Chromodomain Protein Swi6-mediated Role of DNA Polymerase α in Establishment of Silencing in Fission Yeast*

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Although DNA replication has been thought to play an important role in the silencing of mating type loci in Saccharomyces cerevisiae, recent studies indicate that silencing can be decoupled from replication. In Schizosaccharomyces pombe, mating type silencing is brought about by the trans-acting proteins, namely Swi6, Clr1-Clr4, and Rhp6, in cooperation with the cis-acting silencers. The latter contain an autonomous replication sequence, suggesting that DNA replication may be critical for silencing in S. pombe. To investigate the connection between DNA replication and silencing in S. pombe, we analyzed several temperature-sensitive mutants of DNA polymerase α. We find that one such mutant, swn7H4, exhibits silencing defects at mat, centromere, and telomere loci. This effect is independent of the checkpoint and replication defects of the mutant. Interestingly, the extent of the silencing defect in the swn7H4 mutant at the silent mat2 locus is further enhanced in absence of the cis-acting, centromere-proximal silencer. The chromodomain protein Swi6, which is required for silencing and is localized to mat and other heterochromatin loci, interacts with DNA polymerase α in vivo and in vitro in wild type cells. However, it does not interact with the mutant polα and is delocalized away from the silent mat loci in the mutant. Our results demonstrate a role of DNA polymerase α in the establishment of silencing. We propose a recruitment model for the coupling of DNA replication with the establishment of silencing by the chromodomain protein Swi6, which may be applicable to higher eukaryotes.

The well studied system of mating type silencing in the budding yeast Saccharomyces cerevisiae has served as a paradigm for developmental regulation of gene regulation. Although the mating type phenotype of a homothallic strain is determined by the MAT locus depending on whether it harbors the α- or α-specific alleles, two copies of the same genetic information are located at distant sites on the same chromosome, namely HML and HMR, which harbor α and α alleles, respectively. However, these alleles are transcriptionally silent. The silencing is achieved by the cis-acting sequences E (essential) and I (important) that flank both HML and HMR loci (1, 2). In addition, several genes encode factors named mating type regulator/silent information regulator (MAR/SIR) that function in trans through the cis-acting sequences in keeping the HML and HMR loci silent. Extensive studies in S. cerevisiae have suggested that DNA replication is important for repression of the silent mating type loci HML and HMR (see Refs. 1 and 2 for reviews). These findings include a requirement of passage through S phase, a functional autonomous replication sequence (ARS) flanking the silent locus HMR, and a functional origin recognition complex for silencing (reviewed in Ref. 2). However, the requirement of DNA replication for silencing is obviated if the SIR1 silencing protein is recruited by alternative means, although passage through S phase is still essential (3, 4). Thus, the exact connection between DNA replication and silencing is not clear.

In the analogous system in Schizosaccharomyces pombe, the silent loci mat2P and mat3M are repressed by several trans-acting factors, namely Swi6 (5), Clr1-Clr4 (6–8), Clr6 (9), and Rhp6 (10), and cis-acting sequences, which are associated with (ARS) activity (11, 12). In addition, these mutations also affect silencing at centromere and telomere loci (13, 14). Among these, Swi6 contains the conserved chromodomain motif that is associated with proteins involved in the assembly of heterochromatin in a large number of species, including Drosophila, mice, and humans (15), whereas the Clr4 protein performs an evolutionarily conserved function: it methylates the histone H3 at the Lys-9 position (16, 17), an activity that is critical for silencing (17). Together these observations have suggested that DNA replication may play a role in mating type silencing in S. pombe.

To check the possible role of DNA replication in silencing, we analyzed several temperature-sensitive (ts) mutants of DNA polymerase α, which is required for lagging strand synthesis during DNA replication in eukaryotes (18). We find that one such ts mutant, swn7H4 (19), is defective in silencing not only at mat2 and mat3 but also at centromere and telomere loci. Biochemical data show that DNA polα interacts with and regulates the localization of the evolutionarily conserved chromodomain protein Swi6 to the mat loci. These results indicate a direct link between DNA replication and silencing through replication-mediated recruitment of Swi6 to heterochromatin. We believe that this mechanism of heterochromatin assembly may be conserved in all eukaryotes.

EXPERIMENTAL PROCEDURES

Materials and Reagents—Media components were purchased from Difco (Detroit, MI) or SRL (Mumbai, India). MuLV reverse transcriptase, the expression vector pMALp2, and anti-MBP2 antibodies

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1 R. N. Dubey and J. Singh, unpublished data.

