EUKARYOTIC CELL, Sept. 2011, p. 1183–1192 Vol. 10, No. 9
1535-9778/11/$12.00 doi:10.1128/EC.05123-11
Copyright © 2011, American Society for Microbiology. All Rights Reserved.

MINIREVIEW

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

Meleah A. Hickman,[2] Cara A. Froyd,[1] and Laura N. Rusche[1]*

Duke University, Biochemistry Department and Institute for Genome Sciences & Policy, Durham, North Carolina,[1] and University of Minnesota, Department of Genetics, Cell Biology, and Development, Minneapolis, Minnesota[2]

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, *HMR***a** and *HML*α, 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. *HMR***a** and *HML*α 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 (*s*ilent *i*nformation *r*egulator) 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-

* Corresponding author. Mailing address: Box 3382, DUMC, Duke University, Durham, NC 27710. Phone: (919) 684-0354. Fax: (919) 668-0795. E-mail: lrusche@duke.edu.
▽ Published ahead of print on 15 July 2011.

Downloaded from http://ec.asm.org/ on October 24, 2020 by guest

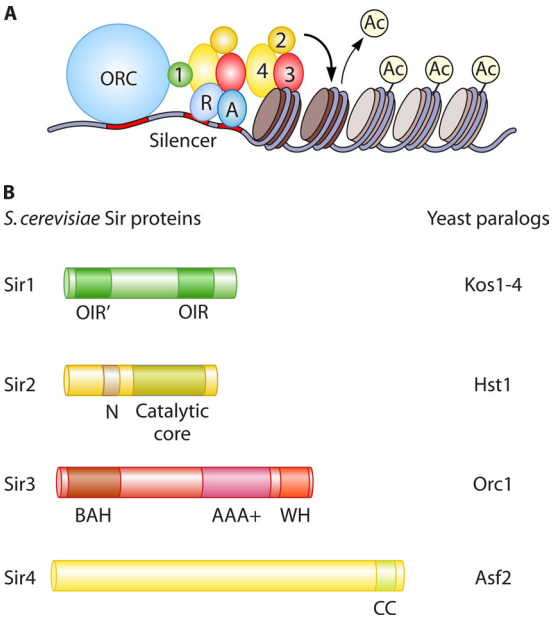


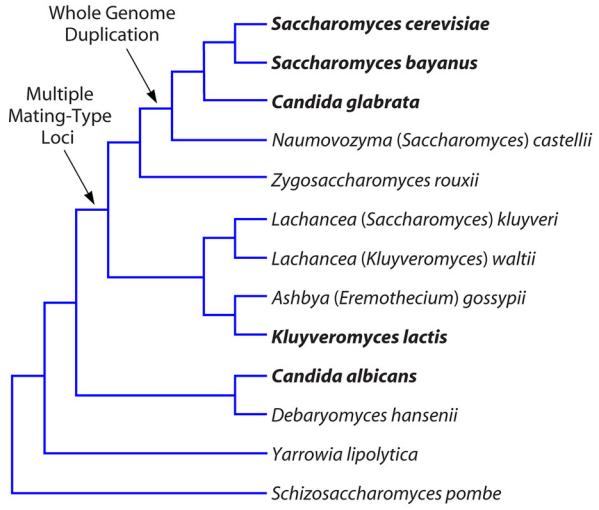
FIG. 1. Functions of Sir proteins. (A) The silencer binding proteins ORC, Rap1 (R), and Abf1 (A) recruit the Sir proteins (1 to 4) to the silencer. Sir2 deacetylates neighboring nucleosomes, generating binding sites for Sir3 and Sir4. Reiterations of this cycle enable spreading. (B) Conserved domains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

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, *Taprhinomycotina*, which is thought to have diverged from *Saccharomycotina* around a billion years ago (53, 54).

SACCHAROMYCOTINA SPECIES LACK KEY COMPONENTS OF HP1-MEDIATED HETEROCHROMATIN

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, Clr4, which specifically methylates lysine 9 of histone H3, and a chromodomain-containing protein, Swi6, which binds preferentially to H3-K9^{me}. These proteins are well-conserved among eukaryotes and are generally known as SuVar3-9 (the methyltransferase) and HP1 (the chromodomain-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 (*R*NA-*i*nduced *t*ranscriptional *s*ilencing) 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.



Paralogs		Paralogs			
Sir2	Hst1	Orc1	Sir3	Sir4	Sir1
+*	+	+*	+*	+*	+*
+*	+	+	+*	+*	++++*
+*	+	+	+*	+*	-
+	+	+	+	++	++++
+	-	+	-	+	+
+	-	+	-	+	-
+	-	+	-	+	-
+	-	+	-	+	-
+*	-	+*	-	+*	-
++	-	+	-	-	-
+	-	+	-	-	-
+	-	+	-	-	-
+*	-	+	-	-	-

Saccharomycetaceae Family
CTG Clade
* known silencing function

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).

