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

Sun-Hee Leem, Philip A. Ropp1 and Akio Sugino*

Department of Molecular Immunology, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-Oka, Suita, Osaka 565, Japan and 1Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, National Institutes of Health, PO Box 12233, Research Triangle Park, NC 27709, USA

Received May 13, 1994; Revised and Accepted July 13, 1994

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 5.) 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)

*To whom correspondence should be addressed
(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 yeast 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 CB01 (MATa leu2 trp1 ura3-215 trpl 246 trpl 248), MR966 (MATa ura3-52 leu2-3, 112 trp1-289 his 1-7), SLH105 (MATa lys2 ho::LYS2 ura3-2 leu2::hisG his4X trpl::hisG), SLH108 (MATa lys2 ho::LYS2 ura3-2 leu2::hisG his4B trpl::hisG), SLD101 (MATa/ MATa lys2/sls2 ho::LYS2 ura3-2 leu2::hisG/leu2::hisG his4X/his4B trpl::hisG/trpl::hisG), SLD113 (the same as SLH105 except for pol4Δ::URA3), SLD119 (the same as SLH105 except for pol4Δ::URA3), SLD124 (the same as SLD101 except for pol4Δ::URA3), SLD145 (the same as SLH105 except for pmslA::LEU2), SLH149 (the same as SLH105 except for pmslA::LEU2), SLD129 (SLH145/SLH149), SLD150 (the same as SLH135 except for pmslA::LEU2), SLH156 (the same as SLH139 except for pmslA::LEU2), SLD113 (SLH150/SLH156), AMY32 (MATa rev3-24 arg4-17 leu2-3, 112 his 3-4 trpl 116 ura3-52) (25), SLD301 (SLH135/AMY32), SLD109 (the same as SLD101 except for swi6Δ::LEU2/swi6Δ::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 trpl-289 pol2-3::LEU2 [YCP pol2-18]) (15), YHA302 (same as YHA301, except for [YCP pol2-9]) (15), 488 (MATa trpl 116 ura3-52 his 1-7 canl pol1-17) (27), H17C1A1 (MATa his7 ura1 cdc7-1) (from B. Garvik), 336 (MATa ade1 ade2 ade2 ura1 his7 tyr1 lys2 gall cdc2-2) (from B. Garvik) and 346 (MATa ade1 ade2 ade2 ura1 his7 tyr1 lys2 gall cdc2-4) (from B. Garvik).

**DNA**

To construct pol4Δ::URA3 allele, The Xhol–BglII DNA fragment containing POL4 (Fig. 1) was amplified from *S. cerevisiae* CB001 genomic DNA by polymerase chain reaction using appropriate primers, digested with Xhol and BglII and subcloned into pUC118 DNA digested with Sall and BamH1 restriction enzymes. The resultant plasmid (pUC118-POL4) was redigested with NdeI and HindIII, followed by replacement of the most part of the POL4 gene (see Fig. 1) with ~1.1kb yeast URAS DNA. Yeast PMS1, PMS1::LEU2, URA3, and URA3 DNA were previously described (13, 28). Yeast swi6::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 Xhol–BglII 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 pmslA::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), H17C1A1 (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 RNA and confirm that equal amount of RNA had been loaded. To make 32P-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-32P]dCTP (> 5,000 Ci/mmol, Amersham Corp.) as described (24).

**Other methods**

Meiosis-specific double-strand breaks were detected as previously described (31) using EcoRV–BglII 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. 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 deletions were mutated by replacing the NdeI–HindIII
diploid cells sporulated and their spore viability was measured. As shown in Fig. 2, no difference in UV-sensitivity of pol4A mutants was observed compared to wild-type cells (data not shown). Even single-mutants of pol2-9, pol2-18, cdc2-2, cdc2-4 exhibited approximately the same UV-sensitivity as wild-type cells (data not shown).

pol4A 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 POLA 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 and pol4Δ mutant cells.

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, BgIII; C, CiaI; E, EcoRI; H, HindIII; K, KpnI; N, NstI; S, SalI; X, XbaI; Xh, Xhol.

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.

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(a), II(e) and/or III(d). To test this possibility, we constructed various double and triple mutants between pol4Δ and other DNA polymerase mutants po/1-77, pol2-17, 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).

