MINIREVIEW

Reinventing Heterochromatin in Budding Yeasts: Sir2 and the Origin Recognition Complex Take Center Stage

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The transcriptional silencing of the cryptic mating-type loci in Saccharomyces cerevisiae is one of the best-studied models of repressive heterochromatin. However, this type of heterochromatin, which is mediated by the Sir proteins, has a distinct molecular composition compared to the more ubiquitous type of heterochromatin found in Schizosaccharomyces pombe, other fungi, animals, and plants and characterized by the presence of HP1 (heterochromatin protein 1). This review discusses how the loss of important heterochromatin proteins, including HP1, in the budding yeast lineage presented an evolutionary opportunity for the development and diversification of alternative varieties of heterochromatin, in which the conserved deacetylase Sir2 and the replication protein Orc1 play key roles. In addition, we highlight how this diversification has been facilitated by gene duplications and has contributed to adaptations in lifestyle.

In Saccharomyces cerevisiae, repressive chromatin forms at the cryptic mating-type loci, HMRa and HMLα, and prevents the expression of extra copies of the genes that determine mating-type (reviewed in reference 99). These two loci enable mating-type switching but must remain silenced to maintain cell type identity and the capacity to mate. A related type of repressive chromatin forms at the telomeres, where it serves a structural role and represses subtelomeric genes. The tandem rDNA array is also embedded in a distinct type of chromatin that serves to suppress unequal sister chromatid exchange. However, the molecular composition of this chromatin is distinct from the cryptic mating-type loci and telomeres.

Silencing is initiated at specific DNA sequences termed silencers. HMRa and HMLα are each flanked by silencers, termed E and I, which have binding sites for the origin recognition complex (ORC), as well as Rap1, Abf1, or both (Fig. 1). Together, these silencer binding proteins recruit the main structural components of silenced chromatin, the Sir (silent information regulator) proteins. At telomeres, Rap1 binding sites embedded in the degenerate telomeric repeat sequence recruit the Sir proteins. The Ku complex also stabilizes the association of Sir proteins with telomeres.

The assembly of Sir proteins into silenced chromatin involves two phases, nucleation and spreading. First, the Sir proteins assemble at the mating-type silencers or chromosome ends through interactions with silencer binding proteins. Subsequently, Sir2, Sir3, and Sir4 spread along the chromosome via interactions with histones (Fig. 1). Sir1 is a silencer-associated protein that stabilizes the other Sir proteins at the mating-type silencers. Sir2 is a NAD⁺-dependent deacetylase, and its enzymatic activity is required for the spreading of the Sir proteins (60, 98). Sir3 and Sir4 bind preferentially to deacetylated histones H3 and H4 (20, 52, 79, 89). Sir4 also serves as a scaffold, interacting with Sir2, Sir3, and silencer-associated proteins. These observations inspire a sequential deacetylation model in which Sir2 deacetylates nearby nucleosomes, creating new high-affinity binding sites for Sir3 and Sir4, which in turn recruit additional Sir2 to the newly deacetylated nucleosome (Fig. 1). However, recent studies suggest that spreading may not always occur in a linear fashion. Instead, assembly may be focused in regions of the chromatin fiber brought together by silencers (75, 76, 118).

THE SUBPHYLUM SACCHAROMYCOTINA

Given that the molecular composition of heterochromatin in S. cerevisiae is distinct from that of other well-studied organisms, it is important to understand when and how this unique silencing mechanism evolved. Thus, we focus on the fungal subphylum Saccharomycotina, which consists primarily of budding yeasts. These species are also referred to as hemiascomycetes. Comparisons of average protein sequence identity suggest that the diversity within this subphylum is slightly greater than that among the chordates (33). The phylogenetic relationships of the species discussed in this review are illustrated in Fig. 2. The family Saccharomycetaceae includes S. cerevisiae and is punctuated by a whole-genome duplication that occurred approximately 100 million years ago. The CTG clade, which includes the opportunistic human pathogen Candida albicans, is charac-
terized by a change in the genetic code, such that CTG encodes serine rather than leucine. The fission yeast *Schizosaccharomyces pombe* belongs to a different subphylum, *Taprinomycotina*, which is thought to have diverged from *Saccharomycotina* around a billion years ago (53, 54).