Downloaded from http://ec.asm.org/ on October 24, 2020 by guest

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 archaea (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 *Saccharomycotina* 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 *Saccharomycotina* 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 *Saccharomycotina*. In *S. pombe*, SpSir2 contributes to silencing at 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 budding yeasts. In addition, Sir2 associates with the rDNA independently of the other Sir proteins, and this function has been conserved in other eukaryotes, including mammals (84).

SIR3. The histone-binding protein Sir3 arose in the whole-genome duplication from *ORC1*, a subunit of the origin recognition complex found in all eukaryotes (12, 19, 28, 65). Consequently, distinct Sir3 proteins are found only in species that descended from the whole-genome duplication (Fig. 2).

Both Sir3 and Orc1 have three conserved domains (Fig. 1). The N-terminal BAH (*b*romo-*a*djacent *h*omology) domain binds nucleosomes and in ScSir3 is required for silencing *in vivo* and the formation of SIR-nucleosome filaments *in vitro* (12, 16, 89, 100). In addition, the BAH domain of ScOrc1 binds ScSir1 (108, 116). The AAA^+ domain (*A*TPases *a*ssociated with diverse *a*ctivities) belongs to a functionally diverse superfamily that hydrolyzes ATP and harnesses the released energy for assembly and disassembly of macromolecular complexes. This domain is poorly conserved in Sir3 and lacks residues critical for binding ATP but nevertheless can be aligned with Orc1 along its entire length, indicating that the integrity of the domain is important. Finally, the winged helix domain is predicted to bind DNA. The AAA^+ and winged helix domains of ScSir3 overlap with regions of the protein shown to make critical contacts with other silencing proteins, including histone tails (20, 52), Sir4 (35, 82, 83, 91), and Rap1 (83).

Orthologs of Sir3 contribute to silencing of subtelomeric domains in *C. glabrata* (21, 26) and associate with cryptic mating-type loci in *S. bayanus* (44, 118), indicating that the silencing function of Sir3 is conserved. Moreover, the nonduplicated Orc1 protein fulfills the role of Sir3 in *K. lactis* and perhaps in *Lachancea kluyveri* (58, 109). However, it is unknown whether Orc1 acts with the deacetylase Sir2 to generate heterochromatin outside the *Saccharomycetaceae* family. Therefore, Orc1 either acquired a Sir3-like role in silencing within the *Saccharomycotina* subphylum or already had such a role in heterochromatin formation and became more critical 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 *Saccharomycetaceae* family and is either absent or highly diverged and nonsyntenic in the CTG 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 telomeres (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 (*p*artitioning *a*nd *a*nchoring) domain enables ScSir4 to associate with the nuclear periphery (3), and other less well-defined regions of Sir4 interact with Sir1, 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 *sir4*Δ mutation in the closely related species *S. bayanus* (118), although both *S. bayanus* Sir4 (SbSir4) and *K. lactis* Sir4 (KlSir4) complement a *sir4*Δ 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, some species, such as *C. glabrata*, have lost Sir1, whereas others, such as *S. bayanus*, encode multiple Sir1-like proteins, termed Kos (*k*in *o*f *S*ir1) 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 (*O*RC-*i*nteracting *r*egion) 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

Downloaded from http://ec.asm.org/ on October 24, 2020 by guest

analyses confirm that it consistently interacts with Orc1 (14). Interestingly, a second OIR-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 (*h*omolog of *S*ir*2*), 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 SIR 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 *hst1*Δ deletion in *S. cerevisiae* (56) and partially suppresses a *sir2*Δ 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, is found throughout eukaryotes, 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 KlOrc1 to spread and promote the spreading of other silencing proteins implies that the common ancestor of KlOrc1 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, KlOrc1 does not appear to act like ScOrc1 to nucleate silencing. KlOrc1 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* (*a*nti-*s*ilencing *f*actor), which occurs

Downloaded from http://ec.asm.org/ on October 24, 2020 by guest

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 (*KOS*) 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, **a** 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 chromosome. However, there is plasticity in the number and placement of mating-type loci. For example, the mating-type loci are not all on the same chromosome in *C. glabrata* and *K. lactis*. *Lachancea waltii* has two adjacent mating-type loci near a single telomere (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 HMR***a** 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 nucleated 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-

Downloaded from http://ec.asm.org/ on October 24, 2020 by guest

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 *Saccharomycetaceae* 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). Thus, there has been a coordinated evolution of Sir1, Sir4, and silencer sequences, resulting in species-specific requirements for the assembly of silenced chromatin. These observations raise the possibility that the coexistence of incompatible proteins in hybrid cells interferes with silencing and thus contributes to reproductive barriers between species.

***Candida glabrata*.** *C. glabrata* is more distantly related to *S. cerevisiae* yet is descended from the whole-genome duplication. This yeast is a commensal, opportunistic pathogen and is a common cause of yeast infection in humans, along with *C. albicans* (37, 64). Although both of these species bear the name *Candida*, they are phylogenetically distant (Fig. 2).

