Molecular Cell, Volume *32*

Supplemental Data

HP1 Proteins Form Distinct Complexes and Mediate Heterochromatic Gene Silencing by Nonoverlapping Mechanisms

Mohammad R. Motamedi, Eun-Jin Erica Hong, Xue Li, Scott Gerber, Carilee Denison, Steven Gygi, and Danesh Moazed

Supplemental Experimental Procedures

Strain construction

Single-step gene replacement strategy was used to generate the strains used in this study, listed in Table S4. PCR products containing kanamycin, hygromycin, or nourseothricin resistance genes flanked by 80 nucleotides of DNA corresponding to the appropriate insertion sites were used to transform logarithmically growing *S. pombe* cells by the lithium acetate method (Bahler et al., 1998). For C-terminal TAP tagging *clr1*$^+$*, clr3*$^+$*, chp2*$^+$ *and swi6*$^+$, the 80-nucleotide sequence flanking the PCR product containing the TAP tag and a drug resistance cassette corresponded to the C-terminus of the gene (80 nts downstream and 80 nts upstream of the stop codon); for replacing the gene with a drug resistance cassette, the 80 nucleotide sequence corresponded to the ends of the gene (80 nts upstream of start and 80 nucleotides downstream of stop codons). C-terminal TAP tagging of Clr2 resulted in a non-functional protein as determined by silencing assays (data not shown). Therefore, we constructed a strain that expresses TAP-Clr2 under the control of the endogenous *clr2*$^+$ promoter. A DNA fragment corresponding to the *clr2*$^+$ promoter (1000 nts upstream of *clr2*$^+$ start codon) was used to replace the P3nmt1 DNA sequence in the pFA6a-KanMS6-P3mnt1-TAP plasmid. This plasmid was used to generate a DNA fragment

containing the drug resistance cassette, *clr2*$^+$ promoter and the TAP tag flanked by DNA sequence corresponding to 80 nucleotides upstream and downstream of *clr2*$^+$ start codon.

RNA preparation

Cells, grown to logarithmic phase in YEA, were spun down and frozen in liquid nitrogen, and their total RNA was isolated using the hot phenol/chloroform method (Leeds et al., 1991).

Real-time RT-PCR

Total RNA isolated from *S. pombe* strains as described above was subjected to the RNA Cleanup protocol as described for the RNeasy mini Kit from QIAGEN. The concentration of the purified RNA was measured, and 50 or 500ng of RNA was used to amplify *act1*$^+$, *matM*, *cen dg*, *cen dh*,, or *ura4*$^+$ and *tlh1*$^+$ transcripts, respectively. The primers and the corresponding annealing temperature used and to amplify *ura4*$^+$, *act1*$^+$, *matM*, *cen dg*, *cen dh*, and *tlh1*$^+$ were mb193/194 (annealing at 61 deg), mb86/87 (annealing at 57 deg), mb310/311 (annealing at 65 deg), mb330/334 (annealing at 61 deg), mb263/264 (annealing at 57 deg), and mb274/276 (annealing at 59 deg), respectively. Primer sequences are listed in Table S5. Quantifications were calculated as described previously (Buhler et al., 2007).

Northern Blots

Northern blots were performed as described previously (Buhler et al., 2006). Briefly, about 1mg of total RNA was size-fractionated using the RNeasy midi columns (QIAGEN) according to the manufacturer's instructions. The flow-through (containing RNA < 200nts) was saved, precipitated with isopropanol and dissolved in RNase free water. 25ug of this RNA was run on a 17.5% TBE polyacrylamid gel containing 7M urea. The RNA was then transferred to a positively charged RNase-free nylon membrane (Roche) using a semi-dry Biorad transfer machine at 3.3mA/cm^2 for an hour in 1X TBE. The transferred RNA was cross-linked to the nylon

membrane by UV, baked for 1-2 hours at 80 deg and incubated with the hybridization buffer (Ambion, UltraHyb-Oligo Hybridization Buffer) at 35 deg for 1 hour. 20pmol DNA oligos, end-labeled with 20pmol of γ-^{32}P ATP (6000 Ci/mmol) with T4 polynucleotide Kinase (Roche) was added to the hybridization buffer. The sequence of the *cen* DNA oligos was described previously (Verdel et al., 2004). A DNA probe made against snoR69 was used to as a loading control. Hybridization and wash conditions were described previously (Buhler et al., 2006).