*S. pombe* has been another important model organism for studying heterochromatin formation (reviewed in reference 48), particularly because many key proteins are conserved between *S. pombe* and metazoans. In *S. pombe*, heterochromatin forms at pericentromeric regions, telomeres, and the cryptic mating-type loci. Two important heterochromatin proteins are a methyltransferase, Ctr4, which specifically methylates lysine 9 of histone H3, and a chromodomain-containing protein, Swi6, which binds preferentially to H3-K9me. These proteins are well-conserved among eukaryotes and are generally known as SuVar3-9 (the methyltransferase) and HP1 (the chromo-domain-containing protein). However, these proteins are missing from the genomes of *S. cerevisiae* and other *Saccharomycotina* species.

The formation of heterochromatin in *S. pombe* is often initiated via a mechanism that involves small, noncoding RNAs. These RNAs are part of a protein-RNA complex known as RITS (RNA-induced transcriptional silencing) that is related to the RISC posttranscriptional silencing complex (88, 111). Indeed, the RNA interference (RNAi) proteins argonaute and dicer are required for heterochromatin formation in *S. pombe*. However, argonaute and dicer are missing in *S. cerevisiae* and many *Saccharomycotina* species (86), although argonaute and noncanonical dicer proteins have recently been identified in a few *Saccharomycotina* species, including *Naumovozyma castellii* and *C. albicans* (31). These RNAi proteins have been suggested to silence retrotransposons, but a potential role in nucleating heterochromatin-like structures, as occurs in *S. pombe* and metazoans, has not been explored.

![FIG. 1.](image1.png)

**FIG. 1.** Functions of Sir proteins. (A) The silencer binding proteins ORC, Rap1 (R), and Abf1 (A) recruit the Sir proteins (1 to 4) to the silencer. Sir2 deacetylates neighboring nucleosomes, generating binding sites for Sir3 and Sir4. Reiterations of this cycle enable spreading. (B) Conserved domains of the four Sir proteins in *S. cerevisiae* are indicated. For each protein, the names of paralogs are listed.

![FIG. 2.](image2.png)

**FIG. 2.** Distribution of Sir proteins in species discussed. For each species, the presence (+) or absence (−) of silencing proteins is indicated. The paralogs Sir2/Hst1 and Orc1/Sir3 are separated for clarity. In other cases, multiple paralogs are indicated by the number of + symbols. Species in boldface have been subject to experimental investigations of silencing. Asterisks identify proteins known to function in silencing. The tree represents the relative relationships of species and is based on the consensus in the field (18, 67, 68, 106).
In summary, many of the key components of heterochromatin in *S. pombe* and other eukaryotes are missing in the Saccharomycotina species. It is unclear what led to the loss of these proteins, but their absence presented an evolutionary opportunity for the development and diversification of alternative silencing mechanisms.

**DISTRIBUTION AND FUNCTION OF Sir PROTEINS AMONG BUDDING YEAST SPECIES**

**SIR2.** The deacetylase Sir2 is the most widespread and well-conserved of the Sir proteins (Fig. 2). In fact, unlike the other Sir proteins, which are restricted to budding yeasts, Sir2 has homologs among all domains of life, including eubacteria and archaebacteria (reviewed in references 47, 102, and 110). Furthermore, many species have multiple Sir2 family members. For example, in *S. cerevisiae* there are five Sir2 deacetylases (Sir2 and HST1 to HST4 [HST1–4]) (15), but only Sir2 functions in silencing.

The Sir2 family is defined by a conserved catalytic domain (Fig. 1), which employs a mechanism distinct from that of other deacetylases. In particular, deacetylation is coupled to the lysis of NAD⁺, potentially linking the activity of these enzymes to the metabolic state of the cell. *S. cerevisiae* Sir2 (ScSir2) and its orthologs in the Saccharomycesota have a second conserved domain, which likely enables these proteins to interact with specific partners, such as Sir4.