A striking difference between *C. glabrata* and *S. cerevisiae* is that silencing is regulated by environmental conditions in *C. glabrata*, whereas it is constitutive in *S. cerevisiae*. Nevertheless, the general mechanism of silencing appears to be conserved (26, 30, 62). The environmental regulation of silencing in *C. glabrata* is possible because *C. glabrata* is auxotrophic for niacin, a precursor of NAD^+ (30), which is required for Sir2 deacetylation. In niacin-poor environments, the cellular level of NAD^+ drops, thereby reducing the function of *C. glabrata* Sir2 (CgSir2) and hence silencing (30, 77). Loss of silencing leads to the induction of several subtelomeric *EPA* adhesin genes, enabling the cells to adhere to epithelial cells (21, 30). Regulating silencing in this way may facilitate colonization of the urinary tract, an environment poor in niacin. Subtelomeric *EPA* adhesin genes are also induced under conditions that favor biofilm formation (62).

C. glabrata reproduces mitotically and is not known to have a sexual stage in its life cycle, despite maintaining three mating-type loci and genes important for meiosis (114). The adaptation to a predominantly asexual mode of reproduction may have enabled Sir-mediated silencing to become regulatable. Perturbations in CgSir2 function would have deleterious effects if cells needed to maintain cell type identity in readiness for mating.

***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.

K. lactis has orthologs of Sir2 and Sir4, which function similarly to their *S. cerevisiae* homologs (5, 6, 57). In contrast, there is no distinct Sir3 in *K. lactis* but rather a single nonduplicated Orc1/Sir3 protein. KlOrc1 acts in conjunction with Sir2 and Sir4 to generate specialized chromatin structures at telomeres and *HML*α. Furthermore KlOrc1 depends on its nucleosome-binding BAH domain to facilitate spreading of these proteins, much as ScSir3 does (58). Thus, a SIR-like complex does exist in this nonduplicated species.

Silencing in *K. lactis* involves additional factors beyond those characterized in *S. cerevisiae*. In particular, the KlSUM1 complex acts in concert with the SIR complex to silence the cryptic mating-type locus *HML*α (57). Since KlSir2 associates with both the SUM1 and SIR complexes, its role in silencing is probably mediated through both complexes. Interestingly, the KlSUM1 complex also represses *HMR***a** 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 KlSum1 (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 *KlSUM1* results in a greater induction of *HML*α genes than does deletion of *KlSIR4* (57). In contrast, the Sir4-Sir2-Orc1 complex, which still assembles in the absence of KlSum1, may fulfill a different function, such as preventing inappropriate switching events at *HML*α or recombination at telomeres.

In addition to repressing the mating-type loci, the KlSUM1 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

Downloaded from http://ec.asm.org/ on October 24, 2020 by guest

required for mating, such as the pheromone *MFα1* 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 *HMR***a** 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?

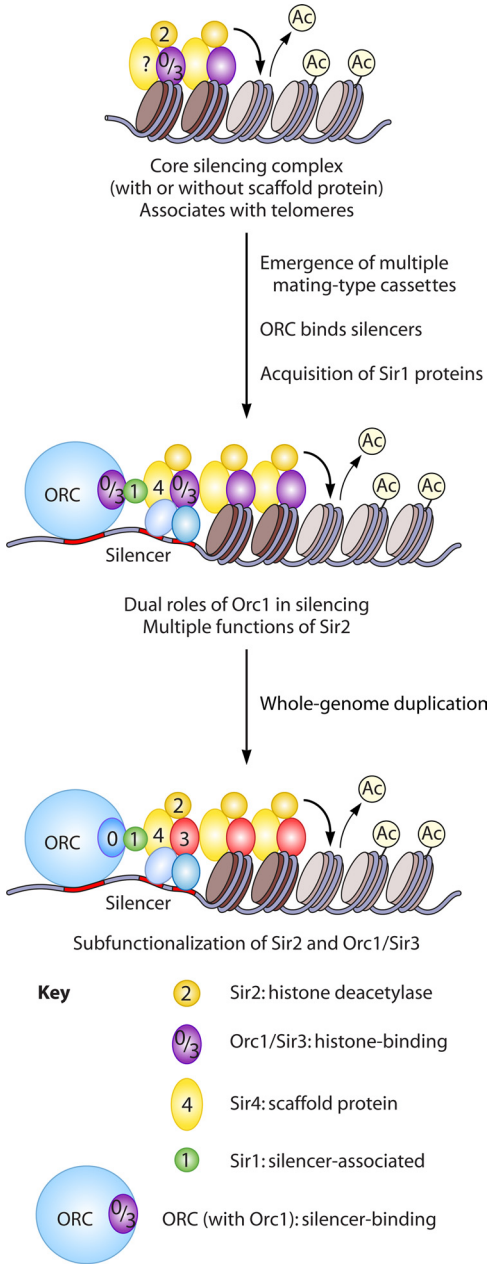


FIG. 3. Model for the evolution of Sir-mediated silencing. (Top) In the ancestral state, the deacetylase Sir2 partnered with the histone binding protein Orc1. (Middle) An intermediate stage involved dual roles for Orc1, binding silencers and nucleosomes. (Bottom) After the whole-genome duplication, subfunctionalization of Sir2/Hst1 and Orc1/Sir3 occurred.

