We have previously shown that DNA polymerase ε (Pol ε) of *Saccharomyces cerevisiae* binds stably to double-stranded DNA (dsDNA), a property not generally associated with DNA polymerases. Here, by reconstituting Pol ε activity from Pol2p-Dpb2p and Dpb3p-Dpb4p, its two component subassemblies, we report that Dpb3p-Dpb4p, a heterodimer of histone-fold motif-containing subunits, is responsible for the dsDNA binding. Substitution of specific lysine residues in Dpb3p, highlighted by homology modeling of Dpb3p-Dpb4p based on the structure of the histone H2A-H2B dimer, indicated that they play roles in binding of dsDNA by Dpb3p-Dpb4p, in a manner similar to the histone-DNA interaction. The lysine-substituted *dpb3* mutants also displayed reduced telomeric silencing, whose degree paralleled that of the dsDNA-binding activity of Pol ε in the corresponding *dpb3* mutants. Furthermore, additional amino acid substitutions to lysines in Dpb4p, to compensate for the loss of positive charges in the Dpb3p mutants, resulted in simultaneous restoration of dsDNA-binding activity by Pol ε and telomeric silencing. We conclude that the dsDNA-binding property of Pol ε is required for epigenetic silencing at telomeres.

In eukaryotic cells, compaction of DNA into the higher order structure of chromatin involves many highly regulated steps and is required when cells go through S-phase in which the chromatin is temporarily unpacked for DNA replication (1). Epigenetic information on maintenance of both silenced and expressed states of chromatin must also be properly propagated during S-phase for these epigenetic states to be stably transmitted to subsequent generations (2). Thus, duplication of both chromosomal DNA and its chromatin states are tightly coupled processes. Proliferating cell nuclear antigen, one of the key components in the replication machinery serving as a platform for recruiting replication proteins (3), is considered to be a factor connecting DNA replication to chromatin assembly, most probably by directing chromatin assembly factor I to replicated DNA (4). As proliferating cell nuclear antigen mutants defective for chromatin assembly factor I interaction show reduced silencing (5), replication-coupled chromatin assembly mediated by these proteins is suggested to be a step required for proper inheritance of epigenetic chromatin structures.

In *Saccharomyces cerevisiae*, DNA polymerase ε (Pol ε), another major component in DNA replication, has recently been shown to participate in the stable inheritance of the silenced state of chromatin (6). Pol ε is a four-subunit complex comprising the catalytic subunit, Pol2p, and three auxiliary subunits, Dpb2p, Dpb3p, and Dpb4p (7–11); this subunit composition is conserved from yeast to humans (12–17). Among the auxiliary subunits, Dpb3p and Dpb4p contain a histone-fold motif (11, 16), a structural motif originally found in histones and involved in histone-histone, and histone-DNA interactions (18). The motifs in Dpb3p and Dpb4p are homologous in sequence to those in NF-YC and NF-YB (two small subunits of the transcription factor NF-Y; also termed CBF), respectively (16). The structure of the NF-YC-NF-YB solved by x-ray crystallography resembles that of the histone H2A-H2B dimer (19). Cells bearing a mutation in *POL2* or lacking *DPB3* and/or *DPB4* are defective in silencing, as demonstrated by examining the expression of genes placed at telomeres. The single-cell telomeric silencing assay, which monitors switching between silenced and expressed states at telomere-proximal regions of a single cell in each cell division, revealed that either maintenance or inheritance, but not establishment, of silencing is impaired in these cells. However, the molecular mechanism underlying these observations is poorly understood.

We have previously shown that Pol ε of *S. cerevisiae* has multiple sites at which it interacts with DNA (20). Besides stable association with single-stranded DNA (ssDNA), one of these sites has a strong affinity for double-stranded DNA (dsDNA), a feature not generally associated with DNA polymerases. Since a
truncated polypeptide comprising the N-terminal half of Pol2p, containing the DNA polymerase and 3′-5′ exonuclease activities, binds only to ssDNA, the dsDNA-binding site must reside either in the C-terminal half of Pol2p or in the auxiliary subunits. Furthermore, simultaneous overexpression in *Escherichia coli* of the two non-essential subunits, Dpb3p and Dpb4p, revealed that these histone-fold motif-containing subunits form a heterodimer and associate with dsDNA. The dsDNA-binding characteristics of the Dpb3p-Dpb4p subassembly resemble those of the intact Pol ε with respect to their lack of dependence on specific sequence context and the presence of DNA ends. However, the affinity of Dpb3p-Dpb4p for dsDNA is extremely weak (KD ≈ 10 nm) to the Dpb3p-Dpb4p subassembly was not possible.

In this study, we show that neither Pol2p-Dpb2p nor Dpb3p-Dpb4p binds stably to dsDNA, but that binding is efficiently reconstituted when the two subassemblies are combined, indicating that both complexes are required for stable association with dsDNA. Further characterization of mutant forms of Pol ε, having amino acid substitutions in Dpb3p, strongly suggests that the Dpb3p-Dpb4p subassembly in intact Pol ε binds dsDNA around its dimeric histone-fold structure in a manner resembling the histone-DNA interaction. A pair of histone-fold motif-containing proteins is known to be present in transcription regulators and chromatin remodeling complexes, such as NF-Y/CBF and CHRAC (21–24). Since the histone-fold pair in NF-Y/CBF and CHRAC resembles the histone-DNA interaction, a pair of histone-fold dsDNA around its dimeric histone-fold structure in a manner resembling the histone-DNA interaction. A pair of histone-fold motif-containing proteins is known to be present in transcription regulators and chromatin remodeling complexes, such as NF-Y/CBF and CHRAC (21–24). Since the histone-fold pair in NF-Y/CBF and CHRAC resembles the histone-DNA interaction.

