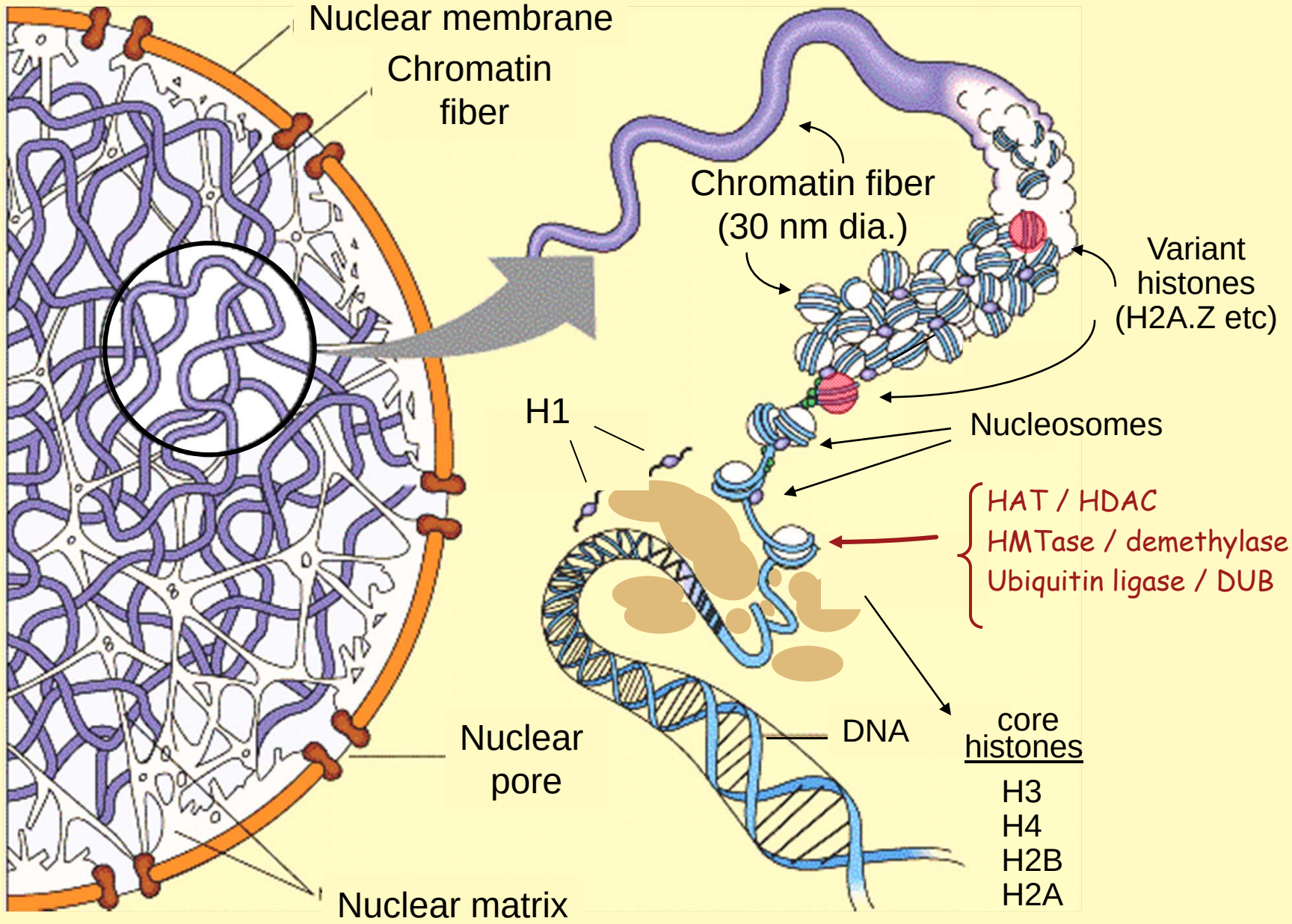
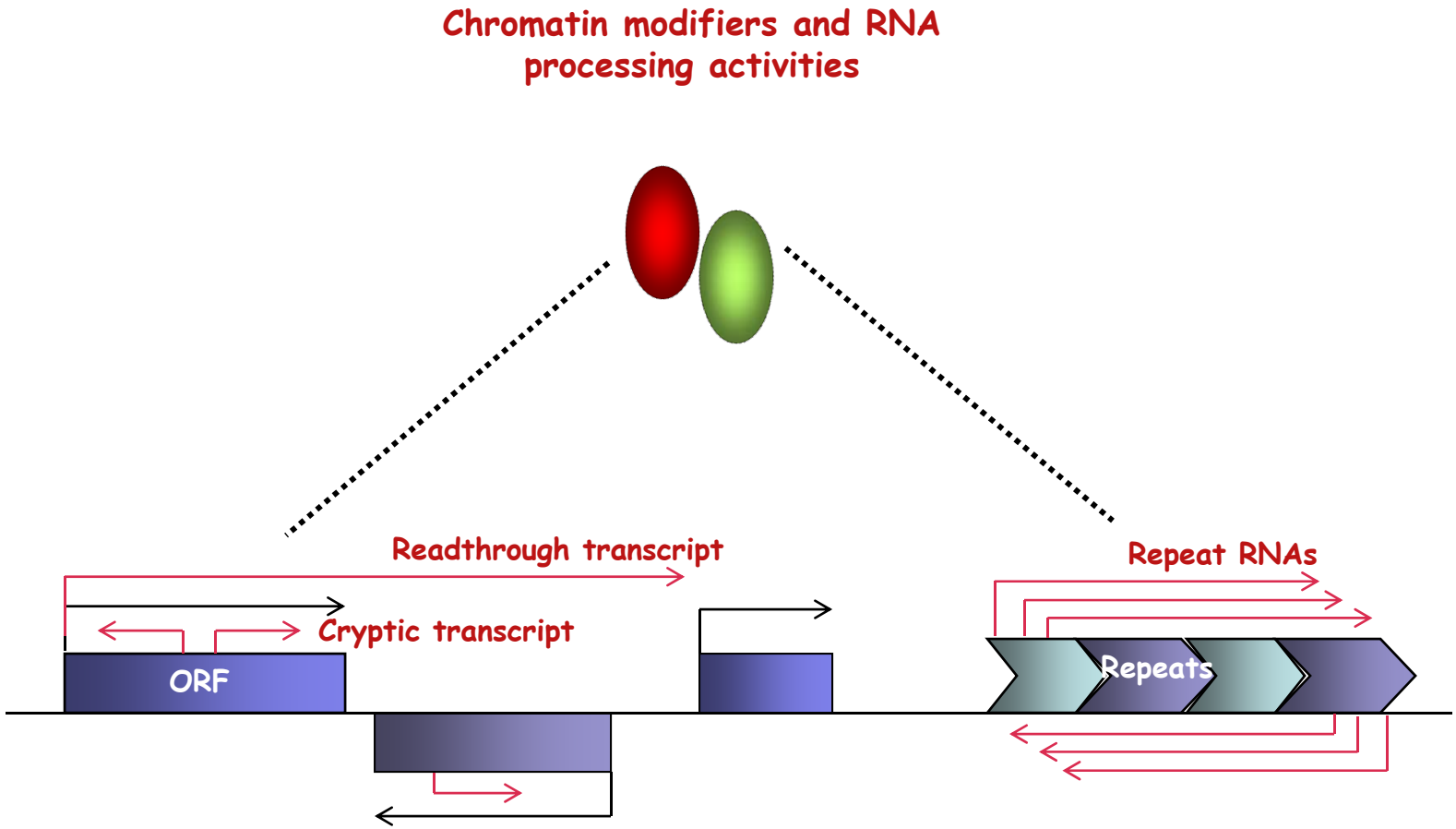
Epigenetic genome control by RNAi and transposon-derived proteins

