Two Ubiquitin-Conjugating Enzymes, Rhp6 and UbcX, Regulate Heterochromatin Silencing in Schizosaccharomyces pombe

Eun Shik Choi,¹ Hyun Soo Kim,¹ Yeun Kyu Jang,¹,²* Seung Hwan Hong,¹ and Sang Dai Park¹*

School of Biological Sciences, Seoul National University, Seoul 151-742,¹ and Branch of Lung Cancer Research, Division of Common Cancer Research, National Cancer Center, Goyang 411-764,² Republic of Korea

Received 5 June 2002/Returned for modification 23 July 2002/Accepted 28 August 2002

Methylation of histone H3 has been linked to the assembly of higher-order chromatin structures. Very recently, several examples, including the Schizosaccharomyces pombe mating-type region, chicken β-globin locus, and inactive X-chromosome, revealed that H3-Lys9-methyl (Me) is associated with silent chromatin while H3-Lys4-Me is prominent in active chromatin. Surprisingly, it was shown that homologs of Drosophila Su(var)3-9 specifically methylate the Lys9 residue of histone H3. Here, to identify putative enzymes responsible for destabilization of heterochromatin, we screened genes whose overexpressions disrupt silencing at the silent mat3 locus in fission yeast. Interestingly, we identified two genes, rhp6 and ubcX (ubiquitin-conjugating enzyme participating in silencing), both of which encode ubiquitin-conjugating enzymes. Their overexpression disrupted silencing at centromeres and telomeres as well as at mat3. Additionally, the overexpression interfered with centromeric function, as confirmed by elevated minichromosome loss and antimicrotubule drug sensitivity. On the contrary, deletion of rhp6 or ubcX enhanced silencing at all heterochromatic regions tested, indicating that they are negative regulators of silencing. More importantly, chromatin immunoprecipitation showed that their overexpression alleviated the level of H3-Lys9-Me while enhancing the level of H3-Lys4-Me at the silent regions. On the contrary, their deletions enhanced the level of H3-Lys9-Me while alleviating that of H3-Lys4-Me. Taken together, the data suggest that two ubiquitin-conjugating enzymes, Rhp6 and UbcX, affect methylation of histone H3 at silent chromatin, which then reconfigures silencing.

In eukaryotic cells, chromosomes can be partitioned into two structurally and functionally distinct domains, called euchromatic and heterochromatic regions (5, 33). Unlike the case for euchromatin, heterochromatic regions are condensed even during interphase, and nearby or embedded genes are transcriptionally repressed (called position effect variegation, or silencing). Assembly of these chromatin structures has been linked to posttranslational modification of histone N-terminal tails, including acetylation and phosphorylation (19). In general, heterochromatin contains hypoacetylated histone H3 and H4 compared to the case for euchromatin (7).

Owing to the findings that mammalian and Schizosaccharomyces pombe homologs of Drosophila Su(var)3-9 encode enzymes that specifically methylate histone H3 on lysine 9, histone methylation has emerged as another important modification that distinguishes heterochromatin from euchromatin (30). Methylation of H3 at Lys4 or Lys9 was shown to be reciprocally associated with euchromatin regions and heterochromatic regions, respectively (8, 12, 21, 22, 27, 29). Recently the mechanism by which the H3 methylations are translated into transcriptional states is delineated by the observation that HP1 proteins can bind to Lys9-methylated H3 via their chromo domains (4, 21).

In fission yeast, at least four loci (centromeres, telomeres, silent mating-type loci, and ribosomal DNA) are silenced by heterochromatin-like structures (3, 36). Of the common silencing factors, Clr4, a homolog of Su(var)3-9, has intrinsic H3 Lys9-specific methyltransferase (HMTase) activity both in vitro and in vivo (25). Furthermore, Clr4 recruits Swi6, a fission yeast homolog of HP1, to heterochromatins, suggesting that heterochromatin formation of fission yeast resembles that of higher eukaryotes (9). In addition, Clr3, an H3-specific deacetylase, and Rik1 are required for H3-Lys9 methylation (25). H3-specific deacetylases, such as Clr3 and Clr6, create circumstances favoring methylation at H3-Lys9 by the Clr4/Rik1 complex. Then, methylation induces binding of Swi6, leading to the establishment of a silent chromatin. Once bound to methylated H3, Swi6 serves as an epigenetic imprint for the inheritance of silent chromatin, possibly by recruiting HMTase or other enzymes required for heterochromatin formation after the completion of DNA replication (26). Supporting this model, it was recently shown that Swi6 remains associated with the heterochromatic mat2/3 region throughout the cell cycle, and the mouse homolog of Swi6, M31, physically interacts with Su(var)3-9 (1, 26).