**EXPERIMENTAL PROCEDURES**

**Plasmid Constructions**—We used PCR to amplify from YCplac111-DPB3 plasmid DNA a fragment containing 278 bp upstream of *DPB3* and the *DPB3* coding region, preceded by a Sall site and followed by a C-terminally fused recognition sequence for PreScission protease (Amersham Biosciences) (PP sequence), which contains a Xhol site. The Sall-Xhol fragment was inserted into the pT7Blue-2 T-vector (Novagen) to yield pT7Blue-DPB3-PP. We then amplified from YTI189 genomic DNA a fragment containing a 5FLAG sequence and a 431-bp region downstream of *DPB3*, preceded by the PP sequence and followed by a SacI site. The resultant fragment was digested with Xhol and SacI and inserted into pT7Blue-DPB3-PP to yield pT7Blue-DPB3-PP-5FLAG, and the Sall-SacI insert from this plasmid was transferred into the YIplac22 integration vector to yield YIplac211-DPB3-PP-5FLAG. We made the plasmid YIplac211-POL2-5FLAG in an analogous fashion by inserting a KpnI-Sphl fragment containing a 596-bp C-terminal portion of *POL2* fused to the PP-5FLAG sequence and a 420-bp region downstream of *POL2*. YIplac211-DPB4 was constructed by inserting a KpnI-EcoRI fragment generated by PCR amplification using pBluescript-DPB4 as a template. This fragment contains the *DPB4* coding region flanked by the 788 bp immediately upstream and 25 bp downstream of the coding sequence. YCplac22-DPB4 was constructed by subcloning a BamHI-KpnI fragment containing *DPB4* and its flanking sequences from pBluescript-DPB4 into the YCplac22 vector. Amino acid substitution mutations in *DPB3* and *DPB4* on YIplac211-DPB3-PP-5FLAG, YIplac211-DPB4, YCplac111-DPB3, and YCplac22-DPB4 were introduced using the QuikChange site-directed mutagenesis kit (Stratagene), and the mutations were confirmed by DNA sequencing. Plasmids YIplac211, YCplac111-DPB3, YCplac22, and pBluescript-DPB4 were gifts from A. Sugino, Osaka University (28).

**Strains and Media**—The yeast strains used in this study are listed in Table 1. YTI189 was transformed with SnaBI-digested YIplac211-DPB3-PP-5FLAG, and the endogenous *DPB3* gene was replaced with *DPB3-3P-SFLAG* by two-step homologous recombination to yield YRT1. YTI189 was transformed with Nael-digested YIplac211-POL2-PP-5FLAG, and the endogenous *POL2* gene was replaced with *POL2-3P-SFLAG* by two-step homologous recombination to yield YRT10. A *TRP1* fragment flanked by the 80 bp just upstream and downstream of *DPB3* and a *LEU2* fragment flanked by the 80 bp upstream and downstream of *DPB4* were generated by PCR amplification using YIplac22(*TRP1*) and YCplac111(*LEU2*) as templates, respectively. To delete *DPB3* and *DPB4*, YRT10 was transformed with these PCR fragments to replace the entire open reading frames of endogenous *DPB3* and *DPB4* with *TRP1* and *LEU2*, respectively, yielding YRT13. Amino acid substitution *dpb3* mutants (YTT7, YSM2, and YSM3) were constructed by transforming YRT1 with plasmid YIplac211-dpb3-PP-5FLAG carrying various amino acid substitution mutations in *DPB3* and replacing the endogenous *DPB3-3P-SFLAG* with mutated *dpb3-3P-SFLAG*. YSM3, which carries the *dpb3L2 + α1* mutation, was transformed with YIplac211-dpb3(K16A/K18D/K19A)-S66K/T37K to replace endogenous *DPB4* with *dpb4(K6A/K16A/K19A)-S66K/T37K*, yielding YSM6. To delete the *RAD9* gene from YTI249 and YTI250, the entire open reading frame was replaced with *HIS3* by transforming the cells with an appropriate PCR fragment to yield YSM8 and YSM9, respectively. YPD (1% Bacto-yeast extract, 2% Bacto-peptone, 2% glucose, 2% Bacto-agar), YPD (1% Bacto-yeast extract, 2% Bacto-peptone, 2% glucose, 2% Bacto-agar) and YPAD (1% Bacto-yeast extract, 2% Bacto-peptone, 2% glucose, 0.004% adenine sulfate) media were prepared as described (29).

### Table 1

**Yeast strains used in this study**

| Strain | Genotype |
|--------|----------|
| YTT189* | MATα prc1–407 prb1–1122 pep4–3 leu2 trpl1 ura3–52 gal2 |
| YTT165* | MATα prc1–407 prb1–1122 pep4–3 leu2 trpl1 ura3–52 gal2 |
| YTT149* | MATα ade2–1 can1–100 his3–11,15 leu2–3 trpl1–1 ura3–1 vih–434–1 HIR3–3 TEL VR–ADE2–TEL (W303 background) |
| YTT250* | YTI249 dpb3-2::KanMX6 |
| YTT268* | YTT149 dpb3-2::KanMX6 dpb4ΔA::H9280 |
| YRT1 | YTT189 dpb3::DPB3-PP-SFLAG |
| YRT10 | YTT189 pol2::POL2-PP-SFLAG |
| YRT13 | YRT10 dpb3::DPB3-PP-SFLAG LEU12 |
| YTT7 | YRT1 dpb3-PP-SFLAG(K62A/K66A) |
| YSM2 | YRT1 dpb3-PP-SFLAG(K62A/K66A) |
| YSM3 | YRT1 dpb3-PP-SFLAG(K62A/K66A) |
| YSM4 | YRT1 dpb3-PP-SFLAG(K62A/K66A) dpb4(S36K/T37K) |
| YSM5 | YTT249 rad9Δ::H9280 |
| YSM9 | YTT250 rad9Δ::H9280 |

*Gifts from H. Araki (National Institute of Genetics, Mishima, Japan) (6).
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thermic complete (SC) medium was prepared as described (29), except that 170 mg leucine per liter and 8.5 mg para-amino-benzoic acid per liter were added.