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

Downloaded from http://ec.asm.org/ on October 24, 2020 by guest

the conserved deacetylase Sir2, which participates in the formation of repressive chromatin in a variety of fungal and nonfungal 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 function 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 Kurtzman and Kenneth Wolfe for guidance on yeast taxonomy.

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

REFERENCES

1. **Aguilaniu, H., L. Gustafsson, M. Rigoulet, and T. Nystrom.** 2003. Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. Science **299:**1751–1753.
2. **Alsford, S., T. Kawahara, C. Isamah, and D. Horn.** 2007. A sirtuin in the African trypanosome is involved in both DNA repair and telomeric gene silencing but is not required for antigenic variation. Mol. Microbiol. **63:** 724–736.
3. **Ansari, A., and M. R. Gartenberg.** 1997. The yeast silent information regulator Sir4p anchors and partitions plasmids. Mol. Cell. Biol. **17:**7061–7068.
4. **Astrom, S. U., T. W. Cline, and J. Rine.** 2003. The *Drosophila melanogaster* sir2+ gene is nonessential and has only minor effects on position-effect variegation. Genetics **163:**931–937.
5. **Astrom, S. U., A. Kegel, J. O. Sjostrand, and J. Rine.** 2000. *Kluyveromyces lactis* Sir2p regulates cation sensitivity and maintains a specialized chromatin structure at the cryptic alpha-locus. Genetics **156:**81–91.
6. **Astrom, S. U., and J. Rine.** 1998. Theme and variation among silencing proteins in *Saccharomyces cerevisiae* and *Kluyveromyces lactis*. Genetics **148:**1021–1029.
7. **Auth, T., E. Kunkel, and F. Grummt.** 2006. Interaction between HP1alpha and replication proteins in mammalian cells. Exp. Cell Res. **312:**3349–3359.
8. **Barsoum, E., P. Martinez, and S. U. Astrom.** 2010. Alpha3, a transposable element that promotes host sexual reproduction. Genes Dev. **24:**33–44.
9. **Barsoum, E., J. O. Sjostrand, and S. U. Astrom.** 2010. Ume6 is required for the MATa/MATalpha-cellular identity and transcriptional silencing in *Kluyveromyces lactis*. Genetics **184:**999–1011.
10. **Baum, M., K. Sanyal, P. K. Mishra, N. Thaler, and J. Carbon.** 2006. Formation of functional centromeric chromatin is specified epigenetically in *Candida albicans*. Proc. Natl. Acad. Sci. U. S. A. **103:**14877–14882.
11. **Bedalov, A., M. Hirao, J. Posakony, M. Nelson, and J. A. Simon.** 2003. NAD^+-dependent deacetylase Hst1p controls biosynthesis and cellular NAD^+ levels in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **23:**7044–7054.
12. **Bell, S. P., J. Mitchell, J. Leber, R. Kobayashi, and B. Stillman.** 1995. The multidomain structure of Orc1p reveals similarity to regulators of DNA replication and transcriptional silencing. Cell **83:**563–568.
13. **Booth, L. N., B. B. Tuch, and A. D. Johnson.** 2010. Intercalation of a new tier of transcription regulation into an ancient circuit. Nature **468:**959–963.
14. **Bose, M. E., et al.** 2004. The Origin Recognition Complex and Sir4 protein recruit Sir1p to yeast silent chromatin through independent interactions requiring a common Sir1p domain. Mol. Cell. Biol. **24:**774–786.
15. **Brachmann, C. B., et al.** 1995. The *SIR2* gene family, conserved from bacteria to humans, functions in silencing, cell cycle progression, and chromosome stability. Genes Dev. **9:**2888–2902.
16. **Buchberger, J. R., et al.** 2008. Sir3-nucleosome interactions in spreading of silent chromatin in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **28:**6903–6918.
17. **Butler, G., et al.** 2004. Evolution of the *MAT* locus and its Ho endonuclease in yeast species. Proc. Natl. Acad. Sci. U. S. A. **101:**1632–1637.
18. **Butler, G., et al.** 2009. Evolution of pathogenicity and sexual reproduction in eight *Candida* genomes. Nature **459:**657–662.