Orthologs of ScSir2 have been identified in all examined Saccharomycesota species (36, 97), and the silencing function of these orthologs is conserved in the few species that have been investigated, namely, *Kluyveromyces lactis* (5, 23, 57), *Candida glabrata* (30, 94, 96), and *Saccharomyces bayanus* (118). In fact, Sir2 has a role in silencing beyond the centromeres, telomeres, and mating-type loci, where it deacetylates H3-K9, thereby promoting methylation of this lysine and the association of Swi6 (39, 103). In *Drosophila melanogaster*, mutations in DmSir2 affect position effect variegation mediated by HP1 (4, 87) and repression mediated by polycomb group proteins (43). Moreover, in distant protozoan species, such as *Trypanosoma brucei* and *Plasmodium falciparum*, Sir2 homologs are also associated with subtelomeric chromatin (2, 34, 40). Therefore, the Sir2 deacetylase most likely had an ancient role in silencing that has gained prominence in the absence of HP1 and other heterochromatin proteins. As discussed below, the second model is consistent with connections between ORC and heterochromatin in a wide range of species.

**SIR4.** The scaffold protein Sir4 displays extremely low sequence conservation (36, 118), and in some genomes the identification of Sir4 is based on synteny rather than homology. Consequently, Sir4 has been identified only in the Saccharomycetae family and is either absent or highly diverged and nonsyntenic in the CGT clade (36, 97) (our unpublished analysis). An unresolved yet important issue is how Sir2 is targeted to silenced domains in species that apparently lack Sir4, as ScSir4 is required for the recruitment of ScSir2 and ScSir3 to silencers and telomerases (72, 98).

A consistent structural feature of Sir4 is a coiled-coil domain (6, 36) (Fig. 1), which interacts with Sir3 and is essential for silencing in *S. cerevisiae* (22, 85). A functionally defined PAD (partitioning and anchoring) domain enables ScSir4 to associate with the nuclear periphery (3), and other less well-defined regions of Sir4 interact with Sir3, Sir2, and Rap1. Orthologs of Sir4 contribute to silencing in *S. bayanus* (44), *C. glabrata* (62) and *K. lactis* (6, 57), indicating a conserved function. However, ScSir4 appears to have lost an ancestral function, as it cannot complement a sir4Δ mutation in the closely related species *S. bayanus* (118), although both ScSir4 and *K. lactis* Sir4 (KISir4) complement a sir4Δ mutation in *S. cerevisiae* (6, 118).

**SIR1.** The silencer-associated protein Sir1 has a restricted distribution, with *Zygosaccharomyces rouxii* being the species most distant from *S. cerevisiae* in which a Sir1-like protein has been identified (Fig. 2). The Sir1 gene family has undergone dramatic expansions and contractions. Consequently, species, such as *C. glabrata*, have lost Sir1, whereas others, such as *S. bayanus*, encode multiple Sir1-like proteins, termed Kos (kin of Sir1) proteins (44). Sir1 and many of the KOS genes are located in subtelomeric regions, and this placement likely contributed to the rapid gains and losses of the Sir1 family.

ScSir1 contains a functionally defined OIR (ORC-interacting region) domain that associates with the ScOrc1 BAH domain and with ScSir4 to stabilize the Sir complex at silencers (45). This domain is conserved across species, and two-hybrid
analyses confirm that it consistently interacts with Orc1 (14). Interestingly, a second ORC-like domain also occurs in Sir1 (Fig. 1), indicating that there was an internal duplication within the SIR1 gene.

In addition to S. cerevisiae, SIR1-like genes have been examined experimentally in S. bayanus (44), where there are four family members, SIR1, KOS1, KOS2, and KOS3. All four paralogs contribute to silencing at the cryptic mating-type loci. However, the exact contributions of the different paralogs remain to be determined.

**IMPACT OF GENE DUPLICATIONS ON SILENCING PROTEINS**

All four of the SIR genes, as defined in S. cerevisiae, have undergone duplications within the Saccharomycetaceae family, and it is important to understand how these duplications have led to partitioning and specialization of the functions of the Sir proteins. The duplications of SIR2 and SIR3 occurred in conjunction with the whole-genome duplication (Fig. 2) (28, 65, 112, 113). Subsequent to this event, most genes returned to single-copy status. However, about 10% of S. cerevisiae genes, including SIR2 and SIR3, are retained paralogs. Consequently, the nonduplicated orthologs of Sir2 and Sir3 have additional functions, as outlined below.

