Theme and Variation Among Silencing Proteins in \textit{Saccharomyces cerevisiae} and \textit{Kluyveromyces lactis}

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\textbf{ABSTRACT}

The cryptic mating type loci in \textit{Saccharomyces cerevisiae} act as reservoirs of mating type information used in mating type switching in homothallic yeast strains. The transcriptional silencing of these loci depends on the formation of a repressive chromatin structure that is reminiscent of heterochromatin. Silent information regulator (Sir) proteins 2-4 are absolutely required for silencing. To learn more about silencing, we investigated mating type and Sir proteins in the yeast \textit{Kluyveromyces lactis}, which contains cryptic copies of the mating type genes. A functional homolog of \textit{SIR4} from \textit{K. lactis} complements the silencing defect of \textit{sir4} null mutations in \textit{S. cerevisiae}. \textit{K. lactis} \textit{sir2} and \textit{sir4} mutant strains showed partial derepression of the silent \textit{a1} gene, establishing that the silencing role of these proteins is conserved. \textit{K. lactis} \textit{sir2} mutants are more sensitive than the wild type to ethidium bromide, and \textit{K. lactis} \textit{sir4} mutants are more resistant phenotypes that are not observed for the corresponding mutants of \textit{S. cerevisiae}. Finally, the deletion of \textit{sir4} in the two yeasts leads to opposite effects on telomere length. Thus, Sir proteins from \textit{K. lactis} have roles in both silencing and telomere length maintenance, reflecting conserved functional themes. The various phenotypes of \textit{sir} mutants in \textit{K. lactis} and \textit{S. cerevisiae} however, revealed unanticipated variation between their precise roles.

\textbf{HAPLOID} strains of \textit{Saccharomyces cerevisiae} contain three loci that encode mating type information. The \textit{MAT} locus is expressed and thus determines the mating type, whereas two additional loci, \textit{HML} (usually encoding the \textit{a} information) and \textit{HMR} (usually encoding the \textit{a} information) are not expressed despite the presence of functional promoters and structural genes. Transcriptional repression of the cryptic mating type loci, known as silencing, occurs by the formation of a repressive chromatin structure (Laurenson and Rine 1992). Evidence for an unusual chromatin structure associated with these loci includes the involvement of histones H3 and H4 in silencing (Kayne et al. 1988; Thompson et al. 1994), as well as the observation that the \textit{HMR} locus is resistant to DNA modifying enzymes both in vivo (Singh and Klarić 1992) and in isolated nuclei (Loo and Rine 1994). Marker genes close to telomeres are also silenced by telomere position effect (Gottschling et al. 1990), and a similar phenomenon was recently observed for marker genes located in the \textit{rDNA} locus (Smith and Boeke 1997; Bryk et al. 1997). Position effects on gene expression are widespread phenomena. Silencing in yeast is reminiscent of heterochromatic gene inactivation, which underlies the phenomenon of X-chromosome inactivation in mammals and position effect variegation in Drosophila (Grigliatti 1991; Rastan 1994). Thus, telomeres and the \textit{HML} and \textit{HMR} loci are presumably the yeast counterpart to heterochromatin.

Silencing of the cryptic mating type loci requires a combination of regulatory sites called silencers, as well as dedicated proteins. The most thoroughly studied silencer, \textit{HMRE}, contains a binding site for \textit{ORC}, a protein complex that is involved in replication initiation, as well as binding sites for two widely used transcriptional activators, Rap1p and Abf1p. Mutations in the genes encoding \textit{Orc2p} (Foss et al. 1993; Mlicki et al. 1993), \textit{Orc5p} (Loo et al. 1995a), Rap1p (Sussel and Shore 1991), and Abf1p (Loo et al. 1995b) lead to derepression of transcription of \textit{HML} and \textit{HMR}. The silent information regulator (Sir) proteins 2-4 are required for both telomere position effect and cryptic mating type loci silencing in \textit{S. cerevisiae} (Aparicio et al. 1991; Ivy et al. 1986). Null alleles of the \textit{Sir2}, \textit{Sir3}, or \textit{Sir4} genes lead to a complete derepression of the silent mating type genes (Rine and Herskowitz 1987), whereas \textit{Sir1} strains show only a partial derepression of \textit{HML} and \textit{HMR} (Pillus and Rine 1989). Moreover, the requirement for a silencer element can be bypassed by fusing \textit{Sir1p}, \textit{Sir3p}, or \textit{Sir4p} to a \textit{Gal}4p DNA binding domain and exchanging the silencer for \textit{Gal}4 binding sites (Chien et al. 1993; Mancard et al. 1996), confirming the central role of Sir proteins in silencing. The precise functions of the Sir proteins are still unknown, but recent evidence suggests that they are structural parts of silent chromatin because silent chromatin can be specifically immunoprecipitated using antibodies against Sir2p and Sir4p (Strahl-Bolsinger et al. 1997).

