1. HC organizing factors

Very often, constitutive **heterochromatin** (HC) is formed on repetitive DNA sequences.

Telomeres are formed by microsatellite repetitive sequences

A characteristic feature of the DNA sequence of constitutive HC found in **pericentromeric** regions is that it is generally composed of long stretches of satellite repeats.

The lengths of the repeats vary widely between species: from the 5- to 7-bp repeats of *Drosophila* to the 1950-bp repeat found in a HC island in *Arabidopsis*.

The predominant satellite in human pericentromeric HC is the 171-bp a-satellite repeat, which shows significant variation between chromosomes.

Mouse pericentromeric heterochromatin is made up largely of the pancentromeric 234-bp -satellite repeat.

Experimental: Tandem repeats of transgenes are often HC.

Subject: noncoding RNA involvement in silencing establishment and maintenance

CMLS, Cell. Mol. Life Sci. 60 (2003) 2295–2302
1420-682X/03/112295-08
DOI 10.1007/s00018-003-3245-y

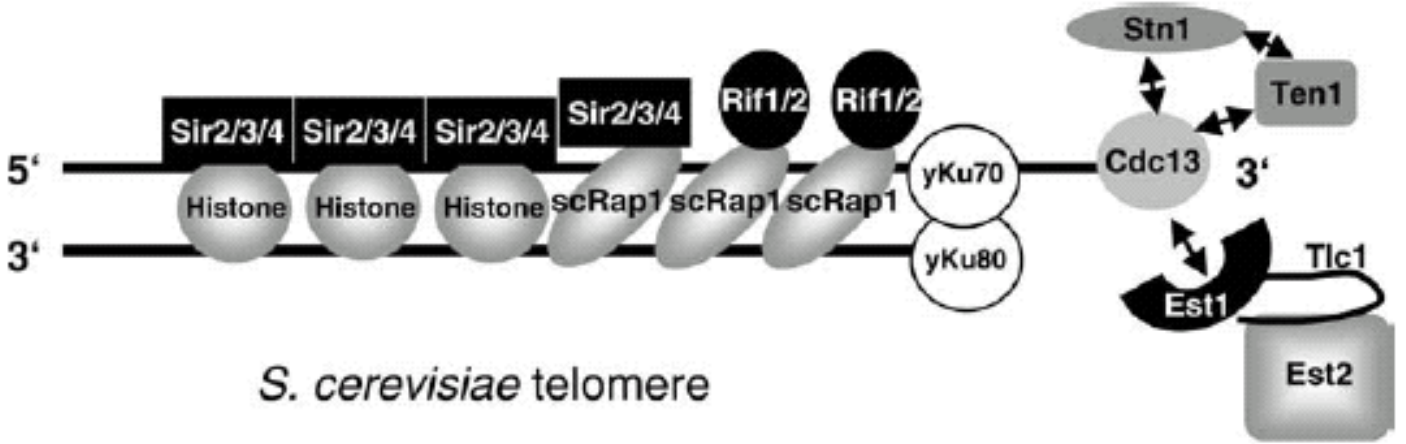
CMLS Cellular and Molecular Life Sciences

Composition and conservation of the telomeric complex

J. Kanoh* and F. Ishikawa

Graduate School of Biostudies, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606-8502 (Japan), Fax: +81 75 753 4197, e-mail: jkanoh@lif.kyoto-u.ac.jp

Abstract. The telomere is composed of telomeric DNA and telomere-associated proteins. Recently, many telomere-associated proteins have been identified, and various telomere functions have been uncovered. In budding yeast, scRap1 binds directly to telomeric DNA, and other telomere regulators (Sir proteins and Rif proteins) are recruited to the telomeres by interacting with scRap1. Cdc13 binds to the most distal end of the chromosome and recruits telomerase to the telomeres. In fission yeast and humans, TTAGGG repeat binding factor (TRF) family proteins bind directly to telomeric DNA, and Rap1 proteins and other telomere regulators are recruited to the telomeres by interacting with the TRF family proteins. Both organisms have Pot1 proteins at the most distal end of the telomere instead of a budding-yeast Cdc13-like protein. Therefore, fission yeast and humans have in part common telomeric compositions that differ from that of budding yeast, a result that suggests budding yeast has lost some telomere components during the course of evolution.



Telomere-associated proteins in budding yeast. Arrows indicate physical interactions.

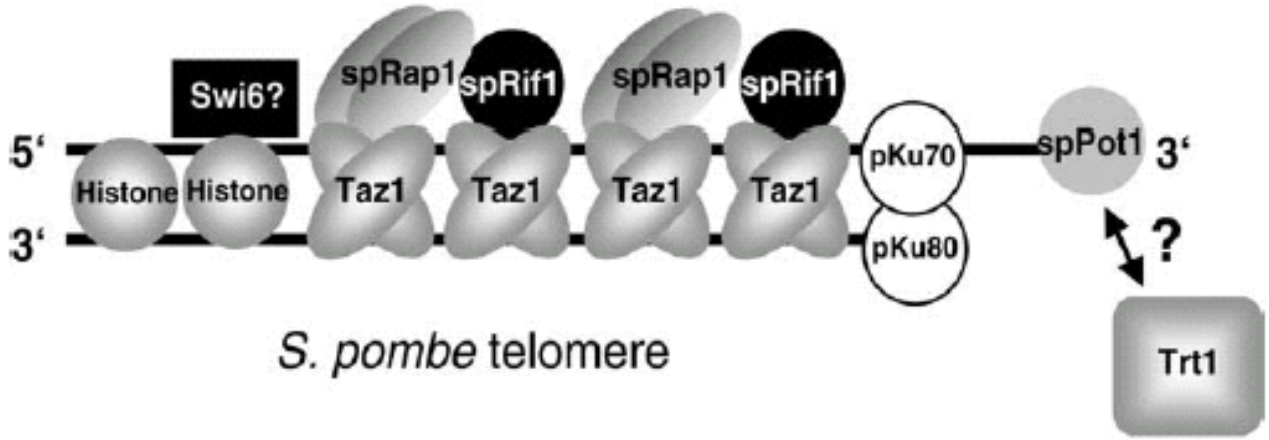


Figure 2. Telomere-associated proteins in fission yeast. Localization of Swi6 is speculative. spPot1 may regulate the access of telomerase (Trt11) to the telomeres.

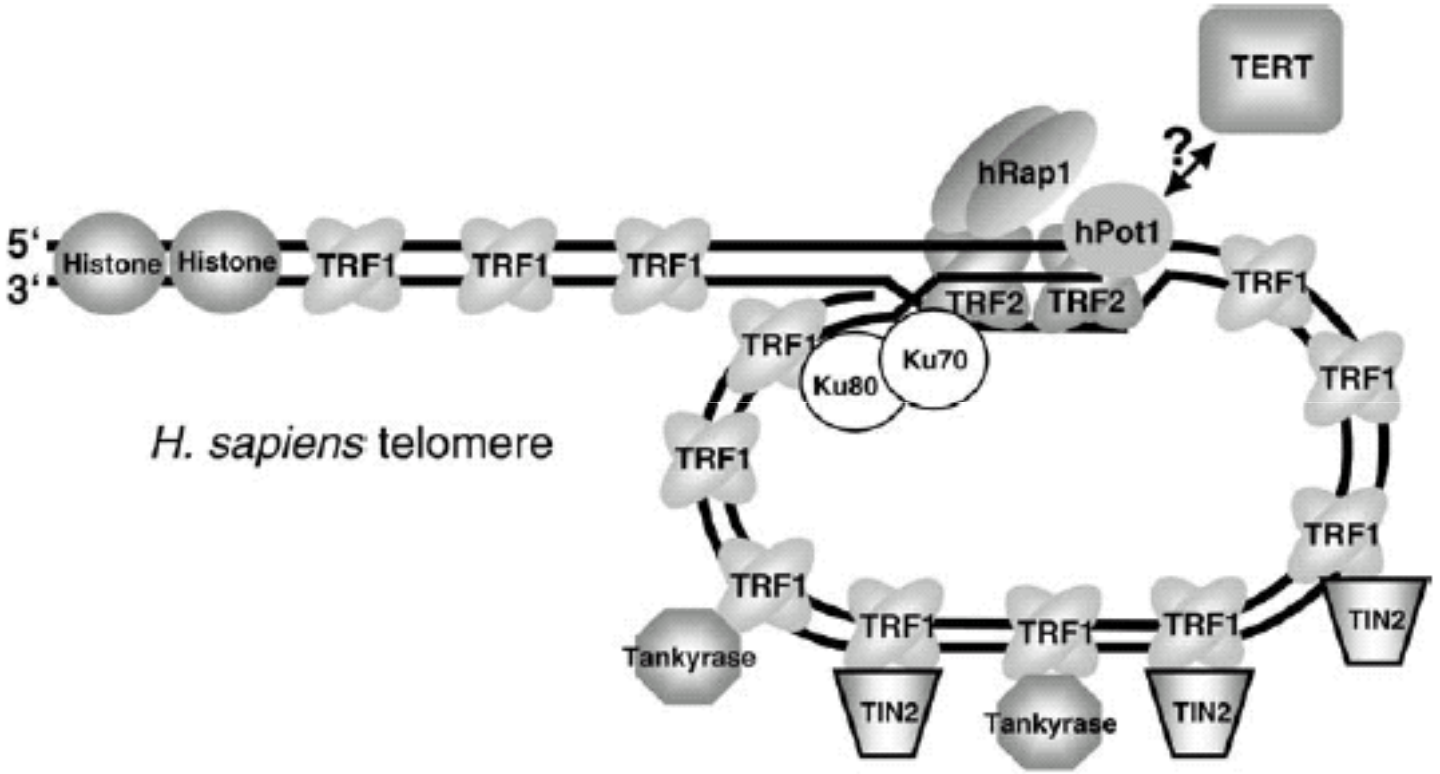
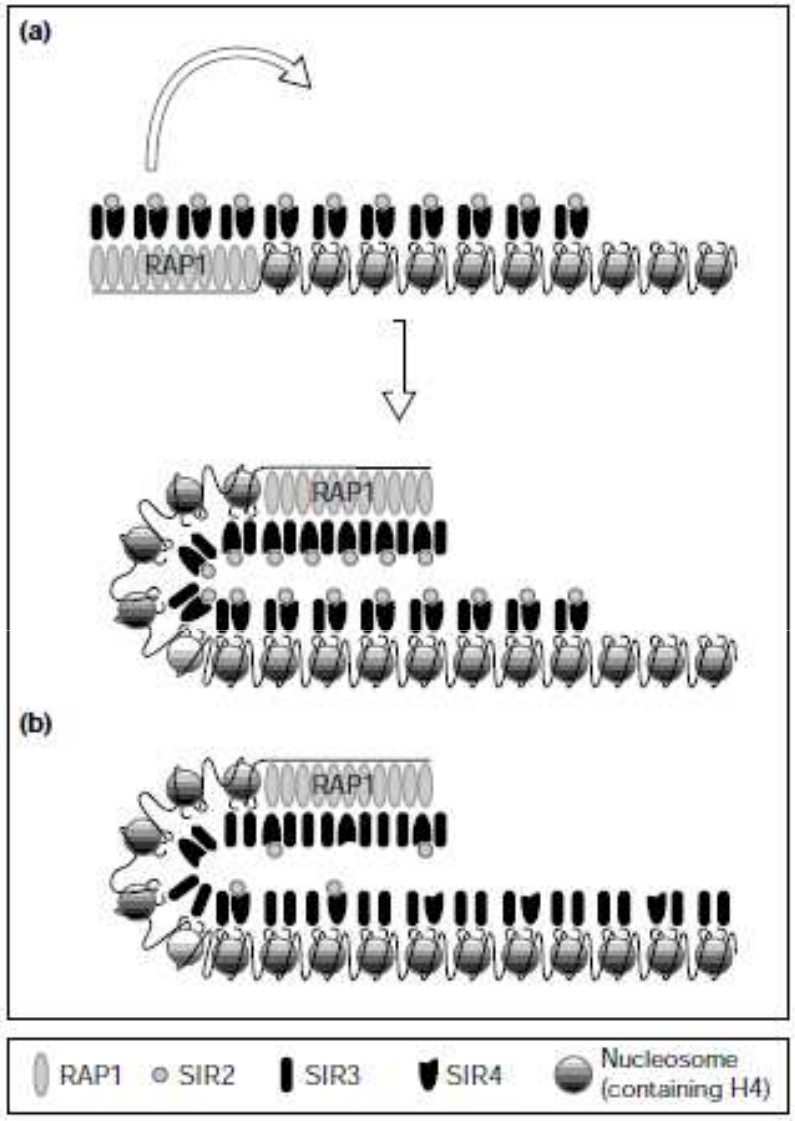


Figure 3. Telomere-associated proteins in human. Relationship between hPot1 and telomerase is not known.



