SURVEY AND SUMMARY

Transcriptional silencing in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*

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ABSTRACT

Transcriptional silencing is a heritable form of gene inactivation that involves the assembly of large regions of DNA into a specialized chromatin structure that inhibits transcription. This phenomenon is responsible for inhibiting transcription at silent mating-type loci, telomeres and rDNA repeats in both budding yeast *Saccharomyces cerevisiae* and fission yeast *Schizosaccharomyces pombe*, as well as at centromeres in fission yeast. Although transcriptional silencing in both *S. cerevisiae* and *S. pombe* involves modification of chromatin, no apparent amino acid sequence similarities have been reported between the proteins involved in establishment and maintenance of silent chromatin in these two distantly related yeasts. Silencing in *S. cerevisiae* is mediated by Sir2p-containing complexes, whereas silencing in *S. pombe* is mediated primarily by Swi6-containing complexes. The Swi6 complexes of *S. pombe* contain proteins closely related to their counterparts in higher eukaryotes, but have no apparent orthologs in *S. cerevisiae*. Silencing proteins from both yeasts are also actively involved in other chromosome-related nuclear functions, including DNA repair and the regulation of chromatin structure.

INTRODUCTION

In addition to the binding of gene-specific repressor proteins to DNA, eukaryotes have also evolved other specialized mechanisms to repress transcription. One such mechanism is transcriptional silencing. Transcriptional silencing involves the establishment of a repressive chromatin structure, called silent chromatin, that can persist throughout the cell cycle and that may be inherited by daughter cells following cell division.

Silencing has been observed in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, as well as in higher eukaryotes. In *Drosophila*, the heterochromatic structure concentrated at centromeric and telomeric regions, variably, but stably represses the expression of nearby genes, a phenomenon termed position-effect variegation (1). A second example is the Polycomb-mediated inactivation of homeotic genes, an event crucial for proper segmentation and differentiation in *Drosophila* (2). A classic example in mammals is X-chromosome inactivation, a process involving dosage compensation of X-linked genes through the transcriptional silencing of one of the X chromosomes in females (1,2). In both *S. cerevisiae* and *S. pombe*, heterochromatin-like silent chromatin is responsible for silencing at silent mating type loci (HM), telomeres and rDNA repeats, as well as *S. pombe* centromeres (3–5). Interestingly, transcriptional silencing has also been observed at sites adjacent to the P1 plasmid centromere in bacteria (6).

*S. cerevisiae* and *S. pombe* represent two attractive models for studying mechanisms of transcriptional silencing (7–19). Over the past few years considerable progress has been made toward the identification of novel factors involved in silencing in both yeasts. Recent discoveries have provided new insight into the role of *S. cerevisiae* Sir proteins in a broad range of cellular processes including DNA double-strand break (DSB) repair, aging, cell cycle control and meiotic checkpoint control. In this review, I discuss the trans-acting factors that orchestrate silencing in *S. cerevisiae* and *S. pombe*, and outline the similarities and differences between the silencing mechanisms of these two yeasts.

TRANSCRIPTIONAL SILENCING IN *S. CEREVISIAE*

Transcriptional silencing at the *HML* and *HMR* loci

Silencing at the *HM* loci is one of best-studied examples of transcriptional silencing. The two silent mating-type loci, *HMR* and *HML*, confer donor information to the *MAT* locus, and are responsible for the mating-type switch (20). The *HM* loci are flanked by ~150 bp cis-acting elements, silencers *E* and *I*, both of which are located ~1 kb from the genes they regulate. The silencers function to initiate assembly of the silent information regulator (Sir) complex. They contain binding sites for the origin replication complex (ORC), repressor and activator protein 1 (Rap1p), and ARS-binding factor (Abf1p) (13,21). Isolated binding sites for any of the silencer binding proteins, termed protosilencers, are unable to act as silencers on their own, but can function to enhance silencing by cooperating with intact, distant silencers (22). *HMR-E* is the best-characterized silencer. While mutations in any one of the protein-binding sites at the *HMR-E* locus does little to affect silencing (23,24), a combination of mutations

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within the binding sites for ORC, Rap1p and Abf1p causes severe defects in HMR silencing (24). The role of silencers is not limited to initiating silencing. Silencer I, for example, also serves as an insulator, separating active and inactive chromatin at the HML locus (25).

Silencing at the HML loci requires the Sir complex, which is composed of Sir1p, Sir2p, Sir3p and Sir4p (26). None of the Sir genes is essential for viability, but deletion of Sir2, Sir3 or Sir4 completely abolishes silencing, whereas disruption of Sir1 partially reduces silencing. The Sir complex is recruited by silencers and their associated proteins. Sir3p and Sir4p are recruited by Rap1p (27–29). Sir4p is also recruited by Sir1p, which binds to ORC (30). Interestingly, the function of Sir1p can be replaced by overexpression of ESC2 (establishment of silent chromatin 2) (31). Esc2p has been proposed to function in the recruitment or stabilization of Sir proteins to silent chromatin (31). Once bound to chromatin, the Sir complex is believed to spread over nucleosomes via interactions between Sir3p and Sir4p with the hypoacetylated N-terminal tails of histones H3 and H4 (32,33).

In addition to the establishment of silent chromatin, the maintenance and stable inheritance of the silenced state are also important for repression of the HM genes (34). Recent studies suggest that silencers are important for the maintenance of silencing during the cell cycle (35,36). In agreement with their function, Sir2p, Sir3p and Sir4p have been shown to be required for the maintenance of silent chromatin (34). Interestingly, a proline to arginine substitution at 898 in Sir3p results in a deficiency in the maintenance of silent chromatin, but not in the establishment of silencing (37). A recent study also suggests that the role of Sir1p, like Sir2p, Sir3p and Sir4p, may not be limited to the assembly of the silent chromatin at the HM loci, but may also play a role in maintaining silent chromatin throughout the cell cycle (38).

Silencing at the HM loci is also preferentially affected by a set of proteins that include Sum1p, Mga2p and Spt2p. Sum1p (suppressor of man or sir) was initially identified in a screen for suppressors of the mating defects caused by deletion of Sir2 (39). Sum1p is a DNA-binding protein that represses expression of meiotic genes involved in sporulation (40). The SUM1-1 mutation suppresses silencing defects caused by deletion of Sir2, Sir3, Sir4, a specific mutation in Rap1, and mutations in the silencer HMR-E that are important for the binding of Abf1p, Rap1p and Orc1p (41–43). The SUM1-1 mutation also suppresses silencing defects caused by mutations in the N-terminal tails of the histones H3 and H4. How does the SUM1-1 mutation bypass the requirement for Sir proteins? Chromatin immunoprecipitation (Chip) and co-immunoprecipitation assays suggest that Sum1p normally does not associate with the HM loci. A threonine to isoleucine change at 988 in Sum1p appears to increase its affinity for ORC which may then be able to recruit Sum1-1p to the HM loci (44,45). Sum1-1p-mediated silencing requires Hst1p, a homolog of Sir2p, that normally forms a complex with Sum1p (40). It is likely that Hst1p plays a role similar to that of Sir2p in Sum1-1p-mediated silencing.

Mga2p and Spt23p are structurally- and functionally-related transcription factors that influence transcription by regulating chromatin structure. Deletion of MGA2 or SPT23 increases the silencing efficiency in the SUM1-1 strain and suppresses the silencing defects caused by deletion of Sir1 or mutations in the HMR silencer (46). These results suggest that MGA2- or SPT23-dependent activating functions compete with the silencing machinery at the silent HM loci. However, deletion of MGA2 or SPT23 causes further decrease in the silencing efficiency of a strain in which the HML silencer is mutated (46). This complex effect on silencing at HML suggests that the activating or repressing activities of MGA2 and SPT23 are context dependent.