19. **Byrne, K. P., and K. H. Wolfe.** 2005. The Yeast Gene Order Browser: combining curated homology and syntenic context reveals gene fate in polyploid species. Genome Res. **15:**1456–1461.
20. **Carmen, A. A., L. Milne, and M. Grunstein.** 2002. Acetylation of the yeast histone H4 N terminus regulates its binding to heterochromatin protein SIR3. J. Biol. Chem. **277:**4778–4781.
21. **Castano, I., et al.** 2005. Telomere length control and transcriptional regulation of subtelomeric adhesins in *Candida glabrata*. Mol. Microbiol. **55:** 1246–1258.
22. **Chang, J. F., et al.** 2003. Structure of the coiled-coil dimerization motif of Sir4 and its interaction with Sir3. Structure **11:**637–649.
23. **Chen, X. J., and G. D. Clark-Walker.** 1994. *sir2* mutants of *Kluyveromyces lactis* are hypersensitive to DNA-targeting drugs. Mol. Cell. Biol. **14:**4501–4508.
24. **Conant, G. C., and K. H. Wolfe.** 2008. Turning a hobby into a job: how duplicated genes find new functions. Nat. Rev. Genet. **9:**938–950.
25. **Connelly, J. J., et al.** 2006. Structure and function of the *Saccharomyces cerevisiae* Sir3 BAH domain. Mol. Cell. Biol. **26:**3256–3265.
26. **De Las Penas, A., et al.** 2003. Virulence-related surface glycoproteins in the yeast pathogen *Candida glabrata* are encoded in subtelomeric clusters and subject to RAP1- and SIR-dependent transcriptional silencing. Genes Dev. **17:**2245–2258.
27. **Deng, Z., J. Dheekollu, D. Broccoli, A. Dutta, and P. M. Lieberman.** 2007. The origin recognition complex localizes to telomere repeats and prevents telomere-circle formation. Curr. Biol. **17:**1989–1995.
28. **Dietrich, F. S., et al.** 2004. The *Ashbya gossypii* genome as a tool for mapping the ancient *Saccharomyces cerevisiae* genome. Science **304:**304–307.
29. **Di Rienzi, S. C., et al.** 2011. Genetic, genomic, and molecular tools for studying the protoploid yeast, *L. waltii*. Yeast **28:**137–151.
30. **Domergue, R., et al.** 2005. Nicotinic acid limitation regulates silencing of *Candida* adhesins during UTI. Science **308:**866–870.
31. **Drinnenberg, I. A., et al.** 2009. RNAi in budding yeast. Science **326:**544–550.
32. **Dujon, B.** 2005. Hemiascomycetous yeasts at the forefront of comparative genomics. Curr. Opin. Genet. Dev. **15:**614–620.
33. **Dujon, B.** 2006. Yeasts illustrate the molecular mechanisms of eukaryotic genome evolution. Trends Genet. **22:**375–387.
34. **Duraisingh, M. T., et al.** 2005. Heterochromatin silencing and locus repositioning linked to regulation of virulence genes in *Plasmodium falciparum*. Cell **121:**13–24.
35. **Enomoto, S., S. D. Johnston, and J. Berman.** 2000. Identification of a novel allele of *SIR3* defective in the maintenance, but not the establishment, of silencing in *Saccharomyces cerevisiae*. Genetics **155:**523–538.
36. **Fabre, E., et al.** 2005. Comparative genomics in hemiascomycete yeasts: evolution of sex, silencing, and subtelomeres. Mol. Biol. Evol. **22:**856–873.
37. **Fidel, P. L., Jr., J. A. Vazquez, and J. D. Sobel.** 1999. *Candida glabrata*: review of epidemiology, pathogenesis, and clinical disease with comparison to *C. albicans*. Clin. Microbiol. Rev. **12:**80–96.
38. **Force, A., et al.** 1999. Preservation of duplicate genes by complementary, degenerative mutations. Genetics **151:**1531–1545.
39. **Freeman-Cook, L. L., et al.** 2005. Conserved locus-specific silencing functions of *Schizosaccharomyces pombe sir2*+. Genetics **169:**1243–1260.
40. **Freitas-Junior, L. H., et al.** 2005. Telomeric heterochromatin propagation and histone acetylation control mutually exclusive expression of antigenic variation genes in malaria parasites. Cell **121:**25–36.
41. **Froyd, C. A., and L. N. Rusche.** 2011. The duplicated deacetylases Sir2 and Hst1 subfunctionalized by acquiring complementary inactivating mutations. Mol. Cell. Biol. **31:**3351–3365.