Divergence of function after duplication can occur through neofunctionalization or subfunctionalization (24, 50), and both ScSIR2 and ScSIR3 are products of subfunctionalization. One mechanism of subfunctionalization is duplication, degeneration, and complementation, in which duplicated genes each lose one of the original functions and together retain the entire set of ancestral functions (38). Subfunctionalization can also occur through specialization, in which the divergence of functions among paralogs also involves the accumulation of advantageous mutations in at least one of the duplicated genes, enabling it to outperform the ancestral gene (24, 50, 51, 74).

**Duplication, degeneration, and complementation of Sir2.** The paralog of the deacetylase Sir2 is Hst1 (homolog of Sir2), a component of the SUM1 transcriptional repressor complex that represses middle sporulation, NAD⁺-biosynthetic, and α-specific genes in S. cerevisiae (11, 115, 117). Similarly, in C. glabrata, Hst1 regulates midsporulation genes as well as genes necessary for high-affinity uptake of NAD⁺ precursors (77). Interestingly, Sir2 and Hst1 generate distinct types of chromatin. Unlike the Sir3 complex, the SUM1 complex does not form extended domains of silenced chromatin but instead functions in a promoter-specific manner to repress its target genes.

Characterization of the nonduplicated Sir2 ortholog from K. lactis reveals that KlSir2 has both Hst1-like and Sir2-like properties, indicating that subfunctionalization occurred after duplication (57). Consistent with this idea, KlSir2 complements an hst1Δ deletion in S. cerevisiae (56) and partially suppresses a sir2Δ mating defect (23). Studies of chimeric ScSir2-Hst1 molecules indicate that distinct regions of these deacetylases enable them to associate with the SIR or SUM1 complexes (41, 56, 80), and these interaction domains are conserved in KlSir2 (41). The most parsimonious model is that the ancestral Sir2 also utilized these interaction domains and that after duplication the paralogs acquired complementary inactivating mutations that reduced their affinities for one of the two complexes. Thus, Sir2 and Hst1 represent an example of the duplication, degeneration, and complementation mechanism of subfunctionalization.

Although the initial subfunctionalization of Sir2 simply retained its ancestral functions, the division may ultimately have been beneficial. For example, ScHst1 has a lower affinity than ScSir2 for the cofactor NAD⁺ (11), and at slightly reduced concentrations of NAD⁺, Hst1-repressed genes are induced but Sir2-repressed genes are not (11, 77). Consequently, as NAD⁺ levels start to fall, ScHst1-repressed NAD⁺ biosynthetic genes are upregulated to restore NAD⁺ pools, without compromising ScSir2 function.

**Potential specialization of Orc1.** The paralog of Sir3 is Orc1, the largest subunit of the origin recognition complex (ORC). ORC binds to origins of DNA replication, and has orthologs in prokaryotes (DnaA) and archaea (Orc1/Cdc6). Orc1 likely had a silencing function long before it gave rise to Sir3, as connections between ORC and heterochromatin have been observed in a wide variety of species (7, 27, 70, 78, 90, 93). It has generally been assumed that ORC acts as a landing pad to recruit silencing factors to heterochromatic domains, based on the paradigm from S. cerevisiae, in which ScOrc1 stabilizes the SIR complex at silencers by interacting with ScSir1. However, Orc1 could also act like ScSir3 to facilitate the spreading of silencing proteins by binding nucleosomes.

Nonduplicated orthologs of Orc1/Sir3 show more sequence similarity to the duplicated Orc1 than to Sir3, initially leading researchers to propose that the silencing functions of Sir3 arose after duplication (65). However, the nonduplicated Orc1 from L. kluyveri weakly complements a sir3 mutation in S. cerevisiae (109), and the nonduplicated Orc1 from K. lactis has the ability to spread across and silence a cryptic mating-type locus in K. lactis in a Sir3-like manner (58). The capacity of KIOrc1 to spread and promote the spreading of other silencing proteins implies that the common ancestor of KIOrc1 and ScSir3 had a similar ability and that subfunctionalization of the replication and spreading functions of Orc1 occurred after duplication. This conclusion is consistent with the existence of an ancient partnership of Orc1 and Sir2 to generate extended heterochromatic domains.