The mechanism by which Sir-mediated heterochromatin represses transcription is not well understood. A recent study suggests that Sir-promoted heterochromatin does not impair the accessibility of RNA polymerase II machinery to DNA, suggesting that it may regulate gene expression by blocking a step downstream of pre-initiation complex recruitment (47).

Transcriptional silencing at the telomeres

Telomeres are protein–DNA complexes formed at the end of chromosomes that are important for chromosome end stability and proper organization of chromosomes within the nucleus (48–50). In S.cerevisiae, telomeric DNA consists of C13A/TG1–3 repeats that are ~300 bp in length at the ends of chromosomes. These repeats are organized into a non-nucleosomal chromatin structure termed the telosome (51). The ends of S.cerevisiae chromosomes also contain a number of subtelomeric repeats including X repeats (52,53). In some organisms, such as S.cerevisiae, S.pombe, Drosophila and humans, marker genes placed near telomeres are repressed in a position-dependent manner, a phenomenon known as telomere position effect (TPE) (54–58). In addition, telomeric silencing is dependent on telomere length (59,60).

Compared with silencing at the HM loci, telomeric silencing is more sensitive to subtle changes in the levels of silencing proteins (61). The telomeres contain multiple Rap1p-binding sites that recruit the Sir complex (62). The interaction of Sir3p with Rap1p is competed by Rap1p-interacting factors 1 and 2 (Rif1p and Rif2p) which act as negative regulators of telomeric silencing and telomeric length (27,29,59,63–65). The inhibitory role of Rif proteins is counteracted by Ku which is also required for telomeric silencing, suggesting that Ku may play a role in the recruitment of Sir proteins (66, and see below). One-hybrid studies have shown that Sir proteins are integral components of the telosome (67). While the association of Sir4p with internal tracts of C13A/TG1–3 DNA is Sir3p independent, the Sir2p association is Sir3p dependent. The association of Sir proteins with internal tracts of C13A/TG1–3 DNA suggests that the Sir proteins may initially be recruited to the internal tracts of telomeric DNA (67). Once assembled on telomeres, Sir proteins propagate over the nucleosome to form a silent chromatin structure at the telomeres. The composition of the silent chromatin structure at telomeres is not uniform. The ‘core’ region contains all three Sir proteins whereas the ‘surrounding’ regions contain mainly Sir3p (60,68).

Orc1p, Abf1p and Sir1p are not required for the assembly of Sir proteins on the telomeres (34,61). However, the binding sites for these proteins have been identified in the core X element of subtelomeric X repeats. Moreover, these sites are important for silencing (69,70). The core X element acts as protosilencer that enhances silent chromatin propagation away from telomeres (70). These subtelomeric silencing elements are separated from the telomeric repeats by subtelomeric anti-silencing
regions (STARS) that create a discontinuity in the propagation of silent chromatin (70).

Telomeres are localized to the nuclear periphery. The myosin-like nuclear pore complex proteins, Mlp1p and Mlp2p, have been proposed to tether telomeres to the nuclear periphery via their interactions with the chromosome end-binding heterodimeric protein, Ku, and the nuclear pore protein, Nyp145p (71). The proper maintenance of nuclear localization and structure of telomeres appears to be important for telomeric silencing. First, the HMR locus with a defective silencer can be silenced if tethered to the nuclear periphery, indicating that perinuclear localization aids in the establishment of silent chromatin (72). Secondly, deletion of YKU70/HDF1 or YKU80/HDF2, the genes encoding Ku, disrupts the perinuclear location of telomeres and telomeric silencing (73,74). Thirdly, deletion of MLP1 or MLP2 also disrupts the organization of silent telomeric chromatin and causes loss of telomeric silencing (71). Finally, the perinuclear localization of telomeres also appears to provide a storage site for Sir3p and Sir4p. Immunostaining studies reveal that Sir3p, Sir4p and Rap1p co-localize with subtelomeric DNA in foci near the nuclear periphery (75,76). Moreover, mutations disrupting telomeric silencing also disrupt the association of Sir proteins with subtelomeric DNA (28,75). Although perinuclear localization of telomeres favors silencing, the localization of telomeres to the nuclear periphery is not sufficient to cause telomeric silencing (77,78).

Silencing proteins are also involved in telomeric functions. Deletion of SIR3 or SIR4 causes shortening of telomeric repeats and mitotic instability of chromosomes (79). This finding, and the fact that there is no known regulatory function for telomeric silencing, suggest that the primary role of silencing proteins at telomeres is to maintain the stability and integrity of the chromosome ends, and that telomeric silencing is a secondary effect.

**Transcriptional silencing at rDNA repeats**

Saccharomyces cerevisiae ribosomal DNA (rDNA) is organized in tandem arrays of approximately 150 repeats that are separated by intergenic spacers (IGS). The rDNA repeats form a unique structure called the nucleolus (80) and are transcribed by RNA polymerase (Pol) I (81). Since rDNA is actively transcribed it was unexpected that Pol II genes would be repressed when inserted at this locus. This repression likely results as a consequence of the suppression of mitotic and meiotic recombination within rDNA repeats since silencing at rDNA repeats and suppression of recombination among rDNA repeats is closely linked and Sir2p dependent (82–84). Unlike silencing at the HMR loci and telomeres, rDNA silencing requires a silencing complex termed RENT (regulator of nucleolar silencing and telophase exit). This complex consists of at least three components: Sir2p, Net1p (nucleolar silencing establishing factor and telophase regulator 1) and Cdc14p (85,86). Sir1p, Sir3p and Sir4p are not required for rDNA silencing and are not part of the RENT complex (83,85). However, Sir4p regulates the efficiency of rDNA silencing by modulating the distribution of a limited amount of Sir2p between telomeres and rDNA repeats (87). Normally, most Sir2p localizes to nucleolus, although a small fraction also localizes to telomeres (76).

Net1p is essential for rDNA silencing, and is responsible for recruiting Sir2p to the nucleolus (85). Net1p also interacts with Pol I and stimulates Pol I transcription presumably by tethering Pol I within the nucleolus (88). Net1p also regulates nucleolar structure. In addition to nucleolar functions, Net1p functions to regulate the cell cycle by sequestering Cdc14p (a protein phosphatase) in the nucleolus until anaphase (86,89). Once released, Cdc14p dephosphorylates nuclear and cytoplasmic substrates that regulate the activity of the anaphase-promoting components, thus destroying the anaphase-promoting components (90,91).

The cis-acting elements responsible for silencing at rDNA repeats are not well understood. Sir2p is believed to be recruited to rDNA repeats by Net1p (85). How Net1p itself is recruited to rDNA repeats is unknown since no apparent DNA-binding motif has been identified in Net1p.

**Competition among three silent loci**

The local concentration of Sir proteins as well as the stoichiometry of Sir proteins in the Sir complex are important determinants in silencing efficiency due to competition among different silent loci for limited Sir proteins (92–97) (Fig. 1). Indeed, Sir3p is limiting at telomeres since overexpression of SIR3 extends telomeric silencing from 4 to 20 kb from telomeric repeats (60,68). Moreover, a rap1-12 mutation specifically reduces silencing at the HMR locus, but improves telomeric silencing, supporting the hypothesis that Rap1p plays a regulatory role in sequestering a limited amount of Sir proteins at telomeres (29,97).