Little is known about whether or not Sir-like proteins
exist in other eukaryotes and what function such potential homologs might have. Sir2 appears to encode a protein of fundamental function because genes highly homologous to Sir2 are found from bacteria to humans (Brachman et al. 1995). The function of these homologs in organisms other than S. cerevisiae remains unknown, except for a K. lactis Sir2 gene homolog. This gene partially complements a S. cerevisiae sir2 null allele and is required for growth of K. lactis in the presence of the DNA intercalating drug ethidium bromide (EtBr; Chen and Clark-Walker 1994). Sequence or functional homologs of the other Sir proteins have not yet been identified.

In this study, we report the identification of a K. lactis functional homolog of ScSir4. The deletion of K. lactis Sir4 had effects on the resistance to EtBr and also affected telomere length. Finally, neither KlSir4p nor KlSir2p are absolutely required for mating in K. lactis, but their absence leads to a partial derepression of the silent a1-gene in MATa strains.

MATERIALS AND METHODS

Cloning of KlSir4: S. cerevisiae strain JRY4577 (MATa can1-100 his3-11 ura3-112 lys2-801 trpl-1 ura3-1 sir4::His3) was transformed with a genomic K. lactis library in plasmid pAB24 (2 μm URA3). Approximately 42,000 transformants were screened by mating to a MATa ura3-52 strain (FY2), after which diploid colonies were recovered. Library plasmids were isolated, and the complementing activity was confirmed by transformation into JRY4577, followed by mating assays. As a result, three plasmids were found with overlapping but nonidentical inserts that could complement the mating deficiency of JRY4577.

Plasmids: From one of the complementing library plasmids, a 7.8-kb SalI-HindIII fragment containing the entire KlSir4 ORF was cloned into the corresponding sites of pSEYC68 (CEN ARS URA3) and pRS426 (2 μm URA3; Emr et al. 1986; Christianson et al. 1992), forming plasmids p199 and p201, respectively. Plasmid p86 contained a 9-kb SalI fragment from one of the library plasmids cloned into the corresponding site of pUC118. Because the insert cloned into p199 and p201 contained two ORFs (Figure 1), three deletion derivatives of p201 were generated to elucidate which ORF was responsible for the complementing activity. The entire upstream ORF in p201 was deleted by a HindIII-MscI digestion, the staggered ends were filled in with T4 DNA polymerase, followed by blunt end ligation, resulting in plasmid p162. Two deletion derivatives of the KlSir4 gene (leaving the upstream gene intact; Figure 1) were generated by SacI digestion of p201 followed by ligation, thus generating plasmid p163. Plasmid p122 was generated by cloning a 5.5-kb XbaI-HindIII fragment from p86 into the SpeI-HindIII sites of pRS426 (Christianson et al. 1992). These procedures removed the sequences encoding amino acids 688-1314 (p162) and amino acids 909-1314 (p122) of the predicted KlSir4 protein. An integrative plasmid, in which the sequences encoding amino acids 87-939 of the KlSir4 gene were exchanged for a functional LEU2 gene, was generated in two steps. First, a 2-kb SalI-XbaI fragment from pJR990 containing a functional LEU2 gene was exchanged for an internal 2-kb SpeI-HindIII fragment in p86, generating plasmid p183. Second, a 5.7-kb BglII fragment from p183 was cloned into the BamHI site of plasmid pRS306 (Sikorski and Hieter 1989), generating plasmid p232. Plasmids capable of replicating in K. lactis containing the KlSir4 and ScSir4 genes were generated by cloning a 6-kb PstI-EcoRI fragment from pRS315-ScSir4 (S. Okamura, unpublished data) into the corresponding sites of pCX18 (Chen 1996) and by cloning a 6-kb SalI-HindIII fragment from p86 into the SalI-HindIII sites of pCX18, thus generating plasmids p248 and p250, respectively.