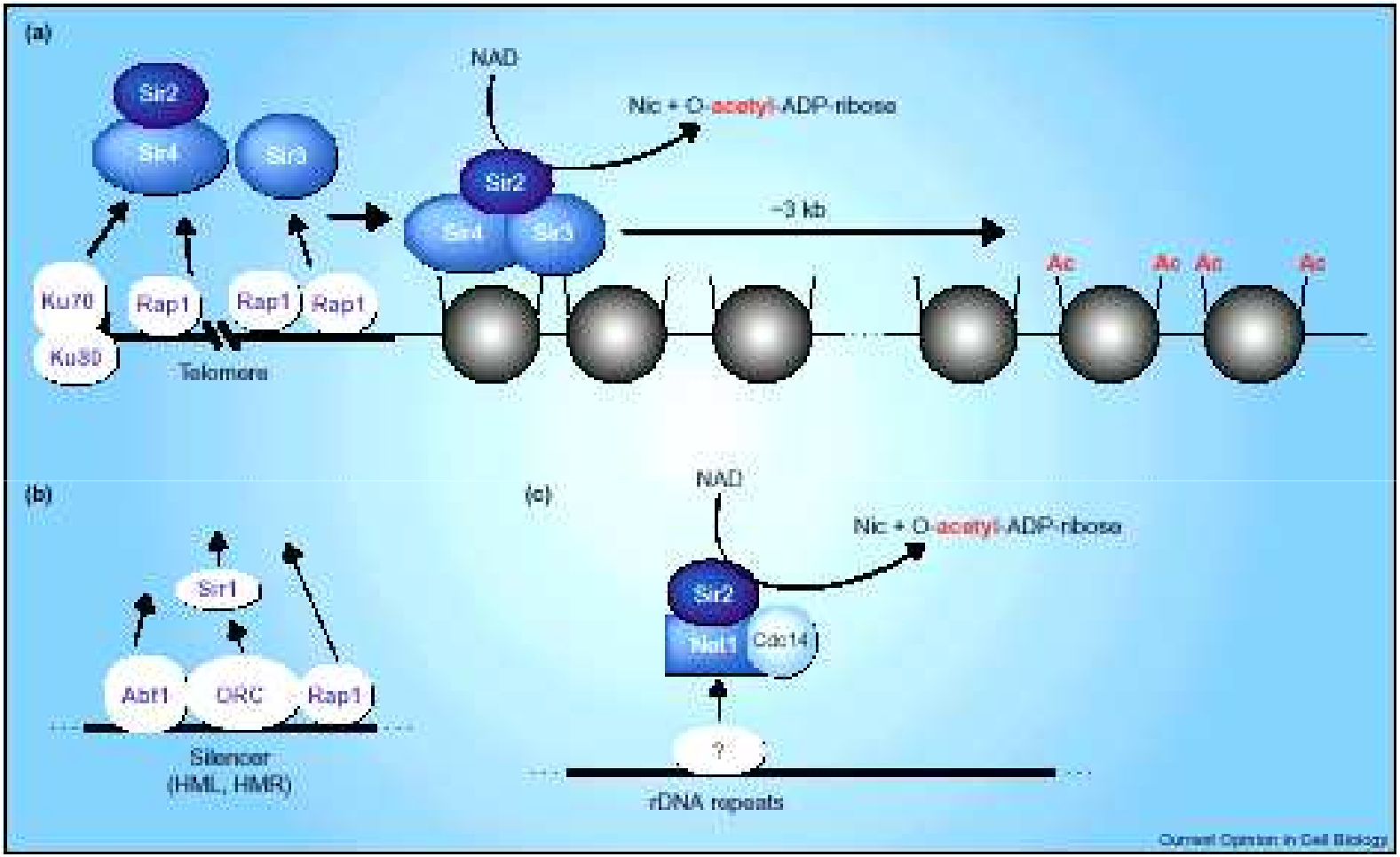
Model for the formation of telomeric heterochromatin. Black lines wrapped around nucleosomes represent DNA.

Core telomeric heterochromatin in wild-type cells containing only a single genomic copy of SIR3.

It is proposed that the RAP1-containing telosome folds back onto subtelomeric regions. In this manner, RAP1–SIR–histone interactions are all required for stability of the complex.

Upon SIR3 overexpression, telomere position effect and the presence of SIR3 is extended **up to some 16–20 kb from the telomere**. SIR3 overexpression causes loss of some SIR4 and most SIR2 from the complex. Due to the interdependence of RAP1–SIR3–H4 interactions, and because all three SIR proteins are required for extension of heterochromatin by SIR3, it is proposed that the complex necessary for the initiation of heterochromatin formation requires RAP1, the SIR proteins and H4.

[Attività di Sir 2]



Enzymatic activities of Sir2 and chromatin silencing
Danesh Moazed
Current Opinion in Cell Biology 2001, 13:232–238

Genetic analysis in yeast for trans-gene suppression at telomeres and HMR mating locus identified several genes that, if expressed a little more or less, affect the extent of a phenomenon known how “Positional Effect Variegation” (PEV) that depends on the extent of the HC regions.

SIR2
SIR3
SIR4 (silencing information regulators)
RAP1 (repressor activator protein 1)
H3
H4 (N-terminal tails)

RAP1 is the only one that binds DNA elements ($C_{1-3}A$ repeats)
RAP-1 C-terminal domain fused to GAL4 DBD can silence GAL4 element-containing genes.
In two-hybrid system, RAP-1 C-terminal domain interacts with SIR3 and SIR4

SIR3 is the limiting factor for chromatin spreading, i.e. increases the distance at which a trans-gene can be silenced.

SIR3 and SIR4 interact with H3 and H4 N-terminal domain in vitro.

Why does Sir2 utilize a high-energy substrate as NAD as the acceptor of acetyl moieties? Most other HDAC just use water!

Sir2 may represent a “sensor” of the metabolic status

Overexpression of Sir2 extends the lifespan in yeast and Caenorabditis elegans

Deletion of Sir2 gene consistently shortens lifespan in yeast

family Sirtuins (wikipedia)

Increased dosage of a *sir-2* gene extends lifespan in *Caenorhabditis elegans*

Heidi A. Tissenbaum & Leonard Guarente

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

In *Caenorhabditis elegans*, mutations that reduce the activity of an insulin-like receptor (*daf-2*)[1] or a phosphatidylinositol-3-OH kinase (*age-1*)[2] favour entry into the dauer state during larval development[3] and extend lifespan in adults[3–6]. Downregulation of this pathway activates a forkhead transcription factor (*daf-16*)[7,8], which may regulate targets that promote dauer formation in larvae and stress resistance and longevity in adults[9]. In yeast, the *SIR2* gene determines the lifespan of mother cells, and adding an extra copy of *SIR2* extends lifespan[10]. Sir2 mediates chromatin silencing through a histone deacetylase activity that depends on NAD (nicotinamide adenine dinucleotide) as a cofactor[11–13]. We have surveyed the lifespan of *C. elegans* strains containing duplications of chromosomal regions. Here we report that a duplication containing *sir-2.1*—the *C. elegans* gene most homologous to yeast *SIR2*—confers a lifespan that is extended by up to 50%. Genetic analysis indicates that the *sir-2.1* transgene functions upstream of *daf-16* in the insulin-like signalling pathway. Our findings suggest that Sir2 proteins may couple longevity to nutrient availability in many eukaryotic organisms.

One interesting aspect of all repetitive (heterochromatic) sequences is that they are transcribed at low frequency.

The same was seen in the centromeric regions of yeast, were rare centromeric transcripts in both senses can be measured by common RNA expression methods (Northern, RT-PCR etc.).

At the beginning of 2000’s
when RNA interference was emerging
researchers wondered whether RNAi could have anything to do with silencing ...

Assigned paper

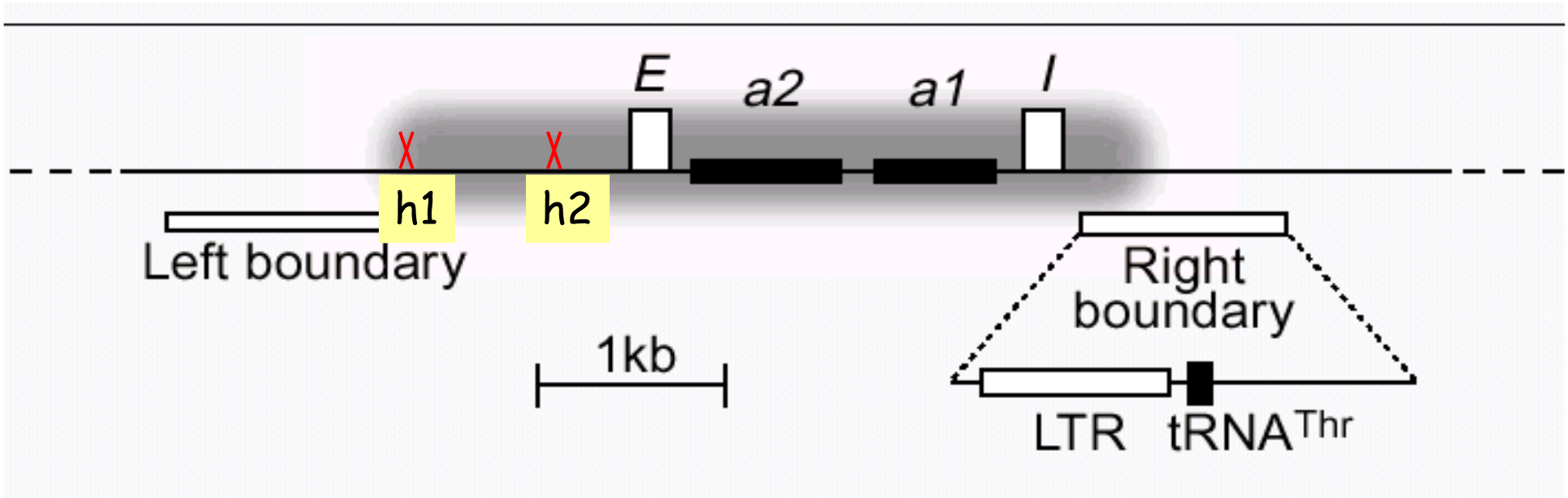
Regulation of Heterochromatic Silencing and Histone H3 Lysine-9 Methylation by RNAi

Thomas A. Volpe,[1] Catherine Kidner,[1] Ira M. Hall,[1,2]
Grace Teng,[1,2] Shiv I. S. Grewal,[1*] Robert A. Martienssen[1*]

Eukaryotic heterochromatin is characterized by a high density of repeats and transposons, as well as by modified histones, and influences both gene expression and chromosome segregation. In the fission yeast *Schizosaccharomyces pombe*, we deleted the argonaute, dicer, and RNA-dependent RNA polymerase gene homologs, which encode part of the machinery responsible for RNA interference (RNAi). Deletion results in the aberrant accumulation of complementary transcripts from centromeric heterochromatic repeats. This is accompanied by transcriptional de-repression of transgenes integrated at the centromere, loss of histone H3 lysine-9 methylation, and impairment of centromere function. We propose that double-stranded RNA arising from centromeric repeats targets formation and maintenance of heterochromatin through RNAi.