Sir2p is believed to be a limiting factor for rDNA silencing (87). Overexpression of SIR2 or disruption of the SIR3 and SIR4 genes enhances rDNA silencing, suggesting that telomeres and rDNA repeats compete for a limited amount of Sir2p (83). Similar, overexpression of SIR4 enhances telomeric silencing, while decreasing rDNA silencing (87). This is likely due to the release of Sir2p from rDNA by overexpression of SIR4. In contrast, overexpression of the N-terminal 214 amino acids of Sir3p (Sir3N) improves silencing at telomeres, while relieving rDNA silencing (98). These effects on silencing coincide with displacement of a portion of Sir2p from the nucleolus (98). Moreover, expression of a C-terminal truncated Sir4p (Sir4C) strengthens silencing at rDNA repeats, but interferes with silencing at the HMR loci and telomeres by titrating the HM and telomere Sir protein recruiting factors (87,99). Consistent with this notion, Sir3p and Sir4p are relocalized to nucleolus when Sir4C is overproduced (100). The relocalization of Sir3p to nucleolus appears to be mediated, at least in part, by Sir2p and two other uncharacterized proteins, Uth4p and Yh1023p (76). In contrast, relocalization of Sir4 proteins from telomeres to the HMR loci seems to be mediated by Sir1p, since increasing Sir1p decreases telomeric silencing (97).

**Chromatin-modulating factors and silencing histones**

Histones that mediate the folding of DNA to form nucleosomes have direct roles in silencing. As noted above, the N-terminal regions of histones H3 and H4 that interact with Sir proteins are important for silencing at HM and telomeres (32,101–105). Moreover, histones H2A and H2B are important for rDNA silencing (106,107). For example, reducing histone H2A-H2B levels by deletion of histone genes HTA1-HTB1 perturbs local chromatin structure and abolishes silencing at
Interestingly, Htz1p, a variant of histone H2A, is also required for efficient silencing at the HMR loci and telomeres (31). Overexpression of HTZ1 increased silencing at the HMR locus, suggesting that silent chromatin at the HMR locus favors Htz1p. Indeed, Htz1p was present at the HMR locus (31).

Chromatin assembly factors

Yeast chromatin assembly factor I, yCAF-I, is one of the proteins that mediates assembly of histones onto the 146 bp of DNA that form a nucleosome (108). yCAF-I is a three-subunit complex (Cac1-3p) encoded by three genes, CAC1, CAC2 and CAC3. Deletion of any of these three genes reduces silencing at HM, telomeres and rDNA repeats because the silent chromatin state cannot be maintained (109,110). The contribution of yCAF-I to silencing can be altered by mutations in proliferating cell nuclear antigen (PCNA) (111). PCNA is believed to recruit yCAF-I to DNA by direct interaction (112). yCAF-I also plays a role in coupling chromatin assembly to DNA replication and repair by preferentially assembling nucleosomes onto newly replicated or repaired DNA (109,110,113,114).

Another yeast chromatin assembly factor, Asf1p (anti-silencer factor 1), was identified as a high-copy disruptor of telomeric silencing (115,116). Unlike the cacΔ mutation, the asf1Δ mutation displays only minor defects in silencing (115,116). However, the asf1Δ mutation exacerbates the silencing defect associated with the cacΔ mutation (111,117). Moreover, combination of the asf1Δ mutation with a mutation in the DNA polymerase processivity factor PCNA (pol30-8) prevents yCAF-I from contributing to telomeric silencing, and eliminates residual telomeric silencing (111). These genetic data suggest that Asf1p and yCAF-I have distinct, yet overlapping functions in the formation of silent chromatin. Importantly, a Drosophila homolog of Asf1p is present in the RCAF complex (replication-coupling assembly factor) that has been proposed to mediate chromatin assembly after DNA replication and repair (117). It would be interesting to know if Asf1p, like yCAF-I, also couples chromatin assembly to DNA replication and repair.

Histone regulators (Hir proteins) that control histone gene expression are also involved in silencing. Although deletion of HIR genes have no observable silencing defects at the HM loci and telomeres in wild-type or asf1Δ cells, they do exacerbate silencing at HM and telomeres in cacΔ cells (105,111). Moreover, Asf1p interacts with Hir proteins in vitro and in cellular extract (111). These biochemical and genetic studies suggest that Asf1p and Hir proteins function in silencing through the same genetic pathway. The contribution of Asf1p, Hir proteins and yCAF-I in silencing can be abolished by mutations in PCNA, suggesting that PCNA is involved in the silencing mediated through these silencing proteins (111).

SET domain proteins, which take their name from their founding members Su(var) 3-9, E(z) and trithorax, are involved in chromatin-mediated transcriptional regulation (118). SET domain proteins have been identified in S.cerevisiae and S.pombe. The S.cerevisiae SET domain protein, Set1p, is a regulator of chromatin structure, DNA repair and telomeric functions. Deletion of SET1 alleviates telomeric silencing (118), and increases the repair capacity of cells after DNA damage by, at least in part, alleviating the repression of repair genes (119). Interestingly, the silencing defect caused by the SET1 deletion can be specifically suppressed by the overexpression of genes encoding human (EZH2) or mouse (Ezh1) SET domain-containing proteins (120). In addition, the silencing defects can also partially be suppressed by disruption of the checkpoint gene MEC3, suggesting that Set1p and
Mec3p may regulate telomeric silencing in opposite ways (121). Recently, it has been shown that Set1p complex contains a homolog of trithorax protein and specifically methylates histone 3 lysine4 (122).

**Histone acetylation and deacetylation**

It is well known that acetylation and deacetylation of core histones plays a role in regulation of gene transcription (123–125). The hyperacetylation of histones activates gene expression, whereas hypoacetylation represses gene expression. Consistent with this model, the transcriptionally silenced regions of genomes are generally hypoacetylated (33). However, in contradiction to this model, deletion of two *S.cerevisiae* histone deacetylase (HDAC) genes, *hda1* and *rpd3*, increases repression at *HM* (126), the telomeric loci (127) and rDNA repeats (107). One possible interpretation is that the *hda1Δ* or the *rpd3Δ* mutations decreases specific nucleosome acetylation in heterochromatin required for its effects on transcription. For example, acetylation of lysine 12 of histone H4 is important for silencing at *HM* (128) and the *rpd3Δ* mutation decreases acetylation of histones H3 and H4 (127). Alternatively, the HDACs may modulate silencing efficiency by regulating the activity of silencing complexes. Rpd3p is a catalytic subunit of the HDAC complex that includes Sin3p, Sap30p, Sds3p and Pho23p (127,129–134).

Histone acetyltransferase (HAT) has also been shown to regulate transcriptional silencing, although silenced regions appear to differ in their sensitivity to acetylation status. For instance, two *S.cerevisiae* HAT genes, SAS2 and SAS3 (something about silencing), behave as both positive and negative regulators of transcriptional silencing at *HM* (135,136). SAS2 or SAS3 deletion suppresses silencing defects at the *HMR* locus. The effect of suppressing silencing at the *HMR* locus caused by deleting SAS2 or SAS3 may be an indirect consequence of disrupting telomeric silencing. In contrast, deleting SAS2 or SAS3 enhances silencing defects at the *HML* locus (135,136). The differential regulation of the *HML* and *HMR* loci suggests that silencing at the *HML* and *HMR* loci may be different and that the repression or activation functions of SAS2 and SAS3 are context dependent. Both Sas2p and Sas3p belong to the MYST family of proteins that includes HATs that are known to catalyze acetylation reactions on histone lysines (121). Recently, it has been shown that Sas2p and Sas3p, as well as weak NAD-dependent ADP-ribosyltransferases (140). Sas3p is the catalytic subunit of a HAT complex and interacts with the Spt16p subunit of FACT (facilitates chromatin transcription), a complex implicated in the regulation of transcription and DNA replication (141).

It appears that Sas4p and Sas5p are functionally related to Sas2p and Sas3p. Like Sas2p and Sas3p, Sas4p and Sas5p are negative regulators of silencing at the *HMR* locus (142), but positive regulators of silencing at the *HML* locus and telomeres (143). It has been proposed that Sas4p and Sas5p may be components of the Sas2p-dependent acetylase complex or are targets of Sas2p-dependent acetylation (143). Sas4p is a novel protein, whereas Sas5p is a member of protein family that includes the uncharacterized *S.pombe* open reading frame YD67 and two human proteins encoded by the AF-9 and ENL genes (142). AF-9 and ENL are fusion partners for the human trithorax homolog MLL and are known to contribute to leukemogenesis (144). Both AF-9 and ENL have been shown to interact with Hpc3, a member of the human Polycomb protein family that functions as a transcriptional repressor (145).