At present, although knowledge of a robust linkage between histone methylation patterns and heterochromatin formation is massively accumulating, it still remains to be understood how the methylation process itself is regulated. Namely, while self-reinforcing mechanisms might be advantageous for the maintenance of silent chromatin, indeed cells may require reconfiguration of silenced chromatin, such as removal of the methyl marker from histone for proper cellular functions, including
DNA replication, and mating-type switching, etc. Since histone demethylases are not found yet, the methyl markers of H3 might be removed through the proteolytic pathway (17, 37).

In this report, we demonstrate that two ubiquitin-conjugating enzymes (Ubc or E2), Rhp6 and UbcX, are required for reconfiguration of silenced chromatin in fission yeast. Expressions of RNA Pol II-transcribed genes at heterochromatin are dependent on the dosage of Rhp6 and UbcX. Interestingly, reconfigured silencing induced by altered dosage of the Ubc correlates with the H3 methylation patterns, suggesting a mechanistic link between ubiquitin conjugation and histone H3 methylation.

MATERIALS AND METHODS

Media. Media were used as described previously (24). For low-adenine medium, YE (2% glucose, 0.5% yeast extract) plates not supplemented with adenine, YE (2% glucose, 0.5% yeast extract) plates not supplemented with adenine were used as described previously (24). For low-adenine minimal plates. For incubation for 4 to 5 days at 30°C, only red-white half-sector colonies were counted to determine the loss rate of the ade6+ marker per division. The minichromosome loss rate was calculated by dividing the number of these half-sector colonies by the total number of white colonies plus half-sector colonies.

Chromatin immunoprecipitation (ChIP) assay. Cells (250 ml) were grown to a density of 10° cells/ml and cross-linked with 1% formaldehyde for 20 min at room temperature. Cross-linking was stopped by adding glycine to a concentration of 380 mM for 5 min. Cells were harvested and washed twice with Tris-buffered saline and lysed with glass beads in a lysis buffer (50 mM HEPES-KOH [pH 7.5], 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Na deoxycholate, 0.5% sodium dodecyl sulfate, 1 mM phenylmethylsulfonyl fluoride). The chromatin was sheared by sonication for 20 s eight times. H3 methylated at Lys9 or Lys4 was immunoprecipitated overnight at 4°C with anti-dimethyl lysine 9 or lysine 3 antibody and protein A-Sepharose beads. From the immunoprecipitates, DNA was released and purified as previously described (20). Purified DNA was PCR amplified with specific primers. PCR was carried with [α-32P]dCTP, and the products were resolved by 6% polyacrylamide gel electrophoresis and exposed to X-ray film.

RESULTS

Identification of cDNAs that disrupt silencing at the matα locus when overexpressed. In fission yeast, prototrophic marker genes placed near the silent matα regions, such as mat2-P and matα-M, are subject to transcriptional silencing. Since strain PG9 was previously constructed to contain the ura4+ gene adjacent to the matα locus (35), the silencing was monitored by using a phenotypic assay for ura4+ expression. Normally, very few cells can grow on uracil-free medium due to the silencing of ura4+ in the reporter cells. Interference with matα silencing increases ura4+ expression and then gives rise to mixed clones exhibiting a unique phenotype of Ura+ and FOA sensitivity.

To identify genes whose overexpression reconfigures the matα silencing, genetic screening was performed using PG9.
and an S. pombe cDNA library. Among 5 x 10^6 transformants screened, 20 displayed plasmid-linked Ura^+ and FOA sensitivity phenotypes. Sequence analysis revealed that 16 of them contained the same sequence encoding Rhp6, which is regarded as an S. pombe homolog of the Saccharomyces cerevisiae E2 enzyme, Ubc2/Rad6 (31). The other four clones contained the same gene encoding a novel putative E2 enzyme that we named ubcX^+ (ubiquitin-conjugating enzyme X). The sequence of ubcX^+ matches perfectly databases released by the S. pombe Genome Project at Sanger Centre. The ubcX^+ sequence has four exons and five introns and encodes a protein of 167 amino acid residues (Fig. 1). The deduced amino acid sequence of UbcX shows 68, 51, and 52% identities with those of human UbcG, S. cerevisiae UBC7, and S. pombe Ubc7, respectively, and also contains a catalytic cysteine residue required for E2-ubiquitin thioester formation. Sequences were aligned using Clustal W, and the output was generated using Genedoc. Identical residues are shown in white text with a black background. Conserved residues are shown with gray shading.

