Heterochromatin assembly and transcriptome repression by Set1 in coordination with a class II histone deacetylase

David R Lorenz, Lauren F Meyer, Patrick J R Grady, Michelle M Meyer, Hugh P Cam*

Department of Biology, Boston College, Chestnut Hill, United States

Abstract

Histone modifiers play essential roles in controlling transcription and organizing eukaryotic genomes into functional domains. Here, we show that Set1, the catalytic subunit of the highly conserved Set1C/COMPASS complex responsible for histone H3K4 methylation (H3K4me), behaves as a repressor of the transcriptome largely independent of Set1C and H3K4me in the fission yeast Schizosaccharomyces pombe. Intriguingly, while Set1 is enriched at highly expressed and repressed loci, Set1 binding levels do not generally correlate with the levels of transcription. We show that Set1 is recruited by the ATF/CREB homolog Atf1 to heterochromatic loci and promoters of stress-response genes. Moreover, we demonstrate that Set1 coordinates with the class II histone deacetylase Clr3 in heterochromatin assembly at prominent chromosomal landmarks and repression of the transcriptome that includes Tf2 retrotransposons, noncoding RNAs, and regulators of development and stress-responses. Our study delineates a molecular framework for elucidating the functional links between transcriptome control and chromatin organization.

DOI: 10.7554/eLife.04506.001

Introduction

The packaging of eukaryotic DNA with histones into chromatin provides ample opportunities for chromatin-modifying factors to exert extensive control over many aspects of genome-based processes (Kouzarides, 2007). In particular, enzymes catalyzing the covalent posttranslational modifications of histones are increasingly seen as critical regulators of transcription and the assembly of chromatin into various functional domains (Henikoff and Shilatifard, 2011; Badeaux and Shi, 2013). Two of the better understood posttranslational modifications of histones are acetylation and methylation. Whereas acetylation of histones by histone acetyltransferases (HATs) is generally associated with gene activation (Rando and Chang, 2009), deacetylation of histones by histone deacetylases (HDACs) tends to correlate with gene repression (Yang and Seto, 2008). Coordinated activities among HATs result in region-wide hyperacetylated chromatin states, leading to the formation of euchromatin domains supporting active transcription, and conversely, hypoacetylated chromatin states catalyzed by HDACs give rise to heterochromatin domains refractory to transcription (Grunstein, 1998; Grewal and Jia, 2007). In contrast, histone methylation is associated with either transcriptional activation or repression, and hence, euchromatin or heterochromatin (Huisinga et al., 2006; Henikoff and Shilatifard, 2011). Two well-characterized methylation marks occurring on two closely spaced residues near the amino-terminal tail of histone H3 exemplify this pattern (Grewal and Jia, 2007). Methylation at lysine 4 of histone H3 (H3K4me) and at lysine 9 (H3K9me) distinguishes euchromatin and heterochromatin, respectively (Litt et al., 2001; Noma et al., 2001). However, studies from the fission yeast Schizosaccharomyces pombe and other systems show that the euchromatic and heterochromatic landscapes are somewhat fluid, with islands of H3K9me transiently assembled within...
**eLife digest** Genes can be turned on or off at different times in an organism’s life. In humans, yeast and other eukaryotes, this is mainly controlled by the way DNA is packaged with proteins—known as histones—in a structure called chromatin. Genes that are switched on, or only temporarily switched off, are associated with areas of the genome where the chromatin is loosely packed. In contrast, genes that remain switched off for long periods of time are found in regions—known as heterochromatin—where the chromatin is tightly packed.

There are many enzymes that can modify histones to change the structure of chromatin. One enzyme—called Set1—adds a methyl tag to chromatin, which is known to be associated with genes being switched on. However, Lorenz et al. found that Set1 also has other roles in modifying chromatin in the yeast *Schizosaccharomyces pombe*.

The experiments found that Set1 helps to keep genes switched off and that this role is largely independent of its ability to add the methyl tag to chromatin. Set1 is recruited to many sites across the genome by another protein called Atf1, which is involved in the cell’s response to environmental stresses. Lorenz et al. believe that this helps to put these genes in a ‘poised’ off state so that they are ready to be switched on rapidly if needed.

Set1 also works with another protein that removes acetyl tags—which encourage chromatin to be less tightly packed—from histones. Together, both proteins contribute to the assembly of heterochromatin and keep genes involved in development and stress responses switched off when they are not required.

Collectively, these experiments reveal unexpected and important insights into how Set1—which plays critical roles in many aspects of human health including aging and cancer—works in cells.

DOI: 10.7554/eLife.04506.002
repressed loci associated with development and stress-response pathways. Furthermore, we demonstrate that the conserved stress-response ATF/CREB Atf1 transcription factor mediates the recruitment of Set1 and modulates the levels of H3K4me3 at the centromere central cores and ribosomal DNA array. We show that Set1 coordinates with the class II HDAC Clr3 to mediate the assembly of H3K9me-associated heterochromatin and genome-wide repression of diverse transcripts, including Tf2 retrotransposons, noncoding RNAs, and developmental and stress-response genes. Our study illuminates a surprising cooperation between two histone-modifying enzymes with seemingly opposing activities in imposing genome-wide repression over the transcriptome and organizing the genome into euchromatin and heterochromatin.

Results

Set1 behaves as a general repressor largely independent of its H3K4me function and other Set1C subunits

Set1 is the catalytic engine of the Set1C complex that includes seven other subunits (Roguev et al., 2003). Except for Shg1, Set1 and six S. pombe subunits (Swd1, Swd2, Swd3, Spp1, Ash2, Sdc1) have orthologs in S. cerevisiae and humans (Roguev et al., 2003; Shevchenko et al., 2008; Shilatifard, 2012). Loss of individual Set1C complex subunits affects differentially the levels and states of H3K4me in S. pombe (Roguev et al., 2003; Mikheyeva et al., 2014). We performed expression profiling analyses in mutant strains deficient in H3K4me or lacking individual subunits of the Set1C complex. Whereas loss of set1 resulted in significant derepression of nearly 1000 of ~42,000 tiling microarray probes (average log2 fold-change vs wild-type >1.5, p < 0.05), H3K4me null mutants H3K4R (histone H3 lysine 4 substituted with arginine) or set1FΔ (H3K4me abolished by Set1 C-terminal FLAG epitope insertion) (Lorenz et al., 2012; Mikheyeva et al., 2014) affected ~100 probes (Figure 1A). Profiling analysis of other Set1C subunits showed a wide range of effects on transcriptional repression, with fewer than 100 probes significantly changed versus wild-type in ash2Δ to ~300 in spp1Δ. Similar to the other H3K4me mutants, most probes affected in Set1C subunit mutants corresponded to upregulated transcripts, consistent with previous observations in budding yeast showing that loss of H3K4me tends to result in derepression (Margaritis et al., 2012; Weiner et al., 2012). Importantly, our results show that the major repressive function of Set1 in S. pombe occurs largely distinct from H3K4me and the Set1C complex. Variations among Set1C/H3K4me mutants in the proportion of affected probes corresponding to sense, antisense, and intergenic transcripts were also observed (Figure 1B), with equal proportions of differentially expressed probes among the three classes of transcripts seen in set1Δ, H3K4R, and set1FΔ mutants. Loss of ash2 primarily resulted in increased sense transcription, and loss of shg1, spp1, or swd3 predominantly affected intergenic transcripts.