Curiously, KIOrc1 does not appear to act like ScOrc1 to nucleate silencing. KIOrc1 is not a silencer binding protein (58, 105) and SIR1 is not detected in the K. lactis genome (36, 44). Therefore, Orc1 either lost its silencer-binding function in the K. lactis lineage or gained this property in the S. cerevisiae lineage. SIR1 is first identifiable in Z. rouxii, a species with a nonduplicated Orc1 (Fig. 2), so Orc1 likely acquired the ability to function as a silencer binding protein prior to the whole-genome duplication.

An important unanswered question is whether SIR3 continued to evolve after duplication, such that it acquired new properties that improved its silencing ability. The accelerated sequence divergence of SIR3 compared to ORC1 may indicate that SIR3 acquired new properties or may reflect relaxed selection. It is also unclear whether there is an adaptive advantage in utilizing both Sir3 and Orc1 in different capacities to achieve silencing.

**An ancient tandem duplication of SIR4.** SIR4 is an ancient paralog of the gene ASF2 (anti-silencing factor), which occurs
in tandem with SIR4 in species of the Saccharomycetaceae family that did not undergo the whole-genome duplication (19). Little is known about the function of Asf2, except that it antagonizes silencing in both S. cerevisiae and K. lactis (57, 69) and copurifies with ScSir2 (16). Thus, Asf2 may compete with Sir4 for binding to Sir2. Studies on the evolutionary histories of these two rapidly changing proteins would be interesting, especially in light of the absence of Sir4-like proteins outside the Saccharomycetaceae family.

**Internal duplication of SIR1.** SIR1 displays two types of duplication, expansions and contractions of subtelomeric SIR1-like genes and an internal duplication resulting in two tandem OIR-like domains. Phylogenetic analysis of OIR-like domains reveals a clear separation of the N- and C-terminal domains, indicating that the internal duplication occurred once during evolution (44). Kos3, which has a single OIR domain, is thought to be the ancestral form of the protein (44). After the internal duplication occurred, the resultant SIR1-like gene was subsequently duplicated in its entirety and diversified, yielding SIR1, KOS1, KOS2, and KOS4.

An important unanswered question is how the tandem duplication of the OIR domain contributes to the function of Sir1. In S. cerevisiae, the C-terminal domain interacts with ScOrc1 and is important for the recruitment of the SIR complex to silencers. In contrast, the function of the N-terminal domain (OIR’) is unclear, although it is hypothesized to interact with Sir3 or another BAH domain-containing protein (25, 61). An intriguing possibility is that the OIR domain duplication was coupled to the duplication and divergence of its interaction partner Orc1/Sir3 (44). A second unanswered question is whether the multiple Sir1-like proteins found in some species have distinct or overlapping functions.

**Genomic Locations Associated with Sir Proteins**

The Sir proteins were originally identified as transcriptional repressors of the cryptic mating-type loci. In budding yeasts, mating-type is determined by the MAT or MTL locus, which has two idiomorphs, α and α, encoding transcription factors that regulate the expression of cell-type-specific genes. Additional copies of mating-type cassettes enable switching of mating-type and emerged in the Saccharomycetaceae family (Fig. 2) (17). In S. cerevisiae, all three mating-type loci are on the same chromosome—two SIR-silenced loci located near the telomeres and an active locus situated more internally on the same chromosome (29). Ashbya gossypii has three mating-type loci near telomeres and a fourth more internally located (Fred Dietrich, personal communication), and L. kluyveri has only a single mating-type locus and can no longer undergo mating-type switching. In addition to this variability in organization of the mating-type loci, the extra mating-type loci are not always silenced as expected. In C. glabrata, MTL2 and MTL3 are both located near telomeres, but only MTL3 is silenced (94), and in K. lactis HMRa is repressed by the SUM1 complex instead of the SIR complex (57, 58).

