RNA interference promotes heterochromatic silencing through replication-coupled release of RNA polymerase II

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Supplementary Information

Supplementary Data

Genetic interactions between dcr1 and replication fork protection factors

	dcr1	mrc1
dcr1	N/A	-11.8
mrc1	-11.8	N/A
ptn1	-3.5	-7.5
tub1	-10.3	-3.6
mcl1	-12.3	-8.6
ask1	-3.5	-8.7
hos2	-4.3	-5.9
spc34	-3.5	-2.9
pob3	-6.1	-7.4
hip1	-5.9	-6.8
clr3	-2.7	-3.0
mug154	-2.9	-3.8
fft3	-3.7	-3.4
swi3	-3.5	-4.1
SPBC29A3.13	-4.0	-5.3
SPAC16C9.05	-2.8	-6.5
SPAC664.02c	-3.9	-2.6
SPBP8B7.10c	-3.7	-2.7
SPBC26H8.05c	-4.5	-2.6
clr4	5.9	-16.6
clr7/dos2	4.8	-16.1
clr8/dos1	5.1	-16.7
klp6	4.2	-4.6
ago1	Suppression[35]	-13.3
chp1	2.6	-8.6
tas3	5.1	-14.7
ers1	3.1	-7.1
swi6	2.4	-10.2
nto1	2.9	-15.1
SPBC13E7.08c	2.1	-5.4
rad26	-2.5	Suppression[36]

Supplemental Table S1. Interactions between RNA interference, DNA replication and DNA repair in *S.pombe* and in higher eukaryotes. Double mutants between strains from a genome-wide deletion library and *dcr1Δ*, or *mrc1Δ* (mediator of replication checkpoint), were assessed for growth relative to single mutants [37]. Positive Z-scores represent phenotypic suppression, while negative scores represent phenotypic enhancement, and genetic interaction. Z-scores above 1.9 are significant ($P<0.05$). Note that there is also a strong negative interaction between *rhp51* and *dcr1*, as well as *rhp51* and *ago1* (Fig. 2d). In *Neurospora* [38] RNAi is induced by HU, as it is in *S. pombe* [2] while in Arabidopsis, mutants in DNA replication and repair, including DNA polymerase Polε lose heterochromatic silencing from transgenes silenced by RNAi, and stimulate recombination [39]. In *Drosophila,* mutations in RNAi are suppressed by replication checkpoint mutants [40], while *dcr-2* mutants have an increased DNA damage response in replicating heterochromatin [41].