Shiv Grewal, Ph.D.
Center for Cancer Research
National Cancer Institute

Distinct levels of chromatin organization

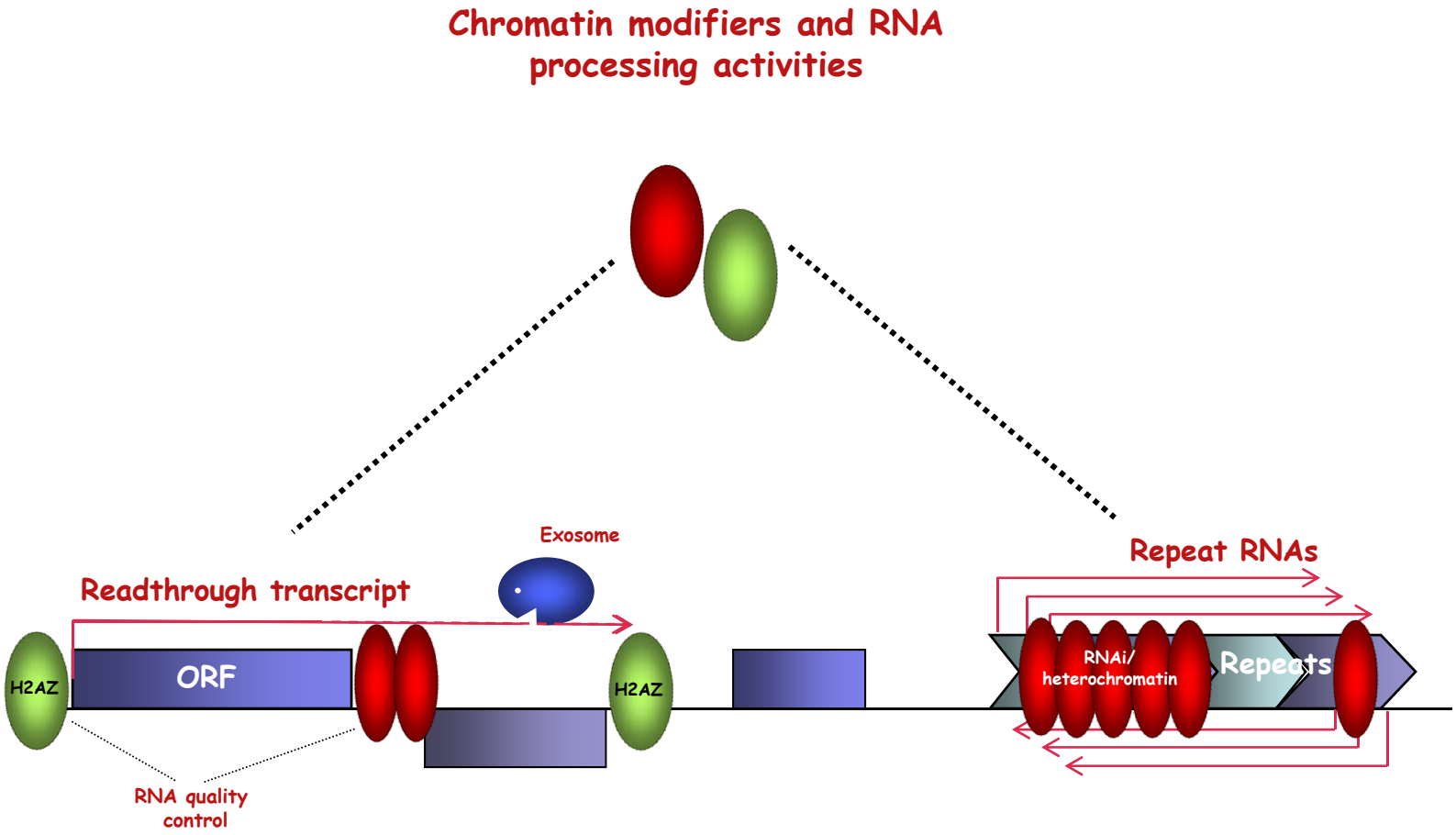


Chromatin modifiers and RNA processing factors suppress transcriptional "noise" across genome



Accumulation of aberrant RNAs can lead to genomic instability

RNAi and heterochromatin factors cooperate with a variant histone H2A.Z to suppress antisense RNAs