FIG. 1. Sequence alignment of UbcX. (A) ubcX^+ open reading frame. Four introns (open boxes) interrupting the open reading frame and filled boxes indicating exons are shown. The ura4^+ gene was used to disrupt the coding region of ubcX^+, as indicated. (B) Comparison of the deduced amino acid sequences of UbcX, human UbcG, S. cerevisiae UBC7, and S. pombe Ubc7. The asterisk indicates the putative cysteine residue required for E2-ubiquitin thioester formation. Sequences were aligned using Clustal W, and the output was generated using Genedoc. Identical residues are shown in white text with a black background. Conserved residues are shown with gray shading.

In summary, these observations demonstrate that overexpression of rhp6^+ or ubcX^+ disrupts silencing at other heterochromatic regions. To determine whether the derepression is gene specific, mat3 silencing was assayed using ade6^+ as a reporter gene. A reporter strain with ade6^+ at mat3 (Hu51) was used as a host, and then ade6^+ expression upon overexpression of rhp6^+ or ubcX^+ was measured. As seen in Fig. 2B (top), the overexpression also derepressed ade6^+ expression, confirming that the effect is not gene specific.

Next, we questioned whether the action of each ubc gene was limited to mat3 silencing. When rhp6^+ or ubcX^+ was overexpressed, the repression of ade6^+ was significantly reduced in telomere reporter cells, as in Hu51, but the effect was slight in centromere reporter cells (Fig. 2B). In addition, this derepression was confirmed by competitive RT-PCR between wild-type ade6^+ mRNA from each heterochromatin and a truncated ade6-DN/N mRNA from the endogenous ade6 locus. As shown in Fig. 2C, ade6^+ transcript from the mat3 locus or telomeres was barely detectable in the wild-type cells containing vector only, but overexpression of each ubc gene increased the level significantly, up to 30% of the ade6-DN/N level. However, there was little effect on derepression of ade6^+ at the centromere, indicating that the derepression was not enough to be detected in our hands.

In summary, these observations demonstrate that overexpression of rhp6^+ and ubcX^+ affects silencing at all heterochromatic regions, suggesting that they act as general silencing regulators, although the effect is somewhat moderate at centromere.

Overexpression of rhp6^+ or ubcX^+ impairs centromeric functions in a dosage-dependent manner. Ade^+ cells form white colonies on low-adenine medium, while Ade^- cells form red colonies, thus allowing the use of colony color as a test of silencing. Thus, a wild-type reporter strain with ade6^+ placed near heterochromatin produces predominantly red colonies.
Overexpression of \( \text{rhp6}^+ \) or \( \text{ubcX}^+ \) was sufficient to turn the red colonies of \( \text{mat3} \) or telomere reporters white (data not shown). In a centromere reporter strain, the overexpressions resulted in very little change in colony color (Fig. 3A) owing to strong silencing at centromeres. However, when the expression of \( \text{rhp6}^+ \) or \( \text{ubcX}^+ \) was enhanced by the introduction of another overexpression vector, derepression of \( \text{ade}6^+ \) was dramatically increased in a dosage-dependent manner (Fig. 3A). Additionally, growth defects were observed in proportion to the level of derepression of the \( \text{ade}6^+ \) gene; the viability of the overexpression cells was decreased to about 10% of the wild-type level (data not shown).

It was previously reported that mutations involved in silencing defects at centromeres are accompanied by defective centromeric function (2, 6, 28). To investigate effects of the overexpression of \( \text{rhp6}^+ \) or \( \text{ubcX}^+ \) on centromeric function, the rate of loss of minichromosome \( \text{Ch}16 \) was measured. The overexpression enhanced the loss rates up to 0.12 and 0.17% per division, which are 6- and 8.5-fold higher than with the wild type (Table 2). When \( \text{ubc} \) expression was enhanced by introduction of another overexpression vector, the loss rate was further increased to 135- and 165-fold, respectively.