**Role of Sir2p and its homologs in silencing**

Sir2p is the only Sir protein that is required for silencing at all silent loci in *S.cerevisiae*. Consistent with its central role in silencing, Sir2p is also the only Sir protein that is evolutionarily conserved in organisms from bacteria to humans (146,147). The Sir2p family is characterized by a highly conserved approximately 200 amino acid core sequence that is required for silencing (146). The human core sequence can readily be substituted for the *S.cerevisiae* core sequence (148).

Sir2p and several Sir2-like proteins are nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylases, as well as weak NAD-dependent ADP-ribosyltransferases (149,150). The HDAC activity of these proteins is unique: it is NAD dependent, and not inhibited by trichostatin A (TSA), which inhibits the deacetylase activity of class I and class II HDACs (151,152).

The HDAC activity of Sir2p is absolutely required for silencing (33,151,153,154). Moreover, overexpression of *SIR2* reduces histone acetylation (33). Surprisingly, deletion of *SIR2* did not change the overall levels of histone acetylation, suggesting that the *in vivo* target of Sir2p deacetylation may not be limited to histones (154). This is supported by the finding that TAFI68, a component of human RNA polymerase I transcription factor, is deacetylated by murine mSir2A, and that this deacetylation impairs rDNA transcription *in vitro* (155).

Co-immunoprecipitation experiments have revealed that Sir4p associates with Sir2p tightly, but does not associate with Sir3p due to a regulatory domain in Sir4p that inhibits its interaction with Sir3p (26,62). This is consistent with recent biochemical characterization of Sir2p which showed that Sir2p exists in two complexes (156). One contains Sir4p, and has an NAD-dependent HDAC activity, whereas the other contains Net1p, and has a HDAC activity largely independent of NAD. It is also interesting that Sir3p is absent from both complexes.

Four Sir2p homologs, Hst1p, Hst2p, Hst3p and Hst4p, exist in *S.cerevisiae*. Among them, Hst1p and Hst2p are the two most well characterized. While deletion of *HST1* does not affect silencing at the *HM* loci, telomeres or rDNA repeats, overexpression of *HST1* partially suppresses the silencing defects at *HMR* caused by deletion of *SIR2* (146,157). This suggests that Hst1p may also regulate chromatin structure (146,157). Indeed, Hst1p is important for Sum1p-mediated silencing in the absence of Sir proteins.

Another Sir2p homolog, Hst2p, is a major HDAC (153,154). While not required for the silencing at the *HM* loci, telomeres or rDNA repeats, overexpression of *HST2* increases silencing at rDNA repeats, but decreases silencing at telomeres (146,157). Unlike Hst1p that is nuclear, Hst2p is cytoplasmic (158). These results led to a model which suggests that Hst2p and Sir2p might share a common ligand that is required for telomeric silencing (158). Overexpression of *HST2* may sequester this ligand, thus releasing Sir2p, which can then reallocate to the nucleolus and enhance rDNA silencing (158).
These studies suggest that in vivo, Hst1p, Hst2p and Hst4p may have roles distinct from Sir2p.

**Role of Sir proteins in other cellular processes**

**Sir proteins and aging.** In addition to their silencing functions, recent studies have led to the proposal that Sir proteins may be determinants of yeast life span and that Sir2p may serve to link silencing, metabolism and aging (159–161). The intracellular level of Sir2p correlates with cellular longevity, suggesting that Sir2p may be a limiting component of the molecular machinery involved in regulating cellular longevity (162). In addition, Sir3p and Sir4p are relocated from telomeres to nucleolus in aging cells, and this relocalization, in the case of Sir3p, correlates with increased longevity (100). Sir3p and Sir4p are also relocated to the nucleolus in a SIR4-42 mutant that displays increased longevity (99). These results suggest that increased levels of Sir proteins at the nucleolus may be crucial for longevity in yeast.

Sir2p has also been proposed to regulate the longevity of yeast cells by suppressing extrachromosomal rDNA circle (ERC) formation, as well as by a mechanism independent of ERC formation. The accumulation of ERCS in mother cells has been shown to be a cause of aging in *S.cerevisiae* (163). Sir2p suppresses the ERCs formation by two mechanisms. First, Sir2p directly suppresses homologous recombination at rDNA repeats (82). Shortened longevity caused by Sir2 deletion could be suppressed by *FOB1* deletion that promotes rDNA recombination, suggesting that *FOB1* and Sir2R may act in the same pathway (162). Secondly, Sir2p acts with Sir3p and Sir4p to repress transcription at the HM loci (162). As noted above, loss of silencing at the HM loci results in co-expression of the a and α genes, which increases recombination between rDNA repeats and as a consequence ERC formation (162). Calorie restriction (CR) also extends longevity in *S.cerevisiae* by reducing rDNA recombination and ERC formation (164,165).

Interestingly, this effect requires Sir2p and Npt1p, an enzyme involved in the synthesis of NAD (164). It is likely that the elevated levels of NAD under CR increases the NAD-dependent HDAC activity of Sir2p and ultimately reduces ERC formation.

**Sir proteins and DNA repair**

Sir proteins have been implicated in Ku-dependent DNA DSB repair by non-homologous end joining (NHEJ) (73,166). Sir proteins and Ku form a complex (Ku/Sir complex) that associates with subtelomeric regions (167). The formation of this complex is likely mediated by interactions between Ku and Sir4p (166). Upon DNA breakage, the Ku/Sir complex is mobilized from a telomeric reservoir to the break sites and functions to facilitate DSB repair (167–169). This mobilization correlates with a loss in telomeric silencing (168). The redistribution of the Ku/Sir complex from the subtelomeric region to the sites of DSB requires the passage of damaged cells from the G1 to S phase of the cell cycle, and is dependent on the MECL1/RAD9 checkpoint pathway (167,169). However, the rad9Δ mutation does not affect telomeric silencing (170). These observations led to a suggestion that, in addition to participating in silencing of telomeric chromatin, Sir proteins may also promote NHEJ of DSB repair by creating a silent chromatin structure at the sites of DSBs (167–169). However, a direct role for Sir proteins in NHEJ has been disputed (171,172). The NHEJ defect caused by deletion of SIR may be a consequence of derepression of the HM loci which leads to co-expression of α and a gene in haploid cells, thus creating a pseudo-diploid state. In pseudo-diploids, homologous recombination of DNA damage is elevated, whereas NHEJ is reduced (173–175). Indeed, the absence of mating-type heterozygosity suppresses the defect in NHEJ caused by the deletion of SIR (171,172).

**Role of Sir proteins in meiotic checkpoint control.** As in the mitotic cell cycle, a meiotic checkpoint, referred to as the pachytene checkpoint, monitors recombination and synaptonemal complex (SC) formation during meiosis to ensure proper meiotic chromosome segregation (176). *Zip1p*, a component of the central region of the SC, is one of the pachytene checkpoint regulators. *zip1* mutants are defective in the pachytene checkpoint and have reduced sporulation frequency and spore viability because of chromosome missegregation (177,178). Pch2p and Sir2p are also regulators of pachytene checkpoint function. However, unlike Zip1p, Pch2p and Sir2p are dispensable for normal meiosis. They are, however, required for checkpoint-induced pachytene arrest. Both Pch2p and Sir2p localize predominantly to nucleolus. The nucleolar localization of Pch2p is important for its checkpoint function and is Sir2p dependent (178). In contrast, the nucleolar localization of Sir2p is dependent on the nuclear protein Dot1p, which is involved in pachytene checkpoint control and silencing at the HM loci and telomeres (116). Pch2p can be relocalized to telomeres by overexpressing SIR4 or deleting rDNA repeats (178). Only when associated with telomeric heterochromatin is the mislocalized Pch2p able to provide some checkpoint function, suggesting that the heterochromatin established at rDNA repeats is important for Pch2p checkpoint function. Alternatively, Pch2p may sequester within the nucleolus an unknown protein that is required for the pachytene checkpoint function. Interestingly, Pch2p and Sir2p also repress meiotic recombination by excluding from the nucleolus meiosis-specific Hop1p that promotes meiotic recombination (178).