Set1C/H3K4me mutants display unique gene expression profiles

Because Set1C/H3K4me mutants displayed varying degrees of transcriptional effects, we performed two-dimensional hierarchical clustering of all differentially expressed probes to gain further insights into their functional relationships. Despite their functions being linked to H3K4me, transcriptional profiles clustered broadly into four distinct groups (Figure 1C, upper panel). The loss of ash2 and sdc1, which affected a higher proportion of sense strand probes than in other mutants (Figure 1B), shared a subset of upregulated transcripts with significant gene ontology (GO) enrichment for terms common to stress response, including ‘response to stress’ (p ≈ 10−3, ash2Δ; p ≈ 10−18, sdc1Δ), ‘oxidoreductase activity’ (p ≈ 10−3, ash2Δ; p ≈ 10−11, sdc1Δ), and ‘generation of precursor metabolites and energy’ (p ≈ 10−5, ash2Δ, p ≈ 10−3, sdc1Δ) (Figure 1—source data 1A). The profiles of shg1 and spp1 mutants formed the second group of predominantly upregulated probes corresponding to diverse intergenic regions and antisense transcripts sharing comparatively weak GO enrichment. The group consisting of swd2Δ, swd3Δ, set1FΔhistone—, and H3K4R mutants included smaller subsets of differentially expressed probes (Figure 1C, upper panel), with modestly significant GO enrichment for upregulated transcripts related to stress response and carbohydrate metabolism (Figure 1—source data 1A). The profile of set1Δ forms its own distinct group, containing a large set of upregulated transcripts including Tf2 retrotransposons, pericentromeric repeats, and long noncoding RNAs (lncRNAs) that were little affected in the other Set1C and H3K4me mutants (Figure 1C, lower panel; Figure 1—source data 1B). These results suggest that loss of individual Set1C subunits produces different effects on the transcriptome that could not be fully accounted for by their known contributory roles to H3K4 methylation.
Set1 localizes to lowly expressed and repressed genes

While H3K4me is known to be enriched at transcriptionally active loci (Cam et al., 2005; Pokholok et al., 2005), we consistently observed transcriptional derepression in the set1Δ mutant at non-active, stress-response genes or heterochromatic repeats. We therefore performed genome-wide mapping of Set1 to gain insights into its repressor function. Consistent with its documented recruitment to active Pol II genes (Ng et al., 2003), Set1 is enriched at sites that correspond to highly active Pol II promoters, including those of the housekeeping gene act1 and the ribosomal protein rps102 (Figure 2A).
Surprisingly, despite little enrichment of Pol II at certain lowly expressed genes (e.g., scr1) and repressed developmental genes (e.g., ste11), noticeable Set1 binding was detected at the promoters of these genes (Figure 2B; Figure 2—figure supplement 1). Set1 localization at active and repressed targets was not hampered by the loss of H3K4me or its catalytic activity. Indeed, the inability of the set1ΔΔH3K4meto methylate H3K4 appears to enhance its association with chromatin.

To discern the relationship between Set1 binding and the transcriptional status of its targets, we ranked 290 protein-coding genes with significant Set1 binding (chromatin immunoprecipitation...
(ChIP) fold enrichment ≥2 at three or more adjacent probes) according to their expression levels (Figure 2C, left panel). While transcript abundance generally correlated with Pol II occupancy levels (Figure 2C, middle panel) and 80% of promoter regions enriched for Set1 corresponded to actively transcribed genes (Figure 2—figure supplement 2), transcript abundance or Pol II occupancy levels did not linearly correlate with the levels of Set1 binding (Figure 2C, right panel). Functional differences between high-abundance and low-abundance Set1-bound genes were assessed by GO analysis of genes rank-ordered by expression levels into quintiles (Figure 2D). Whereas highly expressed genes occupied by Set1 were enriched with expected GO terms associated with rapid exponential growth (ribosome, translation, glycolysis), Set1-bound genes with low abundance transcripts (excluding heterochromatic noncoding RNAs due to limited GO annotation) were enriched for terms related to stress response, cell wall and membrane-bound protein biogenesis, and Pol II transcription factor function (Figure 2—source data 1). Thus, our results suggest that Set1 localization at chromatin is not solely dependent on active Pol II, and that Set1 localization at lowly expressed or repressed loci might be functionally distinct from its canonical role at active Pol II genes.

**Atf1 mediates recruitment of Set1 at the centromere central cores, rDNA array, and developmental and stress-response genes**

