

# Epigenetic Silencing Mechanisms in Budding Yeast and Fruit Fly: Different Paths, Same Destinations

## Minireview

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**Transcriptional silencing in budding yeast and fruit fly is mediated by fundamentally unrelated proteins that assemble very different chromatin structures. Surprisingly, the repressive mechanisms evolved from these very different materials have similar features, including an epigenetic mode of inheritance and a block to transcription based on interference with the assembly or function of the promoter complex rather than with the binding of gene-specific activators.**

Transcriptional silencing represses gene expression by the formation of chromatin complexes that act in a regional, rather than promoter-specific, manner. In the budding yeast *Saccharomyces cerevisiae*, such complexes are formed by silent information regulator (Sir) proteins, and in the fruit fly *Drosophila melanogaster*, by Polycomb group (PcG) proteins. As illustrated in [Figure 1](#), mosaic silencing of reporter genes conferring pigmentation in a yeast colony or in the *Drosophila* eye produces a remarkably similar appearance. In both cases, transcriptional repression is due to a metastable chromatin state that tends to be clonally inherited by progeny cells, producing a characteristic variegated appearance. At their normal physiological targets, the end result of the two mechanisms is strong and stable gene silencing, but under suboptimal situations, it is not the level of repression that is affected but the stability of the silenced state. [Figure 1](#) therefore recapitulates two characteristic features of the two silencing mechanisms: all-or-none repression and epigenetic inheritance. Here, we compare and contrast the molecular bases of these features.

### **Requirement for DNA Response Elements and Sequence-Specific DNA Binding Proteins**

Both Sir- and PcG-mediated silencing are targeted to specific chromosomal domains by *cis*-acting regulatory sequences. In yeast, these sequences are termed silencers: short (~150 bp) elements that flank the silent mating-type loci and contain binding sites for the sequence-specific factors Rap1, Abf1, and the origin recognition complex (ORC). These proteins trigger formation of silenced chromatin through concerted recruitment of a complex comprising Sir2, Sir3, and Sir4. In other contexts, ORC, Rap1, and Abf1 can promote DNA replication or contribute to transcriptional activation. Mechanistically similar, although less robust, si-

lencing occurs at telomeres, where the Sir2/3/4 complex is recruited by Rap1 and yKu70.

Similarly, PcG silencing is generated by composite regulatory elements termed PREs (PcG response elements). These sequences can be several hundred base pairs in length and are believed to harbor binding sites for sequence-specific factors that recruit PcG complexes. Many DNA binding proteins (such as GAGA factor, PHO, and Zeste) are associated with PREs, but their specific roles in the assembly or function of PcG complexes are not yet clear. Like the yeast silencer binding factors, these proteins also bind to many other sites unrelated to silencing and may have activating effects. Moreover, like Sir assembly, the PcG recruitment process is cooperative, involving the concerted action of many partially redundant recruiters. Together, the recruiting proteins assemble at the PRE two kinds of PcG complexes: the PRC1 type, containing a core quartet of proteins, PSC, PH, RING, and the chromodomain protein PC; and the PRC2 type, containing the E(Z) histone H3 methyltransferase.

### **Assembly and Propagation of Chromatin Modification Complexes**

In yeast, the silencer acts as a nucleation site, initially recruiting a Sir2/4 complex. Sir3 is recruited subsequently. The assembled Sir complex then spreads through a network of multivalent interactions between Sir3 and Sir4 and deacetylated lysines in the N-terminal tails of histones H3 and H4. Deacetylation of H4 K16 appears to be critical for spreading, which occurs in sequential cycles of deacetylation catalyzed by Sir2, an evolutionarily conserved NAD<sup>+</sup>-dependent histone deacetylase (reviewed in [Rusche et al. \[2003\]](#)). Sir3 and Sir4 may play additional roles in formation of higher-order structures and in anchoring silenced chromatin to the nuclear periphery, where Sir proteins are locally concentrated. Perinuclear anchoring requires one of two Sir4-interacting proteins, yKu70 or Esc1, although neither is essential for silencing ([Gartenberg et al., 2004](#)). Interestingly, Sir2 orthologs have been found associated with E(Z) complexes in flies and mammals (e.g., [Furuyama et al., 2004](#)), providing an intriguing link between Sir and PcG silencing mechanisms.