Pericentromeric chromatin in fission yeast (S. pombe) reveals a role for RNA interference in HC maintenance


h1
β-gal
h2


E
a2
a1
I
h1
h2
Left boundary
1kb
Right
boundary
LTR
tRNA Thr

Gene targeting by homologous recombination in yeast

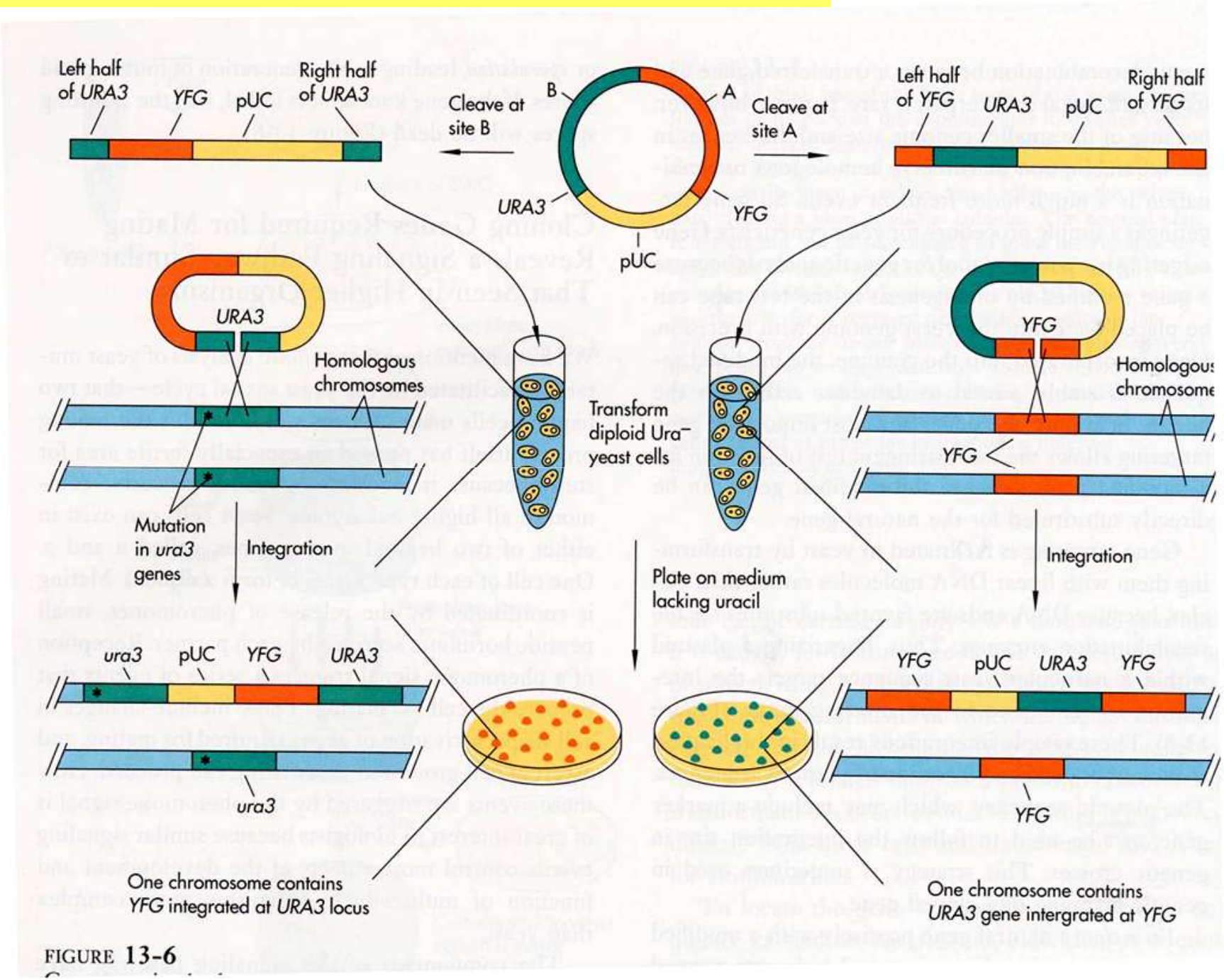
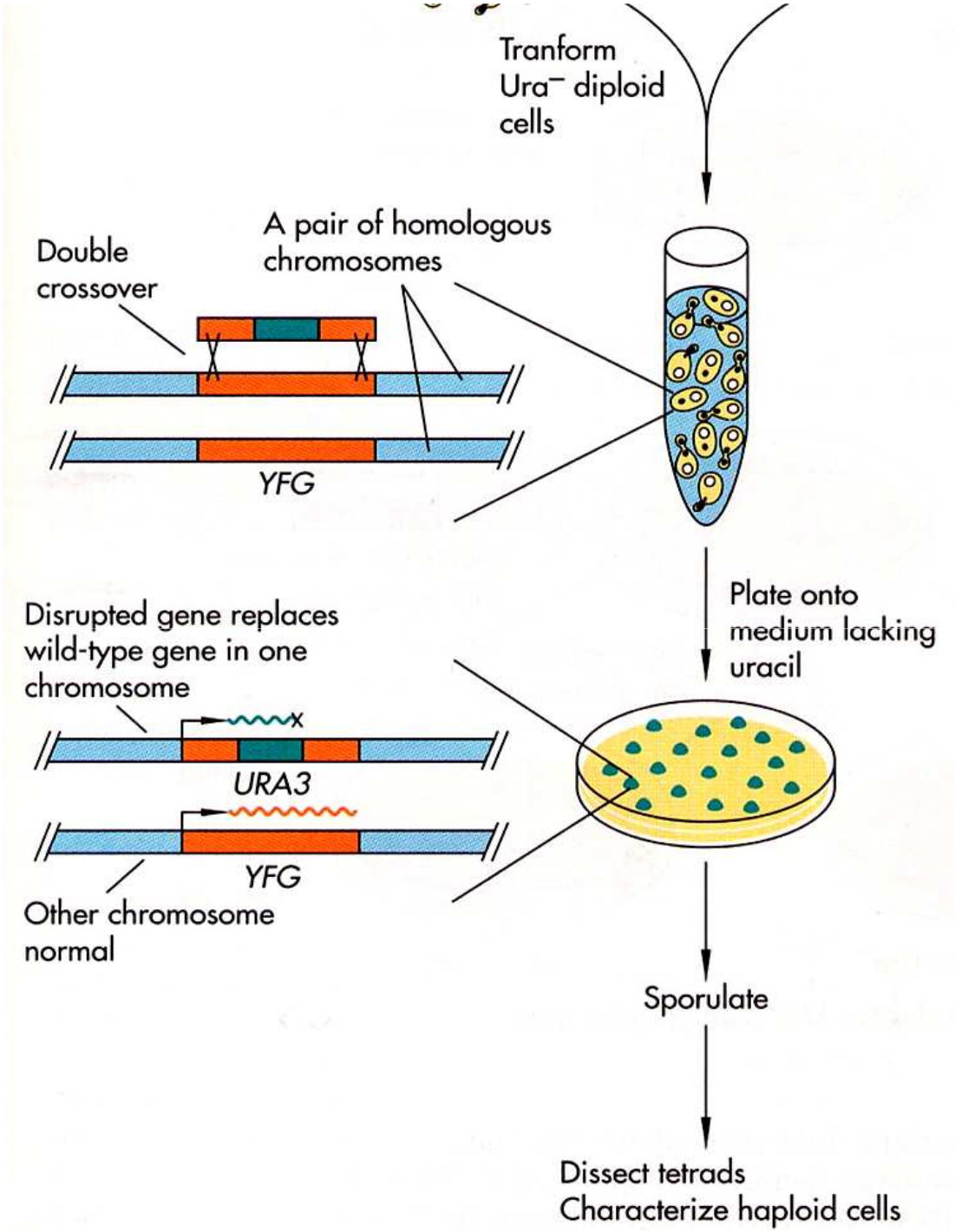


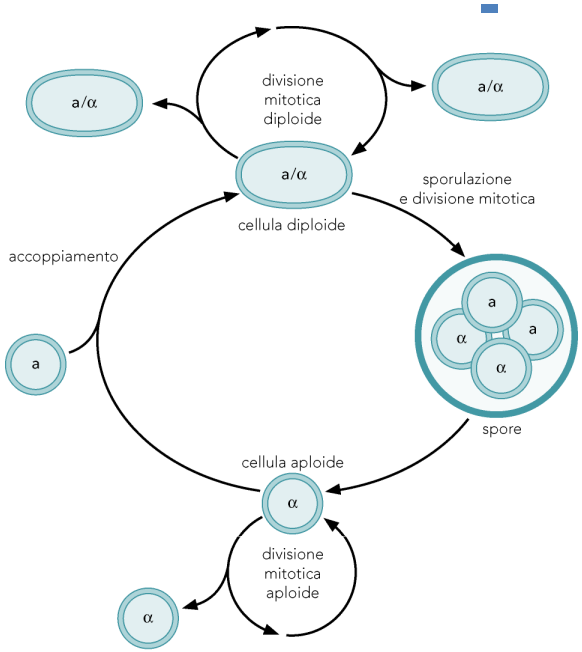
FIGURE 13-6

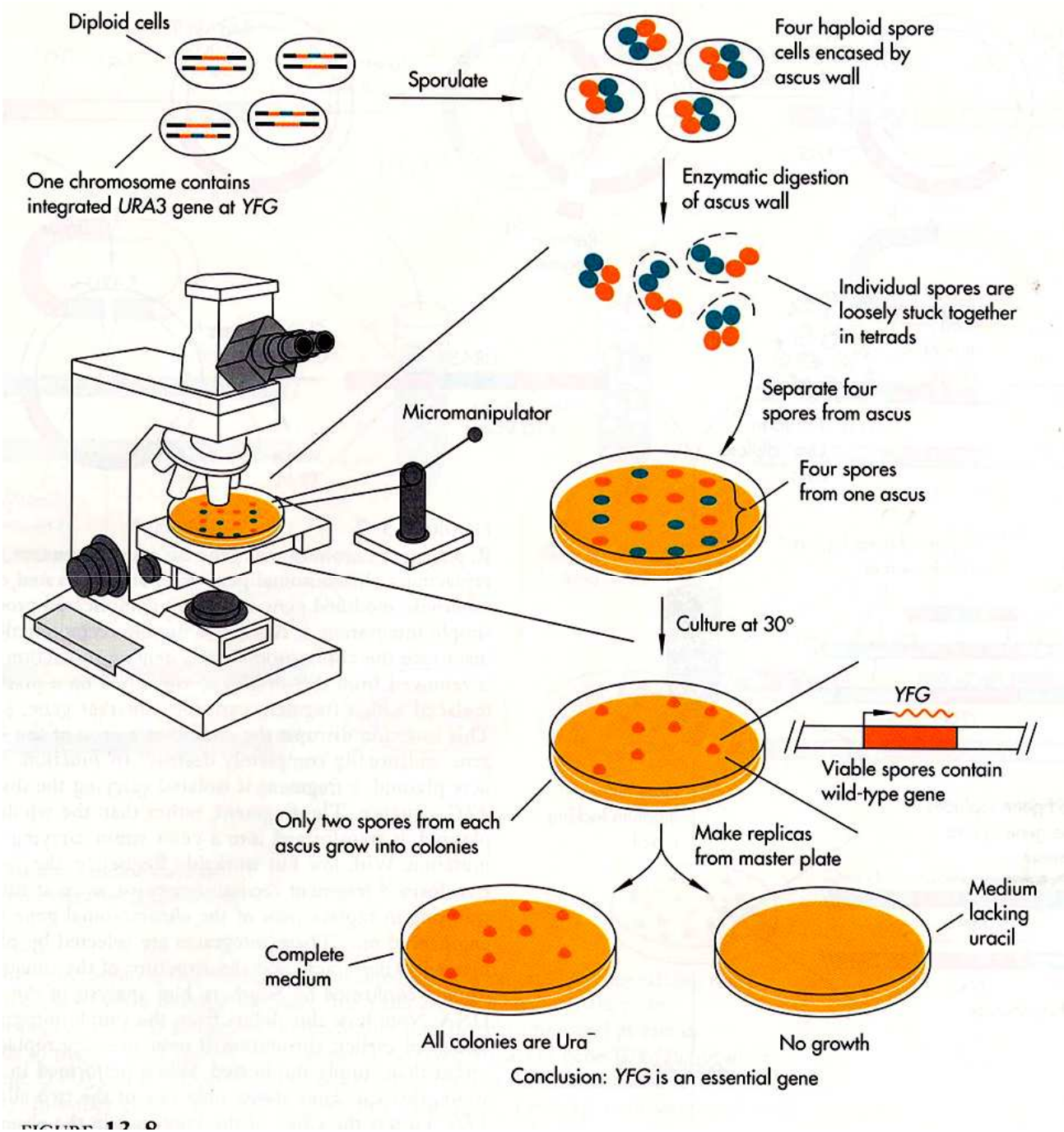


Replacement of a gene with a version of the same gene that lacks important parts or carryes lethal mutations, abolishes the function of the gene.