Western blots

Western blots were performed as described previously (Motamedi et al., 2004). TCA Western blots were performed on Clr1-TAP strains for detection of the full-length protein, according to the following protocol. 10ml of logarithmically growing cells (OD600=0.1-0.5) were frozen in liquid nitrogen and the pellet was suspended in 0.5ml of cold TCA buffer (20mM Tris pH=8.0; 50mM ammonium acetate; 2mM EDTA; 2mM PMSF; 2mM Leupeptin; and 2mM aprotinin), 0.5ml of cold 30% TCA and 0.5ml of cold glass beads. After bead-beating (4X 30 seconds with a 3-minute on ice cooling in between each cycle), the samples were centrifuged for 5 min at 20000x g and washed once with cold acetone. Acetone was removed and the samples were dissolved in 100 ul of SDS-PAGE sample buffer at 65 deg for 10 minutes. The samples were run on 10% polyacrylamide gels, transferred to nitrocellulose membranes, and the protein was detected using anti-TAP antibody.

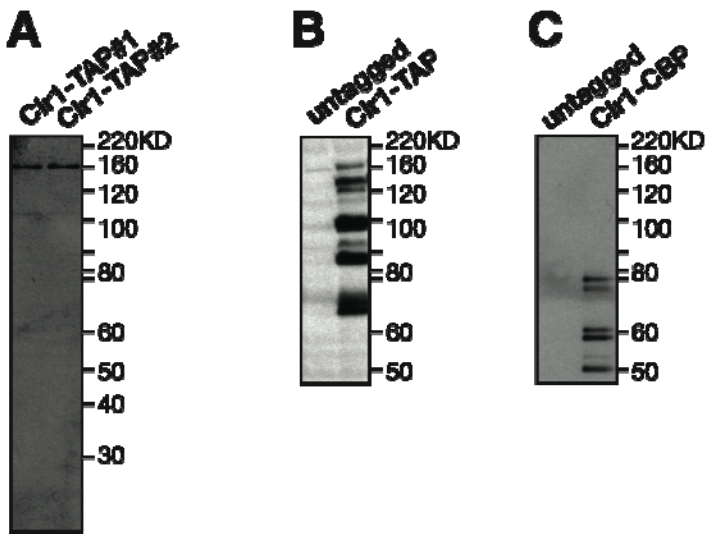

Figure S1. The full-length Clr1-TAP runs at roughly 160 kD, but is degraded rapidly during protein purification.
(A) TCA western blots prepared from whole cell extracts from two independent *S. pombe* clones carrying a C-terminally TAP-tagged Clr1 protein. A band running at roughly 160 kD corresponding to the full-length Clr1-TAP protein was detected using anti-TAP antibody. **(B)** Western blots from whole cell extract prepared after before TAP purification using anti-TAP antibody. Smaller bands corresponding to Clr1-TAP degradation products are detected. **(C)** Western blot using anti-CBP antibody performed on Clr1-TAP extract following the TEV cleavage step. No full-length Clr1-TAP protein is detected at this stage of TAP purification.