A number of low-abundance transcripts shown to be enriched for Set1 in genome-wide binding profiling (e.g., ste11) have previously been shown to be targets of the highly conserved ATF/CREB transcription factor Atf1. In addition to localizing to its targets before their activation (Eshaghi et al., 2010), which is important for subsequent proper response to environmental stresses (Chen et al., 2003), Atf1 contributes to heterochromatic silencing at the silent mating-type locus (Jia et al., 2004). We performed genome-wide binding profiling of Atf1 and compared it with that of Set1 to gain insights into the mechanism of Set1 recruitment to chromatin. We observed colocalization of Atf1 and Set1 at centromeric tRNA clusters flanking the euchromatin/heterochromatin boundaries of centromere II and the inner imr repeats of the central core (Figure 3A, upper panel). Similar colocalization patterns were detected at centromeres I and III (Figure 3—figure supplement 1, upper panels). We also detected colocalization of Atf1 and Set1 at the intergenic region of the rDNA and the promoter of the developmental regulator ste11 (Figure 3B,C, upper panel; Figure 3—figure supplement 2). We assessed the loss of atf1 on Set1 activity by mapping distributions of H3K4me3 at these loci in wild-type and atf1Δ cells. In wild-type cells, H3K4me3 signals could be detected throughout the centromere central cores and the rDNA array but were little enriched at the ste11 promoter (Figure 3A,B, C; Figure 3—figure supplement 1, bottom panels). Loss of atf1 resulted in a sizeable reduction of H3K4me3 levels throughout the central cores and rDNA array. Moreover, genome-wide analysis identified many loci displaying reduced H3K4me3 in atf1Δ compared with wild-type (Figure 3—source data 1). The repressed status of the ste11 gene was not noticeably affected by atf1Δ (Figure 3—figure supplement 4) and hence has little effect on the status of H3K4me3. However, we noticed that several repressed genes whose promoters are occupied by Atf1 exhibited increased H3K4me3 levels in atf1Δ cells (Figure 3—figure supplement 3), probably owing to the loss of Atf1-mediated repression.

To determine whether reduced H3K4me3 levels at the centromere central cores and the rDNA array partly reflect the failure of Atf1 to recruit Set1, we assessed Set1 localization at these loci by ChIP. We found that Set1 enrichment at these loci, including the ste11 gene, was reduced in atf1Δ cells (Figure 3D). At ste11, Atf1 and Set1 appear to act primarily in parallel pathways to keep ste11 expression repressed, as appreciable upregulation of ste11 expression was seen only in mutants deficient for both atf1 and set1 (Figure 3—figure supplement 4). Comparing Atf1 and Set1 localization at the genome scale revealed 217 and 261 distinct bound loci for Atf1 or Set1, respectively, with more than one-third co-occupied by both proteins (p < 0.001, Fisher’s exact test) (Figure 3E). Collectively, our results suggest that Set1 recruitment to certain repressed loci is mediated in part by Atf1, which in turn is important for proper maintenance of H3K4me levels and, depending on genomic context, transcriptional repression.

**Set1 cooperates with the class II HDAC Clr3 in heterochromatic silencing and the assembly of heterochromatin**

To better understand the repressive function of Set1, we sought to identify factors that cooperate with Set1 in heterochromatic silencing. The class II HDAC Clr3 has been shown to contribute to transcriptional silencing of heterochromatin (Grewal et al., 1998; Yamada et al., 2005) Tif2 retrotransposons (Hansen et al., 2005; Cam et al., 2008), and stress-response genes (Lorenz et al., 2012). These classes of
Genetic elements are also regulated by Set1, suggesting a possible functional link between Clr3 and Set1. To explore this idea, we constructed a mutant strain deficient for both set1 and clr3 (set1Δclr3Δ). We observed that in contrast to wild-type or single set1Δ or clr3Δ mutant strains, a double mutant set1Δ clr3Δ strain exhibited a significant synthetic slow-growth phenotype and sensitivity to the tubulin
Figure 4. Set1 and the class II HDAC Clr3 cooperates in heterochromatic silencing and heterochromatin formation. (A) Serial dilution analysis (SDA) of set1 and clr3 mutant strains in nonselective (N/S) media or in the presence of the tubulin inhibitor thiabendazole (TBZ). (B) Uracil minus media (−Ura) or in the presence of the uracil counter selective drug 5-fluoroorotic acid (5-FOA). (C) Transcription of forward and reverse strands at centromere II in indicated mutant strains was analyzed by microarrays. (D) H3K9 dimethylation (H3K9me2) in strains deficient for set1 and clr3 at the pericentromeric dg repeat. H3K9me2 enrichment at the dg repeat in indicated strains was carried out by chromatin immunoprecipitation (ChIP) and quantified by qPCR. (E) H3K9me2

Figure 4. Continued on next page
distribution across the entire centromere II in wild-type and set1Δ clr3Δ strains. H3K9me2 at centromere II was assayed by ChIP–chip. (F) siRNA levels in wild-type, set1 and clr3 mutant strains. Detection of siRNAs was carried out by a northern blot using a probe specific for pericentromeric dg repeats.

DOI: 10.7554/eLife.04506.015

The following figure supplements are available for figure 4:

**Figure supplement 1.** Pol II and Swi6 localization at pericentromeres in set1 and clr3 mutants.

DOI: 10.7554/eLife.04506.016

**Figure supplement 2.** H3K9me2 defects at centromeres I and III, mating type locus and subtelomeric regions in a strain deficient for both set1 and clr3.

DOI: 10.7554/eLife.04506.017

Intriguingly, cells lacking both set1 and clr3 in a strain deficient for both set1 and clr3 (set1Δ clr3Δ) did not produce such a drastic change to the transcriptome compared with set1Δ clr3Δ, but only reduced the proportion of downregulated transcripts seen in the single clr3Δ mutant. Similar proportions of probes corresponding to the sense or antisense strands of known transcripts were differentially expressed across set1Δ and set1Δ clr3Δ mutants, with the exception of clr3Δ cells, which displayed an increased...
proportion of sense strand probes (Figure 5B). Hierarchical clustering showed that transcripts downregulated in set1Δ tended to be downregulated further in set1Δ clr3Δ (Figure 5—figure supplement 1), and transcripts that were upregulated in set1Δ (i.e., Tf2s and subtelomeric regions) were further upregulated in the double mutants (Figure 5C, Figure 5—figure supplement 2). Most notably, loss of both set1 and clr3 resulted in significant expression changes within protein-coding gene regions for a large subset of genes displaying negligible change in individual set1 or clr3 mutants (Figure 5C).
Upregulated transcripts include well-characterized developmental and stress-response regulatory proteins that include fbp1, mei2 and ste11 (Figure 5—figure supplement 3). Gene ontology analysis suggested that most of the upregulated transcripts in set1Δ clr3Δ are associated with stress-response processes that include the Tor2-Mei2-Ste11 pathways (Figure 5D; Figure 5—source data 1). These pathways are known to be activated during the meiotic development program (Otsubo and Yamamoto, 2012). In this regard, we noted that compared with wild-type or single mutant strains, the set1Δ clr3Δ double mutant exhibited considerable meiotic defects (Figure 5—figure supplement 4). Collectively, our results disclose unexpected coordination between Set1 and Clr3 in ensuring genome-wide repression of the fission yeast transcriptome and proper developmental control.

