Heterochromatin Silencing

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Most DNA in eukaryotes consists of repetitive DNA, including retrotransposons, transposable elements. Packaged into a condensed form: **Heterochromatin:** epigenetical inherited, generally maintained after replication and meiosis.

Aminoterminal regions of histones are modified; in heterochromatin: Hypoacetylation and methylation of Histone H3 on lysine 9. H3mK9 is bound by HP1 (heterochromatin Protein1) which is highly conserved. DNA Methylation (5mC) on CpG islands, but also on CpNpG. All three modifications are interrelated.

Constitutive heterochromatin: centromeres and telomeres;Facultative heterochromatin: cell specific and clonally inherited (X-chromosome)

´**Position effect variegation**: Juxtaposition of a gene normally transcribed to a heterochromatic domain (rearrangements, transposition) results in silencing of the gene by spreading of hetero-chromatic packaging. Heterochromatic packaging as defence to minimize activity of repetitive sequences.

Table 1. Characteristics of different chromatin forms.

Feature	Euchromatin	Constitutive heterochromatin
Staining/packaging in interphase	Dispersed	Condensed, prominently stained (heteropycnotic)
DNA sequence	Predominantly unique; gene rich	Repetitive (satellites; derivatives of viruses, transposons, etc.); gene poor
Replication timing	Throughout S phase	Late S phase
Meiotic recombination	Normal frequency	Low frequency
Characteristic modifications	Histone hyperacetylation	Histone hypoacetylation
	Histone H3-mK4 present	Histone H3-mK9 present
	Cytosine hypomethylation	Cytosine hypermethylation
Chromatin structure	HS sites, irregular nucleosome spacing; accessible to nucleases	Loss of HS sites, regular nucleosome array; less accessible to nucleases

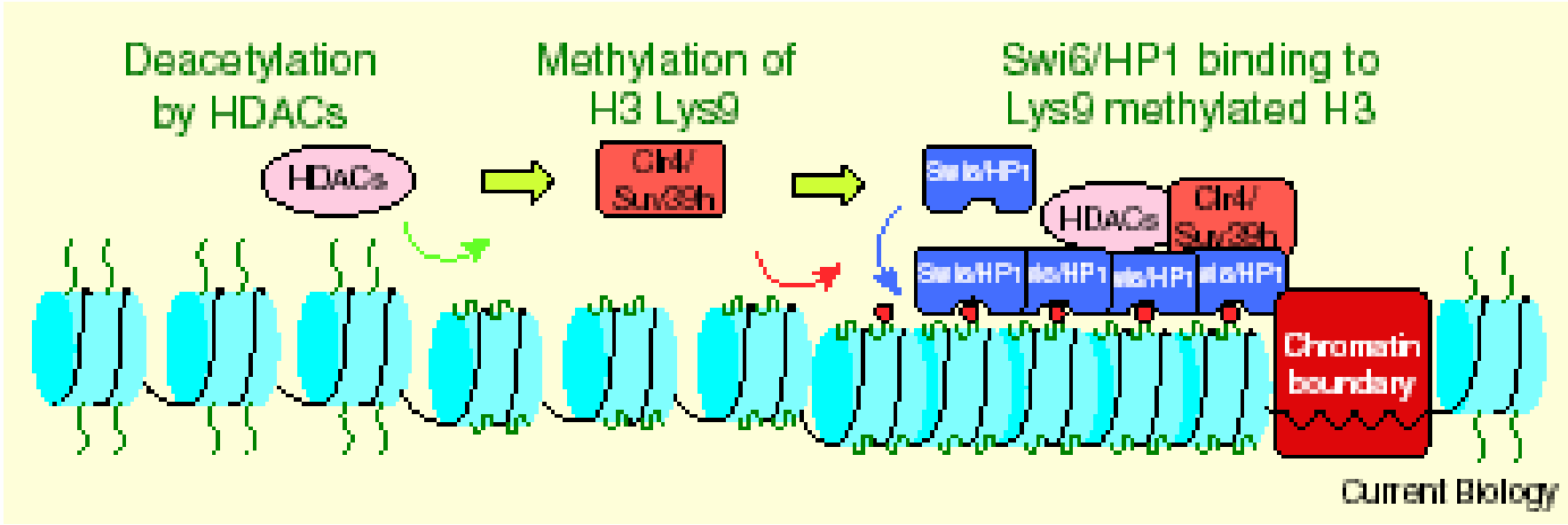


Figure 1. A stepwise model for epigenetic control of heterochromatin assembly in fission yeast (*S. pombe*).