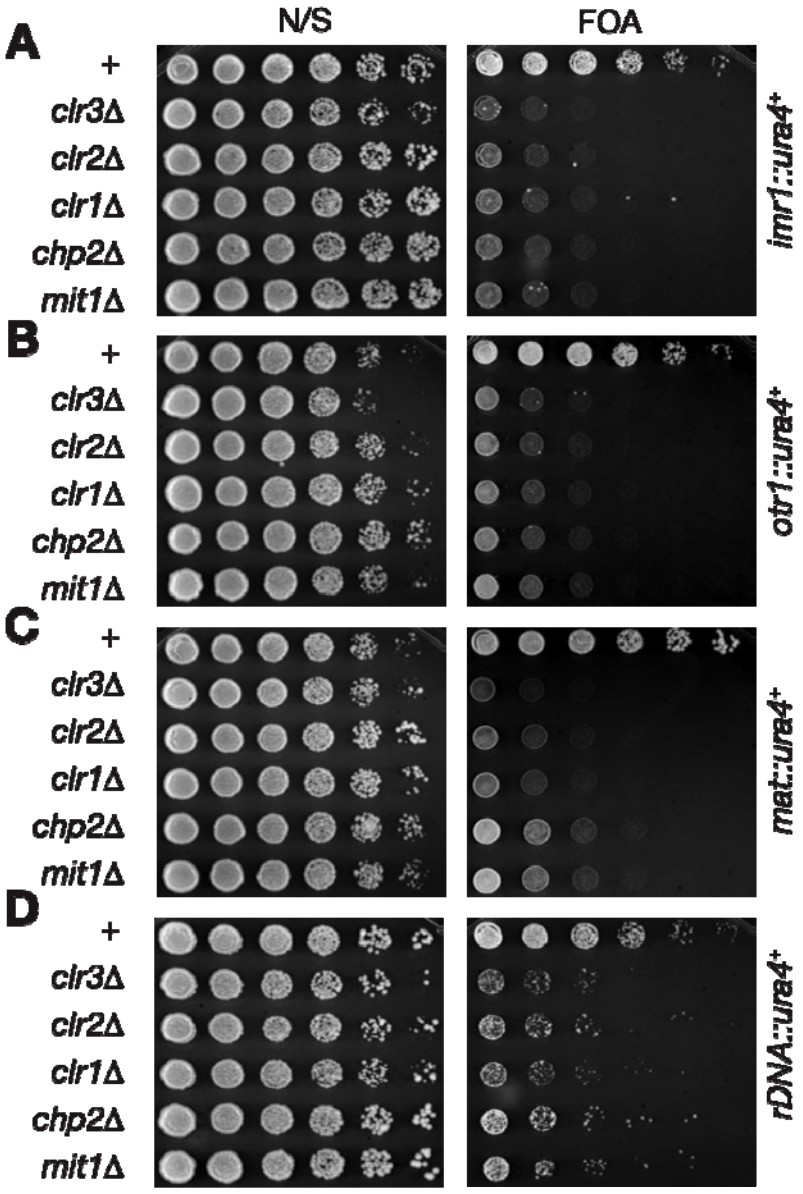

Figure S2. SHREC2 is required for silencing of a transgene at all *S. pombe* heterochromatic loci. Silencing of a *ura4*$^{+}$ reporter gene inserted at **(A)** innermost (*imr1::ura4*$^{+}$) **(B)** outer (*otr1::ura4*$^{+}$) pericentromeric repeats or **(C)** the mating-type (*mat::ura4*$^{+}$), and **(D)** rDNA (*rDNA::ura4*$^{+}$) loci is disrupted in SHREC2 mutants.

