Supplemental Data

RNA Interference Guides Histone Modification during the S Phase of Chromosomal Replication

Anna Kloc, Mikel Zaratiegui, Elphege Nora, and Rob Martienssen

Supplemental References

S1. Petersen, J., and Hagan, I.M. (2003). S. pombe aurora kinase/survivin is required for chromosome condensation and the spindle checkpoint attachment response. Curr. Biol. *13*, 590–597.

S2. Li, F., Goto, D.B., Zaratiegui, M., Tang, X., Martienssen, R., and Cande, W.Z. (2005). Two novel proteins, dos1 and dos2, interact with rik1 to regulate heterochromatic RNA interference and histone modification. Curr. Biol. *15*, 1448–1457.

S3. Hiraoka, Y., Toda, T., and Yanagida, M. (1984). The NDA3 gene of fission yeast encodes beta-tubulin: a cold-sensitive nda3 mutation reversibly blocks spindle formation and chromosome movement in mitosis. Cell *39*, 349–358.

S4. Mellone, B.G., Ball, L., Suka, N., Grunstein, M.R., Partridge, J.F., and Allshire, R.C. (2003). Centromere silencing and function in fission yeast is governed by the amino terminus of histone H3. Curr. Biol. *13*, 1748–1757.

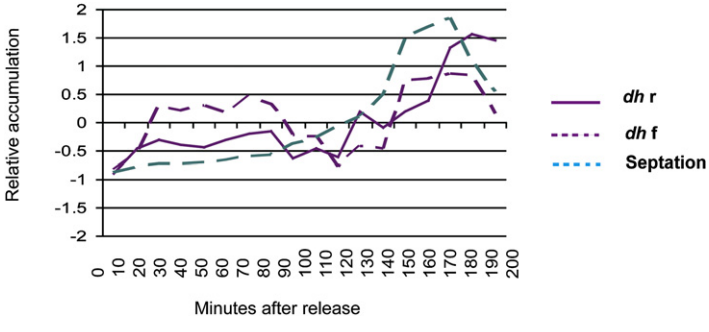

Figure S1. Cell-Cycle Analysis of Centromeric Transcripts in RNAi-Deficient Cells

Quantitative RT-PCR of *dh* centromeric transcripts (violet) during the cell cycle in *dcr1*$^{-}$ mutant cells. The reverse strand transcript (solid) still only increases in S phase and remains high. The forward strand transcript (dashed) is ectopically expressed throughout cell cycle. Septation index (dashed teal line) indicates S phase in *dcr1*$^{-}$ is delayed relative to WT (Figure 3).

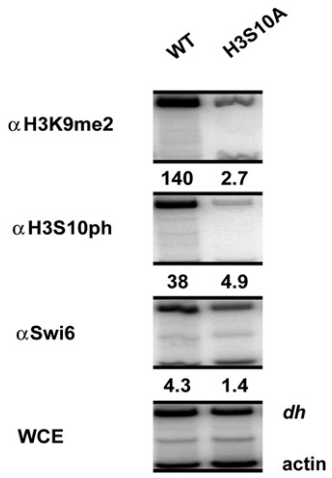

Figure S2. Chromatin Immunoprecipitation of the *dh* Pericentromeric Repeat in H3S10A Strain

In a substitution strain in which H3S10 has been replaced by alanine and cannot be phosphorylated, H3S10ph and H3K9me2 are lost whereas levels of Swi6 are decreased substantially (about three-fold).

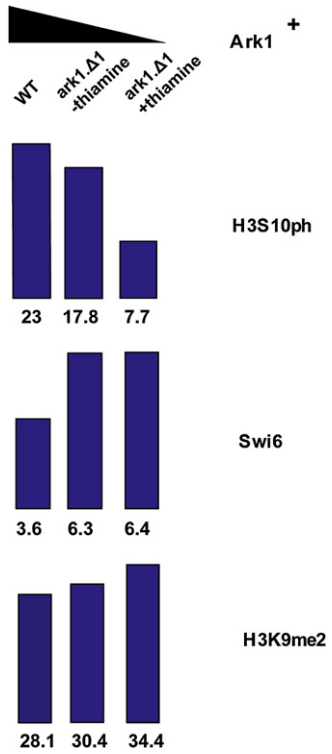

Figure S3. H3 Histone Modification in Serine-10 Kinase Mutants

ark1.Δ1 cells are deficient for the H3S10 Aurora kinase Ark1 and undergo cell-cycle arrest but can be rescued in part by a *nmt1::ark1*$^+$ gene fusion on a complementing plasmid (*ark1.Δ1* − thiamine). When grown in thiamine (*ark1.Δ1* + thiamine), *nmt1::ark1*$^+$ is shut off, and the cells undergo cell-cycle arrest [S1]. In *ark1.Δ1* cells, H3K9me2 and Swi6 were increased, whereas H3S10ph decreased relative to strains with intact *ark1*$^+$ (WT).