The gene is now called "knock-out".

Since the yeast is diploid, his genotype will be YFG +/-
If aploid are produced and mutants isolated, a diploid YFG -/- mutants can be isolated.


a/α
divisione
mitotica
diploide
a/α
a/α
cellula diploide
sporulazione
e divisione mitotica
accoppiamento
a
a
α
α
a
spore
cellula aploide
α
divisione
mitotica
aploide
α


Diploid cells
Sporulate
Four haploid spore cells encased by ascus wall
One chromosome contains integrated URA3 gene at YFG
Enzymatic digestion of ascus wall
Individual spores are loosely stuck together in tetrads
Micromanipulator
Separate four spores from ascus
Four spores from one ascus
Culture at 30°
YFG
Viable spores contain wild-type gene
Only two spores from each ascus grow into colonies
Make replicas from master plate
Medium lacking uracil
Complete medium
All colonies are Ura⁻
No growth
Conclusion: YFG is an essential gene

How do small RNAs generated by the RNAi machinery initiate heterochromatin assembly in fission yeast ?

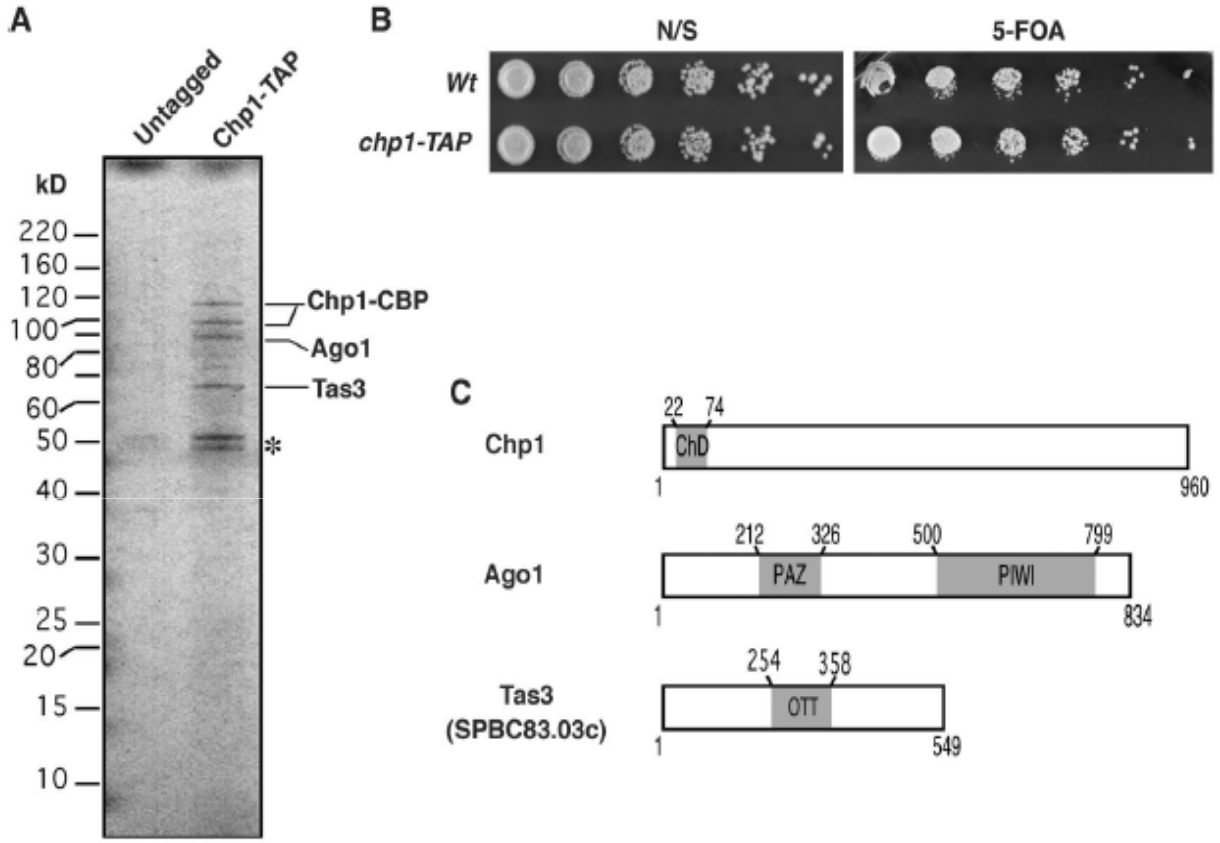
RNAi-Mediated Targeting of Heterochromatin by the RITS Complex

André Verdel,[1] Songtao Jia,[2] Scott Gerber,[1,3] Tomoyasu Sugiyama,[2] Steven Gygi,[1,3] Shiv I. S. Grewal,[2]* Danesh Moazed[1]*

RNA interference (RNAi) is a widespread silencing mechanism that acts at both the posttranscriptional and transcriptional levels. Here, we describe the purification of an RNAi effector complex termed RITS (RNA-induced initiation of transcriptional gene silencing) that is required for heterochromatin assembly in fission yeast. The RITS complex contains Ago1 (the fission yeast *Argonaute* homolog), Chp1 (a heterochromatin-associated chromodomain protein), and Tas3 (a novel protein). In addition, the complex contains small RNAs that require the Dicer ribonuclease for their production. These small RNAs are homologous to centromeric repeats and are required for the localization of RITS to heterochromatic domains. The results suggest a mechanism for the role of the RNAi machinery and small RNAs in targeting of heterochromatin complexes and epigenetic gene silencing at specific chromosomal loci.

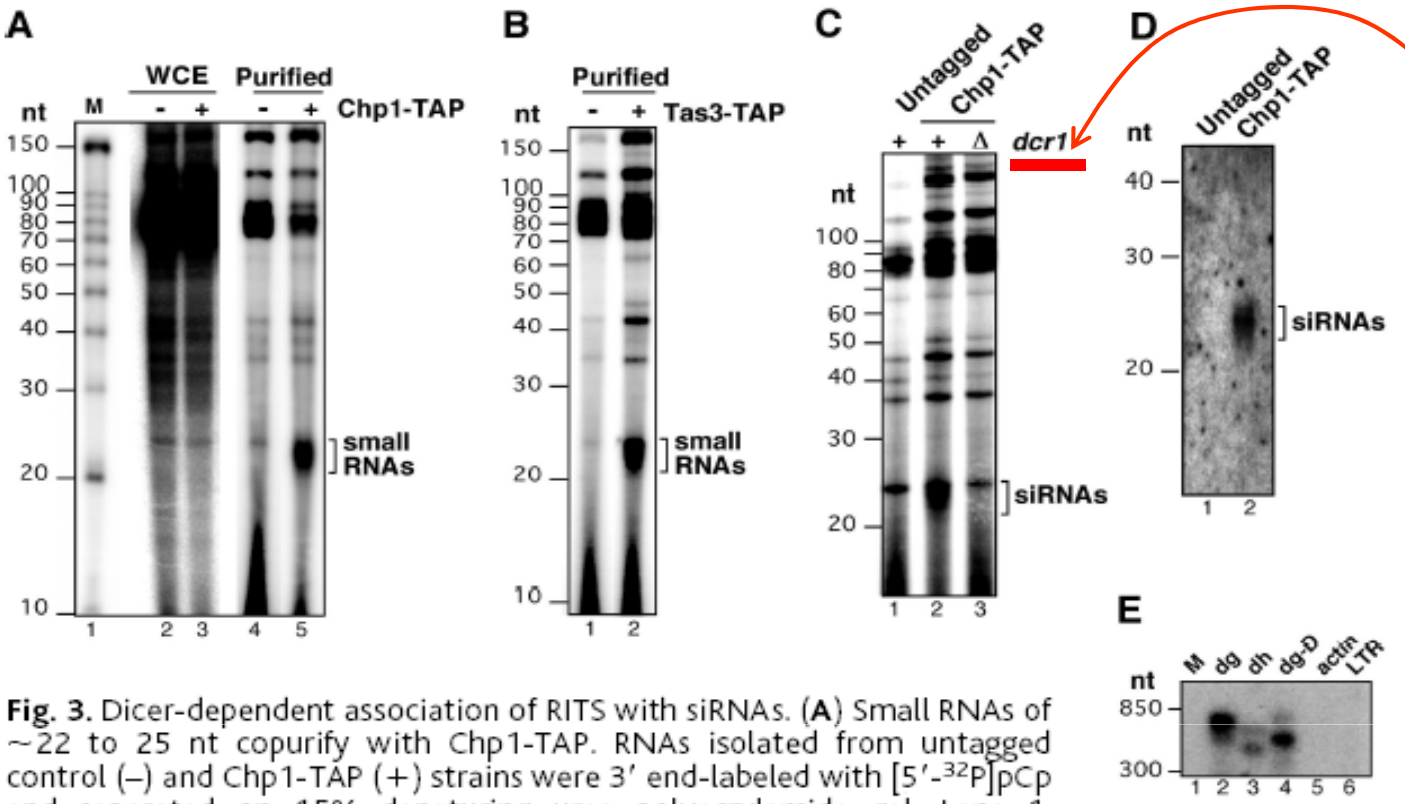
The Chp1 protein binds to centromeric repeats and is required for H3K9 methylation and for Swi6 (hu-HP1 homolog) binding.

Fig. 1. Purification of Chp1-TAP and identification of associated proteins. Extracts from a Chp1-TAP strain and an untagged control strain were purified by the TAP procedure and applied to a 4 to 12% polyacrylamide gel, which was stained with colloidal Coomassie blue (**A**). The bands in the Chp1-TAP purification were excised from the gel and sequenced by tandem mass spectrometry (*22*). The identity of each band is based on multiple sequenced peptides and is indicated on the right. *Residual GST-TEV, the protease used for elution from the first affinity column. (**B**) The Chp1-TAP protein was fully functional for silencing of a centromeric *imr::ura4*$^+$ reporter gene as indicated by wild-type levels of growth on 5-FOA medium, which only allows growth when *ura4*$^+$ is silenced. N/S, nonselective medium. (**C**) Schematic diagram showing the subunits of the RITS complex and their conserved motifs. The chromodomain (ChD) in Chp1, the PAZ and PIWI domains in Ago1, and a region of sequence similarity between Tas3 and the mouse OTT (ovary testis transcribed) protein are indicated.