It is worth noting that Dot1p shows sequence similarity with S-adenosyl-l-methionine (SAM) methyltransferase and comparative modeling suggests that Dop1p contains a methyltransferase fold found in SAM methyltransferase (179). Based on these observations Dot1p has been proposed to be a histone methyltransferase (179).

**TRANSCRIPTIONAL SILENCING IN S.POMBE**

**Transcriptional silencing at the silent mating-type loci**

The mating-type region of *S.pombe* consists of three mating-type loci: *mat1, mat2-P* and *mat3-M*. The *mat2-P* locus is separated from the *mat1* locus by a 15 kb interval designated the L region. The *mat3-M* locus is separated from the *mat2-P* locus by an 11 kb interval designated the K region (180). The mating-type of *S.pombe* is determined by the presence of either *Plus* (*P*) or *Minus* (*M*) information at the *mat1* locus. *mat2-P* and *mat3-M* are donors of *P* or *M* information to the *mat1* locus during mating-type switching (12). These donor loci are normally maintained in a transcriptionally silent state (181). The region subject to silencing at the mating-type region of *S.pombe* is much bigger than that of *S.cerevisiae*. It extends
throughout a large 15 kb chromosomal domain that covers mat2-P, mat3-M, a ~3 kb region at the mat2-P-proximal side of the L region, and the entire K region (182,183). In addition to being subjected to transcriptional suppression, mat2-P, mat3-M and the K region are refractory to meiotic recombination (184,185). The K region also controls the mating-type switching directionality, a non-random selection of switching information (186,187).

Three cis-acting elements responsible for silencing at the silent mating-type loci have been identified and characterized. One, designated REII, is located at the junction between mat2-P and the L region (188). Another element, termed the mat3-M element, lies within 500 bp of mat3-M (189). These two cis-acting elements were proposed to function together with the products of the esp1, esp2 and esp3 genes to suppress silencing at the donor loci (187). The third cis-acting element is located in the K region and bears strong homology to the dg and dh sequences of centromeric repeats (182). Deletion of the K region, REII or the Mat3-M element alone fully derepresses silencing at the donor loci. However, the combination of the REII element or the mat3-M proximal element deletion with mutations in some trans-acting factors (e.g. Swi6, Clr1, Clr2, Clr3 and Clr4) can cause pronounced effects on silencing at the donor loci. This suggests that the REII and mat3-M elements act in synergy with these trans-acting factors to suppress donor loci expression (183,187,189,190). In contrast to the K region, which has a global effect on silencing, the REII and mat3-M elements appear to control local expression around the donor loci (189,191). The REII element is a protosilencer. It is unable to promote silencing on its own, but can cooperate with centromeric-like sequences in the K region to enhance silencing stability (191). Interestingly, the REII element also serves as boundary elements of silent chromatin at the donor loci (191). In addition to the REII element, two 2 kb identical inverted repeats, termed IR-L and IR-R, may also serve as boundary elements of the silent chromatin at the donor loci (192).

Mutations in several trans-acting genes including clr1, clr2, clr4, clr6, swi6 and rik1, have been found to cause partial derepression at mat2-P and mat3-M (12,182,183,186,187,190,193–197). In addition to repressing transcription, these factors also inhibit meiotic recombination (12,182,183,186,187,190,193–197). Genetic data have shown that these genes act in the same pathway (183). Clr6 and Clr3 (cryptic loci regulator) are HDACs (196). Clr6 belongs to class I deacetylases and specifically deacetylates H3 at lysine 9 (198). Clr3 is a member of the class II HDAC family and specifically deacetylates histone H3 at lysine 14 (198). The deacetylase activity of Clr3 is critical to its silencing function (199).

Clr4 and Swi6 are chromo-domain proteins. Clr4 also contains a SET domain and is a member of the SU(VAR)3-9 protein family which includes human SUV39H1, murine Suv39h1 and Drosophila SU(VAR)3-9 (118,200–202). Members of this protein family have been identified as histone H3 methyltransferases that specifically methylate histone H3 at lysine 9 (199,203). These modifications create a binding site for heterochromatin-associated proteins like HP1 (204,205). The SET domain is required for methyltransferase activity (190). Similarly, Clr4 controls silent chromatin assembly at mat2-P, mat3-M and centromeres by specifically methylating H3 at lysine 9 (202). In addition to repressing gene repression, Clr4 also activates a number of genes including cdl3, a gene encoding a homolog of the Escherichia coli thermosensitive glucokinase protein (202). Clr4 has also been implicated to function in switching directionality (202).

The chromo-domain protein Swi6 also contains chromo-shadow domains (206,207) and shares a high degree of homology with chromatin-associated proteins such as Drosophila HP1 and Polycomb proteins that function in assembly of transcriptionally inactive chromatin (207,208). Swi6 is the structural component of silent chromatin at mat2-P, mat3-M, centromeres and telomeres, and its localization to silent chromatin requires Clr4 (193,209). Swi6 is also critical to the maintenance and stable inheritance of silencing at mat2-P and mat3-M during mitosis and meiosis (193,209). In addition to functioning in silencing, Swi6 also regulates the efficiency of mating-type switching (210).

Rik1 contains β-propeller-like domains typically found within WD-40 domain proteins that participate in protein–protein interactions (211). A mutation in Rik1 completely abolishes methylation of H3 at lysine 9 and localization of Swi6 at both the silent mating-type region and centromeres (198). The WD-40 domain proteins have been suggested to function as chaperones that bring chromatin assembly factors, chromatin-remodeling factors, HATs and HDACs to histones (212). Thus, Rik1 may bring histone-modifying enzymes, such as Clr4, and silent chromatin-binding proteins, such as Swi6, to silent chromatin.

Another chromo-domain protein, Chp2, identified by the S.pombe sequencing project, also functions at silent mating-type loci (213,214). Chp2 is highly homologous to Swi6. Similarly to Swi6, Chp2 also participates in silencing at many silent loci (214). However, unlike Swi6, Chp2 is not absolutely required for efficient mating-type switching (214).

In addition to the previously described HDACs Clr3 and Clr6, S.pombe contains a third putative HDAC gene, hda1+ (also termed phd1+ or hoc1+) (215). Hda1 is 52% identical to S.cerevisiae Rpd3p and 58% identical to human HDAC1. Biochemical studies have revealed that although bacterially expressed Hda1 shows no HDAC activity, affinity-purified Hda1 does (216). Moreover, the deacetylase activity of the affinity-purified Hda1 complex is inhibited by TSA, a HDAC inhibitor, albeit at a relatively high concentration (IC50 value of 43 nM) (216). Since the Hda1 complex is not well characterized it is unclear if Hda1 or its associated proteins are responsible for HDAC activity. Similar to the effects observed from mutations in the S.cerevisiae Rpd3p and the Drosophila homolog of Rpd3p, removal of Hda1 enhances silencing at silent mating-type loci, centromeres and telomeres (215). Moreover, deletion of the hda1+ leads to partial inhibition of cell growth at low concentrations of TSA, suggesting that Hda1 is one possible target for inhibition by TSA in vivo (215). In addition to being involved in silencing, Hda1 may also be involved in the early stages of meiotic cell cycle control by regulating the expression of a set of genes whose products function in the meiotic cell cycle (215,216).