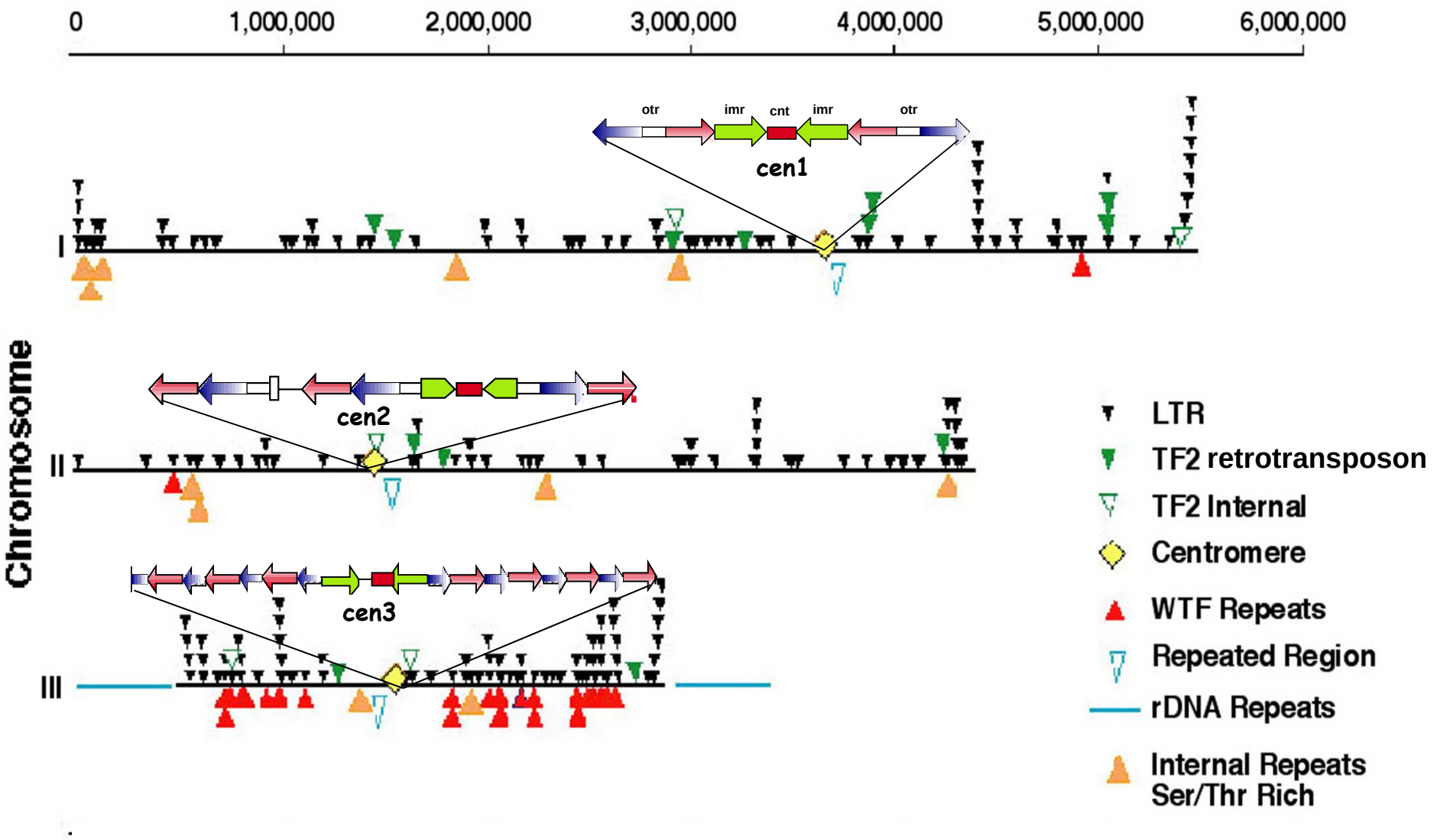
Accumulation of aberrant RNAs can lead to genomic instability

Topics:

- Silencing of retrotransposons and repeat elements by RNAi and chromatin-modifying factors