2 The abbreviations used are: MBP, maltose binding protein; PCR, polymerase chain reaction; polα, DNA polymerase α; swi, switch; clf, cryptic loci regulator; Rhp6, rad6 homologue in S. pombe; ChIP, chromatin immunoprecipitation; ARS, autonomous replication sequence; polα, DNA polymerase α; MBC, maltose-binding protein; RSA, replication-silencing autonomous; MAR, mating-type-silencing autonomous; SIR1, silent information regulator 1.
were purchased from New England Biolabs. Ni-NTA resin was from Qiagen. The nylon membranes for Southern and Western blotting were from Amersham Pharmacia Biotech. The alkaline phosphatase and horseradish peroxidase conjugated antibodies were from Promega (Madison, WI). Glutathione-agarose was from Sigma. Oligonucleotides were from Qiagen. The nylon membranes for Southern and Western blotting were purchased from New England Biolabs. Ni-NTA resin was from BARC, Mumbai, India. The x-ray films were from Hindustan Photo Films. Isopropyl-1-thio-
β-D-galactopyranoside and 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal) were from Promega (Madison, WI). GST-agarose was from Sigma. Oligonucleotides were from Ransom Hill Biosciences. The enhanced chemiluminescence (ECL) kit was purchased from Amersham Pharmacia Biotech. Strains and Media—Media compositions have been described (20). Strains for monitoring expression of ura4 marker at mat2, mat3, and centromere have been described earlier (6, 8, 14). For serial dilution assay, several 10-fold serial dilutions of strains grown overnight were spotted on complete and selective plates. The his3-telo strain, in which the his3 gene is inserted at the telomere, was a gift from P. E. Allshire (21). For iodine staining, the colonies were grown on PMA plates for 3–4 days and stained with iodine (20).

Reverse Transcriptase-PCR and Southern Blot Analysis—The conditions for reverse transcriptase-polymerase chain reaction and Southern blotting for detecting mat2Pc and pola transcripts have been described (10).

Chromatin Immunoprecipitation (ChIP) Assay—A ChIP assay to detect Swi6 localization at the mat region was carried out as described (22). The oligonucleotides used were GGTTAGGAAAAAGAAAGAACAGATGATTGGAGG and CATACTAAATGTTAAAGTGAAG-GACC for mat1M (310 base pairs), GGTCTCTTTATCTTGGATCC and ACTCGTTCTCATAATGTAAGTAGAATGTTGAAGG and CATACTAATAATGTAAGTAGAA-TGCTCTTAATCTTGGATCC. The PCR product was restricted with NotI and cloned at NotI site of the vector pREP1NHA. The MBP-Polo fusion protein was immobilized with Ni-NTA beads (Qiagen, 100 μl of 2% suspension in binding buffer) to which recombinant (His)_6-Swi6 had been immobilized. After collection of the unbound fraction and suitable washings with the binding buffer (50 mM NaH₂PO₄, pH 8.0, 300 mM NaCl), the input, unbound flow through (FT), and bound (EL) fractions (individual beads) were subjected to immunoblotting. Ni-NTA beads (Qiagen) were equilibrated with binding buffer containing 50 mM NaH₂PO₄ (pH 8.0), 300 mM NaCl, and 10 mM imidazole. 300 μg of extract from cells expressing (His)_6-Swi6 was allowed to bind the equilibrated Ni-NTA beads at 4 °C for 30–60 min. The beads were then washed with binding buffer containing 50 mM imidazole (30 min at 4 °C). After the washing step, 500 μg of the concentrated extract from cells of S. pombe (wild type and swi7H4) was added to the (His)_6-Swi6-conjugated Ni-NTA beads and mixed for 2 h at 4 °C. The mixture was centrifuged to obtain the supernatant (FT) fraction. After a single washing, the bound protein was eluted with 35 μl of elution buffer (binding buffer containing 250 mM imidazole). Elution was performed at 4 °C for 30 min. This represented the bound fraction (EL).