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

<table>
<thead>
<tr>
<th>Oligopeptide #</th>
<th>Amino acid sequence determined by automated sequencer</th>
<th>Corresponding sequence of POL4 (Amino acid number of POL4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>DIFQREAGLNDVYD</td>
<td>(67–79)</td>
</tr>
<tr>
<td>2.</td>
<td>ESEISTDEVESER</td>
<td>(138–149)</td>
</tr>
<tr>
<td>3.</td>
<td>RWNLNPESPCVA</td>
<td>(287–299)</td>
</tr>
<tr>
<td>4.</td>
<td>ALRGIDPEQVEQVLQG</td>
<td>(342–356)</td>
</tr>
<tr>
<td>5.</td>
<td>XGDIDLFF</td>
<td>(365–373)</td>
</tr>
</tbody>
</table>

1X in the amino acid sequence represents an unidentified amino acid residue and ( ) indicates an uncertain amino acid residue.
Figure 3. MMS-sensitivity of rad55, pol4Δ, rev3Δ, pms1Δ, pol4Δ, pms1Δ, and pol4Δ rev3Δ mutant cells. (A) Isogenic strains of wild-type cells, pol4Δ, rev3Δ, and pol4Δ rev3Δ mutant cells, as well as rad55, were grown to 1 x 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, pol4Δ, pms1Δ, and pol4Δ pms1Δ mutant cells were measured as (A).

Figure 4. MMS-sensitivity of pol4Δ mutant cells is complemented by the POL4 gene. SLH105 (wild-type) strain containing either vector YCplacl11 (ilihan) or YCplacl11POL4 plasmid and SLH135 strain (pol4Δ ) containing either vector YCplacl11 or YCplacl11POL4 plasmid were grown to 1 x 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.

and pol4Δ mutant cells after UV-irradiation. The intragenic recombination frequency increased 10-fold in pms1Δ mutant cells over wild-type cells, and pms1Δ pol4Δ double mutants exhibited the same levels of intragenic recombination with and without UV-treatment as pms1Δ mutant cells (Fig. 5), suggesting that DNA polymerase IV does not play any role in UV-induced intragenic recombination.

Meiotic intragenic recombination in pol4Δ mutant cells
Meiotic intragenic recombination between his4X and his4B was measured in both wild-type and pol4Δ mutant cells. As shown in Table 2, spore viability of both wild-type and pol4Δ diploid cells was very high. Therefore, meiosis in pol4Δ 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 pol4Δ 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 pol4Δ mutant cells. However, the number of breaks found in pol4Δ 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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Percent of spores at 12h</th>
<th>Percent of spore viability</th>
<th>Frequency of intragenic recombination at his4-Xlhis4-B alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLD101</td>
<td>POL4</td>
<td>72</td>
<td>98 (471/480)</td>
<td>0.05 (6/120)</td>
</tr>
<tr>
<td>SLD125</td>
<td>pol4Δ::URA3</td>
<td>73</td>
<td>99 (1269/1280)</td>
<td>0.29 (93/320)</td>
</tr>
</tbody>
</table>

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.

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 MATα strain at the almost same efficiency as a wild-type strain (Fig. 7). Illegitimate mating of α-cells might occur via true mutations, MATα → MATα, 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).

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 MATα lys2 tester strains were grown at 30°C on YPD, then replica plated to minimal medium and incubated 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 x 10⁷ 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 32P-labelled POL4 probe. Top panels show an autoradiograph of the hybridized filter. Bottom panels 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.

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 intensly 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-, O₃O₄-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-recombination 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 mutation), 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 revΔ mutation increased the MMS-sensitivity of pol4Δ mutant cells (Fig. 3). The effect of revΔ 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, MNNNG, and H₂O₂) (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), UAH₅ (upstream activation site), and TᵥC site (46). Interestingly, a TᵥC site like sequence `-TTTCTCTCCTG`- an URS1 like sequence `-GGAGGGCGGT`- and an UAH₅ like sequence `-GT-CACAGCGGT`- 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.
ACKNOWLEDGEMENTS

We thank Drs Paolo Plevani and Giovanna Lucchini for providing us their unpublished results of UV- and MMS-sensitivities of pol4A mutants. We also thank Dr B. Garvik (University of Washington) for yeast strains and Dr L. H. Johnston for yeast swt4::LEU2 and swi6::TRP1 DNA (National Institute for Medical Research, London). This work was supported in part by Grant-in-aid for Scientific Research of the Ministry of Education, Science and Culture of Japan.

REFERENCES

47. Gieze, R. D. & Sugino, A. Gene 74, 527–534.