-irradiation. The intragenic recombination frequency increased 10-fold in *pms1*Δ mutant cells over wild-type cells, and *pms1*Δ *pol4*Δ double mutants exhibited the same levels of intragenic recombination with and without UV-treatment as *pms1*Δ mutant cells (Fig. 5), suggesting that DNA polymerase IV does not play any role in UV-induced intragenic recombination.

Meiotic intragenic recombination in *pol4*Δ mutant cells

Meiotic intragenic recombination between *his4X* and *his4B* was measured in both wild-type and *pol4*Δ mutant cells. As shown in Table 2, spore viability of both wild-type and *pol4*Δ diploid cells was very high. Therefore, meiosis in *pol4*Δ mutant cells proceeds normally. On the other hand, intragenic recombinants in the mutant cells increased 5-fold over wild-type cells during meiosis.

Elevated levels of meiosis-specific double-strand breaks in *pol4*Δ mutant cells

Meiotic recombination occurs at a high frequency at specific sites, so called hot spots, on the chromosome. In the three loci of meiotic recombination hot spots, *HIS4-LEU2*, *ARG4* and *THR4* genes, in *S.cerevisiae*, meiosis-specific double-strand breaks have been observed by Southern blot analysis (34, 35, 36). As shown in Fig. 6, typical double-strand breaks were detected in both wild-type and *pol4*Δ mutant cells. However, the number of breaks found in *pol4*Δ mutant cells were at least three times as much as those found in wild-type cells.

Table 2. Intragenic recombination in *pol4Δ* mutant cells during meiosis

Strain	Relevant genotype	Percent of spores at 12h	Percent of spore viability	Frequency of intragenic recombination at *hia4-X/his4-B* alleles (asci contained His^+ spores/total asci)
SLD101	POL4 / POL4	72	98 (471/480)	0.05 (6/120)
SLD125	pol4Δ::URA3 / pol4Δ::URA3	73	99 (1269/1280)	0.29 (93/320)

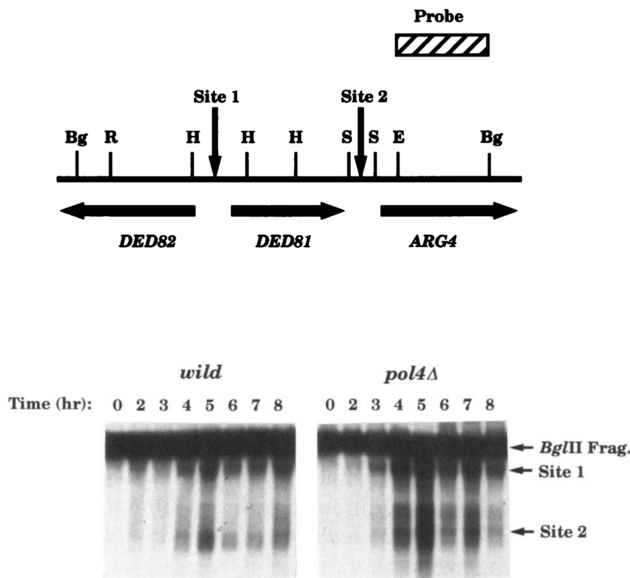

Figure 6. Meiosis-specific double-strand breaks in the wild-type and *pol4Δ* strains. A map of relevant restriction sites in ~ 5.5 kb *ARG4-DED81-DED82* region (34) and the positions of the two major double-strand break sites and the probe used are shown in the top of the Figure. Chromosomal DNA samples from either SLD101 (wild-type) or SLD124 (*pol4Δ*) cells were digested with *BglII* and hybridized with a random primed *EcoRV–BglII* DNA probe.

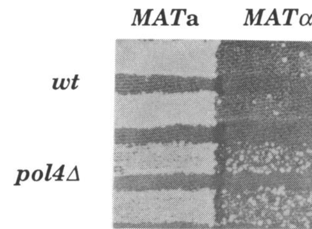

Figure 7. Illegitimate mating increases in *pol4Δ* mutant cells. The crosses of *MATα* strains::*POL4* wild-type (MR966) and its *pol4Δ::URA3* mutant (MR966-1) with the *MATα lys2* and *MATa lys2* tester strains were grown at 30°C on YPD, then replica plated to minimal medium and icubated at 30°C.