Although the Sir proteins were first identified as repressors of mating-type loci, this was probably not their original function. Species outside the Saccharomycetaceae family contain a single, active mating-type locus, for which there is no evidence of regulation by Sir2 (18, 95). Nevertheless, Sir2 is present in these species and must have another function. Two candidate regions at which Sir2 may act are the telomeres and centromeres, which are silenced by HP1-containing heterochromatin in other eukaryotes.

An ancient role for Sir2 in generating subtelomeric heterochromatin seems highly likely given its presence at telomeres in S. pombe (39, 103) as well as S. cerevisiae, S. bayanus (118), C. glabrata (21, 62), and K. lactis (49, 58). However, a considerable reorganization of telomere structure did occur early in the Saccharomycotina subphylum (reviewed in reference 71). Compared to most eukaryotes, these yeasts display longer and more varied telomere repeat units, within which are embedded binding sites for Rap1, the protein responsible for recruiting Sir proteins to telomeres. Thus, the way in which Sir2 is recruited to telomeres is distinct in Saccharomycotina species.

Centromeres are often associated with heterochromatin. In most eukaryotes, including S. pombe, centromeres are flanked by repetitive sequences that are incorporated into heterochromatin, which is required for faithful chromosome segregation. In contrast, Saccharomycotina species lack HP1 and must either employ an alternative type of pericentromeric chromatin or have evolved other mechanisms to preclude the requirement for pericentromeric heterochromatin. There are two types of centromeres observed in budding yeasts. Species in the Saccharomycetaceae family generally have “point” centromeres, in which a relatively short DNA sequence (<500 bp) specifies the centromere (32). A specialized pericentromeric heterochromatin structure has not been observed at the centromeres in S. cerevisiae, and Sir2, 3, and 4 do not associate with centromeres (104). Curiously, Sir1 is found at centromeres (104), although its function is unknown. Thus, the development of point centromeres may have circumvented the requirement for a specialized chromatin structure. In contrast, species in the CTG clade have more complex centromeres that span 3 to 5 kb and are epigenetically inherited (10, 73, 101). Although the flanking chromatin structure of C. albicans centromeres has not been characterized, these centromeres are highly efficient origins of replication and bind ORC (66). It will be interesting to investigate whether the association of ORC facilitates the formation of a specialized chromatin structure containing Sir2.

**Rapid Sequence Evolution of Silencers**

The silencers that recruit the SIR complex to the cryptic mating-type loci have evolved much more rapidly than the Sir proteins themselves. For example, in K. lactis, the identified silencers do not contain binding sites for ORC or Rap1 but instead bind Reb1 and Ume6 (9, 105). Interestingly, Reb1 and Rap1 are related myb domain-containing proteins, suggesting that this family of proteins may be well-suited to function as silencer binding proteins. In C. glabrata, silencing of the MTL3 locus is apparently not nucelated at a silencer sequence at all but is instead subject to subtelomeric silencing (94). This loss of silencers is consistent with the absence of the silencer-
associated Sir1 protein in this species (44). Thus, substitutions of one silencer binding protein for another, and even the complete loss of silencers, have occurred over the course of evolution, but nevertheless the primary role of the Sir proteins in forming silenced chromatin has been preserved in species of the Saccharomyces family.

Comparisons of silencers in S. cerevisiae and closely related (sensu stricto) species provide insights into how silencers diverge. Although the same proteins bind to the silencers in these species, DNA sequences between the protein binding sites display elevated sequence divergence compared to other noncoding regions of the genome (107). This observation suggests that silencing impairs the fidelity of DNA replication or repair, thereby increasing the likelihood of acquiring or losing protein binding sites in these regions.