RESULTS

swi7H4, a ts Mutation in DNA Polymerase α, Alleviates Silencing at the Mating Type Loci—A marker gene ura4, when placed at mat2, mat3, or cen (centromere) and telomere loci, is subject to silencing. Strains harboring such a marker grow poorly on plates lacking uracil (ura^-); 6, 14). However, in silencing defective mutants such as swi6 and clr1-clr4, the expression of the ura4 marker gene is enhanced, as indicated by increased growth level on ura^- plates (6–8, 14). To check the requirement of DNA polo for silencing, several ts mutants of polo were generated in S. pombe, but they showed no silencing defect. However, swi7H4, an independently isolated ts mutant of polo with a replication checkpoint defect (19), elicited enhanced growth of strains carrying ura4 marker at mat2 and mat3 loci on ura^- plates and reduced growth on FOA plates (FOA allows growth of ura^- cells but not ura^- cells).
and b; see also Refs. 6 and 20), indicating a derepression of the ura4 gene. A heterothallic strain in which the centromere-proximal silencer element was deleted (denoted by silencers I and II in Fig. 1a), called Msmto ∆mat2::ura4 (8), also showed a higher growth level on ura plates and no growth on FOA plates because of the swi7H4 mutation (Fig. 1b).

Enhanced Silencing Defect in Silencer Deletion Background in the swi7H4 Mutant—Heterothallic strains such as Msmto and Msmto ∆mat2::ura4 do not switch and express only the minus (M) transcripts from the mat1 locus. Expression of the silent mat2P transcripts in these strains triggers meiosis, leading to sporulation in haploid cells (the phenotype is called haploid meiosis (hm)). The spores contain a starchy compound, which itself does not give iodine staining in the silencer deletion background (Msmto, swi7H4), which itself does not cause any increase in iodine staining and the level of haploid meiosis was due to enhanced expression of the silent transcript mat2P, quantitative reverse transcriptase-PCR analysis was carried out. PCR under logarithmic conditions (10 cycles; see Ref. 10) could not detect the mat2P transcript (10) in Msmto (Fig. 2c, lane 1) and Msmto ∆mat2::ura4 strains (Fig. 2c, lane 2). However, the Msmto, swi7H4 mutant strain expressed detectable mat2P transcript (Fig. 2c, lane 3), which was elevated by ~8-fold in the silencer deletion background (Msmto ∆mat2::ura4 swi7H4; Fig. 2b, lane 4). The level of pola transcript was not affected (Fig. 2c, lower panel), justifying its use as a control. These results indicate that DNA pola is required to establish silencing at the mat2 locus, the efficiency of which is regulated by the cis-acting silencer. Because swi6 and clr1-clr4 mutants also do not give iodine staining in the Msmto background but yield dark staining in the silencer deletion background (Msmto ∆mat2::ura4; Ref. 8), our results suggest that pola and Swi6 (and possibly Cln1-Cln4) may act at the same step in silencing.

**Pola and cds1 Genes Suppress the Checkpoint Defect but Not the Silencing Defect of the swi7H4 Mutation—**All the above assays were performed at 30 °C. It is possible that the swi7H4 mutation, which is reported to exert a checkpoint defect at 36 °C (19), may have a residual growth defect at 30 °C or may have a prolonged S phase. However, we found that the swi7H4 mutant grows at a level similar to wild type strains at 30 °C. Furthermore, both the pola and cds1 genes, which are known to suppress the ts and checkpoint defects of the swi7H4 mutant (19), allowed growth of the swi7H4 mutant at 36 °C (Fig. 3a),
confirming that both the genes suppress the growth defect of the swi7H4 mutant. Similarly, microscopic examination showed that although the mutant strain with the control vector still displayed the "cut" phenotype at 36 °C (chromosomes un-
timely torn; 13% of cells display cut phenotype after growth at 36 °C for 8 h), the pola and cds1 genes suppressed this phenotype completely with 0% of cells displaying the cut phenotype (Fig. 3b). However, most interestingly, the dark staining of the swi7H4 strain in the silencer deletion background was not suppressed by either the pola and cds1 genes (Fig. 3c). Therefore, the silencing defect in the swi7H4 mutant is not due to a prolonged S phase or replication checkpoint defect. On the other hand, the effect appears to be dominant, suggesting that the pola may participate in a silencing multimolecular complex.