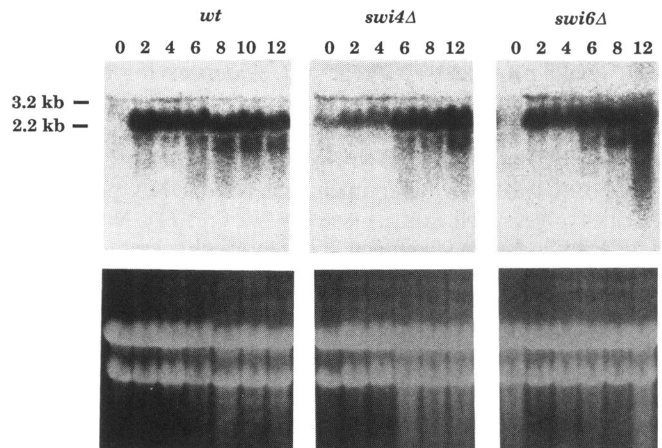

Figure 8. Induction of the *POL4* transcript during meiosis. Either diploid SLD101 (wild-type), SLD109 (*swi4Δ*), or SLD113 (*swi6Δ*) cells were grown in presporulation medium to 1×10^7 cells/ml, transferred to meiotic medium as described (31) and incubated at 25°C. At the indicated time, cells were harvested by centrifugation and total RNA was extracted. Total RNA (20 μg) was separated by agarose gel electrophoresis, transferred onto a Hybond-N nylon membrane and hybridized with ^{32}P-labelled *POL4* probe. Top pannels show an autoradiograph of the hybridized filter. Bottom pannels are photographs of the agarose gel stained with ethidium bromide before RNA transfer on Hybond-N filter. Numbers shown on the top of figure represent the times (hours) after medium change from presporulation medium to meiotic medium. Left side of the figure shows the size (kb) of the transcripts hybridized with the probe.

Increased frequency of illegitimate mating in the *pol4Δ* mutants

Illegitimate mating of *MATα pol4Δ* segregants with a *MATα* tester strain was measured. As shown in Fig. 7, *pol4Δ* mutants had at least 10-fold higher frequency of illegitimate mating with *MATα* strains than wild-type cells. Although the signal was relatively small in comparison with other mutations which are known to have a high frequency of illegitimate mating (37), the results were very reproducible. On the other hand, *MATα pol4Δ* mated with *MAT*a strain at the almost same efficiency as a wild-type strain (Fig. 7). Illegitimate mating of *α-cells might occur via true mutations, MATα* → MATa, or after the inactivation of the MATα locus (38). The inactivation of MATα could be due to point mutations, chromosome III rearrangements, or to the loss of the entire chromosome III. Another process leading to illegitimate mating is the spontaneous or induced transient inactivation of the *MATα* allele (39). However, we could not detect any significant difference between *pol4Δ* mutant cells and wild-type cells in spontaneous mutation frequency or chromosome loss (data not shown).

POL4 transcript is induced during meiosis

To detect *POL4* transcript, Northern blot hybridization was carried out using total RNA extracted from log-phase of *S.cerevisiae* wild-type cells. As shown in Fig. 8, a 3.2kb transcript hybridized to the *POL4* probe. When RNA was extracted from synchronized cells, the same size transcript could

be detected at constant levels throughout the cell cycle (data not shown). When RNA was extracted from a diploid strain synchronously sporulated in meiotic specific medium, a 2.2kb transcript hybridized very intensely with the *POL4* probe and increased during meiosis (Fig. 8). At the same time, the weak 3.2kb message was also detected but stayed at a constant level during meiosis. Induction of the 2.2kb transcript during meiosis was delayed by *swi4Δ* mutation, but not by *swi6Δ* mutation (Fig. 8). On the other hand, both 2.2- and 3.2kb transcripts were not detected in *pol4Δ* mutant cells (data not shown), confirming that they are from *POL4*.