Downloaded from http://ec.asm.org/ on October 24, 2020 by guest

42. **Fu, X. H., F. L. Meng, Y. Hu, and J. Q. Zhou.** 2008. *Candida albicans*, a distinctive fungal model for cellular aging study. Aging Cell **7:**746–757.
43. **Furuyama, T., R. Banerjee, T. R. Breen, and P. J. Harte.** 2004. SIR2 is required for polycomb silencing and is associated with an E(Z) histone methyltransferase complex. Curr. Biol. **14:**1812–1821.
44. **Gallagher, J. E., J. E. Babiarz, L. Teytelman, K. H. Wolfe, and J. Rine.** 2009. Elaboration, diversification and regulation of the Sir1 family of silencing proteins in *Saccharomyces*. Genetics **181:**1477–1491.
45. **Gardner, K. A., J. Rine, and C. A. Fox.** 1999. A region of the Sir1 protein dedicated to recognition of a silencer and required for interaction with the Orc1 protein in *Saccharomyces cerevisiae*. Genetics **151:**31–44.
46. **Greig, D.** 2009. Reproductive isolation in *Saccharomyces*. Heredity **102:** 39–44.
47. **Greiss, S., and A. Gartner.** 2009. Sirtuin/Sir2 phylogeny, evolutionary considerations and structural conservation. Mol. Cells **28:**407–415.
48. **Grewal, S. I., and J. C. Rice.** 2004. Regulation of heterochromatin by histone methylation and small RNAs. Curr. Opin. Cell Biol. **16:**230–238.
49. **Gurevich, R., S. Smolikov, H. Maddar, and A. Krauskopf.** 2003. Mutant telomeres inhibit transcriptional silencing at native telomeres of the yeast *Kluyveromyces lactis*. Mol. Genet. Genomics **268:**729–738.
50. **Hahn, M. W.** 2009. Distinguishing among evolutionary models for the maintenance of gene duplicates. J. Hered. **100:**605–617.
51. **He, X., and J. Zhang.** 2005. Rapid subfunctionalization accompanied by prolonged and substantial neofunctionalization in duplicate gene evolution. Genetics **169:**1157–1164.
52. **Hecht, A., T. Laroche, S. Strahl-Bolsinger, S. M. Gasser, and M. Grunstein.** 1995. Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin in yeast. Cell **80:**583–592.
53. **Heckman, D. S., et al.** 2001. Molecular evidence for the early colonization of land by fungi and plants. Science **293:**1129–1133.
54. **Hedges, S. B.** 2002. The origin and evolution of model organisms. Nat. Rev. Genet. **3:**838–849.
55. **Herman, A., and H. Roman.** 1966. Allele specific determinants of homothallism in *Saccharomyces lactis*. Genetics **53:**727–740.
56. **Hickman, M. A., and L. N. Rusche.** 2007. Substitution as a mechanism for genetic robustness: the duplicated deacetylases Hst1p and Sir2p in *Saccharomyces cerevisiae*. PLoS Genet. **3:**e126.
57. **Hickman, M. A., and L. N. Rusche.** 2009. The Sir2-Sum1 complex represses transcription using both promoter-specific and long-range mechanisms to regulate cell identity and sexual cycle in the yeast *Kluyveromyces lactis*. PLoS Genet. **5:**e1000710.
58. **Hickman, M. A., and L. N. Rusche.** 2010. Transcriptional silencing functions of the yeast protein Orc1/Sir3 subfunctionalized after gene duplication. Proc. Natl. Acad. Sci. U. S. A. **107:**19384–19389.
59. **Hnisz, D., T. Schwarzmuller, and K. Kuchler.** 2009. Transcriptional loops meet chromatin: a dual-layer network controls white-opaque switching in *Candida albicans*. Mol. Microbiol. **74:**1–15.
60. **Hoppe, G. J., et al.** 2002. Steps in assembly of silent chromatin in yeast: Sir3-independent binding of a Sir2/Sir4 complex to silencers and role for Sir2-dependent deacetylation. Mol. Cell. Biol. **22:**4167–4180.
61. **Hou, Z., et al.** 2009. Phylogenetic conservation and homology modeling help reveal a novel domain within the budding yeast heterochromatin protein Sir1. Mol. Cell. Biol. **29:**687–702.
62. **Iraqui, I., et al.** 2005. The Yak1p kinase controls expression of adhesins and biofilm formation in *Candida glabrata* in a Sir4p-dependent pathway. Mol. Microbiol. **55:**1259–1271.
63. **Kaeberlein, M., M. McVey, and L. Guarente.** 1999. The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. Genes Dev. **13:**2570–2580.
64. **Kaur, R., R. Domergue, M. L. Zupancic, and B. P. Cormack.** 2005. A yeast by any other name: *Candida glabrata* and its interaction with the host. Curr. Opin. Microbiol. **8:**378–384.
65. **Kellis, M., B. W. Birren, and E. S. Lander.** 2004. Proof and evolutionary analysis of ancient genome duplication in the yeast *Saccharomyces cerevisiae*. Nature **428:**617–624.
66. **Koren, A., et al.** 2010. Epigenetically-inherited centromere and neocentromere DNA replicates earliest in S-phase. PLoS Genet. **6:**e1001068.
67. **Kurtzman, C. P.** 2003. Phylogenetic circumscription of *Saccharomyces*, *Kluyveromyces* and other members of the *Saccharomycetaceae*, and the proposal of the new genera *Lachancea*, *Nakaseomyces*, *Naumovia*, *Vanderwaltozyma* and *Zygotorulaspora*. FEMS Yeast Res. **4:**233–245.
68. **Kurtzman, C. P.** 2011. Discussion of teleomorphic and anamorphic ascomycetous yeasts and yeast-like taxa, p. 293–307. *In* C. P. Kurtzman, J. W. Fell, and T. Boekhout (ed.), The yeasts, a taxonomic study, 5th ed. Elsevier, Amsterdam, The Netherlands.
69. **Le, S., C. Davis, J. B. Konopka, and R. Sternglanz.** 1997. Two new S-phase-specific genes from *Saccharomyces cerevisiae*. Yeast **13:**1029–1042.
70. **Leatherwood, J., and A. Vas.** 2003. Connecting ORC and heterochromatin: why? Cell Cycle **2:**573–575.