Supplemental Figure 1

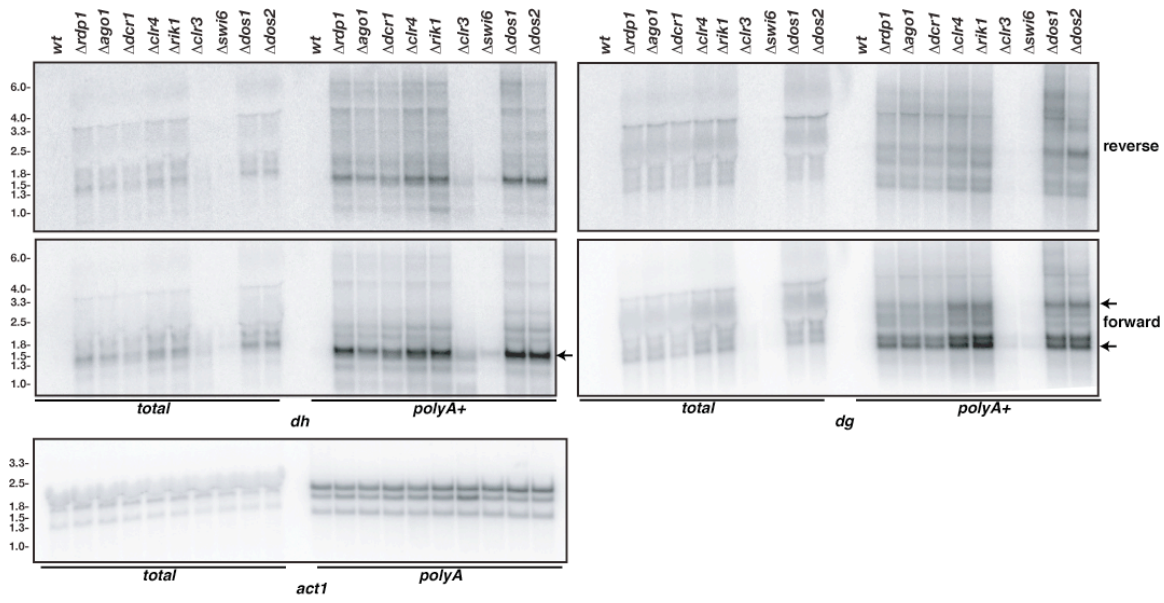
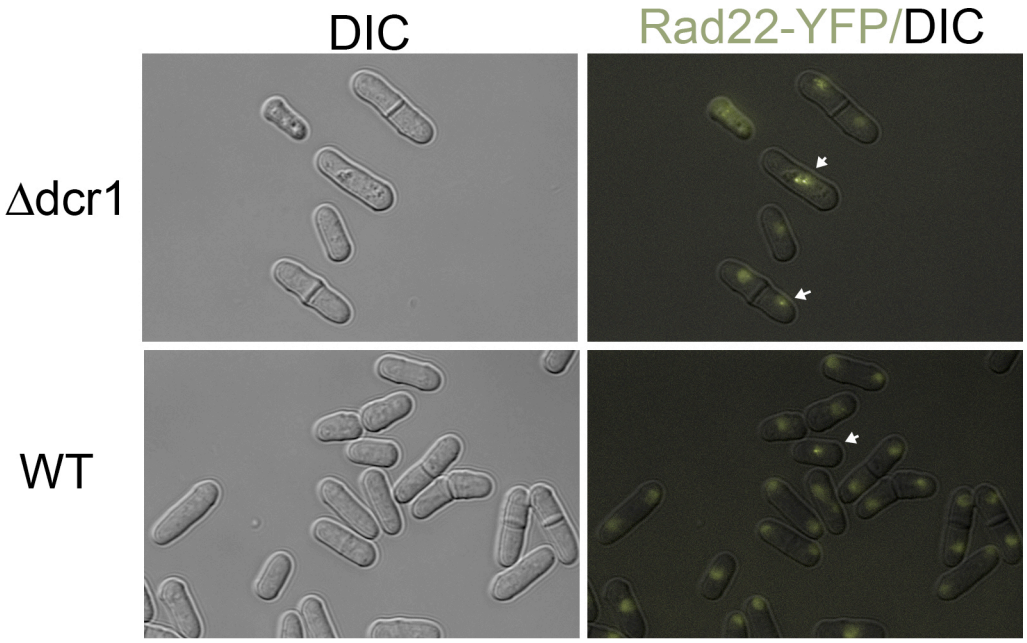


Figure S1. Northern blot analysis of RNA from wild-type and mutant strains. Transcripts corresponding to near full length cDNA clones are indicated by arrows. Blots were reprobed with *act1* as loading control.

Supplemental Figure 2



	WT	Δ*dcr1*
Total cells	13.2%	22.7%
S-early G2 cells	6.3%	34.2%
Late G2 cells (1 focus)	18.0%	10.6%
Late G2 cells (>1 foci)	0.6%	7.1%

Figure S2. RNAi protects from spontaneous DNA damage. Rad22 foci were scored under fluorescence microscopy in unsynchronized cells, which were assessed for cell cycle stage by Differential Interference Contrast (DIC). S phase (binucleate septated cells) could be readily distinguished from G2 (mono-nuclear cells). Double foci in *dcr1Δ* cells (n=132), and single foci in WT cells (n=273) are shown, along with a table of results.

Supplementary Figure 3.