**TABLE 2.** The Effect of \( \text{Rhp6} \) and \( \text{UbcX} \) overexpression on loss of \( \text{Ch}16 \) minichromosome

<table>
<thead>
<tr>
<th>Background</th>
<th>No. of half-sector colonies/ no. of total colonies</th>
<th>Loss rate (%)</th>
<th>Relative loss rate (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT(^b)</td>
<td>0/1418, 1/2137, 0/1369</td>
<td>0.02</td>
<td>1</td>
</tr>
<tr>
<td>pREP3-(\text{rhp6}^+)</td>
<td>0/1709, 2/510, 2/1013</td>
<td>0.12</td>
<td>6</td>
</tr>
<tr>
<td>pREP2-(\text{ubcX}^+)</td>
<td>0/1208, 2/900, 3/826</td>
<td>0.17</td>
<td>8.5</td>
</tr>
<tr>
<td>pREP3-(\text{rhp6}^+), pREP3-(\text{ubcX}^+)</td>
<td>6/158, 5/197, 8/341</td>
<td>2.7</td>
<td>135</td>
</tr>
<tr>
<td>pREP2-(\text{ubcX}^+), pREP2-(\text{ubcX}^+)</td>
<td>5/178, 4/102, 5/143</td>
<td>3.3</td>
<td>165</td>
</tr>
</tbody>
</table>

\(^a\) Increase in rate compared with that of the wild-type control.

\(^b\) WT, wild type.
Mutations interfering with centromeric function affect the interaction of microtubules with kinetochore and show sensitivity to the microtubule-destabilizing drug thiabendazole (TBZ) (9, 10). To determine whether overexpression of *ubcX* affects silencing, we investigated whether Rhp6 or *ubcX* affects silencing at heterochromatic regions. Since histone demethylase has not been reported yet, proteolytic degradation of the entire H3 histone was proposed to be a potential mechanism for the removal of the methyl marker required for reconfiguration of silencing (17, 37). Interestingly, our finding that two E2 enzymes negatively regulate silencing fits well with this idea. To test this hypothesis, we investigated whether Rhp6 or *ubcX* affects methylation of H3 by using a ChIP assay. To observe changes in histone methylation in cells overexpressing or lacking *rhp6* or *ubcX*, we performed the ChIP assay using antibodies recognizing H3-Lys9-Me at the *mat3* locus, telomeres, and centromeres. (Fig. 4A). The *ubcX* cells showed a very slight increase in silencing at the heterochromatic regions tested compared with wild-type cells (Fig. 4B).

Next, we performed a transition assay to measure the effect of *Δrhp6* or *ΔubcX* on inheritance of the repressed state. Interestingly, the *ubcX* deletion as well as the *rhp6* deletion caused a significant decrease in *Ade*− (red)-to-*Ade*+ (white) conversion (Table 3), indicating increased silencing. These results suggest that *rhp6* and *ubcX* both act as a negative regulator of silencing, although the effect of *UbcX* is very slight.

**Table 3.** Effect of *Δrhp6* or *ΔubcX* mutation on inheritance of repressed state

<table>
<thead>
<tr>
<th>Background</th>
<th>Genotype</th>
<th>Rate of half-sectoring (per cell division)*</th>
<th>Relative transition rateb</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>mat3::ade6</em></td>
<td>WT*</td>
<td>1.0 × 10−2 (85/8,333)</td>
<td>1</td>
</tr>
<tr>
<td><em>mat3::ade6</em></td>
<td><em>rhp6::ura4</em></td>
<td>2.3 × 10−4 (1/4,293)</td>
<td>1/44</td>
</tr>
<tr>
<td><em>mat3::ade6</em></td>
<td><em>ubcX::ura4</em></td>
<td>4.7 × 10−3 (4/8,460)</td>
<td>1/21</td>
</tr>
<tr>
<td><em>otr1R::ade6</em></td>
<td>WT</td>
<td>2.5 × 10−3 (23/9,795)</td>
<td>1</td>
</tr>
<tr>
<td><em>otr1R::ade6</em></td>
<td><em>rhp6::ura4</em></td>
<td>1.4 × 10−4 (4/7,040)</td>
<td>1/16</td>
</tr>
<tr>
<td><em>otr1R::ade6</em></td>
<td><em>ubcX::ura4</em></td>
<td>2.3 × 10−4 (2/8,548)</td>
<td>1/10</td>
</tr>
<tr>
<td><em>ade6−::tel</em></td>
<td>WT</td>
<td>8.5 × 10−2 (85/7,821)</td>
<td>1</td>
</tr>
<tr>
<td><em>ade6−::tel</em></td>
<td><em>rhp6::ura4</em></td>
<td>&lt;1.4 × 10−4 (0/7,270)</td>
<td>&lt;1/79</td>
</tr>
<tr>
<td><em>ade6−::tel</em></td>
<td><em>ubcX::ura4</em></td>
<td>1.1 × 10−4 (1/9,333)</td>
<td>1/100</td>
</tr>
</tbody>
</table>

a Number of half-sectored colonies divided by the total number of colonies.

b Increase in rate compared with that of each wild-type control.