The spread of the Sir complex is blocked by certain pol II and pol III promoters, probably through the binding of activators that recruit general transcription factors (GTFs) and coactivators (reviewed in [Rusche et al. \[2003\]](#)). Histone acetylation itself may constitute a barrier, and proteins like Bdf1, which protects H4 AcK16, antagonize the spread of Sir2/3/4. The histone H2A variant Htz1 also contributes to halting the spread of the Sir complex ([Meneghini et al., 2003](#)), as does histone H3 methylated at either K4 or K79 (see below). PcG silencing can operate over distances of several tens of kb; however, in contrast to the Sir complex, PcG proteins remain primarily localized in the region of the recruiting element, the PRE ([Ringrose et al., 2004](#)). Ergo, PcG silencing probably involves a looping mechanism akin to that envisioned for enhancer-promoter interaction. This is also suggested by the fact that ele-

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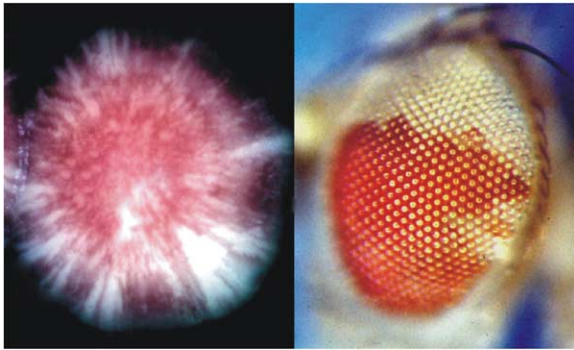


Figure 1. Mosaic Appearance of a Yeast Colony or *Drosophila* Eye Is Due to the Metastable Chromatin State of Their Pigmentation Genes. In yeast (left), the Sir-repressed state confers red coloration; in *Drosophila* (right), the PcG-repressed state confers white coloration. (Yeast photograph kindly provided by Dan Gottschling.)

ments such as the Su(Hw) or the scs insulators can serve as barriers to PcG-mediated silencing.

#### **Histone Modifications Linked to Silencing**

PcG silencing involves at least three kinds of covalent histone modifications, which may occur at both the PRE and promoter of the silenced gene. One is histone deacetylation through Rpd3/HDAC1, associated with both PRC1 and PRC2 types of complexes, and through Sir2, associated with PRC2 (E[Z]) complexes. Another is histone H3 methylation by the E(Z) complex, which trimethylates K9 and K27. Trimethylation of K27 is read directly by the PRC1 type of complex, whose PC component contains a chromodomain that binds selectively to H3 me3K27 in vitro (Czermin et al., 2002). A final modification is H2A ubiquitylation directed by dRing, a core component of PRC1 (Wang et al., 2004). Whether uH2A is related to the presence of the histone variant H2Av, found associated with PcG- and heterochromatin-silenced loci (Swaminathan et al., 2005), is unknown. Paradoxically, Htz1, the yeast ortholog of H2Av, is associated with transcriptional activity.

*S. cerevisiae* lacks the histone H3 meK9 and meK27 methylation marks associated with repression. However, in yeast as in *Drosophila*, methylation of H3 K4 and K79, as well as acetylation at various positions on histones H3 and H4, are associated with transcriptional activity. Indeed, histone methylation at either H3 K4 or H3 K79 antagonizes the binding of Sir proteins, as does acetylation of H4 K16 (reviewed in van Leeuwen and Gottschling [2002]). The enzymes responsible for these modifications—Set1, Dot1, and Sas2, respectively—are continuously required to prevent the inappropriate binding of Sir proteins to euchromatin. Because Sir proteins are present in limiting quantities, mutations that interfere with genomic methylation or acetylation tend to destabilize telomeric silencing.