Discussion

Set1C as a repressor complex of the fission yeast transcriptome

Recent transcriptome studies of chromatin mutants in S. cerevisiae reveal that loss of set1 or any of the other four core Set1C subunits (Swd1, Swd3, Bre2/Ash2, Sdc1) produces comparable expression profiles (Margaritis et al., 2012). Furthermore, loss of set1 has only a modest effect on the transcriptome, mainly towards derepression that could fully be accounted by the loss of H3K4me (Margaritis et al., 2012; Weiner et al., 2012). Similar to these studies, our current study shows that complete loss of H3K4me (i.e., H3K4R, set1FΔH3K4me− mutants) in S. pombe has only a slight impact on the transcriptome, with most differentially expressed transcripts upregulated. However, there are important differences. Except for the expression profiles of H3K4R and set1FΔH3K4me− mutants, the profiles among S. pombe Set1C subunit mutants are notably disparate, which could not be fully explained by their roles as subunits of Set1C or contributions to H3K4me (Roguev et al., 2003). For example, Ash2 and Sdc1 are thought to form heterodimers that together with Swd1 and Swd3 constitute the core of the Set1C complex (Roguev et al., 2001; Dehe et al., 2006; Southall et al., 2009; Kim et al., 2013). Yet, while their expression profiles are most similar to each other, there are even differences between them, with the sdc1 mutant displaying stronger derepression for a subset of genes involved in response to oxidative stress than those seen in the ash2 mutant (Figure 1C). These similarities and differences might reflect their association with other chromatin modifiers such as the Lid2 complex, not present in budding yeast (Roguev et al., 2003; Shevchenko et al., 2008). Most importantly, the expression profile of set1Δ is strikingly different from those of other Set1C/H3K4me mutants, displaying more than eight times the number of upregulated probes relative to those of swd3 or H3K4R mutants. Our findings show that unlike the results reported for S. cerevisiae, Set1 in S. pombe not only exerts more regulatory influence over the transcriptome, but also mediates its repressive function largely independently of the other Set1C subunits and H3K4 methylation—probably, as a consequence of the uncoupling of Set1 protein stability from H3K4me levels (Mikheyeva et al., 2014). Interestingly, S. pombe Set1 has been reported as a component of at least two complexes: a large ~1 MDa complex similar in size to that of S. cerevisiae Set1C and a smaller complex (~800 kDa) containing a shorter version of Set1 (Roguev et al., 2003). Thus, Set1 might mediate its repressive nonH3K4me function via a distinct form of Set1 different from the form associated with the canonical Set1C complex.

Regulation of repetitive elements, developmental and stress-response loci by Set1 and Atf1

Our study reveals extensive functional interactions across the genome between Set1 and the stress-response transcription factor Atf1 at stress-response genes and major chromosomal landmarks, including the tandem rDNA array and centromeres. At the rDNA array and centromere central cores, Atf1 mediates Set1 recruitment and modulates H3K4me3 levels that might contribute to proper chromatin organization rather than transcriptional repression itself. At loci of stress response and developmental regulators such as ste11, Atf1 and Set1 appear to act in parallel pathways that contribute to the repression of ste11 as loss of both atf1 and set1 resulted in significant derepression of ste11 (Figure 3—figure supplement 4). The transcriptional activation of Atf1 is controlled by phosphorylation mediated by the stress-activated mitogen-activated protein kinase (MAPK) Sty1 pathway (Shiozaki and Russell, 1996; Lawrence et al., 2007). It is likely that co-occupancy of Set1 and Atf1 at the promoters of certain developmental and stress-response regulators not only helps keep these genes in a poised transcriptional off-state, but might also contribute to their rapid transcriptional activation in response to proper developmental or environmental stress signals.
Functional cooperation between Set1 and Clr3 in heterochromatic silencing and genome-wide repression of the transcriptome

Pol II activity is known to be required for transcriptional silencing and heterochromatin assembly at pericentromeric repeats (Djupedal et al., 2005; Kato et al., 2005). Other factors associated with active Pol II transcription including components of the Mediator complex have also been shown to contribute to heterochromatin formation (Oya et al., 2013). Our study identifies an important role for Set1 in the assembly of heterochromatin domains such as those present at pericentromeres (Figure 6). Set1 represses transcription on both the forward and reverse strands of the pericentromeric repeats and cooperates with Clr3 to assemble H3K9me-associated heterochromatin. Importantly, this heterochromatic activity of Set1 appears to be independent of its canonical H3K4me function associated with the Set1C complex, consistent with previous observations for the general lack of H3K4me within H3K9me heterochromatin (Noma et al., 2001; Cam et al., 2005). Set1-mediated heterochromatin assembly might involve Set1 methylating a nonhistone substrate similar to that of SUV39H1/Clr4 methylating Mlo3, an RNA processing and nuclear export factor that also contributes to RNAi-mediated heterochromatin assembly (Zhang et al., 2011). The only known nonhistone target of Set1 is the kinetochore protein DAM1 in S. cerevisiae (Zhang et al., 2005). However, the S. pombe dam1 ortholog does not appear to be the target of Set1-mediated heterochromatic silencing as repression of Tf2 retrotransposons and pericentromeric heterochromatin is maintained in dam1 mutant cells (Mikheyeva and Cam, unpublished data).

In addition to heterochromatic repeats, a significant fraction of the transcriptome is under repressive control by Set1 and Clr3. Such genome-wide repressive effect strongly suggests that Set1 behaves largely as a bona fide repressor. At developmental and stress-response loci such as ste11, Set1 may act in concert with transcription factors, including Atf1 together with Clr3 and other HDACs, to keep the target genes repressed in a steady-state condition. However, unlike heterochromatin, the chromatin states of these loci probably support a transcriptionally poised Pol II and in response to appropriate environmental signals enable Pol II to rapidly upregulate transcription.