WT, wild type.
locus (Fig. 5A, lane 5, top) and moderate decreases at centromeres and telomeres (Fig. 5A, lanes 2 and 8, top). A much weaker decrease in the level of H3-Lys9-Me at ade6+ was also observed in cells overexpressing ubcX+ (Fig. 5A, lanes 3, 6, and 9, top). On the contrary, the level of H3-Lys4-Me was significantly increased at the mat3 locus and telomere in cells overexpressing rhp6+ or ubcX+. Unlike the overexpression results, an opposite result was displayed by deletion of the ubc genes. Deletion of rhp6+ greatly increased the level of H3-Lys9-Me at centromeres, the mat3 locus, and telomeres (Fig. 5B, lanes 2, 5, and 8, top). Although deletion of rhp6+ does not cause a detectable change in the level of H3-Lys4-Me at the mat3 locus (Fig. 5B, lane 5, middle), a slight decrease was induced at centromeres and telomeres in Δrhp6 cells (Fig. 5B, lanes 2 and 8, middle). As expected from its weak silencing phenotype, ΔubcX did not affect methylation patterns as greatly as Δrhp6. However, we could observe a significant increase in the level of H3-Lys9-Me at centromeres and telomeres (Fig. 5B lanes 3 and 9, top) and decrease in the level of H3-Lys4-Me at centromeres, the mat3 locus, and telomeres (Fig. 5B, lanes 3, 6, and 9, middle), a result consistent with the increased silencing found in ΔubcX cells. To test if the effects of the ubc genes on H3 methylation are due to the altered ade6+ gene expression, we performed a ChIP assay with primers specific to the nontranscribed mat2-adjacent region, indicated as mat2-r in Fig. 5C (top). Deletion of rhp6+ greatly enhanced the level of H3-Lys9-Me and reduced the level of H3-Lys4-Me, while overexpression reduced H3-Lys9-Me and enhanced H3-Lys4-Me. Deletion of ubcX+ enhanced H3-Lys9-Me and reduced H3-Lys4-Me, but the effect was not as dramatic as that of rhp6+ and we couldn’t detect significant changes in H3 methylation in ubcX+-overexpressing cells at this region (Fig. 5C). This result suggests that the effects of the ubc genes on H3 methylation at heterochromatic regions are not due to the altered expression of ade6+ inserted at these regions.

To test the possibility that these ubc genes directly conjugate ubiquitin to H3-Lys9-Me, we investigated whether H3-Lys9-Me is ubiquitinated using a Western blot. However, we failed to detect H3-Lys9-Me, probably due to its extremely low abundance (data not shown). Thus, at this stage we cannot conclude whether the effects of these ubc genes on histone methylation are direct or indirect.

Collectively, these results suggest that rhp6+ and ubcX+ negatively regulate histone H3-Lys9 methylation either directly or indirectly, which can then affect silencing at heterochromatic regions.

**DISCUSSION**

In this report, we show that two ubiquitin-conjugating enzymes, Rhp6 and UbcX, are negative regulators of heterochromatin structure. Their overexpression disrupted silencing, while a deficiency of each gene enhanced heterochromatic silencing, indicating a dosage-dependent regulation of silencing by Ubc enzymes.

Over the past few decades, several reports strongly argued that components of the ubiquitin pathway are involved in heterochromatin silencing. Deletion of *UBP3*, which encodes a deubiquitinating enzyme interacting with Sir4, greatly increases silencing at both the telomeres and HML, suggesting that UBP3 antagonizes silencing (23). Similarly, mutation in a gene encoding a putative *Drosophila* UBP enhances transcriptional repression at centric heterochromatin (13). On the contrary, the loss of Dot4, another UBP, resulted in a partial loss of silencing (18). Mutation of *S. cerevisiae* *RAD6* disrupted silencing at HM loci and at telomeres (14). Despite these observations, there had been no clear demonstrations on the molecular role of ubiquitin in regulating silencing. However, recently one of the molecular roles of ubiquitin in silencing was demonstrated by Sun and Allis with *S. cerevisiae* (34). In this study, they reported that ubiquitination of histone H2B at Lys123 by Rad6 is required for the methylation of histone H3 at Lys4 and for telomeric silencing in *S. cerevisiae*. If this is also the case for *S. pombe*, we can interpret our results in view of this mechanism. Since Rhp6 and UbcX negatively regulate silencing and H3-Lys9-Me, we can assume that overexpression of these enzymes would lead to more H2B ubiquitination in heterochromatic regions, blocking Lys9 methylation and promoting Lys4 methylation. Conversely, deletion of rhp6+ or ubcX+ would abolish H2B ubiquitination and Lys4 methylation in heterochromatic regions, increasing Lys9 methylation and silencing. Although there has been no report on Rhp6-mediated H2B ubiquitination in *S. pombe*, this model fits very well with our results. However, while deletion of *RAD6* abolishes whole Lys4 methylation in *S. cerevisiae*, deletion of rhp6+ affects Lys4 methylation only at heterochromatic regions in *S. pombe* (Fig. 5 and unpublished results). Therefore, it is assumed that Rhp6 and UbcX are localized exclusively at heterochromatic regions. However, in our ChIP analysis, neither Rhp6 nor UbcX associated with heterochromatic regions (data not shown).

