Supplementary Information

Unique roles for histone H3K9me states in RNAi and heritable silencing of transcription

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Discussion

Our findings reveal unique roles for the methylation states of histone H3K9 in RNAi-dependent and -independent heterochromatin formation. Clr4 is the sole *S. pombe* enzyme responsible for H3K9 di- and tri-methylation. Our generation of active site mutations in Clr4 that impair or completely block H3K9me3 ($Clr4^{I418P}$ and $Clr4^{F449Y}$, respectively), but allow H3K9me2 catalysis, made it possible to investigate the role of each modification in heterochromatin formation.

First, we found that H3K9me2 defines a transcriptionally permissive chromatin state that is sufficient for the H3K9me-dependent recruitment of RNAi, siRNA amplification, and the spreading of H3K9me at pericentromeric DNA repeats (Fig. 4f). The cooperative association of the RITS complex with pericentromeric DNA repeats therefore requires the interaction of its siRNA-programmed Ago1 subunit with nascent noncoding pericentromeric transcripts and its Chp1 subunit with H3K9me2 (Fig 4f). H3K9me2 is also sufficient for the key steps downstream of this cooperative recruitment: RDRC- and Dcr1-mediated siRNA amplification and the spreading of H3K9me throughout siRNA producing domains, although H3K9me3 contributes to efficient siRNA amplification at the dh pericentromeric repeats. Importantly, the above events are restricted to chromosome regions that produce trigger sRNAs[1,2].

Second, in cells lacking H3K9me3, we observed partial but substantial silencing of pericentromeric dg and dh transcripts without a reduction in RNA polymerase II occupancy (Fig. 1). This demonstrates a major role for H3K9me2- and RNAi-dependent co-transcriptional RNA degradation in silencing (RNAi-CTGS) and indicates that H3K9me3 is required for TGS (Fig 4f). Our findings, as well as previous reports[3], provide an explanation for the dependence of CTGS and TGS on different H3K9me states based on the different affinities of downstream chromo domain proteins for H3K9me2 and H3K9me3 as follows. In native fission yeast whole

cell extracts, the Chp1 subunit of RITS binds to histone H3K9me2 and H3K9me3 with a similar efficiency, while Swi6, which is required for efficient TGS[4,5], binds to H3K9me3 with higher efficiency (Fig. 3d-f).

Finally, the ability of Clr4 to catalyze H3K9me3 is required for epigenetic maintenance of silencing and H3K9me domains, even after the establishment of a large domain of heterochromatin containing both H3K9me2 and H3K9me3 (Fig. 4a-d). It was previously shown that ectopically established domains of H3K9me can be epigenetically inherited in the absence of sequence-dependent recruitment in cells in which the rate of H3K9 demethylation is reduced by deletion of *epe1*$^{+}$[6,7]. H3K9me domains and epigenetic states can also be stably transmitted in *epe1*$^{+}$ cells, but only within native heterochromatic domains such as the pericentromeric DNA repeats and the mating type locus[6,8]. Our findings indicate that H3K9me3 is required for epigenetic maintenance of silencing in both *epe1*Δ and *epe1*$^{+}$ cells. In *epe1*Δ cells, neither *ade6*$^{+}$ silencing nor a large domain of H3K9me2/3, induced by the ectopic recruitment of TetR-Clr4-I, could be maintained by Clr4^{I418P}, which has reduced H3K9 tri-methylation activity. This loss of epigenetic information can be explained by the reduced affinity of the Clr4 chromo domain for H3K9me2 (Fig. 3d-f), which would reduce the strength of the positive feedback mechanism based on binding of Clr4 to H3K9me nucleosome and methylation of newly deposited nucleosomes (also referred to as the read-write mechanism). Additionally, the loss of TGS and the resulting transcription-coupled increase in histone exchange may contribute to instability of H3K9me2 domains. In *epe1*$^{+}$ cells, the residual RNAi-independent H3K9me present throughout pericentromeric DNA repeats is maintained epigenetically by a mechanism that requires the chromo domain of Clr4 (ref. [6]) and is lost when Clr4 cannot catalyze H3K9me3 (Fig. 4e, f).