Affinity Purification of Pol ε and Pol2p-Dpb2p—Pol ε and Pol2p-Dpb2p were purified from YRT1 and YRT13 yeast cells, respectively. All operations were performed at 0–4 °C except where noted. Cells (200 g) were grown at 30 °C in YPD medium to late log phase (where noted). Cells (200 g) were harvested by centrifugation, and washed once with H2O. The cells were divided into 10-g aliquots in 50-ml conical tubes, frozen immediately in liquid N2, and stored at −80 °C. Frozen cells (10 g) were resuspended in 10 ml of Buffer H (50 mM HEPES-NaOH, pH 7.5, 300 mM NaCl, 10% glycerol, 1 mM dithiothreitol, 0.05% Tween 20, 0.005% Nonidet P-40) supplemented with 1x complete protease inhibitor mixture (Roche Applied Science), 1% Sigma protease inhibitor (Sigma), 2 mM β-glycerophosphate, 2 mM NaF, 0.4 mM Na3VO4, and 0.5 mM sodium pyrophosphate and disrupted by adding 25 g of glass beads (0.5 mm) to the suspension and homogenizing using a Multi-Beads Shocker-MB458U(S) (Yasu Kikai), which can process 30 g of cells (10 g × 3) at a time. After removal of the glass beads, the extract was cleared of cell debris by centrifugation at 27,000 × g for 20 min. The extract was mixed with 20 ml of Sepharose 4B equilibrated with Buffer H, supplemented with protease inhibitors as above, and incubated for 1 h with gentle rotation followed by two successive filtrations through JCPW04700 (10 μm) and Type HV (0.45 μm) filters (Millipore). The filtrate was loaded on an anti-FLAG antibody (M2)-conjugated agarose (Sigma) column (5 ml), equilibrated with Buffer H containing protease inhibitors, at a flow rate of 0.5 ml/min. After washing with 5 column volumes of Buffer H containing protease inhibitors and 0.1 mg/ml BSA, and then with 10 column volumes of Buffer H, bound proteins were treated in situ with PreScission protease to cleave the 5FLAG tag from Dpb3p or Pol2p, as specified by the manufacturer, and then eluted with 5 column volumes of Buffer H at a flow rate of 0.5 ml/min; 0.5-ml fractions were collected. Pol ε subunits co-eluted in the major protein peak. The peak fractions were pooled and applied directly to a HiTrap Heparin column (1 ml) (Amersham Biosciences) equilibrated with Buffer P (10 mM sodium phosphate, pH 7.0, 1 mM EDTA, 10% glycerol, 1 mM dithiothreitol) containing 300 mM NaCl. After washing with 5 column volumes of the equilibration buffer, proteins were eluted with 10 column volumes of a linear 300 mM to 1 mM NaCl gradient in Buffer P at a flow rate of 0.1 ml/min; 0.5-ml fractions were collected. Pol ε subunits co-eluted in the major protein peak at 590 mN (for Pol ε) or 580 mM NaCl (for Pol2p-Dpb2p). The peak fractions of Pol2p-Dpb2p contained two other polypeptides (50 and 30 kDa) in small amounts. Mass spectroscopic analysis revealed that these polypeptides are C- and N-terminal truncated polypeptides of Dpb2p, respectively. The peak fractions were aliquoted, frozen immediately in liquid N2, and stored at −80 °C. The purity of the samples was estimated by SDS-PAGE to be >95%. Yields of 210 and 230 μg of Pol ε and Pol2p-Dpb2p, respectively, were obtained from 200 g of cells. Since the subunit stoichiometry (1:1:1:1) and the Stokes radius (76.0 Å) determined for Pol ε were in good agreement with the numbers that appear in the previous report in which Pol ε was concluded to be a heterotetramer (30), we assume that Pol ε in our preparation is also a heterotetramer. From the subunit stoichiometry (1:1) and the Stokes radius (68.8 Å) determined for Pol2p-Dpb2p, together with the fact that Pol ε is a heterodimer, it is strongly suggested that Pol2p-Dpb2p is a heterodimer. The specific DNA polymerase activities of Pol ε and Pol2p-Dpb2p were 23,000 and 8,000 units/mg (8.7 and 2.7 units/pmol), respectively. Mutant forms of Pol ε were purified from YTT7, YSM2, YSM3, and YSM6 cells according to the same procedure, except that 40 g of cells were used, and column sizes were reduced proportionally.

Gel Filtration Analysis—Gel filtration was carried out essentially as described (20). Protein samples were prepared in a volume of 50 μl and filtered through a Superose 6 column (2.4 ml) (Amersham Biosciences) equilibrated with Buffer S (25 mM HEPES-NaOH, pH 7.6, 10% glycerol, 1 mM EDTA, 0.005% Nonidet P-40, 400 mM sodium acetate, 5 mM dithiothreitol) at a flow rate of 10 μl/min. Fractions of 30 μl were collected. Molecular masses and Stokes radii of the reference proteins were: thyroglobulin (669 kDa, 85.0 Å), ferritin (440 kDa, 61.0 Å), catalase (232 kDa, 52.2 Å), BSA (67 kDa, 35.5 Å), and ovalbumin (43 kDa, 30.5 Å). Plasmid pKT21 DNA was used to mark the void volume.

Homology Modeling—A previous report that Dpb3p and Dpb4p are remote homologues of histone proteins (16), H2A and H2B, respectively, were confirmed by conducting a PSI-BLAST search. Making use of these weak similarities, we built the complex structure of Dpb3p-Dpb4p-dsDNA using the chicken H2A-H2B-dsDNA complex (31) (Protein Data Bank code: 1eqz, chains E, F, I, and J) as a template. Because Dpb3p/Dpb4p and H2A/H2B cannot be aligned directly, as their similarities are low (8–17% sequence identities), Dpb3p/Dpb4p sequences were first aligned with NF-YC/NF-YB sequences (19) (Protein Data Bank code: 1n1j, chains B and A) by PSI-BLAST. Next, NF-YC/NF-YB structures (19) were aligned with those of H2A/H2B with the three-dimensional structure comparison program MATRAS (32). By combining these alignments, Dpb3p/Dpb4p sequences could be aligned with H2A/H2B structures, and unaligned regions at both termini of the alignments were deleted. Using these alignments, the Dpb3p-Dpb4p complex was modeled by MODELLER version 6v2 (33). Finally, dsDNA was added to the Dpb3p-Dpb4p model in the same configuration as in the H2A-H2B-dsDNA complex.

Gel Mobility Shift Assay—The assay was done as described (20). DNA-binding reaction mixtures (5 μl) containing 35 mM BisTris-HCl, pH 6.3, 10% (v/v) glycerol, 100 μg/ml BSA, 2 mM dithiothreitol, 20 mM potassium phosphate, dsDNA substrate prepared by hybridizing two 61-mer DNA oligomers (5’-fluorescein isothiocyanate-labeled oligomer 7F + complementary oligomer 71), and either wild-type or mutant forms of Pol ε were incubated on ice for 10 min, mixed with 0.5 μl of loading buffer, and subjected to non-denaturing 4% polyacrylamide gel electrophoresis. The amounts of dsDNA and Pol ε used in the reactions were indicated in each figure.