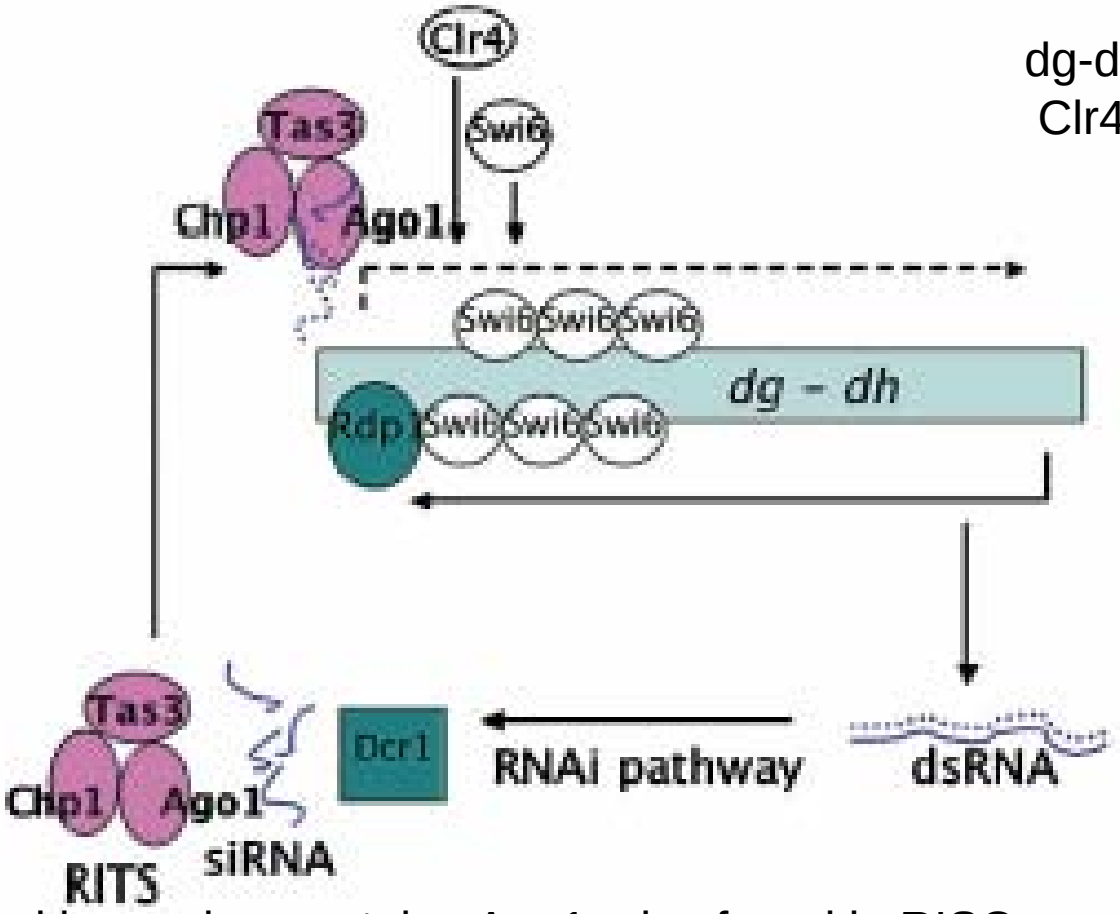
Deacetylation by the histone deacetylases (HDACs) Clr6, Clr3, Sir2, and perhaps others, allows methylation of histone H3 lysine 9 by the Clr4/Rik1 complex. Swi6 (the *S. pombe* homolog of HP1) binds specifically to H3-mK9 to continue heterochromatin assembly. Such progression might be stopped by a boundary, potentially a site that recruits histone acetylases. (Figure adapted from Grewal and Elgin, Figure 5.)