Transcriptional silencing at centromeres

Unlike the centromeres of S.cerevisiae chromosomes, which are relatively small (~125 bp), the centromeres in S.pombe are large (40–100 kb) and contain a 15 kb central region composed of a 4–7 kb central core domain (ctr) flanked by inverted repeats (imr). This central region is surrounded by 10–100 kb
of repeated sequences (otr) containing highly repeated motifs (dg and dh) common to all centromeres (217). This arrangement resembles the arrangement at the centromeres of higher eukaryotes which also contain large arrays of repetitive satellite sequence (4,5). The centromeres of S.pombe, like those of human chromosomes, show characteristics of heterochromatin where the central region is weakly silenced and the outer flanking repeats are more strongly silenced. Similar to the situation at the silent mating-type loci, silencing and suppression of recombination at centromeres are also tightly linked (4,5,184,185).

The trans-acting factors Swi6, Clr1, Clr2, Clr3, Clr4, Rik1, Chp2 and Hda1, which mediate silencing at mat2-P and mat3-M, are also required for silencing at centromeres (5,196,214,215). In particular, Clr4, Rik1 and Swi6 are required for silencing within the outer repeats of centromeres, but not within the central core region. Silencing at centromeres also involves additional centromere-specific factors. The centromere-specific factor Chp1 is a chromo-domain protein identified by the S.pombe sequencing project (213,214). Chp1 is responsible for silencing at the outer repeats of centromeres (218). Like Swi6, Chp1 is localized to the flanking outer repeats, an association that requires Rik1 and Clr4 (218). An additional 12 csp (centromere: suppressor of position effect) genes have been identified genetically. Mutations in these genes have been shown to alleviate silencing in the outer repeats of centromeres, but not at the mating-type region (219).

In contrast to the previously described factors, two other factors, Mis6 and Mis12, are required for silencing within the central core and associate with the central region of centromeres. It is unclear how Mis6 and Mis12 are assembled on centromeres (218). Silencing at centromeres may also be regulated by components of the proteasome 19S, suggesting that these silencing proteins interact with proteolytic degradation (220).

The importance of trans-acting factors for silencing generally correlates with their importance for centromere function. First, mutations in Clr4, Clr6, Rik1 and Swi6 result in increased chromosome loss (5,194,196). In addition, many of these mutants show sensitivity to microtubule-destabilizing drugs, suggesting that these silencing proteins interact with microtubules at the kinetochore, a protein–DNA complex at centromeres that is critical for proper chromosome segregation (194). Secondly, Chp1 shows a genetic interaction with α-tubulin and is required for proper chromosome segregation (213). Finally, consistent with defective centromere structure and function, csp mutants display sensitivity to spindle-destabilizing drugs and elevated chromosome loss rates (219). These results suggest that heterochromatin at centromeres may be crucial to centromere function in S.pombe.

Compared with S.cerevisiae, little is known about the assembly of silent chromatin at mat2-P, mat3-M and centromeres in S.pombe. Recent biochemical studies suggested that histone deacetylases and methylases act in a sequential process (Fig. 2) (198). First, Clr3 and Clr6 deacetylase histone H3 at lysine 14 and lysine 9, respectively. This deacetylation is then followed by methylation of histone H3 at lysine 9 by Clr4 which establishes a ‘histone code’ that can be recognized by Swi6 (221,222). The binding of Swi6 to lysine 9-methylated histone H3 and self-association of Swi6 along nucleosomes result in the establishment of a heterochromatin-like structure at silent loci in S.pombe (198).

Transcriptional silencing at telomeres and rDNA repeats

Silencing has also been observed at telomeres and rDNA repeats in S.pombe, both of which are characterized by repeated sequences (56,214). S.pombe rDNA is organized in tandem arrays of approximately 70–100 repeats in two large domains (500–1000 kb long) (223). These rDNA repeats are located at both ends of chromosome 3, adjacent to the telomeres (223,224). Similarly, S.pombe telomeres consist of ~300 bp of G-rich repeats arranged in tandem arrays. The arrangement of telomeric complexes at the telomeric repeats are apparently different in S.cerevisiae and S.pombe. Whereas S.cerevisiae Rap1p binds telomeric DNA directly, S.pombe Rap1-like protein may be recruited by the TTAGGG Repeat Factor (TRF)-like protein (225).

The cis-acting elements and trans-acting factors responsible for silencing at rDNA repeats and telomeres are not well characterized. Recently, it has been shown that the four chromo-domain proteins, Ch1p, Ch2p, Swi6 and Clr4, are involved in silencing at rDNA repeats (214). Consistent with its role in rDNA silencing, Clr4 is localized in the nucleolus during interphase (226). In addition to these chromo-domain proteins, other S.pombe trans-acting factors including Clr3 are also important for rDNA silencing (214). Mutations in clr1, clr2, clr3, clr4, swi6 and rik1 that affect silencing at mat2-P, mat3-M, centromeres and in some cases rDNA, also affect telomeric silencing (5,196). In addition, telomeric silencing is specifically affected by mutations in taz1, rat1 and lot2 (227). The telomere-binding protein Taz1 is a member of
the TRF family of proteins that are characterized by two domains: a TRF homology (TRFH) domain and a Myb-like domain (225). The TRF family proteins are telomere-associated proteins that negatively regulate telomere length (225). S. pombe Taz1 plays other roles at telomeres. First, Taz1 appears to suppress recombination at telomeres (228). Secondly, Taz1p is required for proper telomere aggregation at the spindle pole body which is crucial for the telomere-led movement of nuclei, chromosome segregation and recombination during meiotic prophase (227). The factors encoded by lot2 and rat1 have yet to be characterized (227).

**Sir homologs in S. pombe**

There are three S. pombe members of the Sir2p family: spSir2, spHst2 and spHst4 (158). Among them, spHst4 is the best characterized. SpHst4 is most closely related in sequence to S. cerevisiae Hst3p and Hst4p, and also appears to be functionally related to Hst3p and Hst4p (229). Overexpression of sphst4 rescues temperature sensitivity and silencing defects caused by deletion of HST3 and HST4 (229). Moreover, sphst4Δ cells exhibit phenotypes (e.g. growth and morphological defects) similar to those of S. cerevisiae hst3 and hst4 mutants. Finally, like Hst4p, spHst4 is concentrated at the nucleolus (229). Thus, the role in the maintenance of chromatin structure and integrity is likely conserved for these Hst proteins.

SpHst4 shares many phenotypes with S. pombe trans-acting factors including Clr4, Clr6, Rik1 and Swi6. Deletion of sphst4 affects silencing at centromeres and telomeres, but not at the silent mating-type loci (229). In addition to silencing, spHst4 is important for centromere function. The sphst4Δ cells display increased chromosome loss rates, fragmented DNA and are sensitive to the microtubule-destabilizing drug thiabendazole (TBZ) (229). However, sphst4Δ cells display growth and morphology defects, and severely fragmented DNA that is not observed in clr4, clr6, rik1 and swi6 mutants. One possible explanation is that spHst4 may function with other silencing factors at some loci, while functioning independently at others.

**SILENCING PROTEINS INVOLVED IN OTHER CELLULAR PROCESSES**

**DNA repair proteins and telomeric silencing**

Although telomeric silencing and DNA repair are controlled by two different cellular pathways, recent studies suggest that DNA repair is related to chromatin structure modulation and silencing. As noted above, the chromatin assembly factor yCAF-1, which couples DNA repair to chromatin assembly, is involved in silencing. Another example comes from studies on Rad6p, an E2 ubiquitin-conjugating enzyme. Rad6p appears to have multiple functions. It is involved in DNA postreplication repair, sporulation, recombination, degradation of proteins by the N-end rule pathway, and silencing (106,230). Ubiquitin-conjugating activity of Rad6p is required for these biological functions (230–232).