Because the silencing defect of the swi7H4 is dependent on the silencer, similar to Swi6 and Clr1-Clr4, we checked whether the silencing defect caused by the swi6 mutation requires DNA pola. Interestingly, we find that overexpression of swi6 gene could suppress the iodine staining of the swi6Δ strain in the silencer deletion background but not if the swi7H4 mutation was also present (Fig. 3d). Thus, Swi6 requires wild type pola to establish silencing, and the effect of the swi7H4 mutation is dominant.

swi7H4 Mutation Abrogates Silencing at Both Centromere and Telomere Loci—Because mutations in swi6 and clr1-clr4 affect silencing at mat, centromere, and telomere loci, we also checked the effect of the swi7H4 mutation on silencing at the cen and telomere loci. The leaky expression of the ura4+ marker gene placed at three different locations within the cen1 locus (14) was enhanced by the swi7H4 mutation, as indicated by reduced growth on FOA plates (Fig. 4a). Likewise, the expression of the his3 gene placed at the telomere locus on chromosome I, which was completely lacking in the wild type strains, was derepressed in the swi7H4 mutant, as indicated by growth on His− plates (Fig. 4b). Thus, similar to the swi6 mutation (14), the swi7H4 mutation also abrogates silencing at all three heterochromatin loci in S. pombe.

Delocalization of Swi6 from the mat Locus in the swi7H4 Mutant—Swi6p has been mainly localized to three heterochro-
matin loci, namely mat, telomere, and centromere, as revealed by fluorescence in situ hybridization analysis (22), but becomes delocalized in clr4 and rik1 mutants (13). Therefore, we checked Swi6 localization in the wild type and swi7H4 mutant by expressing a plasmid containing gfp-Swi6 fusion in place of the endogenous swi6 gene (23, 24). Fluorescence microscopy showed that nearly 66% of nuclei contained three fluorescent foci in wild type cells with 26% of cells containing two foci and only 1% of cells having one foci; the remaining 7% of cells showed four foci. However, in swi7H4 mutant, the number of cells containing three foci was reduced by 50% with an increase in the number of cells with two or one foci by nearly 2 and 15-fold, respectively, as compared with the wild type cells (Fig. 5a).

To directly assess the localization of Swi6 to the mat loci, we carried out the ChIP assay with wild type and swi7H4 mutant strains in which the hemagglutinin-tagged swi6 gene was inserted in place of the normal swi6 gene. The results of ChIP assay confirmed the Swi6 localization at mat1, mat2, and K regions in wild type cells (Fig. 5b, lane 2) but not in the swi7H4 mutant (Fig. 5b, lane 5). Quantitative PCR showed a reduction in Swi6 localization by >10-fold in the swi7H4 mutant as
compared with wild type cells, and the localization at mat1 and mat2 was 5-fold less than that at K region. No localization of Swi6 was detected at the control gene, histone H2B (Fig. 5b, lane 2), even by radiolabeling.

Direct Physical Interaction between Wild Type but Not Mutant DNA pola and swi6 in Vivo and in Vitro—To check whether localization of Swi6 may be because of direct physical interaction between DNA pola and Swi6, we checked the binding of recombinant MBP-Pola fusion protein to the (His)6-tagged Swi6 protein immobilized on Ni-NTA resin. Results showed that MBP-Pola fusion protein was specifically retained by the Ni-NTA resin to which (His)6-tagged Swi6 was immobilized (Fig. 6a, compare lane 5 with lane 6) as the MBP-Pola fusion protein appeared in the bound fraction (Fig. 6a, lane 5, EL) but not in the flow through fraction (Fig. 6a, lane 6, FT).

MBP alone (Fig. 6a, lanes 11–13) did not bind as it appeared only in the flow through fraction (Fig. 6a, lane 11, FT), not in the bound fraction (Fig. 6a, lane 10, EL). Furthermore, MBP-Pola also did not bind to the Ni-NTA resin (Fig. 6c) as it appeared only in the FT fraction not in the bound (EL) fraction when the binding of the MBP-Pola to the Ni-NTA resin was checked (Fig. 6a, lanes 9 and 10). These results indicate that pola binds specifically to Swi6 in vitro.

To check whether pola interacts with Swi6 in vivo, we transformed a construct carrying (His)6-tagged pola gene in the vector pART1 (20) into a strain carrying a disruption of the pola gene, as described earlier (25). The whole cell extract prepared from these cells was fractionated by Ni-NTA chromatography to purify the (His)6-tagged pola protein by elution with 250 mM imidazole and immunoblotted. Interestingly, the bound fraction showed the presence of both pola and Swi6 as probed by the respective antibodies (Fig. 6b, left panel). To check whether the binding of Swi6 to the Ni-NTA was because of copurification of Swi6 with pola and not due to nonspecific retention by the Ni-NTA resin, the binding of recombinant GST-Swi6 fusion protein to the Ni-NTA column was checked.