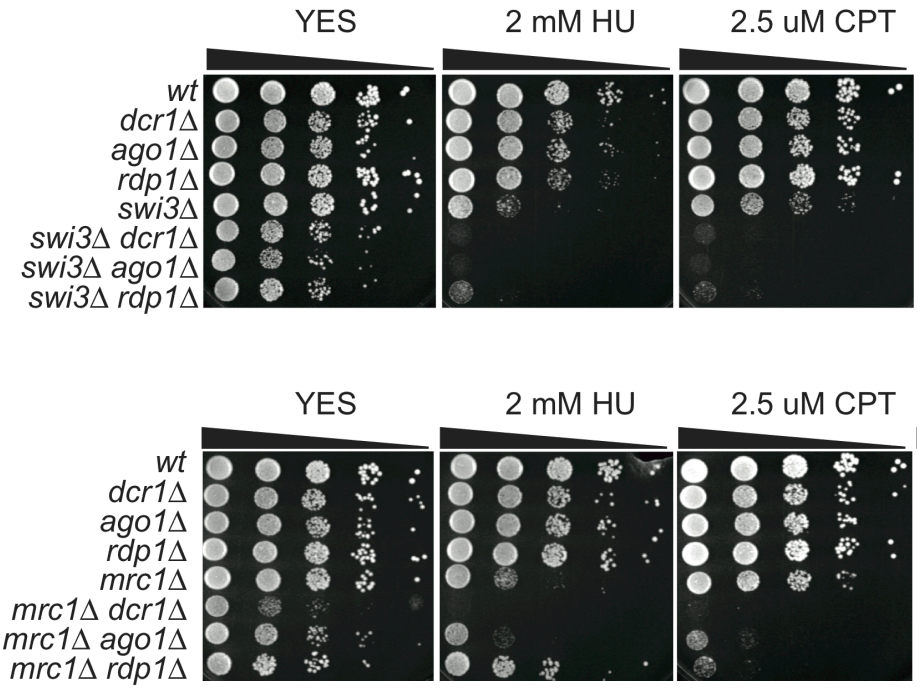


Figure S3. Replication fork protection is essential in the absence of RNAi. Mutant and WT cells were diluted 1:10, plated on 2mM HU, or 2.5μM CPT and analyzed for growth defects. WT and RNAi mutant cells grow normally at these concentrations, but double mutants with *swi3Δ* and *mrc1Δ* do not. Swi3 and Mrc1 are components of the replication fork protection complex.

Supplemental Figure 4.

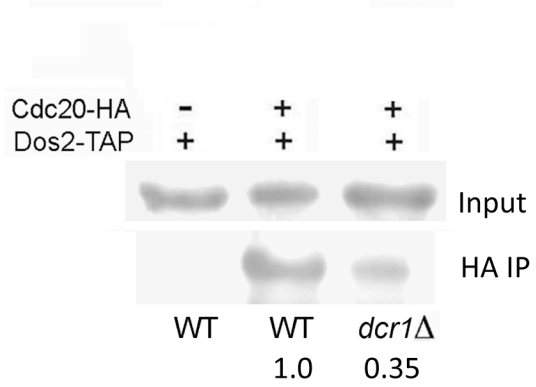


Figure S4. Interaction between DNA polymerase ε and Rik1/CLRC depends on RNAi. Immunoprecipitation from whole cell extracts was performed using antibodies against DNA polymerase Polε (Cdc20-HA), and Westerns were immunoblotted using antibodies against Dos2-TAP tagged protein. Strong interaction in WT cells was reduced in *dcr1Δ* (35-39% in 3 replicates). *cdc20-p7* mutants lacking the Dos2-interacting domain lose heterochromatic histone methylation [3] and are hypersensitive to DNA damage [42]. This Dos2-interacting domain of Polε [3] also interacts with Mrc1 [43], so that loss of Dos2 interaction could account for enhanced requirement for Mrc1 in *dcr1Δ* cells (Supplemental Fig. 2).