RNA-directed targeting of heterochomatin: Repetitive sequences: non-coding RNA plays a key role in targeting and propagating chromatin modification. (As in plant transgene and viral genes, Mammals: Xist, a large non-coding RNA essential for initiation and spreading of Heterochromatin assembly during X chromosome inactivation.

RNAi: Dicer (RNase III), Argonaute proteins and RdRP (rev. transcriptase): Mutants in these proteins cause defects in heterochromatic assembly. H3-mK9 and targeting of HP1 to centromere require these RNAi proteins. siRNAs from the centromeric region are detectable. Retrotransposons (LTRs) recruit heterochromatin complexes in an RNAi dependent manner.

DsRNA transcripts lead to silencing and heterochromatin formation at the genomic locus homologous to dsRNA.

RITS: RNA-induced initiation of transcriptional silencing



dg-dh: centromeric sequences,
Clr4: H3K9 methyltransferase

Purified in pombe: contains Ago1: also found in RISC: mediates contact with mRNA , probably also contact with homologous DNA. Chp1: binds centromers, Tas3: uncharacterized protein. All three proteins are required for methylation of H3K9 and Swi6 binding.

Lit:: Elgin and Grewal: Current Biology, 13, R895.Ekwall: Molecular Cell; 13, 304

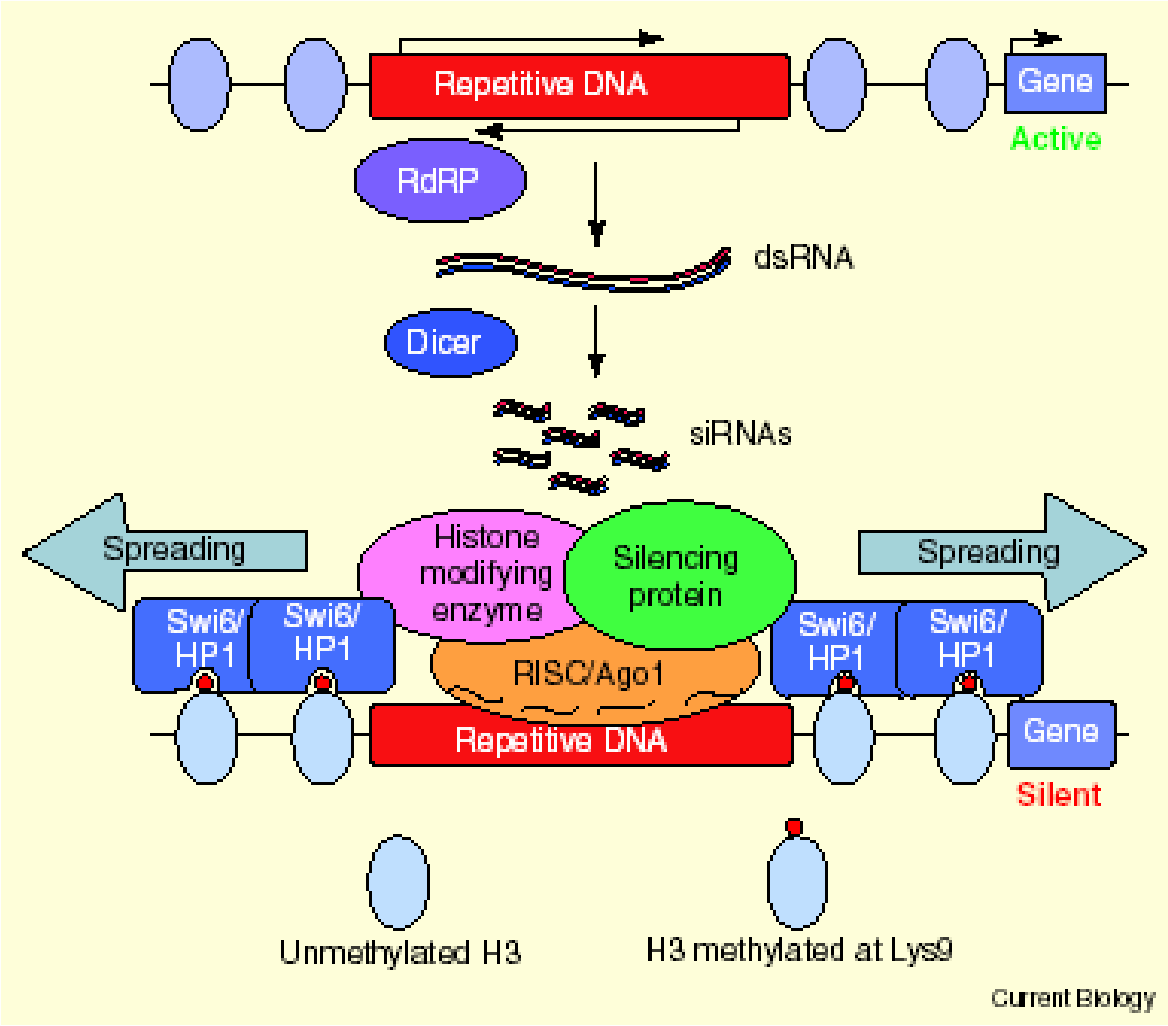
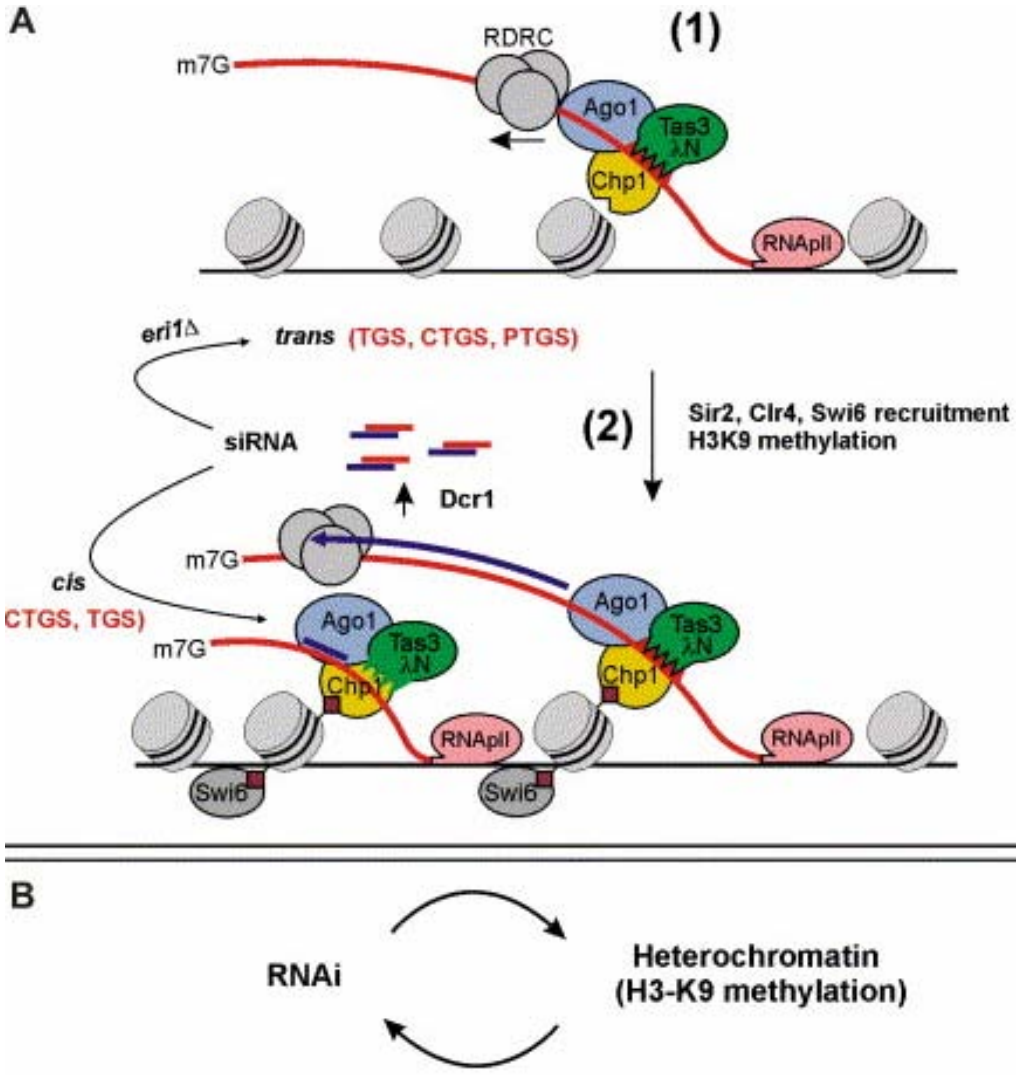