Assay for Silencing of Telomeric URA3 and ADE2—The assay was done essentially as described (6). To assay silencing of telomeric URA3, freshly grown yeast cells (YTI249, YTI250, and YTI268) harboring various YCp plasmids were taken from SC medium depleted of appropriate amino acids and diluted to a
concentration of $2 \times 10^6$ cells/ml. 5-fold serial dilutions ($2 \times 10^6$ to $3.2 \times 10^3$ cells/ml) were then made, and 5-μl cell suspensions from each diluted sample were spotted onto SC-Leu (or SC-Leu- Trp) and SC-Leu 5-fluoro-orotic acid (5-FOA) (or SC-Leu-Trp 5-FOA) plates. The plates were incubated at 30 °C for 36 h. To assay silencing of telomeric $ADE2$, ~100 yeast cells were spread onto SC plates depleted of appropriate amino acids, and the plates were incubated at 30 °C for 3 days and stored at 4 °C for 1 week before pictures were taken.

**DNA Polymerase Activity Assay**—The assay measures incorporation of [α$^{32}$P]dTTP into acid-insoluble material using poly(dA):oligo(dT) as a DNA template. Unit definition and assay conditions were as described (34).

**Other Methods**—SDS-PAGE was carried out with ready-made NuPAGE 4–12% BisTris gels, using MOPS-SDS running buffer. Gels were stained with the Colloidal Blue staining kit (Invitrogen). When needed, protein bands were quantified using NIH Image software. During the course of purification, protein concentrations were determined with a protein assay kit (Bio-Rad). To accurately compare the activities of wild-type Pol ε, mutant forms of Pol ε, and Pol2p-Dpb2p, we determined the concentration of the final preparations by running them on the same SDS-polyacrylamide gel, measuring the intensities of the Pol2p band, and comparing these with the intensities of BSA standard bands.

**RESULTS**

**Reconstitution of dsDNA-binding Activity of Pol ε from Pol2p-Dpb2p and Dpb3p-Dpb4p Subassemblies**—To identify subunits of Pol ε responsible for the dsDNA binding, we purified the Pol2p-Dpb2p subassembly from $dpb3\Delta$ $dpb4\Delta$ yeast cells having a 5FLAG-tagged POL2 gene at the original chromosomal location of POL2. The cell extract was loaded onto an anti-FLAG antibody-conjugated agarose column, and the bound complex containing Pol2p was eluted by in situ cleavage of the tag with PreScission protease at the junction between the C terminus of Pol2p and the FLAG tag. Analysis of the eluted proteins by SDS-PAGE showed that the recovered fraction contained polypeptides with the molecular weights expected for Pol2p and Dpb2p in 1:1 ratio, and the two proteins were confirmed to be Pol2p and Dpb2p by immunoblotting analyses. The Pol2p-Dpb2p complex was further purified to near homogeneity by heparin-agarose column chromatography.

Using Pol2p-Dpb2p and Dpb3p-Dpb4p, which was purified from an E. coli strain that simultaneously overproduces Dpb3p and Dpb4p as previously described (20), we first tried to reconstitute in vitro a four-subunit complex of Pol ε. Upon gel filtration of Pol ε (26 pmol), Pol2p-Dpb2p (26 pmol), and Dpb3p-Dpb4p (300 pmol) separately through a Superose 6 column, each complex was eluted in a single peak centered on fractions 30, 31, and 37, respectively (Fig. 1, A–D). When Pol2p-Dpb2p (26 pmol) and Dpb3p-Dpb4p (300 pmol) were mixed and loaded onto the column, Pol2p, Dpb2p, Dpb3p, and Dpb4p coeluted at the position where native Pol ε eluted (Fig. 1E). The stochiometry of the four polypeptides in SDS-PAGE profiles derived from the peak fraction of either native or reconstituted Pol ε was 1:1:1:1. Thus, almost all the Pol2p-Dpb2p in the mixture seemed to have formed a complex with Dpb3p-Dpb4p.

The Stokes radii of Pol ε (both native and reconstituted), Pol2p-Dpb2p, and Dpb3p-Dpb4p were calculated to be 76.0, 68.8, and 47.1 Å, respectively. Furthermore, the presence of DNA polymerase activity in the peak fractions of either native or reconstituted Pol ε correlated well with the elution profiles of the four polypeptides (Fig. 1A). The amounts of DNA polymerase activity recovered in the peak fractions were also similar between the native and reconstituted Pol ε. These results indicate that a four-subunit complex of Pol ε that is structurally and functionally indistinguishable from native Pol ε was efficiently reconstituted from Pol2p-Dpb2p and Dpb3p-Dpb4p. It is noteworthy that the DNA polymerase activity recovered in the peak fractions of Pol2p-Dpb2p is 3-fold lower than that in the peak frac-
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of Dpb3p-Dpb4p is potentiated when the heterodimer forms a complex with Pol2p-Dpb2p. To gain insights into the DNA-binding sites on Dpb3p-Dpb4p, the three-dimensional structure of histone-fold regions of Dpb3p-Dpb4p complexed with dsDNA was built by homology-based modeling using the H2A-H2B-dsDNA complex structure (31) as a template (Fig. 3A). Since homology between these proteins is too small for a direct alignment, NF-YC and NF-YB were used as bridging proteins to map amino acid sequences of Dpb3p and Dpb4p, respectively, onto the known H2A-H2B structure (see “Experimental Procedures”). We predicted DNA-binding sites of Dpb3p and Dpb4p based on this model. First, the DNA-binding sites of H2A and H2B were assigned; residues whose solvent-accessible area, calculated using the algorithm of Lee and Richards (36), decreased by more than 10 Å² upon complexation with DNA were defined as DNA-binding sites. Next, DNA-binding sites of Dpb3p and Dpb4p were predicted as the residues corresponding to the DNA-binding sites of H2A and H2B in sequence alignments (Fig. 3B).

Isolation of dpb3 Mutants That Produce Pol ε with Reduced dsDNA-binding Activity—The histone-fold of the core histone proteins contains a structural motif, α1-L1-α2-L2-α3, comprising three α helices connected by two loops (35). We use the same nomenclature here for the putative α helices and loops that form the histone-fold structures of Dpb3p and Dpb4p. As with histones, the anti-parallel arrangement of two histone-fold domains in the heterodimeric structure of Dpb3p-Dpb4p, predicted by homology modeling, is likely to form two major types of DNA-binding site, designated L1L2 (two sites) and α1α1 (one site) (Fig. 3A). The L1L2 site comprises the L1 loop of one polypeptide in the histone-fold pair and the L2 loop of the other; likewise, the α1α1 site contains the α1 helices of each of the two polypeptides. As shown in Fig. 3B, calculations of DNA contact areas for each amino acid in H2A and H2B revealed that amino acids in the α1, L1, and L2 regions have large surfaces for interacting with DNA. Thus, substitutions of the corresponding amino acids in Dpb3p and Dpb4p might generate Pol ε with a reduced DNA-binding activity. To determine whether the histone-fold structure of Dpb3p-Dpb4p is responsible for dsDNA binding of Pol ε, we decided to substitute alanines systematically for Lys16, Lys19, Ile29, Lys62, Lys64, and Thr65 in Dpb3p, which correspond to Arg29, Arg32, Arg42, Lys75, Thr76, and Arg77, respectively, in H2A (Fig. 3, A and B). We also tested the K18D substitution (Fig. 3A), because this lysine is one of the conserved amino acids in the histone-fold regions of Dpb3p, p12 (human Dpb3p), and NF-YC, and its replacement in NF-YC with aspartic acid reduces the DNA-binding activity of NF-Y/CFB (37).