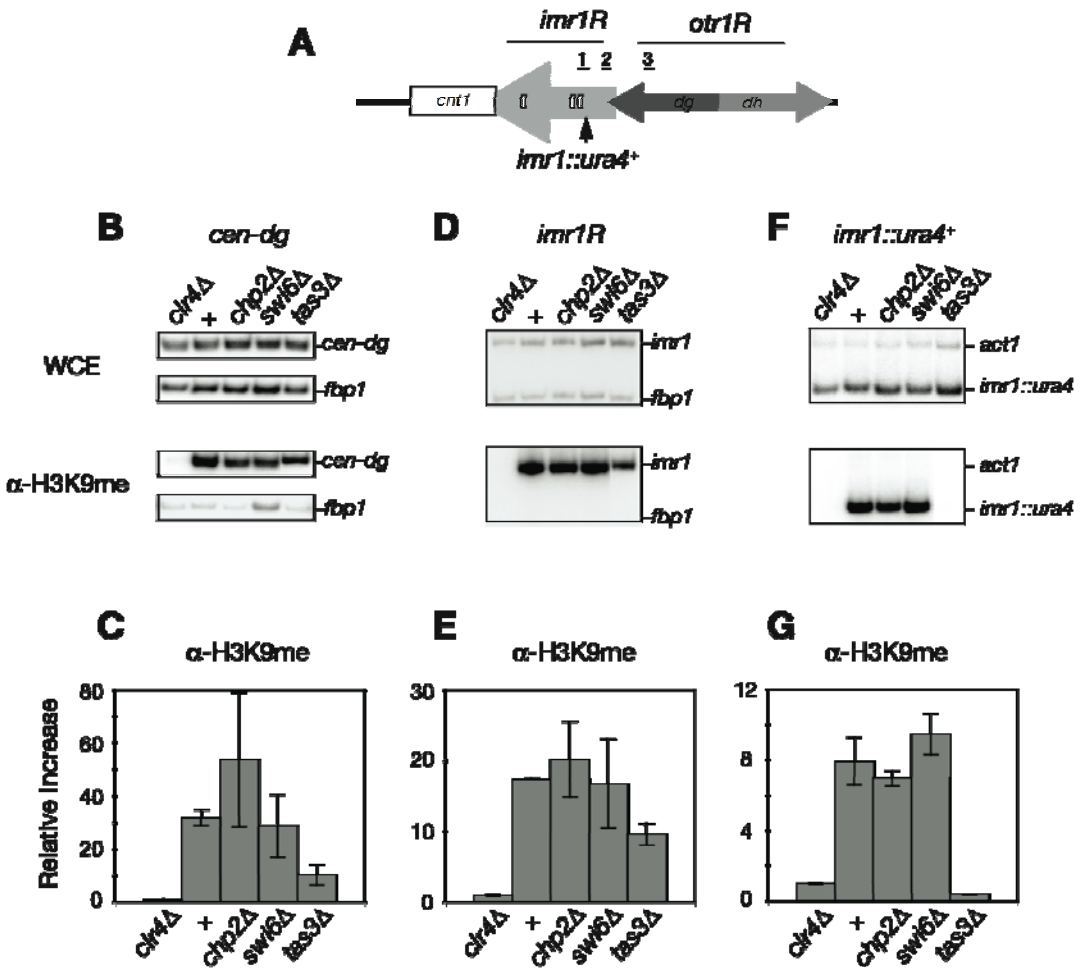

Figure S3. H3K9 methylation at centromeres can occur independently of Swi6 or Chp2.
(A) A diagram representing the pericentromeric DNA repeats on one side of centromere 1 central core region (*cnt1* – white box), including the innermost (*imr1R* -light grey arrow), and outer (*otr1R*) elements. The *dh* and *dg* elements of *otr1R* are shown as light grey and dark grey arrows, respectively. The site of the transgene *ura4*$^+$ insert, three tRNA genes which act as barrier elements to heterochromatin spreading, and the DNA segments amplified in the chromatin immunoprecipitation (ChIP) experiments are shown as a black arrow, small white boxes, and underlined numbers 1 (corresponding to *imr1R::ura4*$^+$), 2 (corresponding to *cen-dg*), and 3 (corresponding to *cen-dh*), respectively. ChIP experiments showing that H3K9 methylation is not affected in the absence of Chp2 or Swi6 at *cen-dg* **(B-D)**, *cen-dh* **(E-G)**, and *imr1R::ura4*$^+$ **(H-J)**. Error bar represent variation from the mean from two independent biological experiments. *fbp1*$^+$ or *act1*$^+$ were used as an internal control for the ChIP experiments.