SPECIES-SPECIFIC ADAPTATIONS OF SILENCING

Saccharomyces bayanus. S. bayanus is the species most closely related to S. cerevisiae in which silencing has been examined experimentally. This yeast has fermentative capabilities similar to those of S. cerevisiae and is often identified in spontaneously fermented wines and ciders. In addition, S. bayanus and S. cerevisiae can mate and subsequently undergo meiosis, although the resulting spores are inviable (46). The maintenance of transcriptional silencing, mediated by Sir2, Sir3, and Sir4, occurs similarly in the two species. However, there are intriguing differences in the nucleation of silencing. S. bayanus has four paralogs of Sir1, whereas S. cerevisiae has one. Curiously, all four paralogs of Sir1 in S. bayanus contribute to transcriptional silencing, with Kos3 playing a distinct role compared to the other paralogs (44). Furthermore, the Sir1-interacting protein Sir4 has properties in S. bayanus not found in ScSir4. ScSir4 cannot efficiently associate with SbHMR, whereas SbSir4 does stably associate with ScHMR (118). Finally, the overall sequences of the silencers have diverged significantly between these two species, although these silencers appear to bind the same proteins (ORC, Rap1, and Abf1) in both species (107, 118).

This species was originally isolated from milk-derived products, although it grows on a wide range of carbon sources. Interest in cultivating K. lactis for biotechnology led to the development of its genetics, and its divergence prior to the whole-genome duplication makes K. lactis a convenient proxy for the ancestral nonduplicated state.

Kluyveromyces lactis. Among species whose genomes were not duplicated, silencing has been most extensively studied in K. lactis. This species was originally isolated from milk-derived products, although it grows on a wide range of carbon sources. Interest in cultivating K. lactis for biotechnology led to the development of its genetics, and its divergence prior to the whole-genome duplication makes K. lactis a convenient proxy for the ancestral nonduplicated state.

In K. lactis, silencing involves additional factors beyond those characterized in S. cerevisiae. In particular, the KISUM1 complex acts in concert with the SIR complex to silence the cryptic mating-type locus HMLα (57). Since KISir2 associates with both the SUM1 and SIR complexes, its role in silencing is probably mediated through both complexes. Interestingly, the KISUM1 complex also represses HMRα in the absence of Sir4 or Orc1. Thus, although the role of Sir2 in silencing mating-type loci is conserved in K. lactis, Sir2 does not always act as part of the SIR complex. In contrast to the mating-type loci, telomeres in K. lactis associate with components of the SIR-like complex but not KISum1 (58). The different protein compositions of the chromatin at these three loci probably confer distinct properties. We speculate that the SUM1-Sir2 complex has a greater role in repressing transcription, because deletion of KISUM1 results in a greater induction of HMLα genes than does deletion of KISIR4 (57).

In contrast to repressing the mating-type loci, the KISUM1 complex acts in a promoter-specific fashion to repress many of the same sporulation genes regulated by the ScSUM1 complex, as well as other cell-type-specific genes.
required for mating, such as the pheromone MFa1 and the G protein γ subunit STE18 (57). Consequently, Sir2 may be a critical factor preventing K. lactis from mating in certain conditions. K. lactis haploid cells delay mating until nutrients become scarce (8, 13, 55) unlike S. cerevisiae cells, which mate in nutrient-rich conditions. This difference is explained in part by a requirement for the transcription factor Rme1 (also known as KLmTs1), which is induced in low-nutrient conditions and activates expression of some genes necessary for mating (13). However, Rme1/Mts1 induction alone may be insufficient to complete mating, as Sir2 represses some cell-type-specific and pheromone-induced genes not induced by Rme1/Mts1. Although Sir2-mediated repression may need to be relieved for progression of mating, Sir2-mediated silencing of HMLα and HMRα favors mating by maintaining cell identity. However, unlike S. cerevisiae, K. lactis cells lacking Sir proteins are not completely sterile. Thus, the time at which Sir2-mediated repression is relieved may govern proper progression of the K. lactis sexual cycle and link it to nutrient availability. An interesting question for future studies is whether variations in Sir2-mediated repression alter the ways species coordinate life cycle transitions with environmental changes.