Sequencing and sequence analysis: The insert of p201 was sequenced on both strands using a Prism sequencing kit (Applied Biosystems, Inc., Foster City, CA) and DNA sequencer (model 373; Molecular Dynamics). Prediction of coiled-coil domain was performed as described (Lupas et al. 1991), and homology searches were performed using the BLAST algorithm (Altschul et al. 1990).

Strain constructions: The strains used in this study are listed in Table 1. K. lactis strain CK213-4C (Chen and Clark-Walker 1994) was transformed with a MsdI-linearized pRS306-sir4::LEU2 (p232) on S-FOA/plates lacking leucine. This procedure resulted in the replacement of Sir4 for sir4::LEU2 (Scherer and Davis 1979), generating strain SAY90. The disruption was confirmed by DNA blot hybridization. Strains SAY90 and CK57-7A (Chen and Clark-Walker 1994) were mated, sporulated, and the resulting tetrads were analyzed. As a result, sir2 sir4 double (SAY97), sir2 single (SAY99, SAY102), and sir4 single mutant (SAY101) strains were obtained for subsequent analysis.

<table>
<thead>
<tr>
<th>A</th>
<th>Complements sir4 mutation?</th>
</tr>
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<tbody>
<tr>
<td>---</td>
<td>+++</td>
</tr>
<tr>
<td>KlSir4p</td>
<td>Delta aa 608-1314</td>
</tr>
<tr>
<td>ScSir4p</td>
<td>Delta aa 910-1314</td>
</tr>
</tbody>
</table>

Figure 1.—(A) Identification of K. lactis Sir4. Plasmids containing the indicated constructs were introduced into a MATa sir4::His3 strain (JRY4577) and tested for complementation of the mating defect. +++ , efficient complementation; -, no complementation. The left-hand ORF shares 20% identity with ScSir4p; the right-hand ORF shares 21% identity. (B) Predicted coiled-coil domains in ScSir4p and KlSir4p. ScSir4p, 1358 amino acids; KlSir4p, 1314 amino acids. The KlSir4 nucleotide sequence appears in the GenBank database under the accession number AF035007.
**TABLE 1**

Yeast strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
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</thead>
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<tr>
<td>A. S. cerevisiae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JRY4577</td>
<td>MAT a can1-100 his3-11 leu2-3-112 lys2Δ trp1-1 ura3-1 sir4::HIS3</td>
<td>This laboratory</td>
</tr>
<tr>
<td>JRY4578</td>
<td>As JRY4577 but MAT a</td>
<td>This laboratory</td>
</tr>
<tr>
<td>RBY15</td>
<td>mataΔ H MLα H MRα leu2-3-112 his3-11,-15 trp1-1 ura3-1 stre4::TRP1</td>
<td>This laboratory</td>
</tr>
<tr>
<td>FY2</td>
<td>MAT α ura3-52</td>
<td>F. Winston, Harvard Medical School</td>
</tr>
<tr>
<td>JRY2726</td>
<td>MAT a his4</td>
<td>This laboratory</td>
</tr>
<tr>
<td>JRY2728</td>
<td>MAT α his4</td>
<td>This laboratory</td>
</tr>
<tr>
<td>B. K. Lactis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK213-4C</td>
<td>MAT a lysA1 trp1 leu2 metA1 uraA1</td>
<td>X. J. Chen and G. D. Clarke-Walker 1994</td>
</tr>
<tr>
<td>WM52V4</td>
<td>MAT α adel ade6 his7 uraA1</td>
<td>X. J. Chen and G. D. Clarke-Walker 1994</td>
</tr>
<tr>
<td>CK57-7A</td>
<td>MAT α adel uraA1 sir2::URA3</td>
<td>X. J. Chen and G. D. Clarke-Walker 1994</td>
</tr>
<tr>
<td>SAY90</td>
<td>MAT a lysA1 trp1 leu2 metA1 uraA1 sir4::LEU2</td>
<td>This study</td>
</tr>
<tr>
<td>SAY97</td>
<td>MAT α uraA1 leu2 or LEU2 adel metA1 sir2::URA3</td>
<td>This study</td>
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<tr>
<td>SAY99</td>
<td>MAT a uraA1 metA1 sir2::URA3</td>
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<tr>
<td>SAT101</td>
<td>MAT a uraA1 lysA1 trp1 leu2 or LEU2 sir4::LEU2</td>
<td>This study</td>
</tr>
<tr>
<td>SAT102</td>
<td>MAT a uraA1 leu2 lysA1 metA1 trp1 sir2::URA3</td>
<td>This study</td>
</tr>
</tbody>
</table>

**Media:** Rich media, sporulation media, and conditions for quantitative matings were as described (Chen and Clark-Walker 1994; Rose et al. 1990).