RITS complex

RNA-induced Initiation of Transcriptional Silencing



Deletion of Dicer abrogate small RNA component of RITS

Fig. 3. Dicer-dependent association of RITS with siRNAs. (**A**) Small RNAs of ~22 to 25 nt copurify with Chp1-TAP. RNAs isolated from untagged control (–) and Chp1-TAP (+) strains were 3′ end-labeled with [5′-^{32}P]pCp and separated on 15% denaturing urea polyacrylamide gel. Lane 1, [γ-^{32}P]ATP–labeled RNA markers (Ambion); lanes 2 and 3, labeling of RNA from whole-cell extract (WCE) (~1/2500 of input); lanes 3 and 4, labeling of RNAs after purification. Bracket on the right side indicates the position of small RNAs specifically associated with Chp1-TAP. (**B**) Copurification of small RNAs with Tas3-TAP. (**C**) No small RNAs are associated with RITS purified from *dcr1*Δ cells. Parallel purifications were performed from an untagged (control, lane 1) strain as well as *chp1-TAP, dcr1*$^{+}$ (lane 2) and *chp1-TAP, dcr1*Δ (lane 3) cells, and the associated RNAs were [5′-^{32}P]pCp labeled (compare lanes 2 and 3, bracket). (**D**) Northern blot showing that siRNAs associated with RITS hybridize to ^{32}P-labeled probes corresponding to centromeric repeat sequences. RNA from untagged control (lane1) and Chp1-TAP cells (lane 2), purified as described in (B), was separated on a denaturing gel and electrotransferred to a nylon membrane (*22*). DNA oligonucleotides with sequence complementary to the 12 heterochromatic siRNAs identified by Reinhart and Bartel (*16*) were 5′ labeled with [γ-^{32}P]ATP and used as probes for the Northern blot. (**E**) Southern blot showing that RITS contains siRNAs complementary to the outer centromeric repeats (*otr*). *dg* (lanes 2 and 4) and *dh* (lane 3) repeats, *actin* (lane 5), and LTRs (lane 6) were amplified by polymerase chain reaction (PCR) from genomic DNA, separated on 1.1% agarose gel, and transferred to nylon membrane. ^{32}P-labeled RITS siRNAs, obtained by labeling RNAs as described in (A), were separated on a denaturing urea gel, eluted, and used as probes for the blot.

Localization of RITS to heterochromatin

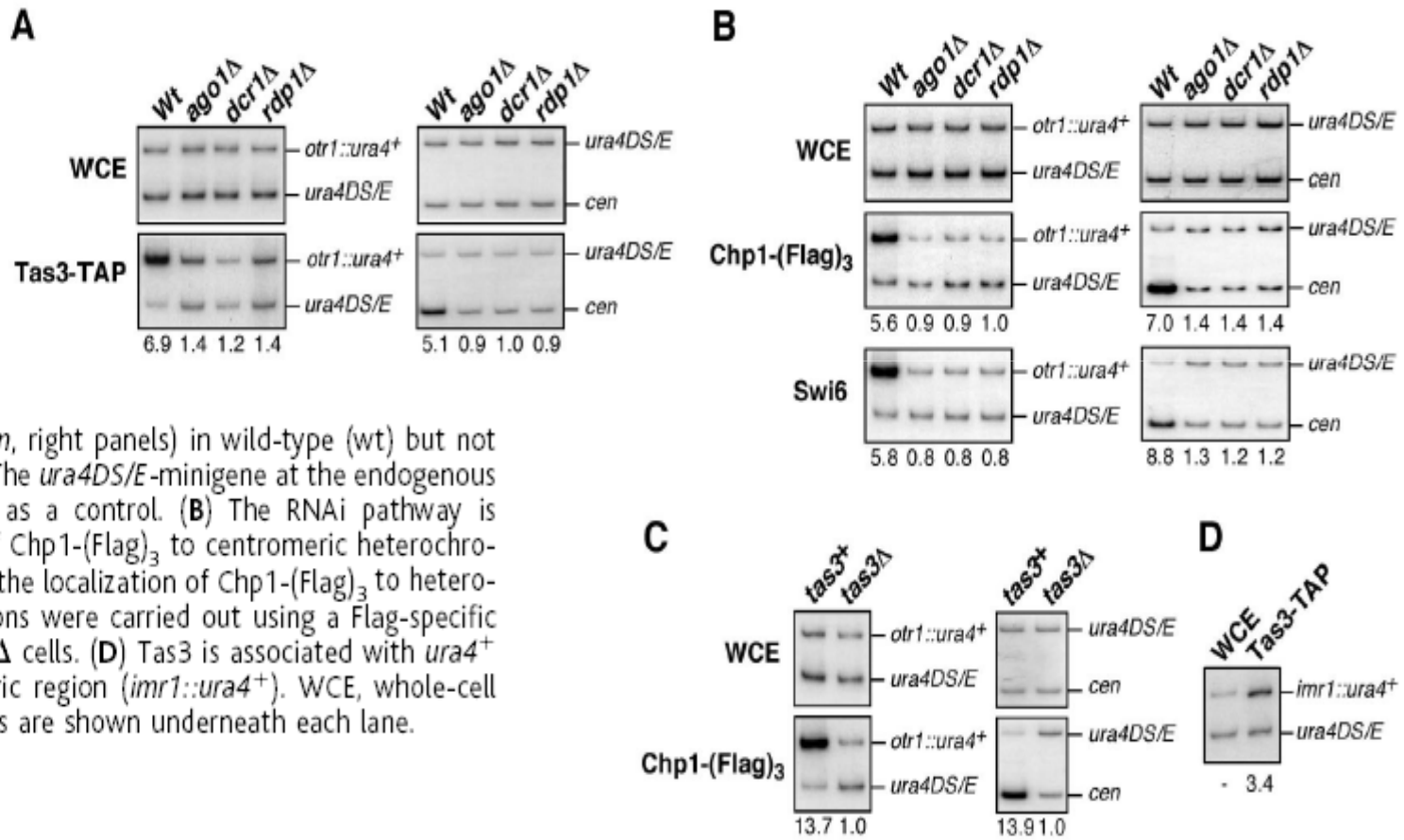


Fig. 4. The RNAi pathway is required for localization of RITS to heterochromatin. (**A**) ChIP experiments showing that Tas3-TAP is localized to centromeric heterochromatin in an RNAi-dependent manner. Tas3-TAP is associated with *ura4*$^+$ inserted at the *otr* centromeric repeats (*otr1::ura4*$^+$, left panels) and with native centromeric repeat sequences (*cen*, right panels) in wild-type (wt) but not *ago1*Δ, *dcr1*Δ, or *rdp1*Δ cells. The *ura4DS/E*-minigene at the endogenous euchromatic location is used as a control. (**B**) The RNAi pathway is required for the localization of Chp1-(Flag)$_3$ to centromeric heterochromatin. (**C**) Tas3 is required for the localization of Chp1-(Flag)$_3$ to heterochromatin. Immunoprecipitations were carried out using a Flag-specific antibody from *tas3*$^+$ and *tas3*Δ cells. (**D**) Tas3 is associated with *ura4*$^+$ inserted at the *imr* centromeric region (*imr1::ura4*$^+$). WCE, whole-cell extract. Fold enrichment values are shown underneath each lane.

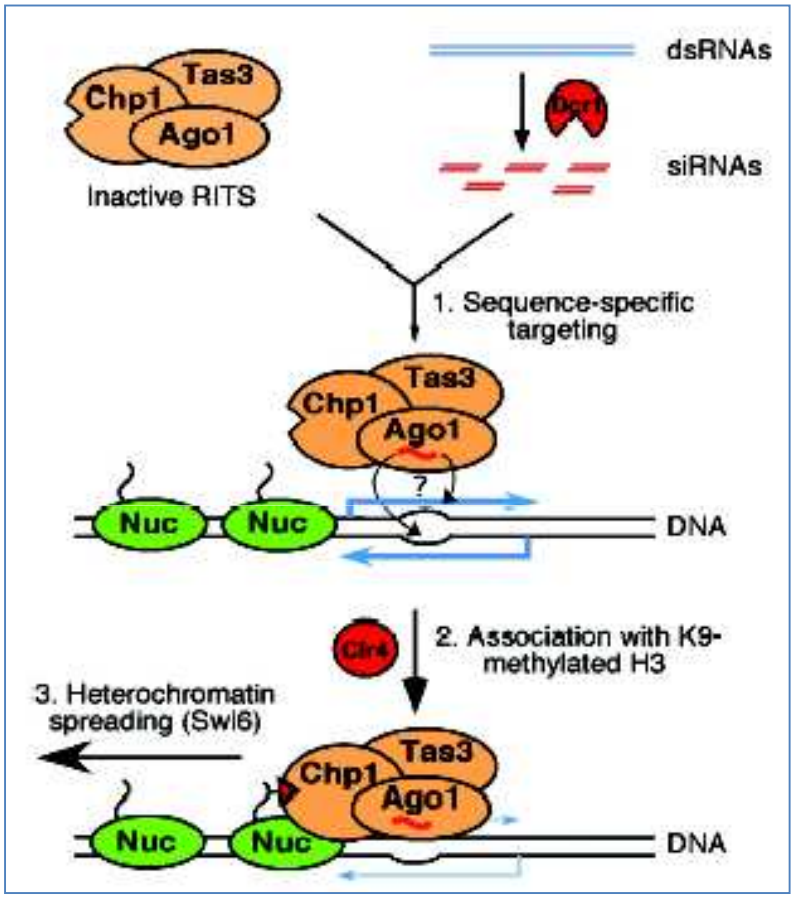


Fig. 5. A model for siRNA-dependent initiation of heterochromatin assembly by RITS. The RITS complex is programmed by Dcr1-produced siRNAs to target specific chromosome regions by sequence-specific interactions involving either siRNA-DNA or siRNA-nascent transcript (blue arrows) base pairing. Nuc, nucleosome; red triangle, K9-methylation on the amino terminus of histone H3. See text for further discussion and references.

Today state-of-art

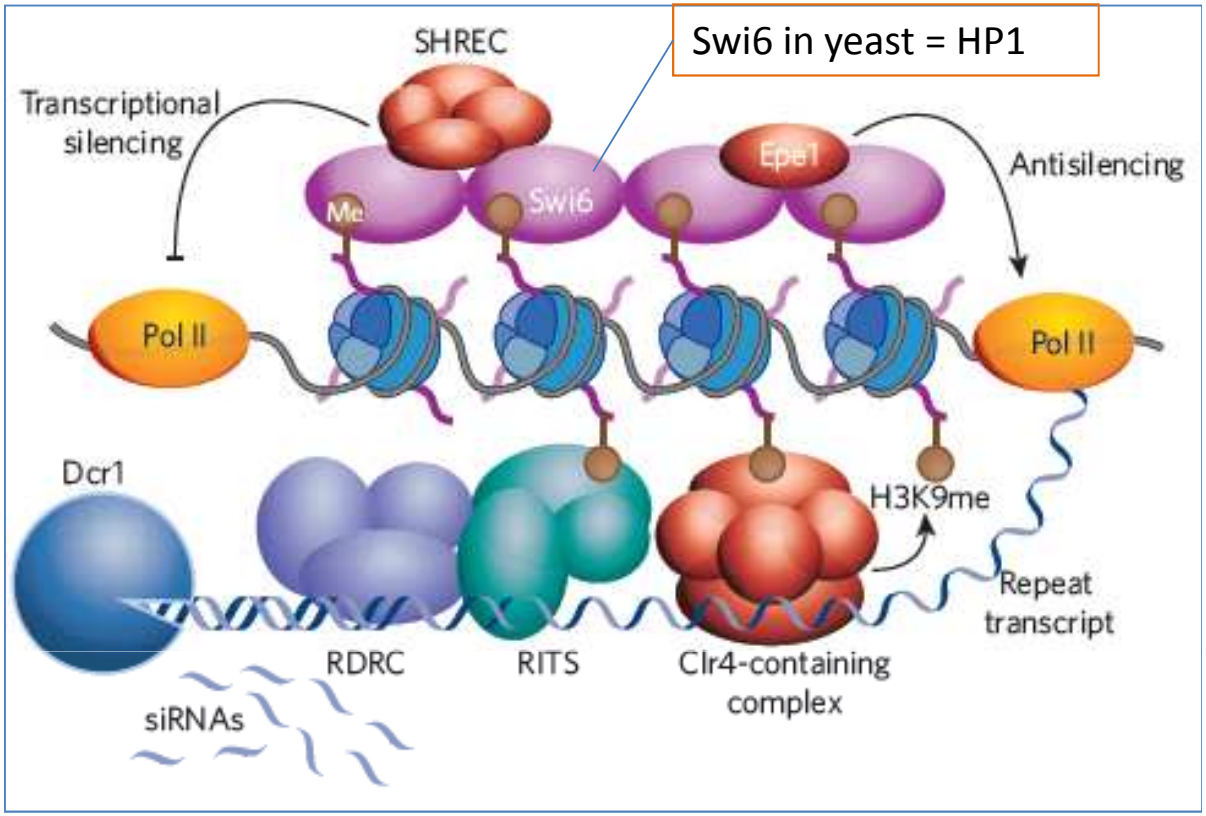
Interaction of RITS with several chromatin-modifying enzymes (Histone Methyl Transferases HMT, DNA Methyl Transferases DMT) was demonstrated using both co-immunoprecipitation experiments and by showing binding of RITS together with HMTs and DMTs to the same regions of DNA, by chromatin immunoprecipitation (ChIP). A second complex containing the H3K9 methyltransferase Clr4 was also found to be recuited to nascent RNA. All these proteins apperently co-exist at the same location.