Several previous studies have described the preferential association of histone H3K9me2 and H3K9me3 with euchromatic versus heterochromatic DNA domains[9-14], have reported a role for H3K9me3 in peripheral nuclear localization of heterochromatin in *C. elegans*[15], and have identified methyltransferases that catalyze H3K9me1 prior to chromatin assembly[16]. Moreover, different H3K4 methylation states are associated with enhancers, promoters, and transcribed regions, and perform distinct functions in recruitment of downstream factors associated with transcription[17-20]. The utility of histone lysine methylation states in signaling downstream events is therefore widespread in chromatin biology.

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Supplementary Table 1 | List of strains used in this study.

Strain	Genotype	Source
SPY137	*h$^+$ leu1-32 ade6-M210 ura4-DS/E otr1R(SphI)::ura4$^+$*	2
SPY815	SPY137 *clr4Δ::kanMX6*	3
SPY1098	SPY137 *swi6Δ::natMX6*	6
SPY1368	SPY137 *chp2Δ::kanMX6*	5
SPY2421	SPY137 *chp1Δ::TAP-kanMX6*	1
SPY4719	SPY137 *ago1Δ::kanMX6*	1
SPY797	SPY137 *natMX6-5'-3xflag-ago1*	4
SPY2337	SPY137 *natMX6-5'-3xflag-ago1, clr4Δ::kan*	1
SPY8072	SPY137 *natMX6-5'-3xflag-ago1, kanMX6-5'(1kb)-clr4^{F449Y}*	1
SPY4636	SPY137 *hphMX6-5'(1kb)-3xflag-clr4*	1
SPY4639	SPY137 *hphMX6-5'(1kb)-3xflag-clr4$^{H410L,\ C412A}$*	1
SPY4642	SPY137 *hphMX6-5'(1kb)-3xflag-clr4^{F449Y}*	1
SPY5562	SPY137 *kanMX6-5'(1kb)-3xflag-clr4^{W31G}*	1
SPY6537	SPY137 *hphMX6-5'(1kb)-3xflag-clr4^{I418P}*	1
SPY6529	SPY137 *hphMX6-5'(1kb)-3xflag-clr4^{I418P}, ago1Δ::kanMX6*	1
SPY5378	SPY137 *hphMX6-5'(700bp)-3xflag-chp2*	1
SPY5380	SPY137 *hphMX6-5'(700bp)-3xflag-chp2, clr4Δ::kanMX6*	1
SPY5382	SPY137 *hphMX6-5'(700bp)-3xflag-chp2, kanMX6-5'(1kb)-clr4^{F449Y}*	1
SPY5593	SPY137 *hphMX6-5'(900bp)-3xflag-clr3*	1
SPY5595	SPY137 *hphMX6-5'(900bp)-3xflag-clr3, clr4Δ::kanMX6*	1
SPY5597	SPY137 *hphMX6-5'(900bp)-3xflag-clr3, kanMX6-5'(1kb)-clr4^{F449Y}*	1
SPY6481	SPY137 *natMX6-5'(0.5kb)-3xflag-swi6*	1
SPY5659	SPY137 *kanMX6-5'(1kb)-clr4^{F449Y}, natMX6-5'(0.5kb)-3xflag-swi6*	1
SPY5086	*h$^-$ leu1-32 ade6? ura4Δ::10xtetO-ade6, clr4Δ:nat-clr4p-NLS-TetR-2xflag-clr4ΔCD, epe1Δ::kanMX6*	7
SPY5244	SPY5086 *trp1::hphMX6-5'(1kb)-3xflag-clr4-3'(1kb)*	1
SPY5245	SPY5086 *trp1::hphMX6-5'(1kb)-clr4^{W31G}-3'(1kb)*	1
SPY5248	SPY5086 *trp1::hphMX6-5'(1kb)-clr4^{F449Y}-3'(1kb)*	1
SPY6326	SPY5086 *trp1::hphMX6-5'(1kb)-clr4^{I418P}-3'(1kb)*	1