**RNA and DNA blot hybridizations:** Standard techniques were used (Sambrook et al. 1989). DNA and RNA preparations were as described (Rose et al. 1990). The α1 probe (Yuan et al. 1993) was obtained by PCR from genomic K. lactis DNA using oligonucleotides 5'-ATGAAATCGAATGCTCCAACC-3' and 5'-CTCAGACTGAGTTCATCAAGG-3'. The telomere probe was an oligonucleotide (5'-GATTAGGTATGTGG-3') specific for the telomeric repeats. Hybridization and washes were performed at 40° for blots probed by the oligonucleotide and at 65° for the α1 probe. Quantification of signals was performed using a Phosphorimager (Molecular Dynamics, Sunnyvale, CA) and Imagequant software.

**RESULTS**

**Cloning of KlSIR4:** Comparisons of similar processes in distantly related organisms offer a powerful approach to identifying themes and variations in evolution from the macroscale (Darwin 1859) to the microscale (Susskind and Botstein 1978). Thus, we chose to explore the similarities and differences between Sir proteins in two budding yeasts, S. cerevisiae and K. lactis. Based on 18S rRNA sequences, these two yeasts are more closely related to each other than either is related to budding yeasts such as Candida albicans or Yarrowia lipolytica (Barns et al. 1991). Previous work by others (Chen and Clarke-Walker 1994) established the existence of a K. lactis Sir2 homolog (KLSir2) that could complement the sir2 mutations of Saccharomyces. To learn if Sir4 was evolutionarily and functionally conserved in K. lactis, we transformed a genomic K. lactis library in a 2 μm plasmid vector into an S. cerevisiae strain carrying a null allele of sir4. Saccharomyces sir4 strains, unable to silence HML and HMR, are sterile because of the simultaneous expression of α and α genes (Laurenson and Rine 1992). Among mating competent transformants that were isolated, there were three plasmids with overlapping but nonidentical inserts. Complementation was specific to the sir4 mutation because the introduction of these plasmids into sir2 or sir3 null strains did not restore mating. The sequence of the insert from one of the complementing plasmids revealed two long ORFs that share a limited homology to ScSir4 (Figure 1). The complementing gene was identified by deletion of the left-hand gene from the complementing insert (as drawn), showing that the intact right-hand gene still complemented the phenotype. A deletion removing the sequences encoding the carboxyl-terminal half of this gene (corresponding to amino acids 688-1314) completely abolished the complementing activity, confirming that this gene was necessary and sufficient to complement the sir4 mutation (Figure 1). A smaller deletion removing sequences corresponding to amino acids 909-1314 of the KLSir4 gene still complemented the phenotype, however, defining a region between amino acids 688 and 909 as important, but excluding amino acids 910-1314 as essential for the complementing activity. This KLSir4 gene encoded a putative peptide of 1314 amino acids that was subjected to a homology search in the GenBank database. No significant homologies were found, and KLSir4p and ScSir4p shared only 21% sequence identity. Because of this limited homology, a meaningful alignment between the two molecules could not be assembled. Despite their limited homology, KLSir4p had a predicted carboxyl-terminal coiled-coil
domain similar to that suggested for ScSir4p (Diffl ey and Stillman 1989; Figure 1). The extreme carboxyl terminus of ScSir4p is essential for silencing (Kennedy et al. 1995), suggesting that this coiled-coil domain plays an important role in Sir4p function. Interestingly, the predicted coiled-coil domain of KlSir4p is more extensive than that of ScSir4p, and the truncation that removed most but not all of the carboxyl-terminal coiled-coil domain still complemented the sir4 null mutation in S. cerevisiae (Figure 1). The truncation that removed the entire coiled-coil domain, however, did not complement the phenotype, which is consistent with a coiled-coil domain being essential for Sir4p function. Thus, these homologs showed low sequence similarity, but they had structural similarity and functional conservation.