*Candida albicans*. Silencing and Sir2 function in the CTG clade are poorly understood. Species from this clade are responsible for the majority of human yeast infections, with *C. albicans* being the most common pathogen. These species are phenotypically diverse and vary in their abilities to mate, sporulate, and colonize mammalian hosts. The only conserved Sir proteins in these species are Sir2 and Orc1. *C. albicans* has five homologs of Sir2. One homolog, *orf19.1992*, was identified prior to genome sequencing and was annotated as *SIR2* (92). However, another Sir2 homolog, *orf19.4761*, has better sequence conservation with Sir2 orthologs. The functions of these two homologs have not been clarified such that one can be definitively annotated as Sir2. Therefore, both genes will be referred to by their systematic names from the SC5314 strain.

Gene dosage of *orf19.4761* correlates with the replicative life span of *C. albicans*, such that cells with more *SIR2* genes have longer life spans and display asymmetric distribution of oxidized and damaged proteins during cell division (42). These observations are consistent with the role of *SIR2* in aging in *S. cerevisiae* (1, 63). Deletion of the other Sir2 homolog, *orf19.1992*, lowers the frequency of phenotypic switching from the opaque to the white state (59). As only *C. albicans* opaque cells are competent to mate (81), *orf19.1992* may reduce mating by favoring the white state. It is not clear whether either of these phenotypes is related to the presumed transcriptional repression activities of Sir2 proteins.

Further investigations into silencing in the CTG clade will answer important questions regarding the evolution of Sir2-mediated silencing. Does silenced chromatin form at the telomeres or near the centromeres? If so, do Sir2 and Orc1 act together to generate this silenced chromatin and are other proteins, perhaps related to Sir4 or Sum1, involved? How have the mechanism of silencing and the loss of mating and meiosis influenced one another? Might the lack of a highly specialized silenced chromatin correlate with the increased genome plasticity observed in these species?

MODEL FOR EVOLUTION OF Sir-MEDIATED SILENCING

Based on the studies outlined above, we propose the following model for the evolution of Sir-mediated silencing (Fig. 3). First, HP1-containing heterochromatin was lost early in the *Saccharomycotina* subphylum. This loss necessitated adaptations, such as the development of alternative chromatin structures at telomeres and centromeres to maintain genome stability. At the core of such alternative silencing mechanisms was...
the conserved deacetylase Sir2, which participates in the formation of repressive chromatin in a variety of fungal and non-fungal species. We speculate that Sir2 partnered with the conserved replication protein Orc1 to generate repressive chromatin, with Sir2 deacetylating nucleosomes that could be bound by the BAH domain of Orc1. It is not clear whether the joint action of Sir2 and Orc1 is evolutionarily ancient and increased in importance after the loss of HP1 or whether it originated after the loss of HP1. However, the participation of Orc1 and Sir2 in subtelomeric heterochromatin in the protozoan parasite Plasmodium falciparum (78) is consistent with an ancient relationship. It is also unclear whether a Sir4-like protein was part of this proposed ancestral silencing complex, as Sir4 is rapidly evolving and could still be found outside the Saccharomycetaceae family. Mechanistic studies of Sir2 functions in Candida species will help resolve these issues by clarifying the role of Orc1 and identifying Sir2-interacting proteins.

In the Saccharomycetaceae family, changes in mating-type architecture and protein function led to the development of Sir-mediated silencing, as characterized in S. cerevisiae (Fig. 2). One important change was the emergence of silent mating-type cassettes in telomere-proximal locations that could exploit the preexisting silenced domains at the ends of chromosomes. The Sir1 family of proteins emerged relatively recently, and the ability of these proteins to interact with Orc1 may have expanded the role of Orc1 in the establishment of silencing. Finally, the whole-genome duplication enabled the partitioning and specialization of Sir2/Hst1 and Orc1/Sir3 functions. In addition, the Sir proteins likely evolved different adaptive functions in yeast species not yet examined. For example, in N. castellii, paralogs of SUM1 and SIR4 have been retained (19), suggesting the existence of multiple varieties of silencing complexes in this species.

ACKNOWLEDGMENTS

We thank Brendan Cormack, Joseph Heitman, and Oliver Zill for comments on this manuscript and Cletus Kurtzmann and Kenneth Wolfe for guidance on yeast taxonomy.

Research in the Rusche lab is supported by a grant from the NIH (GM073991).

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