How do we know this?

(Re-ChIP insert)

In S. pombe, the RdRP enzyme (called RDRC) (not present in higher animals) is also localized to the centromere, thus suggesting a further amplification mechanism for centromeric siRNA.

Figure 4 | Model showing RNAi-mediated heterochromatin assembly and silencing in **S. pombe**. Centromeric repeat (dg and dh) transcripts produced by Pol II are processed by the RNAi machinery, including the complexes RITS and RDRC (which interact with each other and localize across heterochromatic regions).
The slicer activity of Ago1 (a component of RITS) and the RNA-directed RNA polymerase activity of Rdp1 (a component of RDRC) are required for processing the repeat transcripts into siRNAs. The siRNA-guided cleavage of nascent transcripts by Ago1 might make these transcripts preferential substrates for Rdp1 to generate double-stranded RNA, which in turn is processed into siRNAs by Dcr1.



The targeting of histone-modifying effectors, including the Clr4-containing complex, is thought to be mediated by siRNAs. This process most probably involves the base-pairing of siRNAs with nascent transcripts, but the precise mechanism remains undefined. siRNAs produced by heterochromatin-bound RNAi 'factories' might also prime the assembly of RISC-like complexes capable of mounting a classic RNAi response. Methylation of H3K9 by Clr4 is necessary for the stable association of RITS with heterochromatic loci, apparently through binding to the chromo-domain of Chp1. This methylation event also recruits Swi6, which, together with other factors, mediates the spreading of various effectors, such as SHREC. SHREC might facilitate the proper positioning of nucleosomes to organize the higher-order chromatin structure that is essential for the diverse functions of heterochromatin, including transcriptional gene silencing. Swi6 also recruits an antisilencing protein, Epe1, that modulates hetero-chromatin to facilitate the transcription of repeat elements, in addition to other functions. A dynamic balance between silencing and antisilencing activities determines the expression state of a locus within a heterochromatic domain.

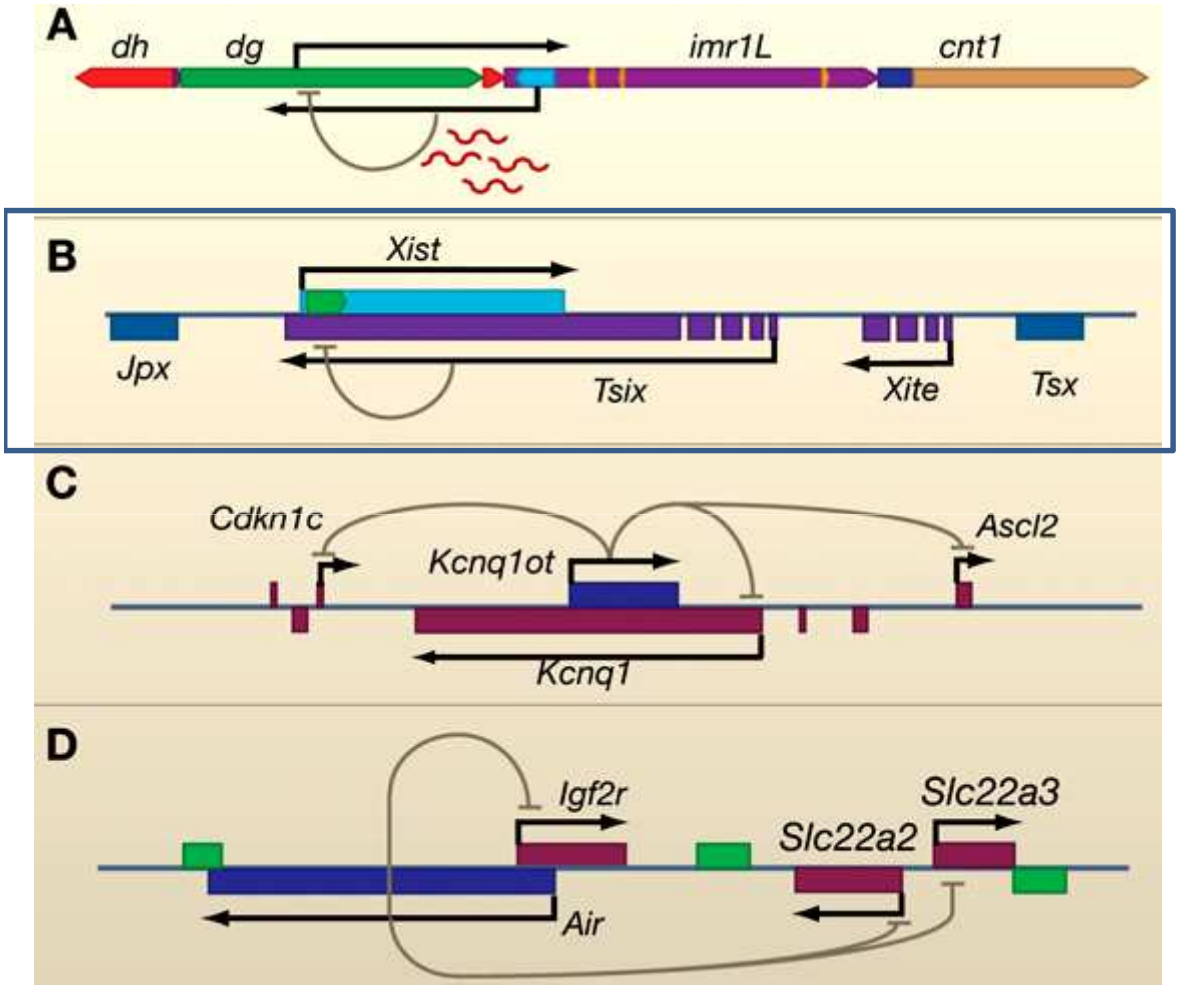
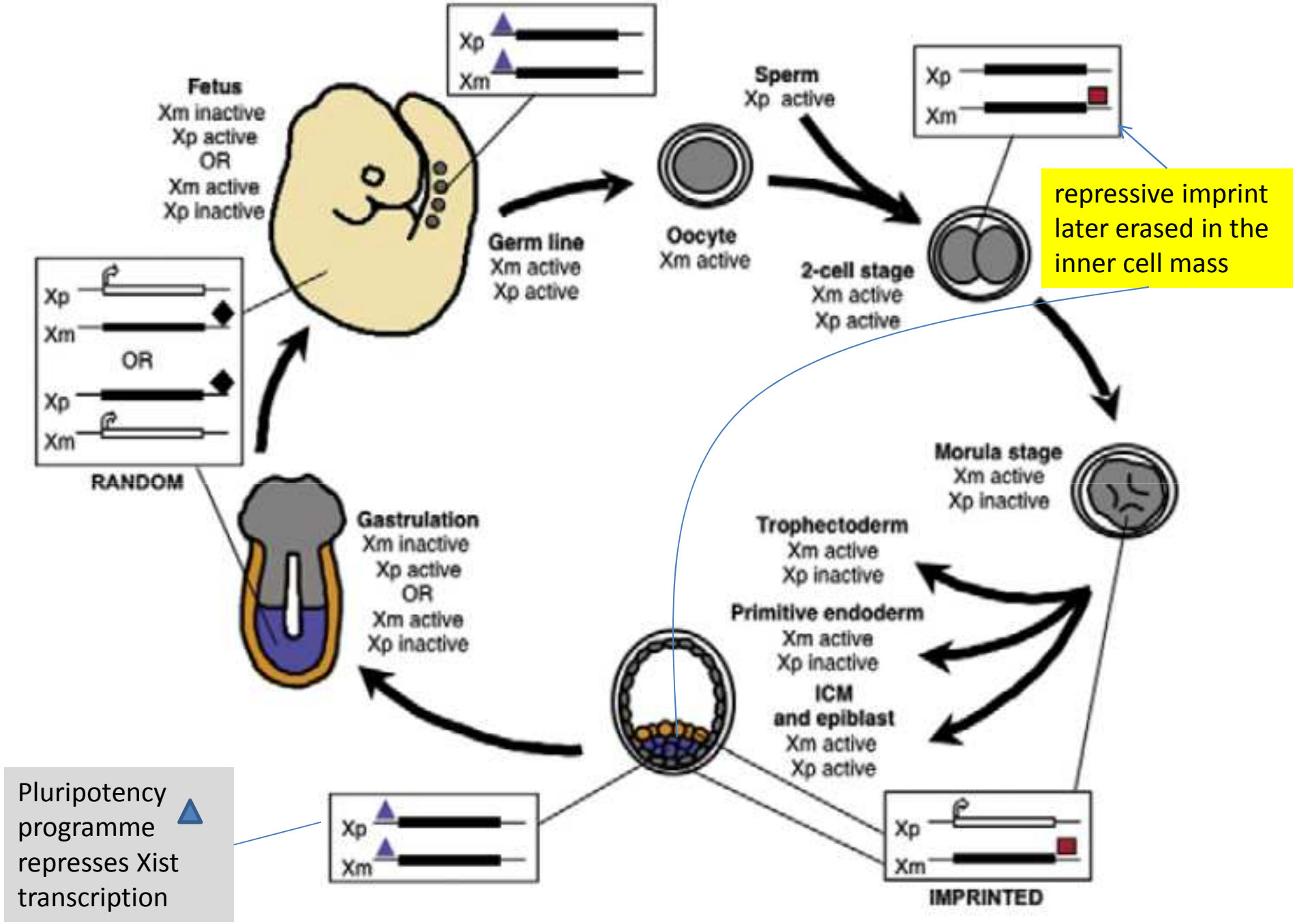


Figure 3. Silencing Transcripts at the S. pombe Centromere and Different Transcript-Mediated Silencing Systems

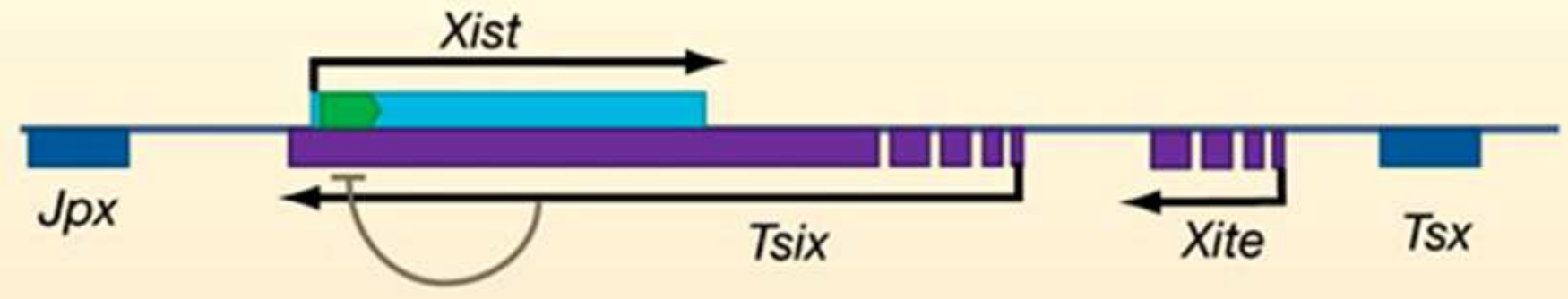
(A) S. pombe dg-imr transcript in centromere IL. The forward promoter is silenced by constitutive transcription and processing into siRNA of the reverse strand.