It has been proposed that the DNA repair activity and silencing activity of Rad6p may involve ubiquitination of different substrates (233). Moreover, ubiquitination of these different substrates by Rad6p may require a different partner protein (233). The hypothesis that Rad6p ubiquitinitates a protein involved in silencing is supported by the finding that deletion of UBP3, encoding a deubiquitinating enzyme that interacts with Sir4p, enhances silencing at telomeres and HM loci (210). It appears that the role of controlling chromatin structure by S. cerevisiae Rad6p is conserved in S. pombe. Rp6p, a homolog of S. cerevisiae Rad6p, has been shown to regulate the expression of silent mating-type loci by modulating chromatin structure (234). Moreover, the silencing defect at mat2-P and mat3-M, and changes in chromatin structure brought on by rp6p mutations, are switching dependent. Thus, Rp6p was proposed to be required for reestablishment of chromatin structure which is disrupted during mating-type switching (234).

Another DNA repair protein involved in silencing is the chromosome end-binding protein, Ku. Ku functions in DNA DSB repair by the NHEJ pathway (235), telomere maintenance (73,236–238) and telomeric silencing (73). Recent genetic studies suggest that Ku may play a direct role in telomeric silencing by overcoming the negative effects of Rif1p and Rif2p, thus helping to recruit the Sir complex to telomeres (66).

Although S. pombe Ku70 (Pku70) is also required for DSB repair by NHEJ and maintenance of telomere length, it does not play a role in telomeric silencing (239). Furthermore, in contrast to S. cerevisiae Ku that is concentrated at telomeres, S. pombe ku70 is distributed throughout nuclei. Consistent with these results, S. cerevisiae Ku associates with Sir proteins (e.g. Sir3p and Sir4p), whereas no apparent homologs of Sir3p and Sir4p exist in S. pombe.

**DNA repair checkpoint proteins and silencing**

Studies of DNA repair checkpoint proteins have demonstrated that there is a connection between checkpoint control and silencing. DNA repair checkpoint control involves a constant surveillance of the state of DNA. When DNA is damaged, DNA repair checkpoint proteins sense the DNA damage, transduce a signal to the DNA repair machinery, and suspend cell cycle progression until the damaged DNA is repaired. Several proteins required for this checkpoint control are also required for telomere function and telomeric silencing.

One of the checkpoint proteins involved in telomeric silencing is Mec3p. Deletion of MEC3 leads to increased telomere length and increased telomeric silencing (121). Mec3p physically interacts with Set1p, a protein involved in the regulation of chromatin structure, DNA repair and telomeric functions. This association links Mec3p to chromatin structure modulation. Interestingly, Mec3p antagonizes Set1p in telomere function and telomeric silencing (121). The SET domain of Set1p participates in telomeric silencing in a MEC3-dependent manner, whereas the non-SET part of Set1p participates in telomeric silencing in a MEC3-independent manner (121).

Another checkpoint protein involved in silencing is Mec1p. Mec1p is a protein kinase that is related to human ATM (Ataxia Telangiectasia) protein kinase family (240). The kinase domain of Mec1p has been shown to be important for DNA damage repair and telomere length maintenance (240), and has been implicated in telomeric silencing (241,242). The transcriptional silencing and telomere length maintenance functions of Mec1p are separable. It has been proposed that Mec1p-dependent phosphorylation of silencing proteins is...
necessary for the telomeric silencing activity of these proteins (241,242). Interestingly, a silencing defect associated with a mec1 mutation could be suppressed by a mutation in SML1 (suppressor of mec1 lethality) (241). SML1 encodes the ribonucleotide reductase inhibitor, the removal of which by Mec1p and Rad53p kinases is required to ensure DNA replication during cell growth and after DNA damage (243). This may explain, at least in part, why no defect in telomeric silencing was observed in the mec1Δ mutant, since this strain also carries a deletion of SML1 (241). Alternatively, the silencing defects associated with overexpression of MECl1 could be suppressed by overexpressing Scs2p (suppressor of choline sensitivity) which was originally identified as a suppressor of the inositol auxotrophy of cse1 (choline sensitive) and ire15 (inositol requiring) genes (244). It is likely that Scs2p regulates telomeric silencing via a pathway different from that used by Mec1p (242). A similar role for Mec1p in telomeric silencing has also been proposed for Dun1p.

The checkpoint protein Rad3, a S. pombe homolog of S. cerevisiae Mec1p, also plays a role in the control of telomere integrity, suggesting that the link between checkpoint proteins and maintenance of telomeres is conserved in S. pombe (245). However, the role of Rad3 in silencing remains to be addressed. While one report has shown that deletion of rad3+ does not affect telomeric silencing (239), another study revealed that the rad3-136 mutation has a moderate effect on telomeric silencing (245).

A connection between checkpoint proteins and telomeric silencing also comes from studies on the checkpoint proteins Mrc1p (Mediator of the DNA replication checkpoint) and Rad53p. Both of these proteins function in the S-phase checkpoint pathway and are required for telomeric silencing (246). Rad53p is an essential Ser/Thr/Tyr protein kinase. This kinase domain of Rad53p seems to be involved in both telomeric silencing and telomere length maintenance since inactivation of the kinase activity of Rad53p increases telomeric silencing and shortening of the telomere (170). Transcriptional silencing and telomere length maintenance represent two separable functions of Rad53p. Recent studies suggest that Rad53p may directly regulate chromatin assembly by releasing Asf1p (Anti-silencing function) in response to DNA damage and DNA replication blockage. Rad53p was found to physically associate with Asf1p, a chromatin assembly factor that has been implicated in deposition of acetylated H3 and H4 during DNA replication and repair (117). The Rad53p–Asf1p association is disrupted in response to replication blocks and DNA damage, and this disruption correlates with Rad53p phosphorylation by Mec1p (246,247). The biological relevance of this association is provided by the findings that a temperature-sensitive allele of the mrc1–rad53–21 mutant and the hydroxyurea (HU; which inhibits DNA replication) sensitivity of rad53–21 mutant can be suppressed by ASF1 overexpression (246). These results suggest that Asf1p physically links Rad53p to chromatin assembly.

DNA replication proteins and silencing

Several lines of evidence suggest that DNA replication is linked to silencing. First, the establishment of silencing requires passage of yeast cells through S phase (111,117). Secondly, silencers contain the DNA replication initiation factor (ORC) binding site and the Orc1p–Sir1p interaction is thought to be important for the recruitment of the Sir complex in the HM loci (30,248). Thirdly, mutations that disrupt silencing have been identified in DNA replication proteins and DNA replication-related proteins such as DNA polymerase α (Pol α, required for both priming and DNA replication) (107,249), DNA helicase Dna2p, PCNA (required for DNA replication and nucleotide excision repair) (250), Rfc1p (PCNA loading factor) and the two replication-coupled chromatin assembly factors yCAF-1 and Asf1p (107,109,113,116,251). In addition, mutations that are capable of suppressing silencing defects are found in DNA replication proteins including DNA polymerase ε (Pol ε, a protein complex required for chromosomal replication and DNA repair) (252), the replication initiation factor Cdc45, PCNA and RF-C (replication factor C, a protein also involved in DNA repair) (253).

However, it appears that DNA replication is not the requisite S phase event for establishment of silencing. First, the requirement for the ORC-binding site and initiation of DNA replication can be bypassed by tethering Sir1p to the silencer, suggesting that DNA replication at the silent site is not required for silencing (254). Secondly, the functions of ORC in replication and silencing are genetically separable, indicating that some replication proteins may have dual roles (255,256). Recent studies have shown that while the establishment of silencing is cell cycle dependent, it can occur independent of DNA replication (257,258). The finding that silencing can be established in the absence of DNA replication raised an interesting question as to how the DNA replication proteins involved in silencing can be uncoupled from DNA replication. It is likely that DNA replication proteins may aid in the establishment of silencing, as well as the inheritance and maintenance of silent chromatin.