Figure 2. A stepwise model for targeting of heterochromatin formation by an RNAi mechanism.

Double-stranded RNA (dsRNA) is generated from a repetitive sequence, either as a consequence of RNA-dependent RNA polymerase activity (RdRP), or because of transcription from internal and external promoters with opposite orientation. The dsRNA is processed by Dicer to generate short interfering RNAs (siRNAs). The siRNAs are utilized to localize histone modifying complexes; heterochromatic packaging spreads due to the combined activity of the H3-K9 histone methyltransferase and associated Swi6/HP1.


A
RDRC
(1)
m7G
Ago1
Tas3
λN
Chp1
RNApII
eri1Δ
trans (TGS, CTGS, PTGS)
siRNA
(2)
Sir2, Clr4, Swi6 recruitment
H3K9 methylation
Dcr1
m7G
cis
CTGS, TGS)
Ago1
Tas3
λN
m7G
Chp1
RNApII
Swi6
Ago1
Tas3
λN
Chp1
RNApII
Swi6
B
RNAi
Heterochromatin
(H3-K9 methylation)

A) Tas3-λN tethering of the RITS complex to a 5BoxB-modified transcript (represented as a tooth saws in the Tas3 protein and the nascent transcript) mimics the association of RITS with the nascent transcript through siRNA-dependent base pairing (1). This leads to nucleation of heterochromatin assembly through the recruitment of chromatin modifying and binding proteins (Sir2, Clr4, Swi6), RDRC (RNA-Directed RNA polymerase Complex), and Dicer to generate siRNAs, which program and direct RITS complexes to nascent transcripts at the site of siRNA generation in *cis* (2). Once dimethylated by Clr4, H3-K9 serves as an anchor for Chp1, tethering the RITS complex to chromatin, which then mediates the degradation of the nascent transcript (CTGS, *co*t*r*anscriptional *g*ene *s*ilencing) and initiates chromatin modifications that can also mediate *t*ranscriptional *g*ene *s*ilencing (TGS). In *eri1*Δ cells, siRNAs are able to act in *trans* to silence the expression of homologous sequences by CTGS, TGS, and/or PTGS mechanisms. Red diamonds denote H3-K9 methylation.

(B) The ability of newly generated siRNAs to act in *trans* in *eri1*Δ cells shows that siRNAs can initiate de novo heterochromatin formation. Conversely, the ability of RITS to bind to H3-K9 methylated heterochromatin and to initiate heterochromatin-dependent, but primer-independent, dsRNA synthesis and siRNA generation suggests that heterochromatin can initiate RNAi to promote the degradation of nascent transcripts. See text for References and det