Table S1. Strain list

Strain Name	Genotype	Source
DG21	*h*$^-$, *otr1R (Sph1)::ura4, ura4-DS/E, ade6-216, his7-366, leu1-21*	Li et al. [S2]
DG124	*h*$^-$, *Δ-rdp1::kanMX6, otr1R(Sph1)::ura4, leu1-32, ade6-216, his7-366, uraDS/E*	Li et al. [S2]
DG445	*h*$^+$, *Δ-rdp1::kanMX6, otr1R(Sph1)::ura4, leu1-32, ade6-216, his*$^+$*, ura4-DS/E*	this study
DG478	*h*$^+$, *Δ-ago1::kanMX6, otr1R(Sph1)::ura4, ade6*$^-$*216, leu1-32, his*$^-$	this study
ZB20	*h*$^-$, *Δ-ago1::kanMX6, otr1R(Sph1)::ura4, ura4*$^-$*DS-E, ade6-216, leu1-32, his*$^-$	Li et al. [S2]
DG690	*h*$^-$, *Δ-dcr1::kanMX6, otr1R(Sph1)::ura4, ade6-210, leu1-32, his7-366*	Li et al. [S2]
DG691	*h*$^+$, *Δ-dcr1::kanMX6, otr1R(Sph1)::ura4, ade6-210, leu1-32*	this study
DG704	*h*$^+$, *cdc25-22, leu1*	Hiraoka et al. [S3]
DG705	*h*$^+$, *nda3-KM311, leu*	Hiraoka et al. [S3]
DG706	*h*$^-$, *cdc10-129, leu1*	Hiraoka et al. [S3]
FY6071	*h?, h3.2-S10A, Δh3.1/ h4.1::his3*$^+$*, Δh3.3/ h4.3::arg3*$^+$*, leu1-32, ura4D18, his3D1, arg3D3, ade6-210, otr1R(SphI):ade6*$^+$	Mellone et al. [S4]
IH2452	*h*$^+$, *ark1. Δ 1::LEU2, ura4D18, ade6*$^-$*, +pRep82Ark1.PkC*	Petersen and Hagan [S1]
AK01	*cdc25-22, Δ-ago1::kanMX6*	this study
AK02	*cdc25-22, Δ-dcr1::kanMX6*	this study
AK03	*cdc25-22, Δ-rdp1::kanMX6*	this study
AK04	*nda3-KM311, Δ-ago1::kanMX6*	this study
AK05	*nda3-KM311, Δ-dcr1::kanMX6*	this study
AK06	*nda3-KM311, Δ-rdp1::kanMX6*	this study
AK07	*cdc10-129, Δ-ago1::kanMX6*	this study
AK08	*cdc10-129, Δ-rdp1::kanMX6*	this study
AK09	*cdc10-129, Δ-dcr1::kanMX6*	this study

Table S2. Oligonucleotide Sequences

Primer Name	Primer Sequence
p30F (*dh* F)	CCTGTTGATTCGGCACCTTTG
p30R (*dh* R)	TGGAGAACGACTGTGAAGAGA
p30F (*dh* F) qPCR	CCATATCAATTTCCCATGTTCC
p30R (*dh* R) qPCR	CATCAAGCGAGTCGAGATGA
p33F (*dg* F)	TGCAAGTGGAAAGTGGCTTCA
p33R (*dg* R)	TCGACCACCCTGACTTGTTCTC
p33F (*dg* F) qPCR	TATCCTGCGTCTCGGTATCC
p33R (*dg* R) qPCR	CTGTTCGTGAATGCTGAGAAA
act1F	TACCCCATTGAGCACGGTAT
act1R	GGAGGAAGATTGAGCAGCAG
act1F qPCR	TGCACCTGCCTTTTATGTTG
act1R qPCR	TGGGAACAGTGTGGGTAACA
ace2F	TCACAAATCCATACCGAGCA
ace2R	GCGCACCGTTTATCTCATCT
hhf2F	ATGTCTGGTCGTGGAAAAGG
hhf2R	ACCATAAATGGTACGGCCTTG
psu1F	CGTGATGTGTCCGTCGTTAC
psu1R	AAGGGAAATCAGTGCAATCG
U6	ATGTCGCAGTGTCATCCTTG