Similarly, the PcG silencing system is closely associated with specific H3 K4 methyltransferase activities whose developmentally important function is to antagonize PcG silencing at target sites that were not silenced in the previous cell cycle. These are ASH1 and TRX, both of which methylate H3 K4 and act as antirepressors or stabilizers of the epigenetic nonrepressed state (Klymenko and Muller, 2004). Histone H3 K79 methylation in *Drosophila* is carried out by an ortholog of yeast Dot1, whose mutations reduce both PcG si-

lencing and gene expression (Shanower et al., 2005). The basis for these effects could be similar to those seen in either *set1* or *dot1* yeast mutants: depletion of PcG proteins from the normal target sites plus less effective elongation of transcription.

#### **Epigenetic Nature of Silencing**

A common feature of chromatin silencing, whether by the yeast Sir complexes or by the Polycomb complexes and heterochromatic complexes in animals, is their epigenetic nature. The silenced state, once established, is metastable from one cell generation to the next, with a high probability of being reestablished in daughter cells after mitosis. In situations in which silencing is relatively weak (such as at yeast telomeres), this gives rise to a characteristic clonal variegation of expression (Figure 1). In the case of the PcG complexes, this tendency to reestablish silencing where it existed in the previous cell cycle (cellular memory) has been attributed to the methylation marks produced by the PRC2 complex. After mitosis, when PcG proteins dissociate from chromatin, the methylated nucleosomes from the previous cell cycle would mark the chromatin region for preferential reassembly of PcG complexes.

Although formally these chromatin modifications may act as a mark, how they maintain silencing is less clear. PcG silencing, as well as the stable binding of PcG complexes, requires the activity of the E(Z) complex, but it is unlikely that H3 K27 methylation suffices to recruit PcG complexes, just as H3 K9 methylation is not sufficient to recruit HP1 (Stewart et al., 2005). It can be argued, however, that a chromatin domain methylated at H3 K27 maintains a high local concentration of PC, thereby ensuring occupancy of the PRE (Ringrose et al., 2004). Furthermore, although the active and repressive chromatin marks are formally antagonistic, we do not know why K4 and K79 methylation antagonize silencing. Possibly, histone acetylation, which together with K4 and K79 methylation accompanies transcriptional activity, might prevent the repressive methylation mark and account for the observation that transcription through a PRE has a derepressing effect.

In *S. cerevisiae* too, Sir silencing of a gene is strongly dependent on whether it was silenced in the previous cell cycle. Because no silencing-specific chromatin modification is known in yeast, the epigenetic mark might be constituted by the absence of the marks associated with transcriptional activity. Thus, histone hypoacetylation, absence of H3 K4 and K79 methylation, and absence of Htz1 could provide high-affinity binding sites for the Sir2/3/4 complex and, by default, favor the propagation of the silencing complex. The Sir complex, bound to parental histones, could deacetylate the N tails of newly deposited core histones, which are acetylated at K5 and K12 of H4 and K9 of H3, removing antagonistic marks and facilitating propagation of the Sir complex (reviewed in Rusche et al. [2003]).

#### **Structural Organization of Silenced Chromatin**

Sir-silenced chromatin is characterized by highly ordered arrays of nucleosomes arranged as closely packed pairs separated by long linkers (Weiss and Simpson, 1998). These silenced domains impair the accessibility of restriction nucleases in vitro and of endonucleases, DNA repair enzymes, and DNA methylases in vivo (reviewed in Rusche et al. [2003]). However, the difference between the silenced and derepressed (*sir2Δ*)

states may be as little as 2-fold (Chen and Widom, 2005). Moreover, completely silenced promoter regions and silencers themselves remain quite accessible (Weiss and Simpson, 1998). Genomic footprinting assays of a model silenced gene reveal the presence of significant Sir-dependent changes in local chromatin structure over UAS and TATA elements, including the presence of novel nucleosome-like structures over each region (reviewed in Sekinger and Gross [2001]). In *Drosophila*, PREs are highly accessible to nucleases, and silenced promoter elements can retain DNase I hypersensitivity. Unlike Sir-repressed chromatin, PcG-silenced domains do not appear to be associated with reductions in accessibility to exogenous endonucleases, and genomic footprinting did not reveal changes in local chromatin structure (Dellino et al., 2004).