71. **Lue, N. F.** 2010. Plasticity of telomere maintenance mechanisms in yeast. Trends Biochem. Sci. **35:**8–17.
72. **Luo, K., M. A. Vega-Palas, and M. Grunstein.** 2002. Rap1-Sir4 binding independent of other Sir, yKu, or histone interactions initiates the assembly of telomeric heterochromatin in yeast. Genes Dev. **16:**1528–1539.
73. **Lynch, D. B., M. E. Logue, G. Butler, and K. H. Wolfe.** 2010. Chromosomal G + C content evolution in yeasts: systematic interspecies differences, and GC-poor troughs at centromeres. Genome Biol. Evol. **2:**572–583.
74. **Lynch, M., and V. Katju.** 2004. The altered evolutionary trajectories of gene duplicates. Trends Genet. **20:**544–549.
75. **Lynch, P. J., and L. N. Rusche.** 2009. A silencer promotes the assembly of silenced chromatin independently of recruitment. Mol. Cell. Biol. **29:**43–56.
76. **Lynch, P. J., and L. N. Rusche.** 2010. An auxiliary silencer and a boundary element maintain high levels of silencing proteins at *HMR* in *Saccharomyces cerevisiae*. Genetics **185:**113–127.
77. **Ma, B., et al.** 2009. High-affinity transporters for NAD+ precursors in *Candida glabrata* are regulated by Hst1 and induced in response to niacin limitation. Mol. Cell. Biol. **29:**4067–4079.
78. **Mancio-Silva, L., A. P. Rojas-Meza, M. Vargas, A. Scherf, and R. Hernandez-Rivas.** 2008. Differential association of Orc1 and Sir2 proteins to telomeric domains in *Plasmodium falciparum*. J. Cell Sci. **121:**2046–2053.
79. **Martino, F., et al.** 2009. Reconstitution of yeast silent chromatin: multiple contact sites and O-AADPR binding load SIR complexes onto nucleosomes *in vitro*. Mol. Cell **33:**323–334.
80. **Mead, J., et al.** 2007. Swapping the gene-specific and regional silencing specificities of the Hst1 and Sir2 histone deacetylases. Mol. Cell. Biol. **27:**2466–2475.
81. **Miller, M. G., and A. D. Johnson.** 2002. White-opaque switching in *Candida albicans* is controlled by mating-type locus homeodomain proteins and allows efficient mating. Cell **110:**293–302.
82. **Moazed, D., A. Kistler, A. Axelrod, J. Rine, and A. D. Johnson.** 1997. Silent information regulator protein complexes in *Saccharomyces cerevisiae*: a SIR2/SIR4 complex and evidence for a regulatory domain in SIR4 that inhibits its interaction with SIR3. Proc. Natl. Acad. Sci. U. S. A. **94:**2186–2191.
83. **Moretti, P., K. Freeman, L. Coodly, and D. Shore.** 1994. Evidence that a complex of SIR proteins interacts with the silencer and telomere-binding protein RAP1. Genes Dev. **8:**2257–2269.
84. **Murayama, A., et al.** 2008. Epigenetic control of rDNA loci in response to intracellular energy status. Cell **133:**627–639.
85. **Murphy, G. A., et al.** 2003. The Sir4 C-terminal coiled coil is required for telomeric and mating-type silencing in *Saccharomyces cerevisiae*. J. Mol. Biol. **334:**769–780.
86. **Nakayashiki, H.** 2005. RNA silencing in fungi: mechanisms and applications. FEBS Lett. **579:**5950–5957.
87. **Newman, B. L., J. R. Lundblad, Y. Chen, and S. M. Smolik.** 2002. A *Drosophila* homologue of Sir2 modifies position-effect variegation but does not affect life span. Genetics **162:**1675–1685.
88. **Noma, K., et al.** 2004. RITS acts in cis to promote RNA interference-mediated transcriptional and post-transcriptional silencing. Nat. Genet. **36:**1174–1180.
89. **Onishi, M., G.-G. Liou, J. R. Buchberger, T. Walz, and D. Moazed.** 2007. Role of the conserved Sir3-BAH domain in nucleosome binding and silent chromatin assembly. Mol. Cell **28:**1015–1028.
90. **Pak, D. T., et al.** 1997. Association of the origin recognition complex with heterochromatin and HP1 in higher eukaryotes. Cell **91:**311–323.
91. **Park, Y., J. Hanish, and A. J. Lustig.** 1998. Sir3p domains involved in the initiation of telomeric silencing in *Saccharomyces cerevisiae*. Genetics **150:** 977–986.
92. **Perez-Martin, J., J. A. Uria, and A. D. Johnson.** 1999. Phenotypic switching in *Candida albicans* is controlled by a *SIR2* gene. EMBO J. **18:**2580–2592.
93. **Prasanth, S. G., K. V. Prasanth, K. Siddiqui, D. L. Spector, and B. Stillman.** 2004. Human Orc2 localizes to centrosomes, centromeres and heterochromatin during chromosome inheritance. EMBO J. **23:**2651–2663.
94. **Ramirez-Zavaleta, C. Y., G. E. Salas-Delgado, A. De Las Penas, and I. Castano.** 2010. Subtelomeric silencing of the *MTL3* locus of *Candida glabrata* requires yKu70, yKu80, and Rif1 proteins. Eukaryot. Cell **9:**1602–1611.
95. **Reedy, J. L., A. M. Floyd, and J. Heitman.** 2009. Mechanistic plasticity of sexual reproduction and meiosis in the *Candida* pathogenic species complex. Curr. Biol. **19:**891–899.
96. **Rosas-Hernandez, L. L., et al.** 2008. yKu70/yKu80 and Rif1 regulate silencing differentially at telomeres in *Candida glabrata*. Eukaryot. Cell **7:**2168–2178.
97. **Rusche, L. N., and M. A. Hickman.** 2007. Evolution of silencing at the mating-type loci in hemiascomycetes, p. 189–200. *In* J. Heitman, L. Casselton, and J. Kronstand (ed.), Sex in fungi: molecular determination and evolutionary implications. American Society for Microbiology, Washington, DC.
98. **Rusche, L. N., A. L. Kirchmaier, and J. Rine.** 2002. Ordered nucleation and spreading of silenced chromatin in *Saccharomyces cerevisiae*. Mol. Biol. Cell **13:**2207–2222.
99. **Rusche, L. N., A. L. Kirchmaier, and J. Rine.** 2003. The establishment,