DISCUSSION

In the previous report (21), we strongly suggested that a newly identified and purified DNA polymerase which exhibited biochemical properties similar to those of mammalian DNA polymerase β is encoded by the *YCR14C* (*POLX*) gene. In this report, we have shown that the partial amino acid sequences determined from the purified polypeptide matched perfectly with the amino acid sequence predicted from the nucleotide sequence of *YCR14C*. Thus, we propose now that *YCR14C* should be renamed *POL4*. As the biochemical properties of the purified DNA polymerase IV are very much like mammalian DNA polymerase β and the amino acid sequence of *POL4* has a significant homology to that of human and rat DNA polymerase β (23), it is very likely that DNA polymerase IV is a yeast homolog of mammalian DNA polymerase β. If this is true, then yeast could provide some genetical evidences for an *in vivo* function of mammalian DNA polymerase β. However, it is still possible that another yet unidentified DNA polymerase is the homolog of mammalian DNA polymerase β, since we have detected at least two other uncharacterized DNA polymerase activities in yeast cell extracts (see Fig. 1 of ref 21). Nonetheless, in order to find an *in vivo* function of DNA polymerase IV, *pol4Δ* deletion mutants were constructed and their sensitivity to either γ-ray, UV-, or MMS-treatment were examined. The mutant cells did not exhibit any significant sensitivity to UV. Combinational mutants of *pol4Δ* with other DNA polymerase mutations did not increase the UV-sensitivity of the *pol4Δ* mutation, suggesting that *POL4* does not participate in repair of UV damaged DNA. Consistent with these *in vivo* results, the yeast *in vitro* repair system described by Friedberg and his associate (32, 40) did not show any significant difference of UV-, OsO_4-treated DNA and Uracil-containing DNA repair between wild-type and *pol4Δ* mutant cells (our unpublished results). Therefore, it is very likely that the UV-repair reaction in yeast requires another DNA polymerase, such as DNA polymerase II (ϵ) and/or DNA polymerase III (δ). It has become clear that the UV-repair reaction in human cells requires either DNA polymerase δ and/or ϵ (41). *pol4Δ* mutant cells were, however, weakly sensitive to MMS- and γ-ray treatment, suggesting that DNA polymerase IV participates in a certain type of DNA repair process, particularly in a double-strand break repair pathway. Consistent with this notion, the mutant cells exhibited a hyper-recombinational phenotype when intragenic recombination between *his4X* and *his4B* was measured during meiosis. It has been believed that double-strand breaks are intermediates of meiosis specific recombination (42). DNA polymerase IV may be involved in the repair of double-strand breaks generated for meiotic recombination. Therefore, there is a potential that DNA polymerase IV (and possibly other DNA repair proteins) may compete with a recombination complex to bind double-strand breaks. If DNA polymerase IV is not present (*pol4Δ* mutant), double-strand breaks would be fully utilized for the recombination process, resulting in the hyper-recombination phenotype. Consistent with this hypothesis is the observation that the amount of double-strand breaks seen during meiosis, which is believed to be an intermediate of homologous recombination (34–36), increases in *pol4Δ* mutant cells (Fig. 6). The mating type switching process also involves double-strand breaks at the *MAT* locus (43). The result of elevated illegitimate mating activity in the *pol4Δ* mutant cells can be explained by the same mechanism of action of DNA polymerase IV. Under normal conditions, double-strand breaks by HO endonuclease at the *MAT* locus are tightly controlled. If the breaks were introduced, they might quickly be repaired by a DNA repair pathway involving DNA polymerase IV. In the absence of DNA polymerase IV the breaks might be more fully utilized for mating type switching.

The weak MMS-sensitivity of *pol4Δ* mutant cells may suggest a partial substitution of DNA polymerase IV with other DNA polymerases. However, neither *pol1*, *pol2*, nor *pol3* mutations had any effect on the MMS-sensitivity. Only mutagenic repair deficient *rev3Δ* mutation increased the MMS-sensitivity of *pol4Δ* mutant cells (Fig. 3). The effect of *rev3Δ* mutation was additive, suggesting that the action of both DNA polymerases [DNA polymerase IV and Rev3 DNA polymerase (this polymerase has to be demonstrated biochemically as DNA polymerase)] are independent and in the different pathway. Prasad *et al.* also constructed *pol4Δ* mutant and tested the sensitivities to various DNA damaging agents (UV radiation, γ radiation, bleomycin, MMS, EMS, MNNG, and H_2O_2) (44). However, they did not detect any altered sensitivity to any of the agents they used. We do not know the reason why they did not see a weak sensitivity of *pol4Δ* to MMS-and γ-ray treatment that we detected. Note that no different sensitivity between *pol4Δ* and wild-type cells was detected at lower concentrations of MMS (0–0.03%) (Fig. 3). Nonetheless, the MMS-sensitivity of *pol4Δ* mutant cells that we observed was complemented by a single copy plasmid containing *POL4*, strongly suggesting that the sensitivity is due to *pol4Δ* mutation.

The message of *POL4* was greatly induced during meiosis (Fig. 8). Furthermore, the size of the message was changed from mitotic cells to cells in meiosis, suggesting it functions during meiosis. However, the *pol4Δ* mutant cells did not exhibit any meiotic phenotype, except for hyper-recombination. It is interesting that the message of *REV3* is also induced during meiosis and *rev3Δ* mutants also do not have any meiotic phenotype (45). Nevertheless, why the *POL4* transcript is induced during meiosis is still remain to be answered. It is known that many genes expressed early during meiosis in yeast have regulatory sequences, URS1 (upstream repression site 1), UAS_H (upstream activation site), and T_4C site (46). Interestingly, a T_4C site like sequence -TTTTCCTCCTG-, an URS1 like sequence -GGAAGGCGGTA- and an UAS_H like sequence -GT-CACAGTGGT- were found in the 5'-untranslated region of *POL4*. These sequences may be responsible for induction of the *POL4* mRNA during meiosis. As induction of the *POL4* transcript was delayed in *swi4Δ* mutant cells during meiosis, it is likely that the *SWI4* gene product is involved in the regulation of this induction. However, further studies are needed to understand the mechanism of this induction.

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