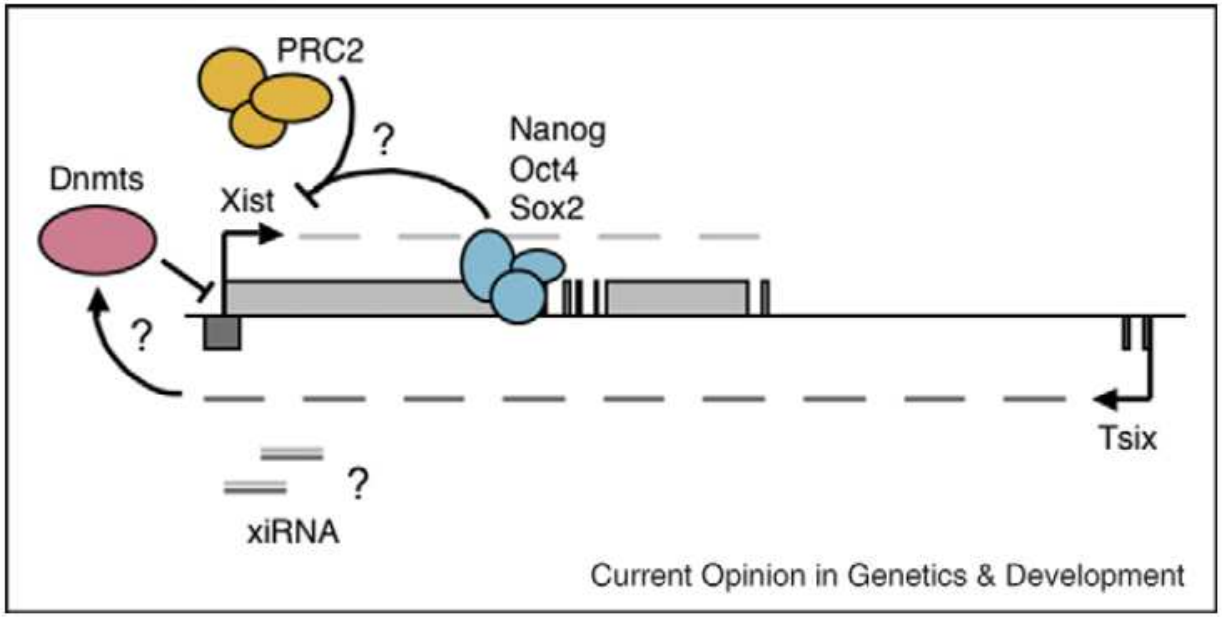
(B) **The X inactivation center has several noncoding transcripts, and transcription of Tsix silences the Xist promoter.**

(C and D) Paternal locus transcription of noncoding transcripts Kcnq1ot and Air influences the expression of overlapping and nonoverlapping
genes in the imprinted gene cluster at the telomeric end of mouse chromosome 7 and the Igf2r locus, respectively. Paternally expressed genes are colored blue, maternally expressed genes are colored red, and ubiquitously expressed genes are colored green.



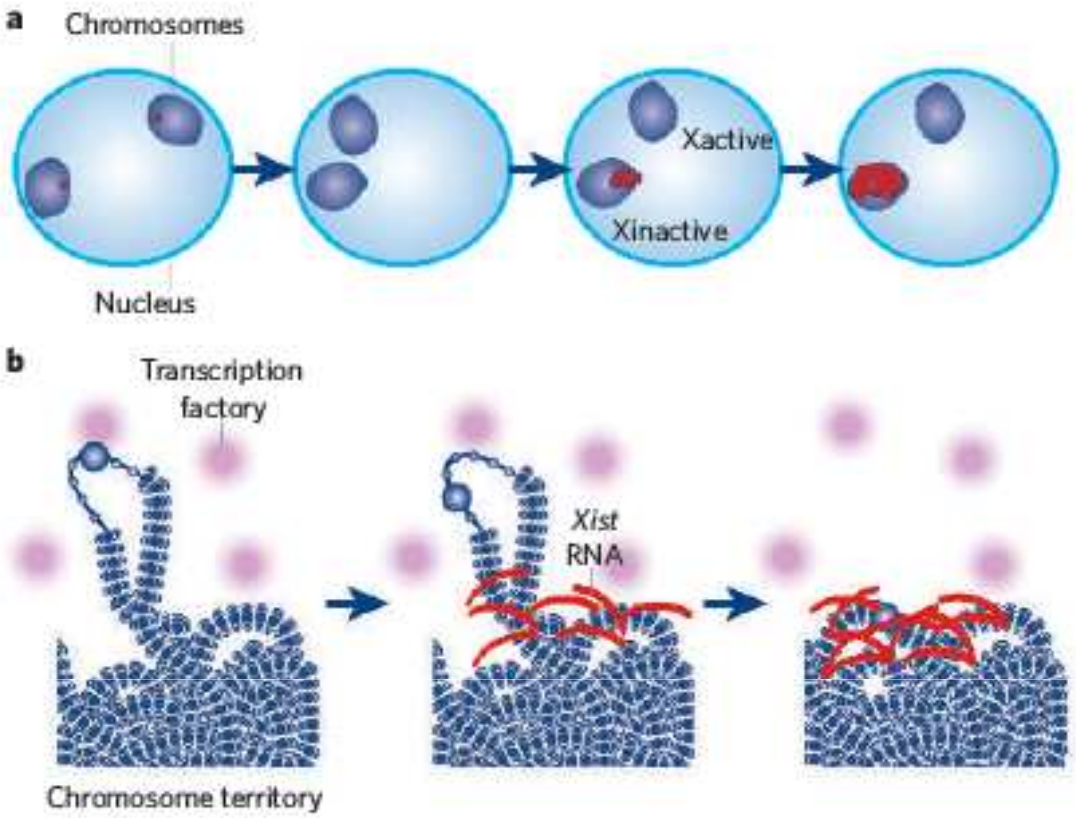
Senner, 2009, 19:122–126 Current Opinion in Genetics & Development


Xist
Jpx
Tsix
Xite
Tsx



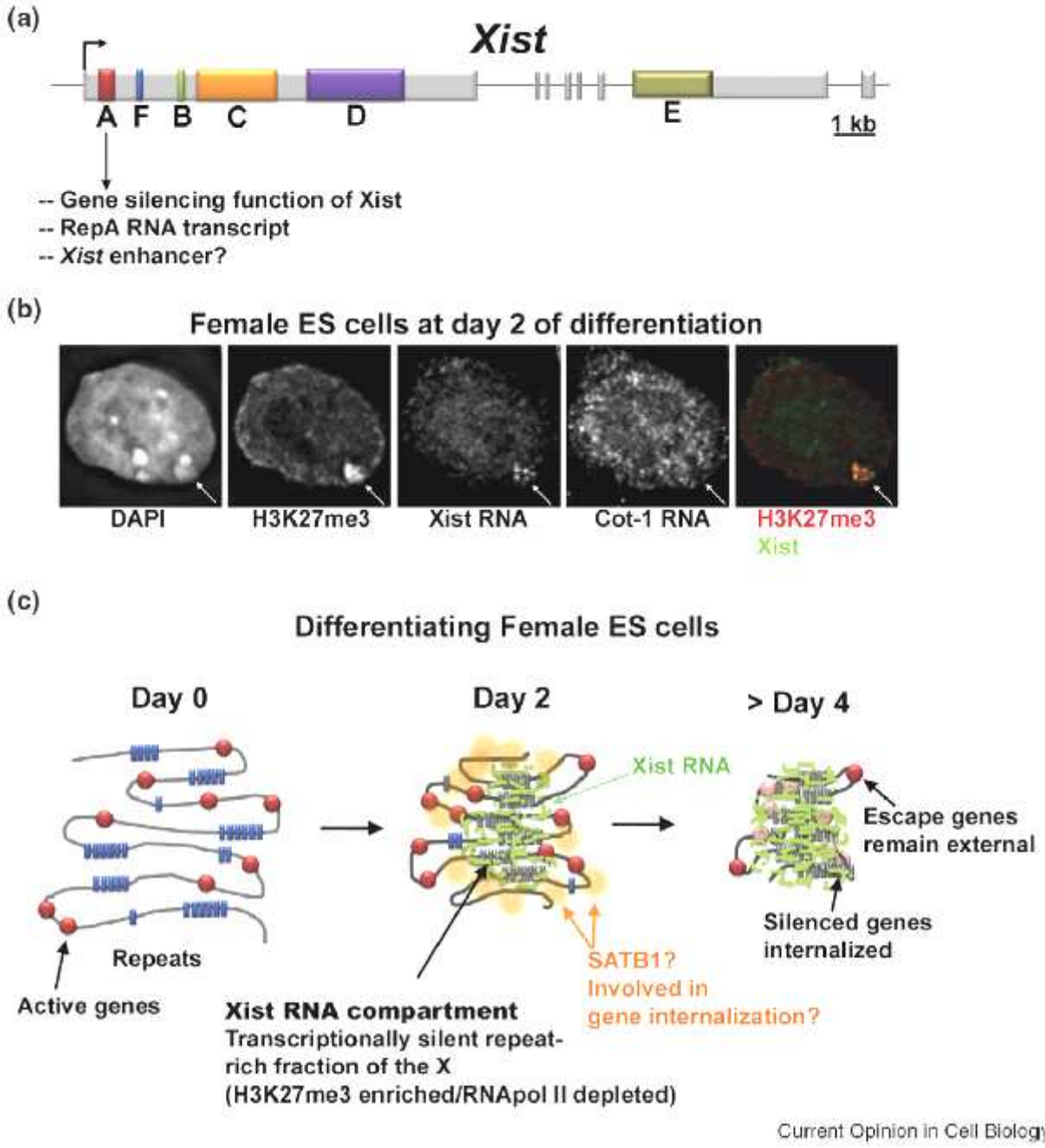
Factors involved in regulation of the Xist promoter. *Xist* (light grey) is negatively regulated by its antisense transcript *Tsix* (dark grey). The balance of sense and antisense transcription across the promoter influences methylation of the *Xist* promoter by Dnmts (pink), through an as yet uncharacterised mechanism. It has been reported that *Xist* and *Tsix* RNAs form duplexes that are processed by RNAi enzyme Dicer to small xiRNAs. Whether or not xiRNAs regulate *Xist* expression is subject to debate. A *Tsix*-independent pathway also negatively regulates *Xist*. The pluripotency factors Nanog Oct4 and Sox2 (blue) have been shown to repress *Xist* and this is suggested to result from a direct interaction between binding sites for these factors in intron 1 of the gene and the *Xist* promoter. Polycomb repressor complex 2 (PRC2) (orange) has been shown to repress transcription from the *Xist* promoter in the absence of *Tsix* transcription, indicating that it may contribute to the *Tsix*-independent pathway.