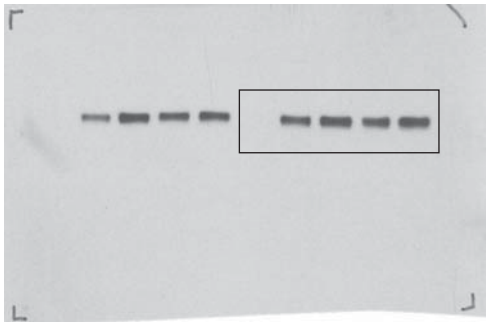
1 = this study; 2 = Karl Ekwall; 3 = ref. [21]; 4 = ref. [22]; 5 = ref. [4]; 6 = ref. [23]; 7 = ref. [6]

Supplementary Table 2 | List of primers used in this study.

Target	Primer	Orientation	Sequence
dg	GJ195	Forward	GGTTAAAGCGGTTGTTTGGCACTG
dg	GJ196	Reverse	TGACGAGGCACATTCCTTATACGC
dg2	GJ736	Forward	GCGAAACGAATGCCAAATAC
dg2	GJ737	Reverse	GGAAAGTGGCTTCACACTATAA
dh	AS133	Forward	GTATTTGGATTCCATCGGTACTATGG
dh	AS134	Reverse	ACTACATCGACACAGAAAAGAAAACAA
dh2	GJ254	Forward	GTCGTTGTCAACCGCACTTCCTTT
dh2	GJ255	Reverse	GCATGCTCCGTTGCTTATCTCGTT
ura4	GJ412	Forward	GGTTTGAGAAGCATACCGATTT
ura4	GJ413	Reverse	CCTTTAACATCCAAGCCGATAC
act1	MB90	Forward	CAACCCTCAGCTTTGGGTCTTG
act1	MB91	Reverse	TCCTTTTGCATACGATCGGCAATAC
fbp1	GJ173	Forward	ATTGACGCCGGTGTTAGTGTAGGT
fbp1	GJ174	Reverse	TGACACGATGACCTGTGGTAAGCA
ade6	KR111	Forward	TTGCAGGAGAGGGTTCAACAGCA
ade6	KR112	Reverse	AATGCATCATCTTGGATGCAGCAA
mug135	KR124	Forward	GAGCCTCATGTCCATACGATCAACCT
mug135	KR125	Reverse	AATCGATGGATGAGTGGAGAAAGTCG