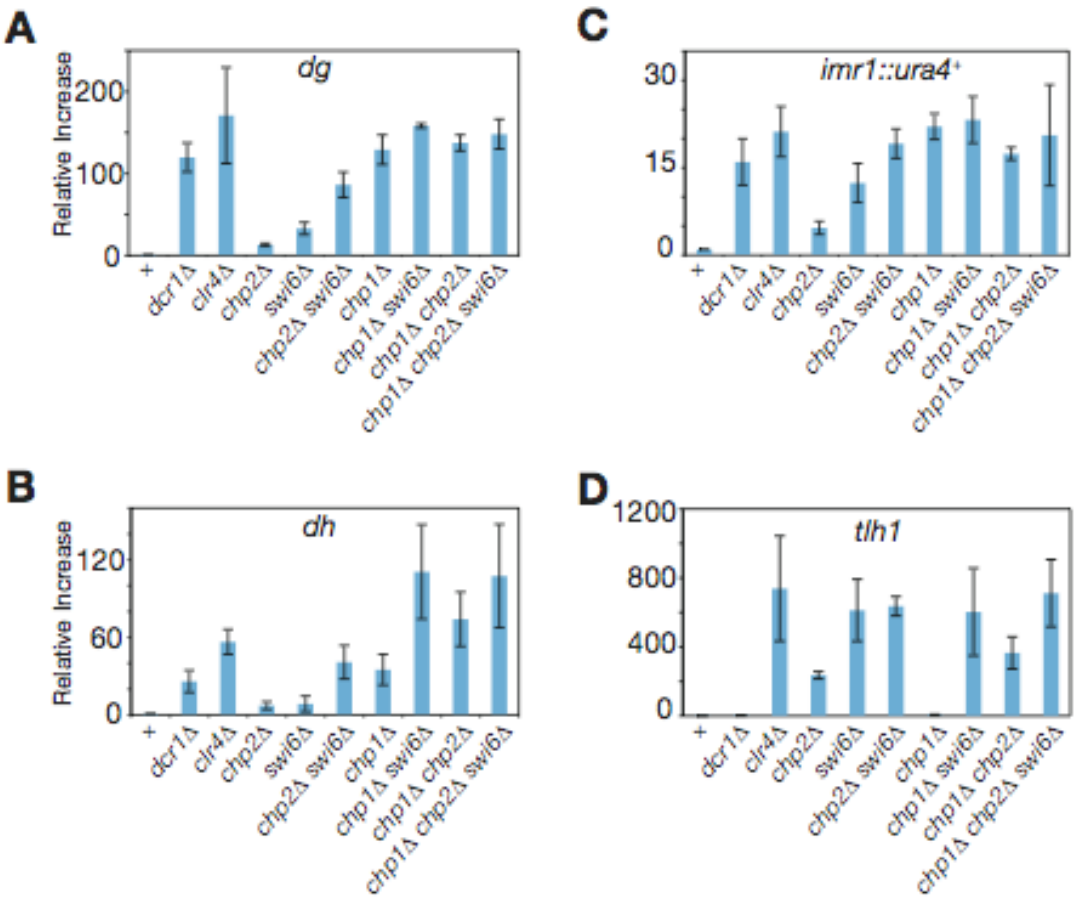

Figure S4. Swi6 and Chp2 contribution to heterochromatic gene silencing is additive. Real-time RT-PCR reactions were performed on total RNA purified from single (*swi6Δ*, *chp2Δ*, *chp1*Δ), double (*chp2Δ swi6Δ*, *chp1Δ swi6Δ*, *chp1Δ chp2Δ*) and triple (*chp1Δ chp2Δ swi6Δ*) mutant cells measuring the steady-state level of **(A)** *dg,* **(B)** *dh,* **(C)** *imr1::ura4*$^{+}$ and**(D)** *tlh1*$^{+}$ transcripts. Error bars represent the standard deviation measured in three independent experiments. The levels of *act1*$^{+}$ transcript were measured and used to normalize all the values obtained from other transcripts.

Table S1. List of unique proteins identified from a representative Clr1-TAP, TAP-Clr2, Clr3-TAP, Chp2-TAP and Swi6-TAP purification

Clr1-TAP purification[a]		
Protein name	# of unique peptides	% coverage (aa)
Clr1	**22**	**20.9**
Clr3	**19**	**26.5**
Clr2	**14**	**33**
Erg5	3	5
Glo3	2	5.6
Ccq1	**1**	**1.9**
Rad24	1	5.2
Histone H2A	1	13.2
Histone H2AZ	1	5.3
Histone H3	1	6.7
Atf1	1	3.9
TAP-Clr2 purification[a]		
Protein Name	# of unique peptides	% coverage (aa)
Clr1	**25**	**26.7**
Clr3	**18**	**29.3**
Clr2	**21**	**32.8**
Prp3	3	8.3
Cdc48	3	5.5
SPCC285.13C	2	4.2
Seb1	2	4
SPBC1539.10	2	13.9
Clr3-TAP purification[a]		
Protein Name	# of unique peptides	% coverage (aa)
Clr3	**29**	**46**
Ccq1	**24**	**43**
Clr1	**23**	**22**
SPAC694.02	13	11
Clr2	**12**	**26**
SPBC646.10c	10	29
Rap1	10	23
Chp2-TAP purification[a]		
Protein Name	# of unique peptides	% coverage (aa)
Chp2	**14**	**44.5**
Mit1	**21**	**16.3**
Clr1	**6**	**7.6**
SPAC32A11.02c	5	8.9
Histone H4	4	42.2
Histone H3	2	14.8
Toc1[b]	2	5.6

Swi6-TAP purification		
Protein Name	# of unique peptides	% coverage (aa)
Swi6	**31**	**65.5**
Hrp1	36	32.6
Top2	27	22.2
Sap1	17	43.3
Ssb1	12	32.8
SPBC83.08 (Rvb2)	11	33.8
Abp2	11	33.2
SPAC1071.06	8	27.7
Cbh1	8	21.8
SPAPB8E5.09 (Rvb1)	8	27.4
Atf1	8	24.6
Snf21	8	7.9
Histone H4	8	53.9
Brd1	7	17.7
SPAC3G6.12	7	14.9
Spt16	7	8.1
Hrp3	6	7.3
SPAC29B12.01	6	6.7
Rsc1	6	12.8
SPCC1281.05	6	21.8
Cbh2	5	13.2
SPAC31G5.19 (Yta7)	5	7.4
Cbp1	5	18.4
SPBP23A10.08 (Arp4)	5	14.5
SPAC17G6.10	5	12.3
SPAC25A8.01c (Fft3)	5	8.2
Histone H2A	5	35.7
Histone H2B1	5	38
Arp5	4	7.8
Pim1	4	14.1
Tdf1	3	19.5
Ssb2	3	24.7
Cdc31	3	12.5
Mto1	3	3.6
SPAC23G3.10C	3	13.2
Pcr1	2	16.4
Pob3	2	5.7
SPAC17G8.03C (Dbp3)	2	13.1
SPBC1703.02	2	3.5
Rpc2 (RNA pol III)	2	3.2
Histone H3	2	14.8
SPBC839.12	2	16.7
SPBC28F2.11	2	7.4

[a] The LC MS/MS data corresponds to a different purification than the one shown in Figure 1E.
[b]Toc1 was only present in this Chp2-TAP purification.