Materials and methods

Strain Construction

Null mutants of Set1C subunits were constructed using a kanamycin cassette (Bahler et al., 1998; Mikheyeva et al., 2014). Double mutants were generated by standard genetic cross methods (Moreno et al., 1991). Liquid cultures were grown at 30°C in standard rich media supplemented with 75 mg/l adenine (YEA).
Chromatin immunoprecipitation (ChIP) and ChIP–chip

ChIP assays were performed as previously described (Lorenz et al., 2012). ChIP enrichment was quantified by qPCR analysis. ChIP–chip was carried out as previously described using Agilent tiling microarrays (Cam et al., 2005). ChIP–chip analysis was performed using the R/Bioconductor ringo package (Toedling et al., 2007). Preprocessing was carried out by loess normalization. ChIP-enriched regions were defined as three or more adjacent microarray probes with fold-enrichment greater than a two-Gaussian null distribution threshold (greater than twofold enrichment). Between-array analysis of H3K4me3 in wild-type and atf1Δ experiments was performed using the limma (linear models for microarray data) package after interarray quantile normalization. Antibodies used for ChIP and ChIP–chip assays were anti-FLAG Set1 (M2; Sigma-Aldrich, St. Louis, MO), anti-Atf1 (sc-53172; Santa Cruz Biotechnology, Inc., Dallas, Texas), anti Pol II (ab5408; Abcam, Cambridge, MA), anti-H3K4me3 (07-473; Millipore, Billerica, MA), anti-H3K9me2 (ab1220; Abcam), and anti-Swi6 (Nakayama et al., 2000).

siRNA detection

Small RNAs were purified from 50 ml culture of logarithmically growing cells using the Ambion mirVana miRNA/siRNA isolation kit (Life Technologies, Grand Island, NY). Small RNAs (60 µg) were loaded onto a 15% denaturing polyacrylamide gel and run at 300 V until the bromophenol blue dye reached the bottom of the gel (~1.5 hr). Northern transfer was done overnight by capillary blotting in Tris-borate-EDTA buffer at room temperature onto Hybond-N+ membrane (GE Healthcare, Pittsburgh, PA). The membrane was subsequently UV crosslinked twice at 1200 J. Hybridization was carried out in 10 ml ULTRAhyb-Oligo buffer (Life Technologies) at 40°C overnight with a 32P-labeled RNA probe specific to pericentromeric dg repeats. The RNA probe was generated by in vitro transcription using a T7 RNA polymerase system and 50 µCi of [α-32P]UTP. Detection of the siRNA signals was carried out using the Storm 820 molecular imager (Molecular Dynamics; GE Healthcare).

Gene expression profiling

Transcriptional profiling analysis was done as previously described (Lorenz et al., 2012). Briefly, RNA was extracted from batch cultures of mid-exponential phase (OD595 ~ 0.3–0.6) from mutant and isogenic wild-type strains, reverse-transcribed into cDNA, and labeled with either Alexa Fluor 555 (wild-type sample) or Alexa Fluor 647 (mutant sample) using Superscript Indirect cDNA labeling system (Life Technologies). Equal amounts of labeled cDNA (200–300 ng) from wild-type and mutant samples were mixed and hybridized on a custom 4 × 44k probe Agilent tiling microarray as previously described (Cam et al., 2005). For hierarchical clustering using the R/Bioconductor hopach package (van der Laan and Pollard, 2003), interarray quantile normalization was performed using the limma package, and transcripts with more than one differentially expressed probe were averaged. The cosine angle function was used for the clustering distance metric. Gene Ontology (GO) enrichment was performed as previously described (Lorenz et al., 2012).

Datasets associated with transcriptional profiling and ChIP–chip experiments in this study can be accessed at the Gene Expression Omnibus under accession number GSE63301.

Acknowledgements

We thank Grace Kim, Daniel Shams, and Betty Slinger for experimental support, Ke Zhang for the siRNA protocol, Shiv Grewal for the Swi6 antibody, Ee Sin Chen, Irina Mikheyeva, and David Layman for critical reading of the manuscript. Work in the Cam laboratory is supported by the Boston College Wielers Faculty Research Fund and the March of Dimes Basil O’Connor Starter Scholar Research Award.

Additional information

Funding

| Funder                  | Author       |
|-------------------------|--------------|
| March of Dimes Foundation | Hugh P Cam   |
| Boston College          | Hugh P Cam   |

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.
**Author contributions**
DRL, HPC, Conception and design, Acquisition of data, Analysis and interpretation of data, Drafting or revising the article; LFM, PJRG, Acquisition of data, Analysis and interpretation of data, Drafting or revising the article; MMM, Conception and design, Acquisition of data, Drafting or revising the article

**Additional files**

**Major datasets**
The following dataset was generated:

| Author(s)                        | Year | Dataset title                                                                 | Dataset ID and/or URL                                          | Database, license, and accessibility information |
|----------------------------------|------|-------------------------------------------------------------------------------|---------------------------------------------------------------|-------------------------------------------------|
| Lorenz DR, Meyer LF, Cam HP      | 2014 | Heterochromatin assembly and transcriptome repression by Set1 in coordination with a class II histone deacetylase | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE63301     | Publicly available at NCBI Gene Expression Omnibus. |

**Reporting Standards:** Standard used to collect data: Microarray datasets are deposited at NCBI GEO according to MIAME 2.0 standards. The following previously published datasets were used:

| Author(s)                        | Year | Dataset title                                                                 | Dataset ID and/or URL                                          | Database, license, and accessibility information |
|----------------------------------|------|-------------------------------------------------------------------------------|---------------------------------------------------------------|-------------------------------------------------|
| Zaratiegui M, Vaughn MW, Irvine DV, Goto D, Watt S, Bähler J, Arcangioli B, Martiensen RA | 2010 | CENP-B preserves genome integrity at replication forks paused by retrotransposon LTR | http://www.ncbi.nlm.nih.gov/sra/?term=SRA024710.2 | Publicly available at NCBI Sequence Read Archive. |
| Rhind N, Chen Z, Yassour M, Thompson DA, Haas BJ, Habib N, Wapinski I, Roy S, Lin MF, Heiman DI | 2011 | Comparative functional genomics of the fission yeasts                         | http://www.ncbi.nlm.nih.gov/sra/?term=SRP005611             | Publicly available at NCBI Sequence Read Archive. |

**References**

Alper BJ, Job G, Yadav RK, Shanker S, Lowe BR, Partridge JF. 2013. Sir2 is required for Clr4 to initiate centromeric heterochromatin assembly in fission yeast. *The EMBO Journal* **32**:2321–2335. doi: 10.1038/emboj.2013.143.

Badeaux AJ, Shi Y. 2013. Emerging roles for chromatin as a signal integration and storage platform. *Nature Reviews Molecular Cell Biology* **14**:211–224. doi: 10.1038/nrm3545.