For purifying Pol ε containing amino acid-substituted forms of Dpb3p, we introduced appropriate mutations into yeast cells carrying a 5FLAG-tagged DBP3 gene at the original chromosomal location of DBP3. As well as single amino acid substitutions in Dpb3p, strains carrying double and triple substitutions in different combinations were constructed. SDS-PAGE and Colloidal Blue staining revealed that the purified mutant forms of Pol ε complexes all contained Pol2p, Dpb2p, Dpb3p, and Dpb4p in 1:1:1:1 stoichiometry (Fig. 4A). For each mutant Pol ε, yield, DNA poly-
putative L2 loop region of Dpb3p. Pol ε with a triple substitution of lysines clustering in the putative α1 helix (K16A/K18D/K19A) showed dramatically reduced band-shift intensity. Notably, titration of this mutant Pol ε in molar excess over the dsDNA gave rise to a smeary up-shift of the original shifted band. We named the former Pol ε (L2) and the latter Pol ε (α1); the corresponding mutations were designated dpb3(L2) and dpb3(α1), respectively (Fig. 3A). Further introduction of I29A or T65A substitutions into any of the combinations had no effect on the dsDNA-binding activity. When the dpb3(L2) and dpb3(α1) mutations were combined to make a quintuple-substitution mutant (K62A/K64A/K16A/K18D/K19A), the resultant Pol ε (L2 + α1) not only showed the severely reduced level in the intensity of the shifted band but also yielded, upon titration, a clear second shifted band (indicated by an asterisk in Fig. 4B), with lower mobility. When a 4-fold higher concentration of dsDNA (150 nM, 0.75 pmol/assay) was used (Fig. 4C), both the smeary up-shift by Pol ε (α1) and the second shifted band by Pol ε (L2 + α1) disappeared: Pol ε (α1) and Pol ε (L2 + α1) showed equally low and dose-dependent levels of the original shifted bands.

These results indicate that while the dpb3(L2) mutation has a minor effect on dsDNA-binding activity by Pol ε, the dpb3(α1) mutation contributes a great deal to a reduction in the strength of dsDNA binding. Accordingly, the dpb3(α1) mutation brings about a change in the mode of DNA binding: two molecules of Pol ε (α1) are able to bind one molecule of 61-mer dsDNA oligomer when the polymerase exists in molar excess over the DNA probe. However, the smeary up-shift pattern indicates that the new entity is not stable enough to be recovered during electrophoresis. Under the same circumstances, wild-type Pol ε or Pol ε (L2) forms almost exclusively the dsDNA-Pol ε complex in 1:1 stoichiometry. The change in the mode of DNA binding becomes more apparent when the dpb3(L2) mutation is combined with dpb3(α1) to create Pol ε (L2 + α1). The complex of DNA bound by two molecules of Pol ε (L2 + α1) is detected as a clear second shifted band, indicating that it is at least as stable as the complex with one molecule of Pol ε (L2 + α1). Thus, the dpb3 mutations (L2, α1, and L2 + α1) alter both the strength and the mode of dsDNA-binding by Pol ε in a stepwise manner (see “Discussion”).

merase activity (Fig. 4A), and thermostability (data not shown) were roughly the same and equivalent to those of wild-type Pol ε. These data indicate that the amino acid-substituted Dpb3p proteins can be incorporated in vivo into four-subunit complexes indistinguishable from that of wild-type Pol ε.

We next assessed the dsDNA-binding activities of the mutant forms of Pol ε and compared them with that of wild-type Pol ε. In the gel-shift assay shown in Fig. 4B, a subsaturating concentration of dsDNA probe (37.5 nM, 0.19 pmol/assay) was used to detect changes in the activity sensitively. Titration of each Pol ε in the assay revealed that any single amino acid substitution in Dpb3p had little effect on dsDNA-binding activity (data not shown). However, a subtle but reproducible reduction in the intensity of the shifted band was observed with Pol ε with K62A/K64A substitutions, both of which are located in the
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In summary, it is highly likely that binding of Pol ε to dsDNA involves the histone-fold structure of Dpb3p-Dpb4p.

**Defects in the dsDNA-binding Activity of Pol ε Correlate with the Degree of Transcriptional Silencing at Telomeres**—It has recently been reported that the silenced state of telomeric genes is not stably inherited in cells lacking Dpb3p or Dpb4p (6). We tested the possibility that the absence of silencing in dpb3Δ cells is due to the lack of dsDNA-binding activity of Pol ε by assessing the silencing ability of cells carrying the dpb3(L2), dpb3(α1), or dpb3(L2 + α1) mutation. The expression of two reporter genes, URA3 and ADE2, placed adjacent to TELVII and TELVII, respectively, was examined.

Expression of URA3 leads to cell death on medium containing the metabolic poison 5-FOA (38). Thus, growth on 5-FOA medium reflects silencing, whereas growth inhibition is indicative of a defect in silencing of URA3. While wild-type cells carrying a low-copy-number vector (YCp) could grow on 5-FOA medium, the dpb3Δ cells with the vector could not (Fig. 5A), confirming the previous results that the lack of Dpb3p causes severe impairment in silencing at telomeres (6). Introduction of low-copy-number DPB3 into dpb3Δ cells restored silencing to the level seen in wild-type cells. Growth on 5-FOA medium was slightly inhibited when YCp vector carrying dpb3(L2) was introduced but severely inhibited on introduction of dpb3(α1) and even more so when dpb3(L2 + α1) was introduced. These results show that the dpb3 mutations found to cause defects in the dsDNA-binding activity of Pol ε also affect the expression of URA3 and that the degree of impairment in dsDNA-binding activity correlates with that of de-repression of telomeric URA3 in the corresponding dpb3 mutants.