**Phenotypic characterization of KISIR4 in S. cerevisiae:** The ability of the KISIR4 gene to complement the Sir4p mutation depended on the copy number of the plasmid carrying the KISIR4 gene in an unusual way. Quantitative mating determinations (a measure of silencing) revealed that KISIR4 on a low-copy plasmid complemented the mating deficiency very well—in fact, more efficiently than the same gene in high gene dosage (Figure 2). This 10-fold difference in complementation efficiency between the high- and low-copy plasmids was observed only in the MATα strain. In MATα strains, both plasmids only partially restored silencing, suggesting that KlSir4p silenced HMLα more efficiently than HMRα. Moreover, KlSir4p was unlikely to be expressed at levels significantly different from ScSir4p because LacZ gene fusions to ScSir4 and KISIR4 produced similar levels of β-galactosidase, 5 units and 7 units, respectively, when assayed in S. cerevisiae.

The inefficient silencing in MATα strains by the high-copy KISIR4 plasmid was reminiscent of the ability of ScSir4p to derepress HML and HMR when overexpressed (Marshall et al. 1987). We tested to see if

![Figure 2](image-url)
overproduction of KISir4p could disrupt silencing at HML and HMR by using a strain in which loss of silencing leads to the ability to mate as an α strain. KISIR4 in high-copy number disrupted silencing in this strain efficiently, and no interference was seen when the gene was present on a low-copy plasmid (Figure 2). Because this strain had wild-type copies of all the Saccharomyces SIR genes, it was likely that high levels of KISir4p interfered with the function of a Saccharomyces protein required for silencing.

**K. lactis strains had stable mating types and silent mating type genes:** To characterize the mating-specific functions of KISir2p and KISir4p, we had to determine if mating in general in K. lactis and S. cerevisiae is similar. The ability of KISIR4 to silence HML and HMR in Saccharomyces implied that K. lactis might also have cryptic copies of mating type genes. To determine if K. lactis contained both silent and expressed mating type genes, we used DNA blot hybridizations. The K. lactis α1 gene (Yuan et al. 1993) was used as a probe in genomic DNA hybridization experiments. If the S. cerevisiae model were to be recapitulated in K. lactis, we would expect to see one invariantly sized restriction fragment hybridizing in strains of either mating type, as well as a second α1-specific band present only in MATα strains. Indeed, this was the observed result (Figure 3). Because wild-type MATα strains did not express the α1-transcript (see below), we deduced that the invariant band seen on the DNA blot must represent a cryptic locus, and that the unique locus found only in MATα strains was expressed. Therefore, K. lactis did have a cryptic copy of α1, and its expression most likely resulted from a genomic rearrangement. Moreover, the presence of a single hybridizing band in the genome of multiple MATα strains indicated that the rate of mating type interconversion in these strains must be low.

**Mutations in KISIR2 and KISIR4 did not abolish mating in K. lactis:** Others have shown that K. lactis sir2 strains have a moderate mating defect (Chen and Clark-Walker 1994), but the phenotype is not as dramatic as that seen in S. cerevisiae sir2 strains. In our hands, the mating defect of sir2-null strains was barely detectable in quantitative mating determinations in which dilutions of the strains tested were spread on a high density lawn of the opposite mating type. We found, however, that the sir2 strains required a high density of the mating partner to mate efficiently. Patch matings, in which approximately equal numbers of the two mating partners were mixed, resulted in less mating of the sir2 strains (Figure 3).

To study the role of the SIR4 gene in K. lactis, we deleted the gene in a MATα strain. The resulting mutant was viable and showed no growth defect compared to the wild-type parent. Moreover, mating in both mating types appeared unaffected by the deletion (Figure 3, data not shown).
Sir proteins of K. lactis have a role in silencing: Although mutations causing defects in silencing in S. cerevisiae lead to decreased mating efficiency, only a small subset of mutations affecting mating efficiency do so through defects in silencing. To investigate if mutations in sir2 and sir4 lead to derepression of the silent mating type loci, we performed an RNA blot hybridization on RNA from various MATα strains probing for the α1-transcript (Figure 3). The control MATα strain, as expected, did not express the α1 transcript. The sir2 and sir4 mutant strains, however, showed derepression of the silent α1-locus, but not to the same extent as the control MATα strain. In Saccharomyces, the α1 gene is partially repressed by the α1/α2 repressor in sir strains, so we would not necessarily expect the α1 levels in sir2 and sir4 mutants to be equal to that of the MATα strains. The difference between the impact of a sir2 mutation and a sir4 mutation on α1 levels in K. lactis, however, was unexpected. The silent α2 transcript was also partially derepressed in sir strains (data not shown). Therefore, null alleles of sir2 and sir4 caused a partial derepression of silent mating type genes in K. lactis that did not abolish mating ability, revealing a surprising difference between K. lactis and S. cerevisiae.