Supplemental Figure 5

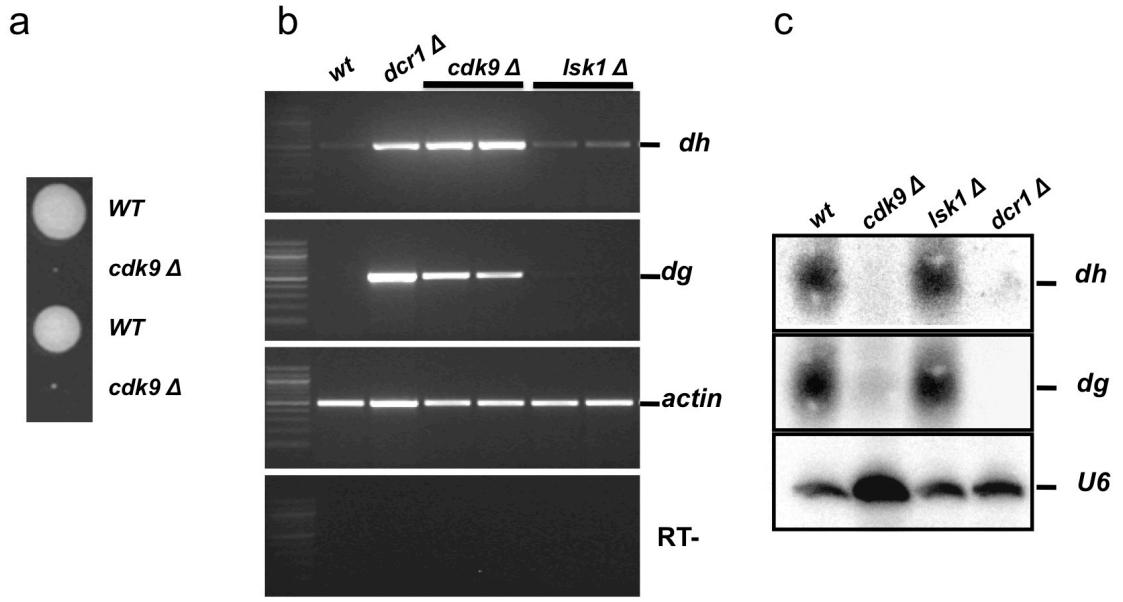


Figure S5. Cdk9, but not the RNA PolII CTD kinase Lsk1, is necessary for sRNA production and pericentric repeat silencing. a. A segregating tetrad dissected from a sporulating *cdk9::Hyg*^{r}*/cdk9*$^{+}$ diploid, growing in rich media. b. RT-PCR of pericentric sRNA precursors in *dcr1*, *cdk9* and *lsk1* KO mutants. c. sRNA northern blot in *dcr1*, *cdk9* and *lsk1* KO mutants.

Table S2. Strains used in this work.

Strain name	Genotype	Reference
DG21	*h-, otr1R(SphI)::ura4+, ura4-DS/E, leu1-32, ade6-216, his7-366*	44
DG690	*h-, delta-dcr1::kanMX6, otr1R(SphI)::ura4+, ura4-DS/E, leu1-32, ade6-210, his7-366*	45
DG124	*h-, delta-rdp1::kanMX6, otr1R(SphI)::ura4+, ura4-DS/E, leu1-32, ade6-216, his7-366*	this study
ZB20	*h-, delta-ago1::kanMX6, otr1R(SphI)::ura4+, ura4-DS/E, leu1-32, ade6-216, his7-366*	this study
DG760	*h-, delta-clr4::kanMX6, otr1R(SphI)::ura4+, ura4-DS/E, leu1-32, ade6-216, his7-366*	this study
DG763	*h-, delta-rik1::kanMX6, otr1R(SphI)::ura4+, ura4-DS/E, leu1-32, ade6-210, his7-366*	this study
DG784	*h-, delta-clr3::kanMX6, otr1R(SphI)::ura4+, ura4-DS/E, leu1-32, ade6-216, his7-366*	this study
DG756	*h-, delta-swi6::kanMX6, otr1R(SphI)::ura4+, ura4-DS/E, leu1-32, ade6-216, his7-366*	this study
FL70	*h-, delta-dos1::kanMX6, ura4-D18, leu1-32, ade6-210, his3-D1*	44
FL71	*h-, delta-dos2::kanMX6, ura4-D18, leu1-32, ade6-210, his3-D1*	44
ZB515	*h-, rad22-YFP::kanMX6, ade6+*	46
AK69	*rad22-YFP::kanMX6, delta-dcr1::kanMX6, ade6-M210*	this study
BG_3416H	*h+, delta-swi3::kanMX, ade6-210*	Bioneer library
AK68	*h-, delta-dcr1::kanMX6, delta-swi3::kanMX6, ade6-216*	this study
DI394	*delta-ago1::kanMX6, delta-swi3::kanMX6, ade6-216*	this study
DI396	*delta-rdp1::kanMX6, delta-swi3::kanMX6, ade6-216*	this study
BG_1948H	*h+, delta-mrc1::kanMX, ade6-210*	Bioneer library
DI408	*h+, delta-mrc1::kanMX, delta-dcr1::kanMX6*	this study
DI412	*delta-mrc1::kanMX, delta-ago1::kanMX6*	this study
DI417	*h+, delta-mrc1::kanMX, delta-ago1::kanMX6, ade6-216*	this study
FL393	*h^{90} , cdc20-GFP-HA::kanMX6, dos2-TAP::ura4+, ura4-D18, ade6-216, leu1-32*	this study
FL400	*h^{-}, dos2-TAP::ura4+ , ura4-D18, ade6-216, leu1-32*	this study
FL421	*h-, cdc20-GFP-HA::kanMX6, dos2-TAP::ura4+, delta-dcr1::kanMX6, ura4-, ade6-216, leu1-32*	this study
ZB580	*h+, delta-mms19::kanMX6, otr1R(SphI)::ura4+, ura4-DS/E, leu1-32, ade6-216, his7-366*	this study
FY14135	*h+, h+ ade6-M210 leu1-32 ura4-D18 rhp51::ura4+*	NBRP, Japan
AY337	*h+, cdk9::hyg, otr1R (sph1)::ura4, ura4 DS/E, ade6 216, leu1-32*	this study
AY453	*lsk1::KanMX6, leu1-32*	this study
PB178	*M smt-0, rhp51::his3, his3-D1*	this study