Supplementary Figure 1 - Uncropped scans

Extended Data Fig 1a, upper panel



Extended Data Fig 1a, lower panel

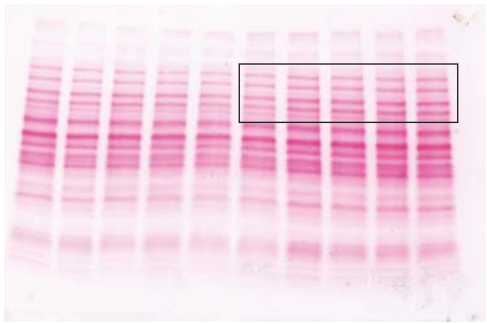


Fig 2b, upper panel

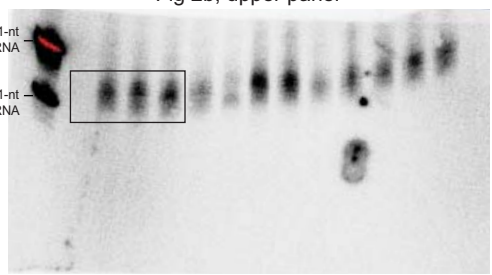


Fig 2b, middle panel

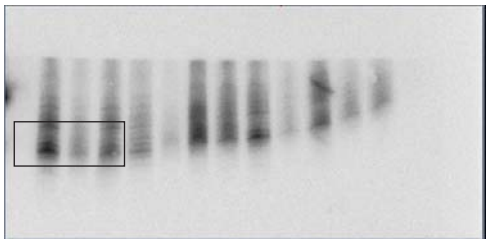
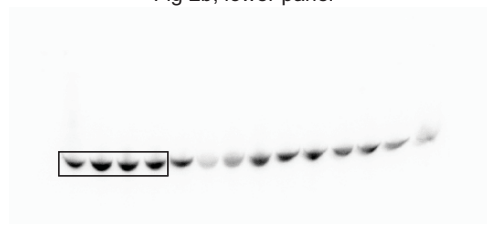
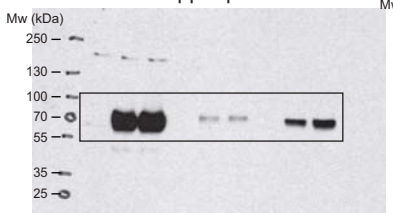


Fig 2b, lower panel

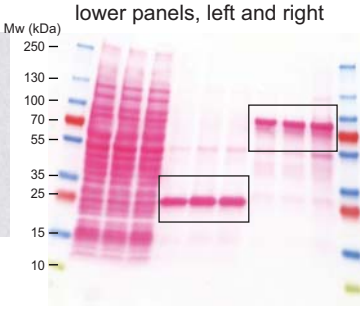


Extended Data Fig 1c

upper panel

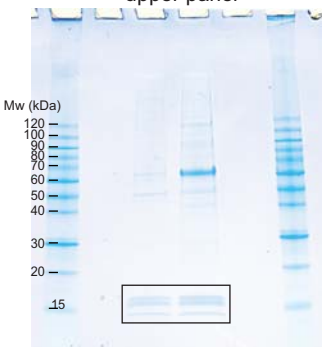


lower panels, left and right

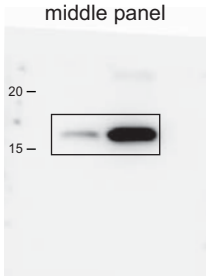


Extended Data Fig 1d

upper panel



middle panel



lower panel

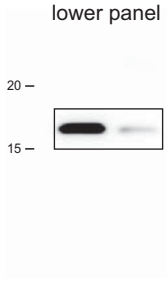


Fig 3e, upper panel

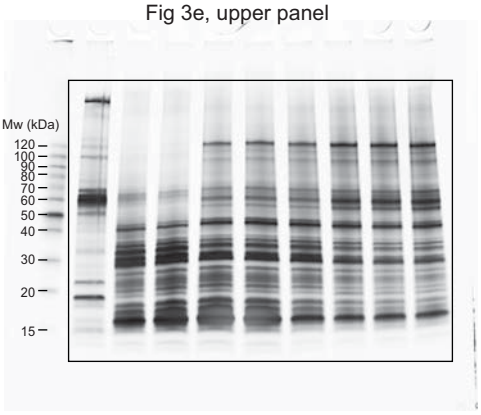


Fig 3e, middle panel

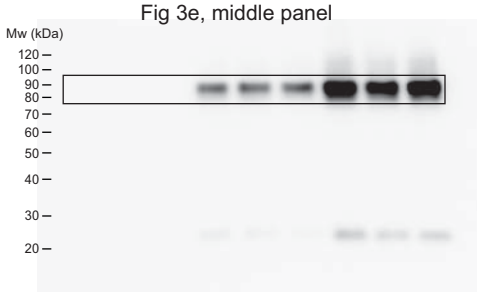


Fig 3e, lower panel