Table S2. Contribution of SHREC2 to total H3K9me-dependent gene silencing

Mutant[a]	Locus	RNA abundance relative to *clr4Δ* cells[b]
SHREC2Δ	*dg*	0.11
SHREC2Δ	*dh*	0.20
SHREC2Δ	*imr1::ura4*$^+$	0.21
SHREC2Δ	*otr1::ura4*$^+$	0.13
SHREC2Δ	*tlh1*$^+$	0.23
clr1Δ, 2Δ, 3Δ	*mat::ura4*$^+$	0.74
chp2Δ, mit1Δ	*mat::ura4*$^+$	0.25
clr1Δ, 2Δ, 3Δ	*matM*	0.54
chp2Δ, mit1Δ	*matM*	0.09

[a]For *cen dg*, *dh*, *imr1::ura4*$^+$, *otr1::ura4*$^+$, and telomeric *tlh1*$^+$ loci, the average RNA level measured for all *SHREC2Δ* (*clr1Δ, 2Δ, 3Δ* , *chp2Δ, mit1Δ*) cells as measured by quantitative real-time RT-PCR (see Figure 2) was compared to *clr4Δ* cell using the following formula: average RNA level in *SHREC2Δ* cells/RNA level in *clr4Δ* cells. For *mat::ura4*$^+$, and *matM* transcripts, the average RNA level for *clr1Δ, 2Δ, 3Δ* cells was measured separately from *chp2Δ,* and *mit1Δ* average, as indicated.
[b]The RNA level in *clr4Δ* cells for each transcript was set to 1.00.

Table S3. Individual and combined contributions of Chp2, Swi6, and Chp1 to heterochromatic gene silencing

Locus	RNA abundance relative to *clr4Δ* cells[a]						
	chp2Δ	*swi6Δ*	*chp2Δ swi6Δ*	*chp1Δ*	*chp1Δ swi6Δ*	*chp1Δ chp2Δ*	*chp1Δ chp2Δ swi6Δ*
Dg	0.10[b]	0.17[b]	0.51	0.76	0.92	0.80	0.87
Dh	0.18[b]	0.24[b]	0.72	0.62	1.96	1.31	1.90
imr1::ura4$^+$	0.18[b]	0.52[b]	0.90	1.04	1.09	0.82	0.97
tlh1$^+$	0.32[b]	0.86[b]	0.86	0.008	0.82	0.50	0.96
matM	0.53	0.12	ND	ND	ND	ND	ND

[a]The RNA level in *clr4Δ* cells for each transcript was set to 1.00. The steady-state RNA levels of *dg*, *dh*, *imr1::ura4*$^+$, *tlh1*$^+$ and *matM* (only for single *chp2Δ* and *swi6Δ* cells) transcripts, as measured by quantitative real-time RT-PCR (see Figure 5 and Figure 4S), in cells carrying mutations in *chp2*, *swi6* and *chp1* and all possible mutant combinations of these genes were compared to *clr4Δ* cells by dividing the RNA level in each mutant by the RNA level in *clr4Δ* cells.
[b]Average values for corresponding experiments shown in Figure 5A-E and Figure 4S.
ND = Not determined.