Bähler J, Wu JQ, Longtine MS, Shah NG, McKenzie A III, Steeuer AB, Wach A, Philippsen P, Pringle JR. 1998. Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. *Yeast* **14**:943–951. doi: 10.1002/(SICI)1097-0061(199807)14:10<943::AID-YEA292>3.0.CO;2-Y.

Berretta J, Pinskaya M, Morillon A. 2008. A cryptic unstable transcript mediates transcriptional trans-silencing of the Ty1 retrotransposon in *S. cerevisiae*. *Genes & Development* **22**:615–626. doi: 10.1101/gad.458008.

Bähler M, Haas W, Gygi SP, Moazed D. 2007. RNAi-dependent and -independent RNA turnover mechanisms contribute to heterochromatic gene silencing. *Cell* **129**:707–721. doi: 10.1016/j.cell.2007.03.038.

Cam HP, Noma K, Ebina H, Levin HL, Grewal SI. 2005. Host genome surveillance for retrotransposons by transposon-derived proteins. *Nature* **451**:431–436. doi: 10.1038/nature06499.

Cam HP, Sugiyama T, Chen ES, Chen X, FitzGerald PC, Grewal SI. 2008. Comprehensive analysis of heterochromatin- and RNAi-mediated epigenetic control of the fission yeast genome. *Nature Genetics* **37**:809–819. doi: 10.1038/ng1602.

Camblong J, Beyrouthy N, Guffanti E, Schlaepfer G, Steinmetz LM, Stutz F. 2009. Trans-acting antisense RNAs mediate transcriptional gene cosuppression in *S. cerevisiae*. *Genes & Development* **23**:1534–1545. doi: 10.1101/gad.458008.

Chen D, Toone WM, Mata J, Lyne R, Burns G, Kivinen K, Brazma A, Jones N, Bähler J. 2003. Global transcriptional responses of fission yeast to environmental stress. *Molecular Biology of the Cell* **14**:214–229. doi: 10.1091/mbc.E02-08-0499.

References
Chen ES, Zhang K, Nicolas E, Cam HP, Zoellner M, Grewal SI. 2008. Cell cycle control of centromeric repeat transcription and heterochromatin assembly. *Nature* 451:734–737. doi: 10.1038/nature06561.

Dehé PM, Dichtl B, Schaft D, Rogue A, Pambianco M, Lebrun R, Rodríguez-Gil A, Mkandawire L, Landsberg K, Shevchenko A, Shevchenko A, Rosalanye LE, Tordera V, Chávez S, Stewart AF, Géli V. 2006. Protein interactions within the Set1 complex and their roles in the regulation of histone 3 lysine 4 methylation. *The Journal of Biological Chemistry* 281:35404–35412. doi: 10.1074/jbc.M603099200.

Djupedal I, Portoso M, Spáth H, Bonilla C, Gustafsson CM, Allshire RC, Ekwall K. 2005. RNA Pol II subunit Rpβ7 promotes centromeric transcription and RNAi-directed chromatin silencing. *Genes & Development* 19:2301–2306. doi: 10.1101/gad.344205.

Eshaghi M, Lee JH, Zhu L, Poon SY, Li J, Cho KH, Chu Z, Karuturi RK, Liu J. 2010. Genomic binding profiling of the fission yeast stress-activated MAPK Sty1 and the bZIP transcriptional activator Atf1 in response to H2O2. *PLOS ONE* 5:e11620. doi: 10.1371/journal.pone.0011620.

Grewal SI, Bonaduce MJ, Klar AJ. 1998. Histone deacetylase homologs regulate epigenetic inheritance of transcriptional silencing and chromosome segregation in fission yeast. *Genetics* 150:563–576.

Grewal SI, Jia S. 2007. Heterochromatin revisited. *Nature Reviews Genetics* 8:35–46. doi: 10.1038/nrg2008.

Grunstein M. 1998. Yeast heterochromatin: regulation of its assembly and inheritance by histones. *Cell* 93:325–328. doi: 10.1016/S0092-8674(00)81160-5.

Gullerova M, Proudfoot NJ. 2008. Cohesin complex promotes transcriptional termination between convergence genes in *S. pombe*. *Cell* 132:983–995. doi: 10.1016/j.cell.2008.02.040.

Hansen KR, Burns G, Mata J, Volpe TA, Martienssen RA, Bähler J, Thon G. 2005. Global effects on gene expression in fission yeast silencing and RNA interference machineries. *Molecular and Cellular Biology* 25:590–601. doi: 10.1128/MCB.25.2.590-601.2005.

Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, Hawkins RD, Barrera LO, Van Calcar S, Qu C, Ching KA, Wang W, Zeng Z, Green RD, Crawford GE, Ren B. 2007. Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nature* 434:861–867. doi: 10.1038/ng1666.

Henikoff S, Shilatifard A. 2011. Histone modification: cause or cog? *Trends in Genetics* 27:389–396. doi: 10.1016/j.tig.2011.06.006.

Huisinga KL, Brower-Toland B, Elgin SC. 2006. The contradictory definitions of heterochromatin: transcription and silencing. *Chromosoma* 115:110–122. doi: 10.1007/s00412-006-0052-x.

Jia S, Noma K, Grewal SI. 2004. RNAi-independent heterochromatin nucleation by the stress-activated ATF/CREB family proteins. *Science* 304:1971–1976. doi: 10.1126/science.1099035.

Kanehira J, Francesconi S, Collura A, Schramke V, Ishikawa F, Baldacci G, Géli V. 2003. The fission yeast spSet1p histone methyltransferase is required for silencing at telomeres and transcriptional silencing at the mating type locus. *Molecular and Cellular Biology* 23:259–272. doi: 10.1016/j.molcel.2007.02.005.

Kato H, Goto DB, Martienssen RA, Urano T, Furukawa K, Murakami Y. 2005. RNA polymerase II is required for histone H3 lysine 4 methylation. *Molecular Cell* 19:2301–2306. doi: 10.1016/j.molcel.2005.05.012.

Kim T, Buratowski S. 2009. Dimethylation of H3K4 by Set1 recruits the Set3 histone deacetylase complex to 5' transcribed regions. *Cell* 137:259–272. doi: 10.1016/j.cell.2009.02.043.

Kim J, Kim JA, McGinty RK, Nguyen UT, Muir TW, Allis CD, Roeder RG. 2013. The n-SET domain of Set1 regulates H2B ubiquitination-dependent H3K4 methylation. *Molecular Cell* 49:1111–1123. doi: 10.1016/j.molcel.2013.01.034.