Results showed that GST-Swi6 does not bind to the Ni-NTA column by itself as it appeared in the flow through (Fig. 6b, right panel, lane 2, FT) but not in the bound (Fig. 6b, right panel, lane 3, EL) fraction. These results strongly argue that pola also interacts with Swi6 in vivo.

The delocalization of Swi6 in the swi7H4 mutant may be because of a lack of interaction between the swi7H4 mutant protein and Swi6, or it may be an indirect effect. To check this, we prepared extracts from the wild type and swi7H4 mutant cells and incubated them with Ni-NTA column to which (His)6-tagged Swi6 had been immobilized. After collecting the FT and the bound (EL) fractions were eluted with 250 mM imidazole. The fractions were subjected to immunoblotting with anti-Pola antibody. Interestingly, we observed that although nearly 40% of the pola is bound to the Swi6 in the wild type extracts (Fig. 6c, lane 4, EL), no detectable band was observed in the bound fraction of the swi7H4 mutant extract (Fig. 6c, lane 6, EL) as almost all of it appeared in the flow though fraction (Fig. 6c,

Interaction between Polα and Swi6 in Silencing

Fig. 6. Polα interacts with Swi6 both in vitro and in vivo. a, in vitro interaction. Extracts from uninduced and induced cultures of TB1 cells expressing the MBP-Polα fusion protein were immunoblotted with preimmune (lanes 1 and 2) or anti-Polα antibody (lanes 3 and 4). Binding of extracts prepared from cells expressing MBP-Polα (lanes 5–7) and MBP (lanes 11–13) to (His)6-Swi6 immobilized on Ni-NTA column and MBP-Polα fusion protein to Ni-NTA resin (lanes 8–10). The input (lanes 7, 8, and 13), FT fraction (lanes 6, 9, and 12), and bound fraction (EL, lanes 5, 10, and 11) were immunoblotted with anti-Polα (lanes 5–7 and lanes 8–10) or MBP antibody (lanes 11–13). b, left panel, copurification of Polα and Swi6 by Ni-NTA chromatography of extracts prepared from cells carrying polα gene disruption and harboring the plasmid pART1 containing the (His)6-tagged polα gene. The extract was subjected to Ni-NTA chromatography. The bound fraction was immunoblotted with anti-Polα and Swi6 antibodies. Right panel, binding of GST-Swi6 fusion protein to Ni-NTA beads. The input (lane 1), FT (lane 2), and bound fractions (EL, lane 3) were immunoblotted with anti-GST antibody. c, binding of wild type but not the mutant Polα to Swi6 in vitro. Extracts from wild type (lane 1) and swi7H4 mutant (lane 2) were incubated with Ni-NTA resin to which the extract from cells expressing (His)6-Swi6 was immobilized. FT (lanes 3 and 5) and bound fractions (EL, lanes 4 and 6) for the wild type (lanes 3 and 4) and swi7H4 mutant (lanes 5 and 6) were subjected to immunoblotting with anti-Polα antibody.

lane 5, FT). Thus, although the wild type polα interacts with Swi6, the swi7H4 mutant polα does not give any detectable interaction with Swi6 in vitro.

Silencing Defect Is Displayed by Other polα Mutants Localized in Conserved Regions of DNA Polymerase—To find out whether mutations in certain regions of polα are required for silencing, we tested two other ts mutants of polα, namely, ats11 and ats13 (26) and the viable mutant of polα namely swi7–1 (25). Interestingly, both ats11 and ats13 mutants also exhibited iodine staining (Fig. 7a) and haploid meiosis in the Msmto background, indicating a silencing defect, with ats11 showing a stronger effect (Fig. 7a). However, the swi7–1 mutant, which is defective in generating the double strand break at mat1 locus (23), did not show such a defect (Fig. 7a). The ats11 and swi7H4 mutations map to the homology boxes II and VI, respectively, that are conserved in all DNA polymerases and are located within the nucleotide-binding domain (26), whereas ats13 maps close to the domain D, which is conserved in the α class of DNA polymerases (Fig. 7a; see also Ref. 26). However, the swi7–1 mutation is not located in any conserved region (Fig. 7a; see also Refs. 25 and 27). Thus, the residues involved in silencing are localized to regions that are conserved in all DNA polymerases.