Table S4. List of strains used in this study

Strain	Genotype	Source
SPY28	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI*	1
SPY33	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI chp1-TAP- kan^R*	2
SPY40	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI chp2-TAP-kan^R*	3
SPY137	*h$^+$ leu1-32 ade6-M210 ura4DS/E otr1(SphI)::ura4$^+$oriA*	3
SPY139	*h^{90} leu1-32 ade6-M210 ura4DS/E mat4::ura4$^+$ oriA*	4
SPY141	*h$^+$ leu1-32 ade6-M216 ura4DS/E leuYlp2.4pUCura4$^+$-7*	4
SPY145	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI clr3Δ::TAP-kan^R*	3
SPY155	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI clr1Δ::TAP-kan^R*	3
SPY216	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI chp1-TAP- kan^R swi6Δ:: nat^R*	5
SPY281	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI chp1-TAP- kan^R dcr1Δ:: nat^R*	5
SPY473	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI dcr1Δ:: nat^R*	6
SPY508	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI clr1-TAP- kan^R*	3
SPY552	*h$^+$ leu1-32 ade6-M210 ura4DS/E otr1(SphI)::ura4$^+$oriA dcr1Δ:: nat^R*	3
SPY815	*h$^+$ leu1-32 ade6-M210 ura4DS/E otr1(SphI)::ura4$^+$oriA clr4Δ:: kan^R*	7
SPY816	*h^{90} leu1-32 ade6-M210 ura4DS/E mat4::ura4$^+$ oriA clr4Δ:: kan^R*	7
SPY825	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI clr4Δ:: kan^R*	7
SPY1041	*h$^+$ leu1-32 ade6-M210 ura4DS/E otr1(SphI)::ura4$^+$oriA clr1Δ::TAP-kan^R*	3
SPY1042	*h$^+$ leu1-32 ade6-M210 ura4DS/E otr1(SphI)::ura4$^+$oriA clr2Δ::TAP-kan^R*	3
SPY1044	*h$^+$ leu1-32 ade6-M210 ura4DS/E otr1(SphI)::ura4$^+$oriA clr3Δ::TAP-kan^R*	3
SPY1048	*h^{90} leu1-32 ade6-M210 ura4DS/E mat4::ura4$^+$ oriA clr1Δ::TAP-kan^R*	3
SPY1050	*h^{90} leu1-32 ade6-M210 ura4DS/E mat4::ura4$^+$ oriA clr2Δ::TAP-kan^R*	3
SPY1053	*h^{90} leu1-32 ade6-M210 ura4DS/E mat4::ura4$^+$ oriA clr3Δ::TAP-kan^R*	3
SPY1063	*h$^+$ leu1-32 ade6-M216 ura4DS/E leuYlp2.4pUCura4$^+$-7 clr1Δ::TAP-kan^R*	3
SPY1065	*h$^+$ leu1-32 ade6-M216 ura4DS/E leuYlp2.4pUCura4$^+$-7 clr2Δ::TAP-kan^R*	3
SPY1067	*h$^+$ leu1-32 ade6-M216 ura4DS/E leuYlp2.4pUCura4$^+$-7 clr3Δ::TAP-kan^R*	3
SPY1084	*h^{90} leu1-32 ade6-M210 ura4DS/E mat4::ura4$^+$ oriA dcr1Δ:: hph^R*	3
SPY1130	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI clr3-TAP- kan^R*	3
SPY1158	*h$^+$ leu1-32 ade6-M210 ura4DS/E otr1(SphI)::ura4$^+$oriA kan^R - TAP-clr2*	3
SPY1290	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI mit1Δ:: kan^R*	3
SPY1297	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI chp2Δ:: kan^R*	3
SPY1300	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI swi6-TAP- hph^R*	3
SPY1325	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI clr2Δ::TAP-kan^R*	3
SPY1336	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI swi6Δ:: kan^R*	3
SPY1368	*h$^+$ leu1-32 ade6-M210 ura4DS/E otr1(SphI)::ura4$^+$oriA chp2Δ:: kan^R*	3
SPY1369	*h$^+$ leu1-32 ade6-M210 ura4DS/E otr1(SphI)::ura4$^+$oriA mit1Δ:: kan^R*	3
SPY1374	*h$^+$ leu1-32 ade6-M216 ura4DS/E leuYlp2.4pUCura4$^+$-7 chp2Δ:: kan^R*	3
SPY1376	*h$^+$ leu1-32 ade6-M216 ura4DS/E leuYlp2.4pUCura4$^+$-7 mit1Δ:: kan^R*	3
SPY1430	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI hrp1Δ:: kan^R*	3
SPY1442	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI hrp3Δ:: kan^R*	3
SPY 1682	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI chp1Δ:: hph^R*	3
SPY 1683	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI swi6Δ:: kan^R chp1Δ:: hph^R*	3
SPY 1691	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI chp1Δ:: hph^R chp2Δ:: kan^R*	3
SPY 1693	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI swi6Δ:: kan^R chp1Δ:: hph^R chp2Δ:: kan^R*	3
SPY 1707	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI swi6Δ:: kan^R chp2Δ:: kan^R*	3
SPY 1710	*h$^+$ leu1-32 ade6-216 ura4-D18 imr1*R*(NcoI)::ura4$^+$ oriI swi6-TAP- hph^R clr4Δ:: kan^R*	3

1 = Shiv Grewal; 2 = (Verdel et al., 2004); 3 = This study 4 = Karl Ekwall; 5 = (Motamedi et al., 2004); 6 = (Colmenares et al., 2007); 7 = (Hong et al., 2005)

Table S5. List of oligonucleotides used in this study.