Kouzarides T. 2007. Chromatin modifications and their function. *Cell* 128:693–705. doi: 10.1016/j.cell.2007.02.005.

Krogan NJ, Dover J, Khorrami S, Greenblatt JF, Schneider J, Johnston M, Shilatifard A. 2002. COMPASS, a histone H3 (Lysine 4) methyltransferase required for telomeric silencing of gene expression. *The Journal of Biological Chemistry* 277:10753–10755. doi: 10.1074/jbc.C200023200.

Krogan NJ, Dover J, Wood A, Schneider J, Heidt J, Boateng MA, Dean K, Ryan OW, Golshani A, Johnston M, Greenblatt JF, Shilatifard A. 2003. The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. *Molecular Cell* 11:721–729. doi: 10.1016/S1097-2765(03)00911-1.

Kusch T. 2012. Histone H3 lysine 4 methylation revisited. *Transcription* 3:310–314. doi: 10.4161/trns.21911.

Lawrence CL, Maekawa H, Worthington JL, Reiter W, Wilkinson CR, Jones N. 2007. Regulation of *Schizosaccharomyces pombe* Atf1 protein levels by Sty1-mediated phosphorylation and heterodimerization with Pcr1. *The Journal of Biological Chemistry* 282:5160–5170. doi: 10.1074/jbc.M608526200.

Litt MD, Simpson M, Gaszner M, Allis CD, Felsenfeld G. 2001. Correlation between histone lysine methylation and developmental changes at the chicken beta-globin locus. *Science* 293:2453–2455. doi: 10.1126/science.1064413.

Lorenz DR, Mikheyeva IV, Johansen P, Meyer L, Berg A, Grewal SI, Cam HP. 2012. CENP-b cooperates with Set1 in bidirectional transcriptional silencing and genome organization of retrotransposons. *Molecular and Cellular Biology* 32:4215–4225. doi: 10.1128/MCB.00395-12.

Margaritis T, Oreal V, Brabers N, Maestroni L, Vitaliano-Prunier A, Benschop JJ, van Hooff S, van Leenen D, Dargeumont C, Géli V, Holstege FC. 2012. Two Distinct Repressive Mechanisms for Histone 3 Lysine 4 Methylation through Promoting 3'-End Antisense Transcription. *PLOS Genetics* 8:e1002952. doi: 10.1371/journal.pgen.1002952.

Mikheyeva IV, Grady PJ, Tamburini FB, Lorenz DR, Cam HP. 2014. Multifaceted genome control by Set1 dependent and independent of H3K4 methylation and the Set1C/COMPASS complex. *PLOS Genetics* 10:e1004740. doi: 10.1371/journal.pgen.1004740.
Moazed D. 2011. Mechanisms for the inheritance of chromatin states. Cell 146:510–518. doi: 10.1016/j.cell.2011.07.013.

Moreno S, Klar A, Nurse P. 1991. Molecular genetic analysis of fission yeast Schizosaccharomyces pombe. Methods in Enzymology 194:795–823. doi: 10.1016/0076-6879(91)94059-L.

Nakayama J, Klar AJ, Grewal SI. 2000. A chromodomain protein, Swi6, performs imprinting functions in fission yeast during mitosis and meiosis. Cell 101:307–317. doi: 10.1016/S0092-8674(00)80840-3.

Nakayama J, Rice JC, Strahl BD, Allis CD, Grewal SI. 2001. Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. Science 292:110–113. doi: 10.1126/science.1060118.

Ng HH, Robert F, Young RA, Struhl K. 2003. Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. Molecular Cell 11:709–719. doi: 10.1016/s1097-2765(03)00092-3.

Nislow C, Ray E, Pillus L. 1997. SET1, a yeast member of the trithorax family, functions in transcriptional silencing and diverse cellular processes. Molecular Biology of the Cell 8:2421–2436. doi: 10.1091/mbc.8.12.2421.

Noma K, Allis CD, Grewal SI. 2001. Transitions in distinct histone H3 methylation patterns at the heterochromatin domain boundaries. Science 293:1115–1120. doi: 10.1126/science.1064150.

Noma K, Sugiyama T, Cam H, Verdel A, Zofall M, Jia S, Moazed D, Grewal SI. 2004. RITS acts in cis to promote RNA interference-mediated transcriptional and post-transcriptional silencing. Nature Genetics 36:1174–1180. doi: 10.1038/ng1452.

Otsubo Y, Yamamoto M. 2012. Signaling pathways for fission yeast sexual differentiation at a glance. Journal of Cell Science 125:2789–2793. doi: 10.1242/jcs.094771.

Oya E, Kato H, Chikashige Y, Tsutsumi C, Hiraoka Y, Murakami Y. 2013. Mediator directs co-transcriptional heterochromatin assembly by RNA interference-dependent and -independent pathways. PLOS Genetics 9:e1003677. doi: 10.1371/journal.pgen.1003677.

Pokholok DK, Harbison CT, Levine S, Cole M, Hannett NM, Lee TI, Bell GW, Walker K, Rolfe PA, Herbslebner E, Zeitlinger J, Lewitter F, Gifford DK, Young RA. 2005. Genome-wide map of nucleosome acetylation and methylation in yeast. Cell 122:517–527. doi: 10.1016/j.cell.2005.06.026.

Rando OJ, Chang HY. 2009. Genome-wide views of chromatin structure. Annual Review of Biochemistry 78:245–271. doi: 10.1146/annurev.biochem.78.071107.134639.

Reyes-Turcu FE, Zhang K, Zofall M, Chen E, Grewal SI. 2011. Defects in RNA quality control factors reveal RNAi-independent nucleation of heterochromatin. Nature Structural & Molecular Biology 18:1132–1138. doi: 10.1038/nsmb.2122.

Rhind N, Chen Z, Yassour M, Thompson DA, Haas BJ, Habib N, Wapinski I, Roy S, Lin MF, Heiman DJ, Young SK, Furuya K, Guo Y, Picoux A, Chen HM, Robbertse B, Goldberg JM, Aoki K, Bayne EH, Berlin AM, Desjardins CA, Dobbs E, Dukaj L, Fan L, FitzGerald MG, French C, Gujja S, Hansen K, Keifenheim D, Levin JZ, Mosher RA, Muller CA, Pfiffner J, Priest M, Russ C, Smialowska A, Swoboda P, Sykes SM, Vaughn M, Vengrova S, Yoder R, Zeng Q, Allshire R, Baulcombe D, Birren BW, Brown W, Etkin K, Kellis M, Leatherwood J, Levin H, Margalit H, Martienssen R, Nieduszynski CA, Spatafora JW, Friedman N, Dalgaard JZ, Baumann P, Niki H, Regev A, Nubaum C. 2011. Comparative functional genomics of the fission yeasts. Science 332:930–936. doi: 10.1126/science.1203357.