Mutations in sir genes affected the sensitivity of K. lactis to EtBr: sir2 mutant strains in K. lactis, but not S. cerevisiae, are hypersensitive to DNA intercalating agents such as the drug EtBr (Chen and Clark-Walker 1994). We investigated the relative EtBr sensitivities of a sir2 strain, a sir4 strain, and a sir2 sir4 double-mutant strain. As expected, the sir2 mutant was at least four orders of magnitude more sensitive to EtBr than were the wild-type strains (Figure 4). Surprisingly, the sir4 mutant was at least 10-fold more resistant to EtBr than the parental strain. The sir2 sir4 double-mutant strain was EtBr sensitive, showing that the sir2 mutation was epistatic to sir4 by this phenotype. Thus, both Sir proteins were involved in the response of K. lactis to EtBr, but in opposite directions.

The role of Sir proteins in telomere metabolism in K. lactis: The deletion of SIR4 in S. cerevisiae leads to shorter telomeres (Kennedy et al. 1995; Palladino et al. 1993), so we investigated the size of the telomeres in K. lactis sir2, sir4, and sir2 sir4 double-mutant strains. The deletion of SIR4 in K. lactis led to telomeres that were ~200 bp longer (Figure 4). This phenotype could be partially complemented by a plasmid carrying KlSIR4, but not by a plasmid carrying ScSIR4. Increased gene

![Figure 4](image-url)
DISCUSSION

In this report, we have studied mating-type and the genes encoding Sir2p and Sir4p in K. lactis. We found that the K. lactis laboratory strains had stable mating types and contained cryptic mating type genes. Moreover, based on the DNA blot hybridization data, mating type interconversion in K. lactis presumably involves genomic rearrangements.

K. lactis Sir proteins were involved in transcriptional silencing of silent mating type genes, as their Saccharomyces counterparts. The modest effect that sir2 and sir4 mutations had on the mating efficiency, however, was surprising. We cannot exclude that K. lactis might encode redundant functions for Sir2p and Sir4p, which could explain why strains mutant for the corresponding genes still mate with reasonable efficiency. However, DNA blot hybridizations using probes against these genes at high stringency indicate that closely related sequences do not exist in the K. lactis genome (Chen and Clark-Walker 1994; data not shown). Moreover, the phenotypes of sir2 and sir4 mutants with respect to EtBr sensitivity, telomere length, and silencing indicate that K. lactis lacks any genes whose function is completely redundant with those of KLSIR2 and KLSIR4.

Given the low sequence similarity between KLSIR4 and SCSir4, it was possible that a gene sharing more sequence identity to SCSir4 was present in the K. lactis genome. The gene that we identified in this study, however, encodes a protein that is structurally similar to SCSir4p, and this gene complements the mating deficiency of S. cerevisiae sir4 strains to almost wild-type levels when present on a single copy vector. Moreover, in complementation experiments of S. cerevisiae sir4 strains with Saccharomyces plasmid libraries, only plasmid-borne Sir4p has been found to complement the mutation. These arguments suggest that the gene we identified indeed encodes the K. lactis equivalent to SCSir4, but we cannot exclude that this gene is in fact a low-copy number suppressor.

Because both KLSir2p and KLSir4p are required for complete silencing of the silent a locus, we expected MATa sir2 and sir4 mutants to have a dramatic mating defect if haploid specific genes are repressed by an a1/a2 repressor, as observed for S. cerevisiae. We tested this assumption by determining whether a plasmid-borne copy of a K. lactis a locus would block mating of a MATa strain. A plasmid encoding the K. lactis a1 and a2 genes, when introduced into MATa strains, almost abolished mating under conditions with a limited number of mating partners. Surprisingly, these strains still mated efficiently when a large surplus of mating partners were present (data not shown). Thus, K. lactis was able to mate despite the simultaneous expression of both a and alpha information, at least under these conditions. This behavior is fundamentally different from comparable S. cerevisiae mutants. The a1/a2 repressor of K. lactis is perhaps unable to completely repress the transcription of haploid-specific genes.