Name	Sequence
AV196	5’-TCCTACGTTGGTGATGAAGC-3’
DM556	5’-GAGTCATCTTCTCACGGTTGG-3’
DM554	5’-AATGACAATTCCCCACTAGCC-3’
DM555	5’-ACTTCAGCTAGGATTCACCTGG-3’
DM566	5’-TTATTGATGGCGAAGCTAGATCCG-3’
DM567	5’-AACTCCATAACCACCACCATGCTC-3’
DM558A	5’-GAAAACACATCGTTGTCTTCAGAG-3’
DM559A	5’-CGTCTTGTAGCTGCATGTGAA-3’
mb86	5’-AACCCTCAGCTTTGGGTCTT-3’
mb87	5’-TTTGCATACGATCGGCAATA-3’
mb193	5’-AATACCGTCAAGCTACAATATGCATCTGGTG-3’
mb194	5’-GGTTTTCTCTGTGTAGGAACCAGTAGCC-3’
mb263	5’-TGAATCGTGTCACTCAACCC-3’
mb264	5’-CGAAACTTTCAGATCTCGCC-3’
mb274	5’-ATGGTCGTCGCTTCAGAAATTGC-3’
mb276	5’-CTCCTTGGAAGAATTGCAAGCCTC-3’
mb310	5’-GTCTACTGAACGTACTCCGAGAC-3’
mb311	5’-GCTGGTACTTATAACCAGGGTACATT-3’
mb330	5’-CTGTTCGTGAATGCTGAGAAAGTTTATGTCTAATGTATTAGC-3’
mb334	5’-CTCCATTCTTGTCATTATTGGCTTGTTGTACGTTGTTC-3’
pMO247	5’-GAAGTACCCCATTGAGCACGG-3’
pMO248	5’- CAATTTCACGTTCGGCGGTAG-3’
pMO283	5’- GTCGAGGATTTCGACCAGGATATG-3’
pMO284	5’- AGCTCCATAGACTCCACGACCAAC-3’

Supplemental References

Bahler, J., Wu, J.Q., Longtine, M.S., Shah, N.G., McKenzie, A., 3rd, Steever, A.B., Wach, A., Philippsen, P., and Pringle, J.R. (1998). Heterologous modules for efficient and versatile PCR-based gene targeting in Schizosaccharomyces pombe. Yeast *14*, 943-951.

Buhler, M., Haas, W., Gygi, S.P., and Moazed, D. (2007). RNAi-dependent and -independent RNA turnover mechanisms contribute to heterochromatic gene silencing. Cell *129*, 707-721.

Buhler, M., Verdel, A., and Moazed, D. (2006). Tethering RITS to a nascent transcript initiates RNAi- and heterochromatin-dependent gene silencing. Cell *125*, 873-886.

Colmenares, S.U., Buker, S.M., Buhler, M., Dlakic, M., and Moazed, D. (2007). Coupling of double-stranded RNA synthesis and siRNA generation in fission yeast. *Mol. Cell* 27, 449-461.

Hong, E.E., Villen, J., Gerace, E.L., Gygi, S.P., and Moazed, D. (2005). A Cullin E3 ubiquitin ligase complex associates with Rik1 and the Clr4 histone H3-K9 methyltransferase and is required for RNAi-mediated heterochromatin formation. RNA Biology *2:3*, 106-111.

Huang, J., and Moazed, D. (2003). Association of the RENT complex with nontranscribed and coding regions of rDNA and a regional requirement for the replication fork block protein Fob1 in rDNA silencing. Genes Dev *17*, 2162-2176.

Leeds, P., Peltz, S.W., Jacobson, A., and Culbertson, M.R. (1991). The product of the yeast UPF1 gene is required for rapid turnover of mRNAs containing a premature translational termination codon. Genes Dev *5*, 2303-2314.

Motamedi, M.R., Verdel, A., Colmenares, S.U., Gerber, S.A., Gygi, S.P., and Moazed, D. (2004). Two RNAi complexes, RITS and RDRC, physically interact and localize to noncoding centromeric RNAs. Cell *119*, 789-802.

Verdel, A., Jia, S., Gerber, S., Sugiyama, T., Gygi, S., Grewal, S.I., and Moazed, D. (2004). RNAi-mediated targeting of heterochromatin by the RITS complex. Science *303*, 672-676.