#### **Permissiveness to Activator and GTF Recruitment**

A striking and unexpected feature of Sir- and PcG-mediated silencing is the fact that both are permissive to the recruitment of sequence-specific activators. These findings run counter to the notion that silenced chromatin represses transcription by sterically hindering access of positive regulators of transcription. However, they are consistent with the aforementioned DNase I hypersensitivity of silenced promoter and enhancer regions as well as the fact that, in yeast, transposases and recombinases can gain access to silenced DNA. Consistent too are studies showing that strong activation can derepress genes silenced by the yeast telomeric complexes, albeit during a restricted window within the cell cycle. In *Drosophila*, strong activation can likewise override silencing by a PRE or even by heterochromatin.

Heat shock gene promoters were used as models to explore the mechanism by which Sir2/3/4 and PcG complexes mediate silencing. In the yeast analysis, the *HSP82* gene was brought under Sir regulation by targeting *HMRE* silencers to the gene's chromosomal locus (Sekinger and Gross, 2001). When integrated 5' and 3' of the gene, the ectopic silencers efficiently recruited Sir proteins and repressed basal transcription 50- to 100-fold. Although HSF and TBP genomic footprints were altered, the abundance of HSF, TBP, and pol II recruited to the hyperrepressed *HSP82* promoter was only slightly diminished. Likewise, heat shock-induced expression of the *Drosophila hsp26* promoter was reduced 10-fold when it was flanked by a PRE (Dellino et al., 2004). Despite this, recruitment of HSF, TBP, and pol II was not much affected. These observations imply that cohabitation of PcG and GTFs at silenced promoters is possible and that silencing can still occur even after the recruitment of promoter complexes.

The use of heat shock promoters for these studies is very instructive, because these promoters are comparatively well understood and manipulated. However, heat shock promoters are unusual in that their chromatin configuration is constitutively held in a state of readiness with pol II and GTFs already recruited. These findings show nevertheless that a component of silencing acts directly upon the promoter complex. They do not exclude the likelihood that either or both silencing systems utilize additional mechanisms to interfere with transcription.

#### **Block to Pol II Function**

Perhaps the most intriguing parallel between the Sir and PcG systems is the fact that both permit pol II re-

cruitment to a heat shock promoter, yet each acts on the promoter complex (or at a point subsequent to its formation) to block transcription. In the case of Sir silencing of the *HSP82* promoter, the transcriptional block must occur early because even very short transcripts were undetectable (Sekinger and Gross, 2001). In the PcG case, silencing blocks formation of a productive open complex (Dellino et al., 2004). At promoters lacking the preset character of heat shock promoters, the failure to form a preinitiation complex might render pol II recruitment labile and account for the polymerase depletion observed in yeast at silenced *URA3* transgenes, whose promoters are nonetheless bound by the activator Ppr1 (Chen and Widom, 2005). The mechanism of the transcriptional block is not yet clear. It is possible that direct interaction between some Sir or PcG component and one of the GTFs interferes with a transition required for transcription initiation. Given the multiplicity of GTFs and of the steps involved in transcription initiation, there is no reason to expect that the Sir and PcG complexes have the same target. Moreover, given the enzymatic activities that are associated with the silencing complexes, the involvement of deacetylation, methylation, or ubiquitylation of a GTF cannot be excluded.

#### **Comparison with Other Examples of Chromatin Repression**

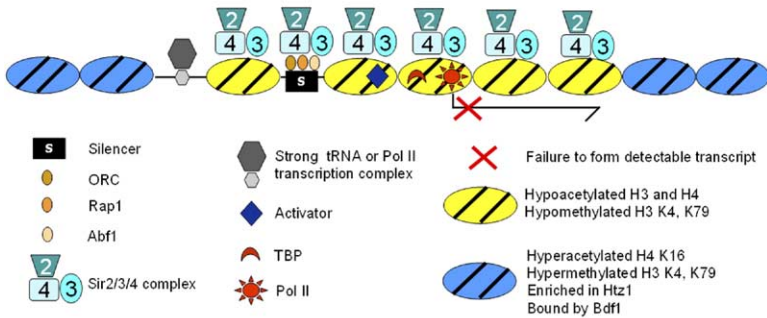
Are Sir and PcG unique in their mechanism of chromatin repression? In *S. cerevisiae*, the Tup1-Ssn6 corepressor is recruited to a large number of genes, including those subjected to glucose-, oxygen- and cell type-mediated repression. Like Sir, Tup1-mediated repression involves the targeted recruitment of histone deacetylases, yet in contrast to Sir, Tup1-Ssn6 represses transcription by blocking recruitment of TBP (Kuras and Struhl, 1999). In *Drosophila*, heterochromatin appears to block recruitment of GAGA factor, TBP, and pol II to a noninduced *hsp70* heat shock transgene (Cryderman et al., 1999), in contrast to PcG-mediated silencing. Thus, in both yeast and fly, alternative forms of repression appear to use distinctly different mechanisms.