Roguev A, Schaft D, Shevchenko A, Aslanlou R, Stewart AF. 2003. High conservation of the Set1/Rad6 axis of histone 3 lysine 4 methylation in budding and fission yeasts. The Journal of Biological Chemistry 278:8487–8493. doi: 10.1074/jbc.M209562200.

Roguev A, Schaft D, Shevchenko A, Pijnappel WW, Wilm M, Aslanlou R, Stewart AF. 2001. The Saccharomyces cerevisiae Set1 complex includes an Ash2 homologue and methylates histone 3 lysine 4. The EMBO Journal 20:7137–7148. doi: 10.1093/emboj/20.24.7137.

Shevchenko A, Roguev A, Schaft D, Buchanan L, Habermann B, Sakalar C, Thomas H, Krogan NJ, Shevchenko A, Stewart AF. 2008. Chromatin Central: towards the comparative proteome by accurate mapping of the yeast proteomic environment. Genome Biology 9:R167. doi: 10.1186/gb-2008-9-11-r167.

Shilatifard A. 2012. The COMPASS family of histone H3K4 methylases: mechanisms of regulation in development and disease pathogenesis. Annual Review of Biochemistry 81:65–95. doi: 10.1146/annurev-biochem-051710-134100.

Shiozaki K, Russell P. 1996. Conjugation, meiosis, and the osmotic stress response are regulated by Spc1 kinase through Atf1 transcription factor in fission yeast. Genes & Development 10:2276–2288. doi: 10.1101/gad.10.18.2276.

Southall SM, Wong PS, Oehdo Z, Roe SM, Wilson JR. 2009. Structural basis for the requirement of additional factors for MLL1 SET domain activity and recognition of epigenetic marks. Molecular Cell 33:181–191. doi: 10.1016/j.molcel.2008.12.029.

Sugiyama T, Cam HP, Sugiyama R, Noma K, Zofall M, Kobayashi R, Grewal S. 2007. SHREC, an effector complex for heterochromatic transcriptional silencing. Cell 128:491–504. doi: 10.1016/j.cell.2006.12.035.

Tashiro S, Asano T, Kanoh J, Ishikawa F. 2013. Transcription-induced chromatin association of RNA surveillance factors mediates facultative heterochromatin formation in fission yeast. Genes to Cells 18:327–339. doi: 10.1111/gtc.12038.

Toedling J, Skylar O, Krueger T, Fischer JJ, Sperling S, Huber W. 2007. Ringo—an R/Bioconductor package for analyzing ChIP-chip readouts. BMC Bioinformatics 8:221. doi: 10.1186/1471-2105-8-221.

van der Laan MJ, Pollard KS. 2003. A new algorithm for hybrid hierarchical clustering with visualization and the bootstrap. Journal of Statistical Planning and Inference 117:275–303. doi: 10.1016/S0378-3758(02)00388-9.
van Dijk EL, Chen CL, d’Aubenton-Carafa Y, Gourvenec S, Kwapisz M, Roche V, Bertrand C, Silvain M, Legoix-Né P, Loeillet S, Nicolas A, Thermo C, Morillon A. 2011. XUTs are a class of Xrn1-sensitive antisense regulatory non-coding RNA in yeast. Nature 475:114–117. doi: 10.1038/nature10118.

Volpe TA, Kidner C, Hall IM, Teng G, Grewal SI, Martienssen RA. 2002. Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. Science 297:1833–1837. doi: 10.1126/science.1074973.

Weiner A, Chen HV, Liu CL, Rahat A, Klien A, Soares L, Gudipati M, Pfeffner J, Regev A, Buratowski S, Pleiss JA, Friedman N, Rando OJ. 2012. Systematic dissection of roles for chromatin regulators in a yeast stress response. PLOS Biology 10:e1001369. doi: 10.1371/journal.pbio.1001369.

Xhemalce B, Kouzarides T. 2010. A chromodomain switch mediated by histone H3 Lys 4 acetylation regulates heterochromatin assembly. Genes & Development 24:647–652. doi: 10.1101/gad.1881710.

Yamada T, Fischle W, Sugiyama T, Allis CD, Grewal SI. 2005. The nucleation and maintenance of heterochromatin by a histone deacetylase in fission yeast. Molecular Cell 20:173–185. doi: 10.1016/j.molcel.2005.10.002.

Yamanaka S, Mehta S, Reyes-Turcu FE, Zhuang F, Fuchs RT, Rong Y, Robb GB, Grewal SI. 2013. RNAi triggered by specialized machinery silences developmental genes and retrotransposons. Nature 493:557–560. doi: 10.1038/nature11716.

Yang XJ, Seto E. 2008. The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. Nature Reviews Molecular Cell Biology 9:206–218. doi: 10.1038/nrm2346.

Zaratiegui M, Vaughn MW, Irvine DV, Goto D, Watt S, Bähler J, Arcangioli B, Martienssen RA. 2010. CENP-B preserves genome integrity at replication forks paused by retrotransposon LTR. Nature 469:112–115. doi: 10.1038/nature09608.

Zhang K, Fischer T, Porter RL, Dhakshnamoorthy J, Zofall M, Zhou M, Veenstra T, Grewal SI. 2011. Ctr4/Suv39 and RNA quality control factors cooperate to trigger RNAi and suppress antisense RNA. Science 331:1624–1627. doi: 10.1126/science.1198712.

Zhang K, Lin W, Latham JA, Riefler GM, Schumacher JM, Chan C, Tatchell K, Hawke DH, Kobayashi R, Dent SY. 2005. The Set1 methyltransferase opposes Ipl1 aurora kinase functions in chromosome segregation. Cell 122:723–734. doi: 10.1016/j.cell.2005.06.021.

Zofall M, Yamanaka S, Reyes-Turcu FE, Zhang K, Rubin C, Grewal SI. 2012. RNA elimination machinery targeting meiotic mRNAs promotes facultative heterochromatin formation. Science 335:96–100. doi: 10.1126/science.1211651.