With respect to telomere length, sir4 mutations lengthened telomeres in K. lactis and shortened telomeres in S. cerevisiae. In S. cerevisiae, the DNA-binding protein Rap1p binds to telomeric sequences (Longtine et al. 1989; Lustig et al. 1990) and interacts with Sir4p via its carboxyl terminus (Moretti et al. 1994). Recently, Shore and co-workers suggested a model for telomere length regulation in S. cerevisiae that involved a protein-counting mechanism (Marcand et al. 1997). In this model, the precise number of Rap1p molecules bound to the telomere negatively regulates telomere length. Furthermore, interaction between Sir4p and Rap1p limits the amount of Rap1p available for the counting mechanism. Thus, in the absence of Sir4p, more Rap1p molecules bound to the telomeres are counted, explaining the shorter telomeres observed in sir4 null strains. This model cannot explain the data from this related yeast, however, because K. lactis sir4-null strains have long telomeres.

In K. lactis, Rap1p also binds telomeres, and mutations that change the sequence of the repeats, and thus decrease Rap1p binding, lead to telomere lengthening (Krauskopf and Blackburn 1996). Moreover, K. lactis strains that contain a carboxyl terminal deletion of Rap1p have long telomeres (Krauskopf and Blackburn 1996), similar to the phenotype obtained here in KLSir4 strains. Perhaps a counting mechanism in K. lactis maintains the number of Sir4p/Rap1p heteromers that are bound to telomeres to regulate telomere length. Further investigations are required, however, to elucidate if KLSir4p has a direct interaction to KLRap1p and telomeres.

In contrast to S. cerevisiae Sir proteins, KLSir2p and KLSir4p controlled the sensitivity of cells to EtBr. Surprisingly, sir2 and sir4 mutations had opposite effects on EtBr sensitivity, indicating that Sir2p and Sir4p have opposing functions by this phenotype. One explanation for the observed phenotypes would be that Sir2p was required for resistance to EtBr, and that Sir4p limited the effective level of Sir2p. Thus, sir2 mutants would be sensitive to EtBr, and in the absence of Sir4p, there would be more Sir2p available for conferring resistance, thereby making sir4 mutants resistant to EtBr. We have observed that wild-type cells grown in the presence of EtBr exclude the drug from the cells, whereas sir2 cells
accumulate EtBr, suggesting that this accumulation causes the EtBr sensitivity (our unpublished observation).

In Saccharomyces, Sir2p, Sir3p, and Sir4p have been found together in a multicomponent complex (Moazed and Johnson 1996). In contrast, the data from K. lactis implies that Sir2p has a function that was different from Sir4p. Recently, ScSir2p was shown to be required for efficient rDNA silencing, whereas Sir4p appears to interfere with rDNA silencing (Smith and Boeke 1997). Thus, in both Saccharomyces and K. lactis, the Sir proteins appear to have functions that are independent of each other. These data imply either that some Sir2p or Sir4p function independently of the Sir2p-Sir3p-Sir4p complex, or that the complex has multiple different roles.

Each species shares fundamental processes with other species and differs from other species by certain specializations. At least one silencing protein (Sir2p) is conserved among many phyla and kingdoms, indicating that its function is important. Studies like the one reported here have helped us discriminate between fundamental themes and species-specific variations, and they should prove equally valuable across a broad range of phenomena.

We thank all the members of the laboratory for interesting discussions, Dr. Clark-Walker for the gift of K. lactis strains, Dr. S. Fields for plasmids containing the K. lactis locus, Dr. A. Johnson and Dr. C. Hull for sharing sequence information of the K. lactis a2 gene before publication, and Chiron Corporation for the K. lactis library. This study was supported by The European Molecular Biology Organization postdoctoral fellowship, the Swedish Institute (S.U.A.), and a grant from the National Institute of Health (GM-31105 to J.R.).

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Kluyveromyces lactis SIR Genes


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