Table S3. Oligonucleotides used in this study.

p30F_T7	TAATACGACTCACTATAGGGAGcctgttgattcggcacctttg
p30R_T3	AATTAACCCTCACTAAAGGGAGAtggagaacgactgtgaagagacc
p33F_T7	TAATACGACTCACTATAGGGAGtgcaagtggaaagtggcttca
p33R_T3	AATTAACCCTCACTAAAGGGAGAtcgaccaccctgacttgttctc
act1F	TACCCCATTGAGCACGGTAT
act1R_T7	TAATACGACTCACTATAGGGAGAGGAGGAAGATTGAGCAGCAG
p30qPCR_F	CCATATCAATTTCCCATGTTCC
p30qPCR_R	CATCAAGCGAGTCGAGATGA
p33qPCR_F	TATCCTGCGTCTCGGTATCC
p33qPCR_R	CTGTTCGTGAATGCTGAGAAAG
p20F	CCGGCGATTGAGAAAGACTACAA
p20R	TCGAAAAGATACGGCCAATAACA
act1qPCR_F	TGCACCTGCCTTTTATGTTG
act1qPCR_R	TGGGAACAGTGTGGGTAACA
dgIIIrevPolC	CGGCAACTCTGGTCCGATTTA
dgIIIrevPolD	TCTCACATAACCGTTGCATTCATT
dgIIIforPolC	CACAATGAAAACCGATATGTGG
dhIforPolA	TGCTGAGGTAGATGCGTTGTTCA
dhIrevPolA	TGCCGAATCAACAGGCCATA
ura4DS/EF	GCCTCAAAGAAGTTGGTTTACC
ura4DS/ER	CGCTACCGCAGTTTACAATC

Table S4. Sequencing statistics for PolII ChIP-Seq

Strain	Treatment	IP	# Reads	% Aligned	# Aligned Reads
dg21	Exp	S2	1.07E+07	82.14	8.77E+06
dg21	Exp	S5	7.69E+06	88.77	6.82E+06
dg690	Exp	S2	2.85E+06	87.35	2.49E+06
dg690	Exp	S5	4.03E+06	87.94	3.55E+06
dg21	HU	S2	1.01E+07	81.53	8.22E+06
dg21	HU	S5	6.78E+06	85.86	5.82E+06
dg690	HU	S2	3.18E+06	86.75	2.76E+06
dg690	HU	S5	2.36E+06	87.29	2.06E+06

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