DISCUSSION

Passage through a distinct number of replication cycles is the hallmark of several developmental systems (reviewed in Ref. 28), indicating that replication may help in the setting up of developmentally regulated switches of expression or repression of specific loci or genes. The main objective of this study was to investigate the involvement of DNA replication in the establishment of silencing in fission yeast. Our results, obtained mostly with swi7H4, a replication checkpoint mutant of DNA polα, show that this mutation affects silencing at the three main heterochromatin loci in fission yeast namely, mat, cen, and telomere. At the mat loci, the silencing defect is accentuated by the deletion of the cis-acting silencer flanking the mat2 locus. Because this silencer-dependent phenotype is also exhibited by swi6 and clr1-clr4 mutants, we inferred that Polα and Swi6/Clr1-Clr4 may participate in the same pathway. Accordingly, we observe that Polα and Swi6 interact with each other both in vivo and in vitro. The localization of Swi6 to the mat loci in wild type cells is abolished in the swi7H4 mutant. These findings suggest that DNA polα may be directly involved in the localization of Swi6 to the heterochromatin loci. This interpretation is supported by two results. First, the mutant polα does not interact with Swi6 in vitro. Second, the effect of the swi7H4 mutation on silencing is not reversed by either polα or cds1 genes. The swi7H4 mutation is reported to be defective in the replication checkpoint. Thus, it is possible that an altered chromatin structure generated in the swi7H4 mutant, which signals the checkpoint defect, may lead to delocalization or mislocalization of the heterochromatin-associated proteins such as Swi6. However, this possibility is discounted by the fact that the mutant exhibits normal growth and no residual cut phenotype, indicative of the lack of checkpoint defect at 30 °C, the condition under which the silencing defect is observed. Furthermore, although the cut phenotype observed at 36 °C is suppressed by cds1 and polα genes, the silencing defect is not suppressed, indicating that the delocalization of Swi6 is not due to an altered chromatin structure generated by replication checkpoint defect. Lastly, the suppression of the silencing defect in the swi6 mutant by the swi6 gene occurs only if wild type
that localization of Swi6 is disrupted in the H3 at Lys-9 position (to which Swi6 binds specifically) must have been shown that the function of Clr4, which methylates the histone, suggesting an imprinting function of Swi6 in establishing the chromatin state that is present. Thus, the mutant pola may recruit Swi6 through direct interaction. After binding to the methylated Lys-9 of histone H3 in the nucleosomes (16, 17), Swi6 may form multimers (24), leading to a cooperative unfolding of the heterochromatin structure at the mat, telomer, and centromere regions. Because Swi6 is bound to the mat region throughout the cell cycle (29), the role of pola may be to recruit Swi6 to the newly replicated DNA strands. In addition, silencing is associated with the hypoacetylation of histone H4 (29). It remains to be determined whether the Lys-9 methylation in histone H3 or acetylation level of histone H4 are altered in the swi7H4 mutant. Further studies will help to decipher the order of events involved in heterochromatin assembly with respect to DNA replication.

The role of DNA replication in silencing has been actively investigated in S. cerevisiae. Recently, it was shown that in a setup where the rate-limiting silent information regulator Sir1p was recruited independently of the cis-acting silencer/ARS, silencing could be decoupled from DNA replication (3, 4), although passage through S phase was still essential. However, in normal cells, the recruitment of Sir1p is presumably through the origin recognition complex (ORC), which suggests that at least in normal cells, the assembly of the functional replication origin is critical for silencing (2). Likewise, several reports have linked replication and chromatin assembly with silencing. For example, mutations in proliferating cell nuclear antigen, replication factor-C, Polα, and Polα are shown to affect silencing in S. cerevisiae (32–34). Proliferating cell nuclear antigen has been shown to be important for proper positioning of nucleosomes in the in vitro chromatin assembly function of CAF1 (35). Similarly, mutations in the chromatin assembly factor CAF1 affect the inheritance of the marked epigenetic states in S. cerevisiae (36).

In view of the above, our results for the first time provide evidence for a direct role of DNA replication in the assembly of the heterochromatin state. Our recent results show that the dark phenotype of the swi7H4 mutant exhibits stable inheritance during mitosis and converts to the silent state at a low rate (37, 38). In the meiotic cross as well, these alternate states behave similar to stable Mendelian genetic markers (37, 38). Thus, our studies suggest that DNA polymerase α may perform an imprinting function in establishing a chromatin state that is competent to recruit the components of the heterochromatin machinery, similar to Swi6. Because Polα and Swi6 are important and conserved components of DNA replication and heterochromatin assembly, respectively, the Polα-Swi6 interaction may be highly conserved during evolution and may serve as a model for gene regulation during development in higher eukaryotes.

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