Alternatively, Rhp6 or UbcX may conjugate ubiquitin directly to H3-Lys9-Me, leading to its proteolysis by the 26S proteasome. Since no histone demethylase has been reported yet, these E2 enzymes may serve as histone demethylation machinery by destabilizing the entire H3 histone. In this model, the overexpression of rhp6+ or ubcX+ is assumed to promote proteolysis of H3-Lys9-Me, leading to disruption of silencing, while their deletions prevent its proteolysis and thus stabilize silencing. This model, which emphasizes a more direct role of ubiquitin, is supported by a report that Rad6 can conjugate ubiquitin to H3 in vitro (11).

Another possible model is that Rhp6 or UbcX may conjugate ubiquitin to a component of the HMTase complex, leading to its proteolysis. This model needs the following speculation: increased activity of HMTase by deletion of the ubc gene would induce increased methylation of H3 at Lys9 and subsequent enhancement in silencing. Supporting this assumption, overexpression of Clr4, an H3-Lys9 HMTase, indeed increases *mat3* silencing in fission yeast (26). Histone methylation by Clr4 requires Rik1, a beta propeller domain-containing protein that is assumed to form a complex with Clr4 (25). Thus, given that Rhp6 or UbcX regulate H3-Lys9-Me via the HMTase complex, the most likely candidate for the substrate would be Clr4 or Rik1. However, since the epitope-tagged Clr4 was very stable and Rik1 did not undergo ubiquitination (our unpub-
lished observations), it is unlikely that the ubiquitin pathway degrades Clr4 or Rik1.

Among these models, the first model is the most plausible, but we cannot exclude the other models at this point. To determine which model is correct requires further studies, including a study of whether ubiquitination of H2B by Rhp6 occurs and regulates H3 methylation in S. pombe, whether H3-Lys9-Me is directly ubiquitinated by Rhp6 and UbcX, and whether the 26S proteasome is involved. Indeed, a recent finding reported that mutations in the genes encoding components of the 26S proteasome enhance silencing within centromeres in S. pombe (16).

In contrast to a negative role of Rhp6 in silencing, presented here, a recent study suggested its positive role in silencing (32). According to the study, rhp6Δ is required to maintain the repression of silent mat genes in switching-competent cells, suggesting its role in the reestablishment of silencing. Consistent with this result, we found that simultaneous deletion of rhp6Δ and ubcXΔ caused a marked derepression of ade6 and mat3 in switching-competent cells (Fig. 6). To accommodate this phenotypic discrepancy for the role of Rhp6, we assume that Rhp6 and UbcX may target more than one substrate in regulating silencing.

ACKNOWLEDGEMENTS

We thank K. Ekwall, G. Thon, and M. Yanagida for providing yeast strains and B. Edgar, C. Norbury, L. Prakash, J. Pringle, and R. Rowley for providing S. pombe cDNA library and plasmids. Also, we thank Gwen Sancar and O. Hwang for their critical readings of the manuscript.

This work was supported in part by the Grants for Leading Scientist from the Korea Science and Engineering Foundation (2001) (to S.D.P.), by Research Fellowship BK21 from the Korean Ministry of Education (to E.S.C. H.S.K. S.D.P.), and by Korea Research Foundation (2001; DP0401) (2002; to S.D.P.). This study was also supported in part by a research grant from the National Cancer Center, Ministry of Health & Welfare (to Y.K.J.).

REFERENCES


3. Allshire, R. C. 1996. Transcriptional silencing in the fission yeast; a mani-