Downloaded from http://ec.asm.org/ on October 24, 2020 by guest

inheritance, and function of silenced chromatin in *Saccharomyces cerevisiae*. Annu. Rev. Biochem. **72:**481–516.
100. **Sampath, V., et al.** 2009. Mutational analysis of the Sir3 BAH domain reveals multiple points of interaction with nucleosomes. Mol. Cell. Biol. **29:**2532–2545.
101. **Sanyal, K., M. Baum, and J. Carbon.** 2004. Centromeric DNA sequences in the pathogenic yeast *Candida albicans* are all different and unique. Proc. Natl. Acad. Sci. U. S. A. **101:**11374–11379.
102. **Sauve, A. A., C. Wolberger, V. L. Schramm, and J. D. Boeke.** 2006. The biochemistry of sirtuins. Annu. Rev. Biochem. **75:**435–465.
103. **Shankaranarayana, G. D., M. R. Motamedi, D. Moazed, and S. I. Grewal.** 2003. Sir2 regulates histone H3 lysine 9 methylation and heterochromatin assembly in fission yeast. Curr. Biol. **13:**1240–1246.
104. **Sharp, J. A., D. C. Krawitz, K. A. Gardner, C. A. Fox, and P. D. Kaufman.** 2003. The budding yeast silencing protein Sir1 is a functional component of centromeric chromatin. Genes Dev. **17:**2356–2361.
105. **Sjostrand, J. O., A. Kegel, and S. U. Astrom.** 2002. Functional diversity of silencers in budding yeasts. Eukaryot. Cell **1:**548–557.
106. **Souciet, J. L., et al.** 2009. Comparative genomics of protoploid *Saccharomycetaceae*. Genome Res. **19:**1696–1709.
107. **Teytelman, L., M. B. Eisen, and J. Rine.** 2008. Silent but not static: accelerated base-pair substitution in silenced chromatin of budding yeasts. PLoS Genet. **4:**e1000247.
108. **Triolo, T., and R. Sternglanz.** 1996. Role of interactions between the origin recognition complex and SIR1 in transcriptional silencing. Nature **381:**251–253.
109. **van Hoof, A.** 2005. Conserved functions of yeast genes support the duplication, degeneration and complementation model for gene duplication. Genetics **171:**1455–1461.
110. **Vaquero, A.** 2009. The conserved role of sirtuins in chromatin regulation. Int. J. Dev. Biol. **53:**303–322.
111. **Verdel, A., et al.** 2004. RNAi-mediated targeting of heterochromatin by the RITS complex. Science **303:**672–676.
112. **Wolfe, K. H., and D. C. Shields.** 1997. Molecular evidence for an ancient duplication of the entire yeast genome. Nature **387:**708–713.
113. **Wong, S., G. Butler, and K. H. Wolfe.** 2002. Gene order evolution and paleopolyploidy in hemiascomycete yeasts. Proc. Natl. Acad. Sci. U. S. A. **99:**9272–9277.
114. **Wong, S., M. A. Fares, W. Zimmermann, G. Butler, and K. H. Wolfe.** 2003. Evidence from comparative genomics for a complete sexual cycle in the 'asexual' pathogenic yeast *Candida glabrata*. Genome Biol. **4:**R10.
115. **Xie, J., et al.** 1999. Sum1 and Hst1 repress middle sporulation-specific gene expression during mitosis in *Saccharomyces cerevisiae*. EMBO J. **18:**6448–6454.
116. **Zhang, Z., M. K. Hayashi, O. Merkel, B. Stillman, and R. M. Xu.** 2002. Structure and function of the BAH-containing domain of Orc1p in epigenetic silencing. EMBO J. **21:**4600–4611.
117. **Zill, O. A., and J. Rine.** 2008. Interspecies variation reveals a conserved repressor of alpha-specific genes in *Saccharomyces* yeasts. Genes Dev. **22:**1704–1716.
118. **Zill, O. A., D. Scannell, L. Teytelman, and J. Rine.** 2010. Co-evolution of transcriptional silencing proteins and the DNA elements specifying their assembly. PLoS Biol. **8:**e1000550.

Downloaded from http://ec.asm.org/ on October 24, 2020 by guest