Expression of the telomere-linked ADE2 gene can be monitored by looking at colony color (39). Cells with ADE2 in the expressed state form white colonies, and those having the gene in the silenced state form red colonies. As shown in Fig. 5B, dpb3Δ cells harboring wild-type DPB3 on a YCp vector form colonies with both red and white sectors, indicating that switches occur between the silenced and expressed transcriptional states of ADE2 during colony development. The clear sectors of red and white reflect semi-stable inheritance of each epigenetic state. dpb3Δ cells carrying YCp vector form homogeneous white colonies, indicating that cells lacking Dpb3p cannot inherit ADE2 stably in a silenced state. When dpb3(L2) was introduced into dpb3Δ cells, sectors were visible but the red regions were slightly paler. On introduction of dpb3(α1), multiple narrow sectors of pink instead of the clear red sectors became dominant, and almost homogeneous white colonies formed on introduction of dpb3(L2 + α1). Thus, correlation is again observed between the level of dsDNA-binding activity of Pol ε and the ability to inherit telomeric ADE2 in the silenced state.

**Simultaneous Restoration of dsDNA-binding Activity of Pol ε and Silencing by Suppressor Amino Acid Substitutions in Dpb4p**—The parallel relationship between defects in dsDNA-binding activity of Pol ε and transcriptional silencing of the telomeric genes strongly supports our hypothesis that dsDNA-binding activity is required for Pol ε-associated silencing. We decided to connect these two observations by introducing suppressor mutations into strain dpb3(L2 + α1) that might overcome the defects in dsDNA binding by Pol ε and then examining whether the suppressor mutants simultaneously became competent in repressing transcription of the telomeric genes.

As shown above (Fig. 4B), substitutions of lysines (K16A/K18D/K19A) in the putative α1 helix of Dpb3p revealed a major contribution of these residues to stable dsDNA binding by Pol ε. These lysines are likely to constitute one of the major DNA binding sites, α1α1, which uses two neighboring α1 helices of Dpb3p and Dpb4p to hold the two DNA backbone segments at the center of the bound DNA (Fig. 3A). We reasoned that replacement of amino acids in the α1 helix of Dpb4p predicted to have large DNA contact areas with positively charged amino acids might overcome the defective dsDNA binding in the dpb3(L2 + α1) mutant by compensating for the loss of positive electrostatic charges. We examined whether substitutions of lysines for Ser16 and Thr37 in Dpb4p, which correspond to Ile39 and Tyr40, respectively, in histone H2B (Fig. 3, A and B), might alleviate the defect in dsDNA-binding activity of Pol ε (L2 +
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The Silencing Defect in dpb3 Mutants Is Not Caused by DNA Damage Checkpoint Response—It has been reported previously that DNA damage, specifically dsDNA breaks, triggers disruption of telomeric silencing by relocalization of telomeric proteins, such as Ku and Sir3, in a manner dependent on DNA damage checkpoint genes (40–42). Since a slight delay in S-phase progression is known to occur in dpb4Δ cells (11), we carried out fluorescence-activated cell sorting analysis to see if there is also such a delay in dpb3 mutants. As shown in Fig. 7A, the S-phase is prolonged slightly in dpb3Δ cells, and to a lesser extent in dpb3(L2 + a1) cells. Thus, the silencing defect we observe in the dpb3 mutant cells could occur, at least in part, via induction of the DNA damage checkpoint mechanism in response to damage that might be produced as a result of abnormal progression of the replication fork. To examine this point, telomeric silencing was characterized in cells with a rad9Δ background, lacking a gene crucial for the DNA damage checkpoint response (Fig. 7B). rad9Δ cells grew as well on 5-FOA medium as wild-type cells, indicating that the deletion of the gene itself does not affect silencing. Growth inhibition of dpb3Δ rad9Δ cells on 5-FOA medium was indistinguishable from that seen with dpb3Δ cells. Thus, the silencing defect observed in dpb3Δ cells was shown not to be occurring through a process requiring the DNA damage checkpoint response. The

the dpb3(L2 + a1) and dpb4(S36K/T37K) mutations were combined, the colonies formed had narrow pink sectors, unlike the homogeneous white of dpb3(L2 + a1) DPB4 cell colonies. Colonies formed by dpb3Δ dpb4(S36K/T37K) cells remained white, indicating that the appearance of narrow pink sectors was dependent on the presence of the mutant form of Dpb3p (L2 + a1). These results indicate that derepression of telomere-linked URA3 and ADE2 observed in the dpb3(L2 + a1) mutant is partially restored by the additional dpb4(S36K/T37K) mutation. The expression levels of URA3 and ADE2 in the suppressor mutant fall somewhere between those in the dpb3(L2) and dpb3(a1) cells.

The extent of both telomeric silencing and Pol ε dsDNA-binding activity decreased in the order wild-type > dpb3(L2) > dpb3(a1) > dpb3(L2 + a1). Furthermore, the dpb4(S36K/T37K) mutation brought about simultaneous restoration of silencing ability and Pol ε dsDNA-binding activity in the dpb3(L2 + a1) mutant; the degree of restoration was comparable in both cases. Thus, we conclude that the dsDNA-binding activity of Pol ε, provided by its histone-fold motif-containing subunits Dpb3p and Dpb4p, is required for proper silencing in S. cerevisiae.

FIGURE 5. An orderly reduction in telomeric silencing in the dsDNA-binding-defective dpb3 mutants.

A. expression of URA3 at the telomere (V:U:URA3-TEL). Yeast strains YTI249 (DPB3) harboring YCplac111 and YTI250 (dpb3Δ) harboring either YCplac11, YCplac11-DPB3, YCplac11-dpb3(L2), YCplac111-dpb3(a1), or YCplac11-dpb3(L2 + a1) were used. A set of the cell suspensions of all the strains was spotted onto a single SC-Leu or SC-Leu 5-FOA plate, as described under “Experimental Procedures.” B, expression of ADE2 at the telomere (V:ADE2-TEL). Freshly grown yeast cells of strain YTI249 (DPB3), YCplac111, YCplac11-DPB3, YCplac111-dpb3(L2), YCplac111-dpb3(a1), YCplac11-dpb3(L2 + a1), and YCplac111 were spread onto SC-Leu plates, as described under “Experimental Procedures.”