In human chromatin, TFIID and TFIIIB are retained at active gene promoters that are mitotically silenced, although pol II is displaced. Mitotic chromatin condensation does not prevent access of transcription factors or exchange of chromatin proteins (Chen et al., 2005). Likewise, the heterochromatin protein HP1 exhibits both physical and genetic interaction with hTAF<sub>II</sub>30, suggesting that TFIID may be present at some genes silenced by heterochromatin. Consistent with this, activated HSF1 has been observed to bind nucleosomal satellite III repeats within human heterochromatin (Jolly et al., 2002), providing an intriguing parallel to the Sir and PcG systems. Thus, cohabitation of positive regulators with repressors also appears to be a feature of certain forms of mammalian silenced chromatin.

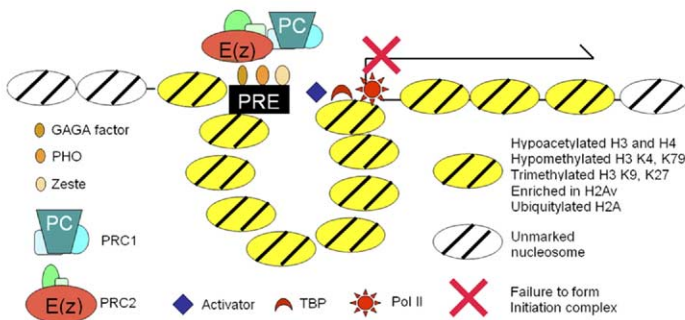
#### **Summary and Prospects**

In summary, Sir and PcG represent evolutionarily distinct forms of silencing that nevertheless converge to share many features, including recruitment of chromatin modification complexes by *cis* elements and sequence-specific DNA binding proteins, hypoacetylation of core histones and hypomethylation of H3 K4 and K79, and an epigenetic mode of inheritance. In addition, both Sir- and PcG-silenced promoters are, at least

### A Sir silencing mechanism



### B PcG silencing mechanism



in certain contexts, permissive to the binding of activators, GTFs, and even pol II (see Figure 2). Although Sir-silenced domains display decreased accessibility, this may be more significant for the nontranscriptional functions of silenced chromatin such as inhibiting HO cutting at *HMR* and *HML*, inhibiting ERC formation at the rDNA repeats, and inhibiting nonhomologous end joining at telomeres. Furthermore, the fact that PcG complexes have direct effects on transcription initiation does not exclude that they may deploy additional repressive mechanisms such as interference with remodeling or with transcriptional elongation.

Principal unresolved issues include distinguishing what is a primary effect of silencing and what is a secondary consequence of long-term silencing. Chromatin condensation has traditionally been considered the cause of silencing, but it could equally be thought of as a consequence of transcriptional repression. The present evidence does not exclude the possibility of silencing mechanisms that operate by inducing a more compact, higher-order structure of the chromatin fiber, but they strongly suggest that, at least in the cases examined here, the primary effects of silencing are on the transactions occurring at or near the promoter. Transcriptional inactivity might then allow the eventual establishment of more highly ordered chromatin structures such as have been reported for heterochromatic sites. Clearly, additional work is required to unravel these issues.

#### Selected Reading

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Figure 2. Basic Features of Sir and PcG Silencing as Suggested by Studies of Model Systems

For both mechanisms, the presence of TBP and pol II at the silenced promoter may depend on promoter context and, particularly in *Drosophila*, may not apply to the general case. The detailed distribution of methylation and ubiquitylation over the PcG-repressed gene is still controversial; the figure assumes that both marks are present over a broad domain.

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