A role for DNA replication proteins in transcriptional silencing has recently been demonstrated in S. pombe (234,259,260). For example, a mutation in S. pombe Pol α (also named Swi7) suppresses repression at the mating-type region and centromeres (259,260). Moreover, CHIP assays have revealed that the Pol α mutant is defective in Swi6 localization at the mating-type region, centromeres and telomeres. Pol α interacts directly with Swi6, suggesting that Pol α may affect Swi6 localization directly. These data imply that Pol α may participate directly in silent chromatin assembly through its interaction with Swi6. Alternatively, mutations in Pol α might impair nucleosome assembly by S. pombe CAF-1 (259,260). It is worthy to note that like other replication proteins in S. cerevisiae, the silencing activity of Pol α is separable from its replication activity (259,260).

CONCLUSION

Transcriptional silencing is one of several molecular mechanisms in the cell that is dependent on the establishment and maintenance of repressed or silent chromatin. Other mechanisms include recombination, chromosome maintenance and segregation, nuclear organization and possibly DNA repair. With this in mind, it is not surprising to see that many silencing proteins from S. cerevisiae and S. pombe are also involved in several chromosome-related nuclear functions. Conversely, many proteins that are directly or indirectly involved in chromatin structure modulation also regulate silencing.
Table 1. Conservation of silencing proteins in *S. cerevisiae* and *S. pombe*

<table>
<thead>
<tr>
<th><em>S. cerevisiae</em></th>
<th>Functions in <em>S. cerevisiae</em> in addition to silencing</th>
<th>Homolog in <em>S. pombe</em></th>
<th>Silencing function in <em>S. pombe</em></th>
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</thead>
<tbody>
<tr>
<td>Sir1p</td>
<td>None</td>
<td></td>
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</tr>
<tr>
<td>Sir2p</td>
<td>NAD-dependent HDAC; aging; DNA repair; Pachytene checkpoints; telomere structure; mating-type switching</td>
<td></td>
<td>+</td>
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<td>DNA repair; aging; telomere structure</td>
<td>Sir2 (158)</td>
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<tr>
<td>Sir4p</td>
<td>DNA repair; aging; telomere structure</td>
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<td></td>
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<tr>
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<td>Homolog of Sir2p, cell cycle; chromosome stability</td>
<td>SpHst2, spHst4 (158)</td>
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<tr>
<td>Pho23p</td>
<td>Component of Rpd3 HDAC complex</td>
<td>Pg1, Png2 (267)</td>
<td></td>
</tr>
<tr>
<td>Sas2p, Sas3p</td>
<td>Histone acetylase homolog</td>
<td>SpSas (135)</td>
<td></td>
</tr>
<tr>
<td>yCAF-I</td>
<td>Chromatin assembling; DNA repair</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Asf1p, Asf2p</td>
<td>Chromatin assembling</td>
<td>SPCC663.05C</td>
<td></td>
</tr>
<tr>
<td>Set1p, Set2p</td>
<td>DNA repair; regulation of chromatin structure</td>
<td>Clr4, SP CCSO6.04C</td>
<td>+</td>
</tr>
<tr>
<td>Rad6p</td>
<td>DNA repair; sporation; recombination; ubiquitination</td>
<td>Rhp6 (234)</td>
<td>+</td>
</tr>
<tr>
<td>Ku70, Ku80</td>
<td>DNA repair; telomere function</td>
<td>Pkd70 (239)</td>
<td></td>
</tr>
<tr>
<td>Mec3p</td>
<td>DNA damage checkpoint; telomere functions</td>
<td>Hus1 (268)</td>
<td></td>
</tr>
<tr>
<td>Mec1p</td>
<td>DNA damage checkpoint; telomere functions</td>
<td>Rad3 (245)</td>
<td></td>
</tr>
<tr>
<td>Mrc1p</td>
<td>DNA damage checkpoint</td>
<td>SPAC694.06C</td>
<td></td>
</tr>
<tr>
<td>Rad53p</td>
<td>DNA damage checkpoint; telomere functions</td>
<td>Cds1 (269)</td>
<td></td>
</tr>
<tr>
<td>Pch2p</td>
<td>DNA damage checkpoint</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Zip1p</td>
<td>DNA damage checkpoint</td>
<td>None</td>
<td></td>
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<tr>
<td>Dot1p</td>
<td>DNA damage checkpoint</td>
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<td></td>
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<tr>
<td>PCNA</td>
<td>DNA replication and repair</td>
<td>Pcn1 (270)</td>
<td></td>
</tr>
<tr>
<td>Rfc1p (RF-C)</td>
<td>DNA replication and repair points; checkpoints</td>
<td>Rfc1 (271)</td>
<td></td>
</tr>
<tr>
<td>Pol2p (scPolc)</td>
<td>DNA replication and checkpoints</td>
<td>spPolc (272)</td>
<td></td>
</tr>
<tr>
<td>Pol1p (scPolxt)</td>
<td>DNA replication</td>
<td>Polxt (spPolxt) (257,258)</td>
<td>+</td>
</tr>
<tr>
<td>Net1p</td>
<td>Cell cycle; nucleolar structure; rDNA synthesis</td>
<td>Unknown</td>
<td></td>
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<tr>
<td>None</td>
<td>Swi6</td>
<td>+</td>
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</tr>
<tr>
<td>None</td>
<td>Rik1</td>
<td>+</td>
<td></td>
</tr>
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*Genes encoding putative *S. pombe* homolog of *S. cerevisiae* silencing protein are listed by their access numbers, which are accessible at http://www.sanger.ac.uk/cgi-bin/yeastpub/comp_class_search.p.

*SPAC694.06C is probably the *S. pombe* ortholog of Mrc1p (V. Wood, personal communication).

*Set2p contains the SET domain and cysteine-rich regions shared by Clr4 and SUV39H1 (199).*
Silent chromatin in the budding yeast *S.cerevisiae* and the fission yeast *S.pombe* are governed by sets of different cis-acting elements and trans-acting factors, suggesting that silent chromatin in these two yeasts may be structurally and biochemically different. Whereas Sir proteins are essential for transcriptional silencing and constitute the ‘core’ component of the silencing machinery in *S.cerevisiae*, the chromodomain protein Swi6 and chromatin modifying proteins Clr3 and Clr4 are crucial for transcriptional silencing in *S.pombe*. The absence of homologs of these *S.pombe* silencing factors in *S.cerevisiae* is likely due to co-elimination of functionally linked genes that encode chromodomain proteins and other chromatin-associated proteins (261). The *S.cerevisiae* Sir proteins that have no apparent orthologs in *S.pombe* and higher eukaryotes have likely been functionally displaced by the chromatin-associated proteins that promote silencing in *S.pombe*. Remarkably, Sir proteins have diverse functions, including DNA DSB repair, regulation of the cell cycle and meiosis, and aging. Many of the proteins that function as the regulators of silent chromatin structure in *S.cerevisiae* have their homologs in *S.pombe* (Table 1). However, some of these *S.pombe* homologs (e.g. Pku70) are not involved in silencing. Despite these significant differences, silent chromatin in these two yeasts shares a number of similarities between them and with the heterochromatin structure of higher eukaryotes. For example, silent chromatin is predominantly composed of repetitive DNA and contains a low density of genes. It is also relatively inaccessible to DNA-modifying reagents, replicates late during S phase, and is inherited during the cell cycle (234,262,263). Finally, many *S.cerevisiae* and *S.pombe* silencing proteins have functional orthologs in higher eukaryotes. Therefore, the study of transcriptional silencing in these two yeasts should provide insight into the mechanisms of transcriptional silencing in higher eukaryotes.

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