A1. Subunit composition, yield, DNA polymerase activity, and thermostability of the Pol ε purified from cells carrying the dpb4(S36K/T37K) mutation in addition to the dpb3(L2 + a1) mutation were similar to those of wild-type Pol ε, indicating that the further amino acid substitutions in Dpb4p affected neither the formation nor the function of the complex (Fig. 4A, data not shown). Pol ε (L2 + a1 + dpb4(S36K/T37K)) showed a dsDNA-binding pattern similar to that of Pol ε (a1) rather than to that of Pol ε (L2 + a1) (Fig. 4B); stable formation of the second shifted band characteristic of dsDNA binding by Pol ε (L2 + a1), as noted in the preceding section, was not observed. We interpret these changes as an alleviation of the defects in dsDNA-binding activity, attributable to the additional amino acid substitutions in Dpb4p.

We then examined whether the dpb4(S36K/T37K) mutation suppresses the silencing defects of the dpb3(L2 + a1) mutant. dpb3Δ dpb4Δ cells carrying dpb3(L2 + a1) on YCp vector were provided with either DPB4 or dpb4(S36K/T37K), also on YCp vector, and cell growth on 5-FOA medium was examined. As shown in Fig. 6A, growth inhibition of the dpb3(L2 + a1) DPB4 cells was partially suppressed by the dpb4(S36K/T37K) mutation. DPB3 dpb4(S36K/T37K) cells grew just as well as the DPB3 DPB4 cells, indicating that the dpb4 mutation itself does not affect silencing. Growth inhibition of dpb3Δ dpb4(S36K/T37K) cells was indistinguishable from that of dpb3Δ DPB4 cells.

Expression of the telomere-linked ADE2 gene was also examined (Fig. 6B). dpb3Δ dpb4Δ cells harboring DPB3 and dpb4(S36K/T37K) on YCp vectors formed colonies with clear red and white sectors that were indistinguishable from those of DPB3 DPB4 colonies, indicating that the dpb4 mutation alone does not affect the expression state of ADE2. However, when
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### A

| Strain       | Plasmids                      | SC-Leu-Trp | SC-Leu-Trp 5-FOA |
|--------------|-------------------------------|------------|------------------|
| dpb3Δ dpb4Δ  | YCplac111- DPB3               |            |                  |
|              | YCplac22- DPB4                |            |                  |
|              | dpb4 (S36K/T37K)              |            |                  |
|              | dpb4 (S36K/T37K)              |            |                  |
|              | vector                        |            |                  |
| dpb3 (L2 + α1) | dpb3 (L2 + α1)                |            |                  |

**FIGURE 6.** Partial restoration of telomeric silencing by additional amino acid substitutions in Dpb4p. A, expression of URA3 at the telomere (VII.:URA3-TEL) in yeast strains YT1268 harboring YCp plasmids as indicated in the figure was examined. B, expression of ADE2 at the telomere (VR::ADE2-TEL) was examined. Experiments were done as in Fig. 5, except that SC-Leu-Trp and SC-Leu-Trp 5-FOA plates were used.

Silencing defect in *dpb3*(L2 + α1) was alleviated slightly in *dpb3*(L2 + α1) *rad9Δ* cells, suggesting that the damage checkpoint response induced in *dpb3*(L2 + α1) cells may partially explain the silencing defect, although its contribution is small.

### DISCUSSION

Our previous finding that Pol ε binds stably to dsDNA was unexpected because it is unusual for DNA polymerases to remain associated with dsDNA, a structure analogous to their end product after DNA polymerization. The finding posed a question as to the role of dsDNA binding in the cellular processes in which Pol ε is involved. In this paper, we show that the dsDNA-binding property of Pol ε is attributable to its histone-fold motif-containing subunits Dpb3p and Dpb4p, which form a heterodimer, most likely through head-to-tail alignment of the motifs. Isolation and characterization of *dpb3* mutants defective in dsDNA binding of Pol ε demonstrated that the binding is required for the mechanism of epigenetic silencing in which Pol ε is involved (6, 43).

Only when Dpb3p-Dpb4p was combined with Pol2p-Dpb2p to form Pol ε complex was stable dsDNA binding observed (Fig. 2). Although we cannot rule out the possibility that Pol2p-Dpb2p constitutes an integral part of the dsDNA-binding site, the putative three-dimensional structure of Dpb3p-Dpb4p-dsDNA, based on the known x-ray structure of H2A-H2B co-crystallized with DNA (31), suggests that complex formation with Pol2p-Dpb2p is required to potentiate the weak dsDNA-binding activity of Dpb3p-Dpb4p, which we previously discovered, by fixing its conformation. Three arginines in the α1 helix of H2A, which are calculated to be in contact with DNA, all correspond to lysines in Dpb3p in the alignment (Fig. 3B). Substitution of two of these lysines (Lys₁⁶ and Lys₁⁹) and an adjacent lysine (Lys₂⁰) contributed to a severe reduction in dsDNA binding when Pol ε containing the mutant form of Dpb3p was tested (Fig. 4, B and C). Furthermore, the alignment assigned two other lysines (Lys₂² and Lys₆⁴) in the putative L2 loop of Dpb3p as DNA-binding sites, and they were also shown experimentally to contribute to the dsDNA binding. These data strongly suggest that the Dpb3p-Dpb4p subassembly in an intact Pol ε organizes dsDNA around its dimeric histone-fold structure through contacts with positively charged residues distributed along the DNA-binding surface, resembling the H2A-H2B-DNA interaction (18, 35). The assumption is in agreement with the finding that binding by Pol ε to dsDNA oligomers shorter than 30 bp is barely detectable (data not shown), and that 61-bp dsDNA allows only a single Pol ε molecule to bind (Fig. 4B). Substitution of a cluster of lysines, such as one in the putative α1 helix or one in the L2 loop of Dpb3p, not only lowers the overall strength of DNA binding but also makes Pol ε less effective in holding a stretch of DNA around the outer surface of its histone-fold structure. This probably enhances the chance for entry of two molecules of Pol ε (α1) or Pol ε (L2 + α1) on a 61-mer DNA (Fig. 4B).