From: Senner, Current Opinion in Genetics & Development 2009, 19:122–126 REVIEW



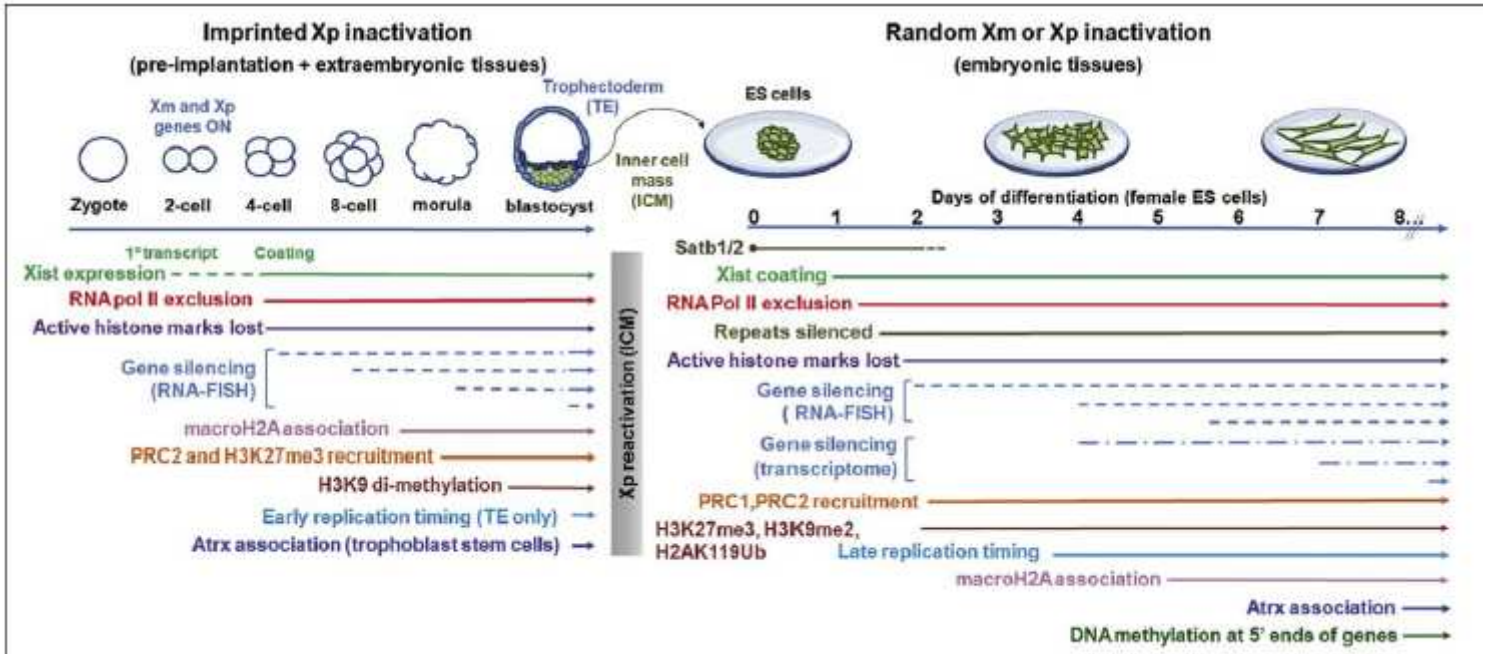
from Frasor, 2007

Figure 1 | Events of nuclear reorganization during X-chromosome inactivation. **a**, Soon after female embryonic stem cells start to differentiate, the two X chromosomes (purple) come together in the nucleus, and the X-inactivation centres, which initiate X-chromosome inactivation, interact[9,10]. These events occur concomitantly with the process of X-chromosome counting and choice[38] and lead to upregulation of *Xist* transcription (red) from the future inactive X chromosome (Xinactive). **b**, The coating of the inactive X chromosome by *Xist* RNA molecules excludes Pol II and the transcriptional machinery (pink) from the inactive X-chromosome territory[22]. Genes initially located outside the domain (purple circles) coated by *Xist* RNA are retracted back inside the *Xist* compartment as they become silenced through a mechanism dependent on the A repeats of *Xist* RNA[22].



(a) Structure of the Xist gene with the conserved repeat regions labeled A–F. The A region (red) denotes the conserved A-repeat region essential for gene silencing.
(b) Combined RNA-immuno-fluorescence on day 2 differentiated female ES cells, showing the Xist-coated transcriptionally silent compartment which is enriched for H3K27me3.
(c) Model. Xist coating induces the formation of a transcriptionally silent repetitive compartment. As genes are silenced they are recruited into this compartment. A possible mediator for this internalization may be the matrix-associated protein SATB1/2.

from Chow, Current Opinion in Cell Biology 2009, 21:359–366
REVIEW



Xi Feature	Factors involved	Reference
RNA polymerase II exclusion	Xist RNA coating (A-repeat independent); repetitive elements?	[25]
Loss of active histone marks (H3 and H4 acetylation; H3K4me2/3)	Xist RNA coating (A-repeat independent); Histone deacetylases? histone demethylases? histone exchange, proteolysis...?	Reviewed in [62]
PRC2 recruitment	Xist RNA coating (A-repeat independent) + ? RepA RNA?	[7,34,55]
HeK27me3	Ezh2 (PRC2)	[63]
H3K9me2	?	
PRC1 recruitment	Xist RNA (A-repeat independent) + ? PRC2/ H3K27me3	[35,64-66]
Cbx7 (mammalian Polycomb protein homolog—PRC1 component)	H3K27me3 + RNA + ?	[67]
H2AK119Ub	Ring1a/b (PRC1)	Reviewed in [63]
H4K20me1	PrSet7 (?)	[7]
Gene silencing	Xist RNA (A-repeat dependent); relocation into silent compartment?	[25,52]
Macro H2A association	Xist RNA (A-repeat independent) + downstream heterochromatin changes/factors?	[31,68-70]
Late replication timing	heterochromatin changes?	
Nucleolar localization	Xist RNA + downstream heterochromatin changes/factors?	[30]
Smchd1 localization	Xist RNA + downstream heterochromatin changes/factors?	[40]
DNA methylation at CpG islands	Smchd1? Dnmt3a/3b (de novo MTases)? Dnmt1 (maintenance MTase)	[40,71]
Satb1	?	[57]
Atrx	Xist RNA + downstream heterochromatin changes/factors?	[50]

What about DICER ?

RNAi in X inactivation: contrasting findings on the role of interference

*Satya K. Kota**

Institute of Molecular Genetics, CNRS, UMR5535, 1919 Route de Mende, 34293 Montpelier Cedex 5, France

X inactivation is the process that brings about the dosage equivalence of X-linked genes in females to that of males. This complex process initiated at a very early stage of female embryonic development is orchestrated by long non-coding RNAs transcribed in both sense and antisense orientation. Recent studies present contradicting evidence for the role of small RNAs and RNase III enzyme Dicer in the X inactivation process. In this review, I discuss these results in the overall perspective of X inactivation and gene silencing.

reported in plants and *Drosophila*. Up to now, unlike the case for lower eukaryotes, in mammals there is no direct evidence linking RNAi machinery proteins and small RNA to the establishment or maintenance of either constitutive or facultative heterochromatin. It has long been speculated that several non-coding RNAs in both the sense and antisense orientations transcribed from the mammalian X inactivation centre (XIC) might be substrates for RNAi machinery to initiate the heterochromatin on one of the X chromosomes.

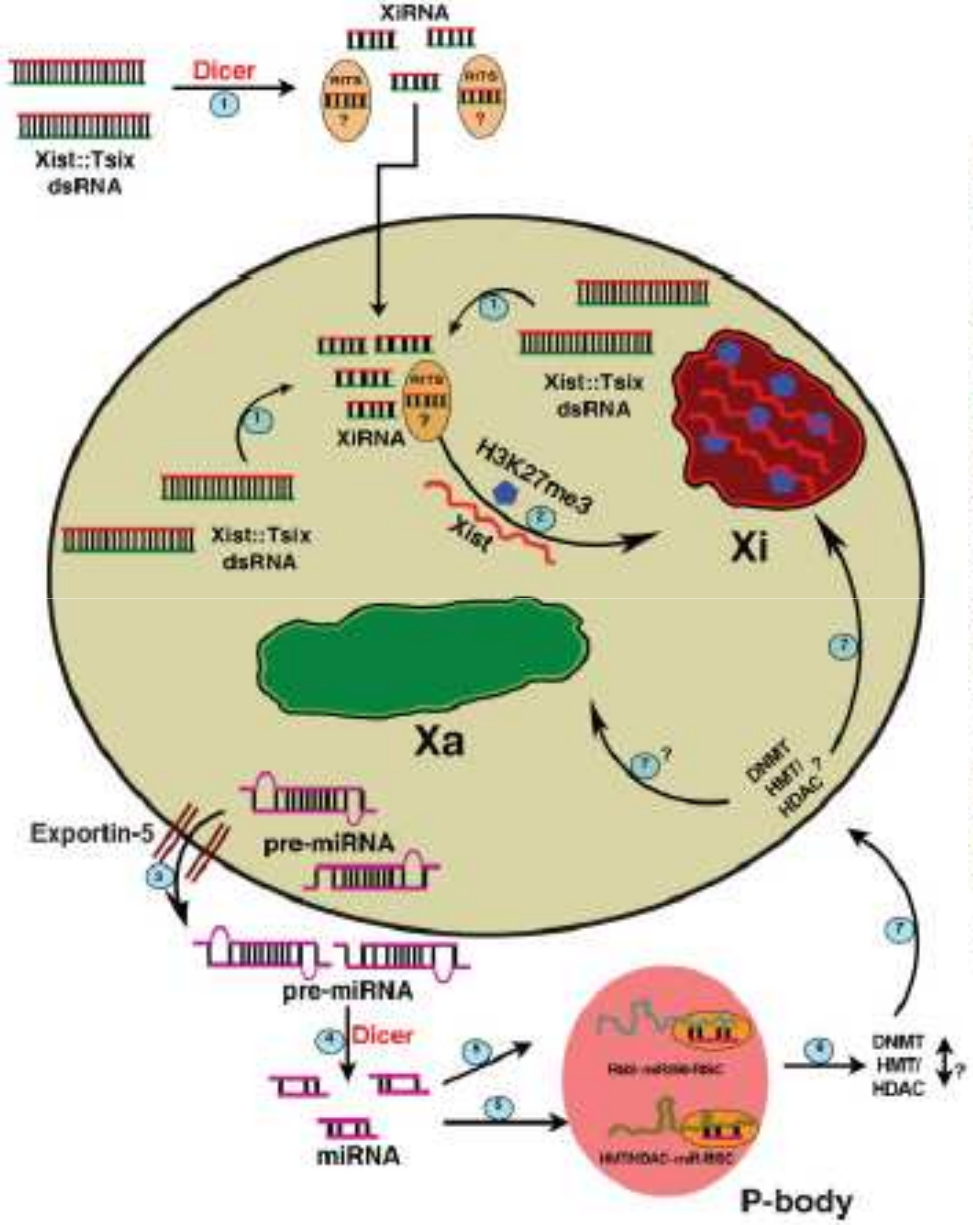


Figure 1. RNase III enzyme Dicer can have either direct or indirect effects on X inactivation. Direct: Xist and Tsix form dsRNA; these Xist::Tsix duplexes are (**1**) processed by Dicer either in the nucleus or in the cytoplasm into small 24–42-nucleotide xiRNA. xiRNA alone or in complex with proteins similar to RITS (RNA-induced transcriptional silencing) of yeast that contain Argonaute-1 and/or Argonaute-2 proteins can mediate (**2**) the localization of Xist and histone methyl transferases like EZH2 to inactive X chromosome (Xi). Indirect: Precursor microRNA (pre-miRNA) transcribed and processed by the Drosha-containing microprocessor complex within the nucleus are (**3**) exported into cytoplasm *via* Exportin-5. These pre-miRNAs are (**4**) processed into mature 21–23-nucleotide miRNA by Dicer in the cytoplasm. **5:** These miRNAs like the miR-290 cluster or other miRNAs can regulate the protein levels of Dnmt transcriptional repressor proteins like Rbl-2 (retinoblastoma-like-2) and heterochromatin-modulating proteins like histone methyl transferases (HMTase) and histone deacetylases (HDAC) or their regulators *via* post-transcriptional repression that localize to processing bodies (P-body). **6** and **7:** Altered levels of Dnmts and/or HMTase and HDACs due to impaired miRNA biogenesis in the absence of Dicer can differentially regulate the inactive X chromosome and its heterochromatic modifications (? indicates that experimental proof is needed).