Besides a very small amount of the free form of Dpb3p-Dpb4p, Pol ε was the only complex purified on an affinity column from yeast cell extracts containing FLAG-tagged Dpb3p, strongly suggesting that Dpb3p exists almost exclusively in the Pol ε complex in yeast cells. Thus, the apparent correlation between the level of dsDNA-binding activity of Pol ε and the silencing ability in wild-type and *dpb3* mutant cells (*dpb3*(L2), *dpb3*(α1), and *dpb3*(L2 + α1)) revealed in this study argues that the interaction between Pol ε and dsDNA is an integral part of the silencing mechanism in which Pol ε is involved. It is likely that the loss of dsDNA-binding activity of Pol ε explains, at least in part, the silencing defect previously reported for the *dpb3*Δ mutant (6). In cells lacking Dpb3p, it has been shown that switching specifically from the silenced (off) to the expressed (on) state at telomere-proximal regions occurs more frequently than it does in wild-type cells. The on-to-off switching rate is not affected. Thus, Pol ε has been proposed not to
regulate assembly of silenced chromatin but rather to participate in maintenance of the proper configuration of chromatin for duplication of silenced chromatin during DNA replication. The dsDNA-binding property of Pol ε is likely to play a role in this process. As the dsDNA-binding activity of Pol ε decreases in the dpb3 mutants (L2 > α1 > L2 + α1), the clear red sectors in a colony, indicative of successive inheritance of the telomeric ADE2 gene in a silenced state, become narrower until distinct sectors are no longer visible (Fig. 5B). This observation implies that it is the frequency with which cells transmit silenced chromatin to subsequent generations, rather than the extent of gene repression the cells attain, that correlates with the level of dsDNA-binding activity of Pol ε; this is consistent with the above hypothesis.

In addition to Pol ε, Dpb4p has been shown to form a distinct complex with three other polypeptides, Isw2p, Itc1p, and Dls1p (6). A similarity in subunit composition with a conserved chromatin-remodeling complex suggests that this second complex is yCHRAC. Dls1p, present exclusively in yCHRAC, shares significant sequence similarity with Dpb3p, and most likely exists as a histone-fold dimer with Dpb4p in this complex. From an analysis of the switching rates between the on and off states of chromatin in cells lacking Dls1p, yCHRAC has been proposed to operate in maintaining the expressed state. Thus, Pol ε and yCHRAC constitute a pair of counteracting regulators which maintain specific epigenetic states. Interestingly, it has recently been reported that a specialized set of protein complexes including Pol ε and yCHRAC are assembled on chromatin at boundary regions throughout the yeast genome that separate silenced chromatin from surrounding active regions (43). It is tempting to speculate that it is these chromatin-bound Pol ε and yCHRAC complexes that organize the chromatin configuration appropriate for faithful duplication of specific epigenetic states and ensure its transmission to subsequent generations. Consistent with this view, a silencing defect has also been observed in the HMR mating type locus in dpb3Δ cells, indicating that the role of Pol ε in silencing is not limited to the telomere region.

Whether the dsDNA-binding property of Pol ε has a role coupled to DNA synthesis or operates independently of DNA synthesis is a central question to be answered to understand how Pol ε exerts influences on the chromatin structure. Our biochemical observations indicate that the DNA polymerase activity of Pol ε is affected by Dpb3p-Dpb4p. The activity of Pol2p-Dpb2p was 3-fold lower than that of Pol ε but recovered to a level equivalent to that of intact Pol ε upon addition of Dpb3p-Dpb4p (Fig. 1). Dpb3p-Dpb4p thus has a stimulatory function for the DNA polymerase. On the other hand, the activity of mutant Pol ε carrying amino acid-substituted forms of Dpb3p gradually increases, up to 1.9-fold, as the dsDNA-binding activity decreases (Fig. 4A), implying an inhibitory effect of the dsDNA binding on DNA synthesis by Pol ε. The same increase in activity was observed with independently purified Pol ε preparations, so we regard this small increase to be significant. These results suggest that Dpb3p-Dpb4p controls Pol ε through a process involving multiple functions including its dsDNA-binding activity. One possibility is that Dpb3p-Dpb4p contributes to the processivity of Pol ε by binding to a double-stranded primer DNA region, as recently suggested by both structural and biochemical analyses (44). However, in experiments using a singly-primed ssDNA circle as a template, we found that, under our conditions, the rate and processivity of DNA chain elongation are not perceptibly affected by the absence of either Dpb3p-Dpb4p or the dsDNA-binding activity. Instead, the dynamic behavior of Pol ε during DNA synthesis upon encountering higher-order DNA structures, such as a tract of dsDNA, on the template is affected differently.5 The apparent dispensability of the DPB3 and DPB4 genes for cell viability suggests that whatever Dpb3p-Dpb4p does to affect DNA synthesis by Pol ε, as inferred from these in vitro results, is not an obligatory process for cells to replicate chromosomal DNA (8, 11). However, the prolonged S-phase observed in dpb3Δ and dpb3Δ(L2 + α1) cells implies that

5 R. Tajima and S. Maki, unpublished results.
movement of the replication fork is achieved in different ways in the presence and absence of Dpb3p-Dpb4p functions. Taken together, we think that Dpb3p-Dpb4p is not itself involved in the catalysis of DNA polymerization but rather confers on Pol ε an extra role that is performed in close association with DNA synthesis and that the dsDNA binding activity plays some part in this process. Currently, we are far from being able to say whether or not the silencing events accomplished by Pol ε require its action as a DNA polymerase. However, the additional level of control that Dpb3p-Dpb4p exerts over the behavior of Pol ε during DNA synthesis may play a key role in the proper maintenance of silencing.

Since deletion of the RAD9 gene does not abolish the defect in telomeric silencing in dpb3 cells, it should be emphasized that the defect is not caused via a mechanism described earlier (40–42) involving the DNA damage checkpoint response, which may be induced as a result of abnormal progression of the replication fork. How might the dsDNA-binding activity of Pol ε then participate in silencing? In recent work on Drosophila CHRAC (45), it has been shown that a dynamic interaction between its component subassembly, p14-p16, and DNA facilitates efficient nucleosome sliding. X-ray crystallography revealed that the two histone-fold subunits, p14 and p16, form a heterodimer whose structure closely resembles that of the H2A-H2B histone dimer. The DNA-binding surface provided by p14-p16 is speculated to contribute to transient disruption of the histone-DNA interaction by promoting distortion of DNA, which aids nucleosome sliding mediated by ACF, the catalytic component of CHRAC. We propose that Dpb3p-Dpb4p also acts in a similar way as a built-in DNA chaperone to support Pol ε during DNA synthesis on its physiological template, chromatin. Such a chaperone activity may be especially helpful for efficient DNA replication through highly condensed heterochromatic regions (46). The requirements for proper duplication of silent chromatin may be fulfilled only when the DNA is replicated with the aid of Dpb3p-Dpb4p.

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