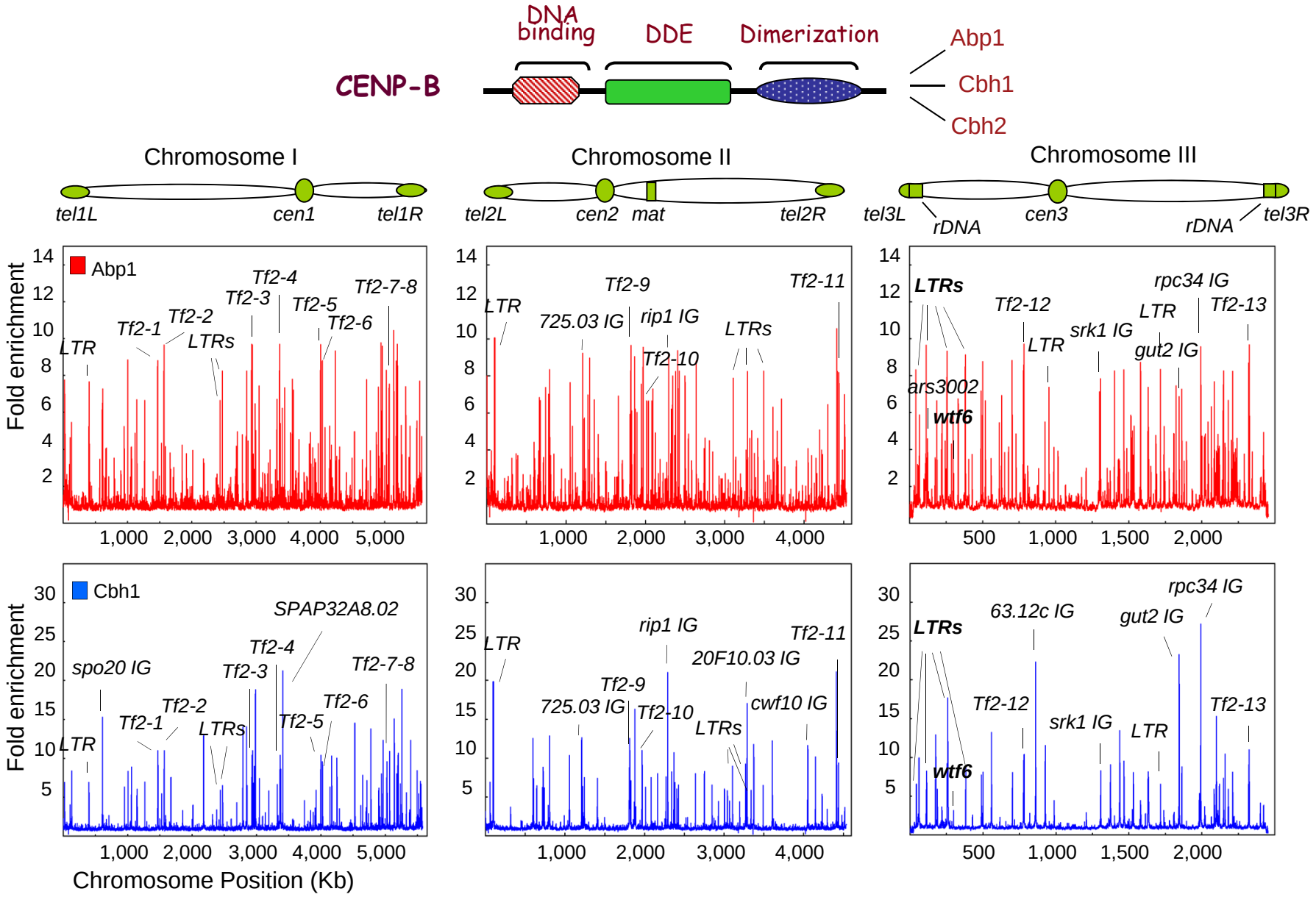
- Genome-wide suppression of antisense RNAs by a variant histone and the RNAi machinery

S. pombe genome contains several classes of repeat elements that are assembled in repressive chromatin

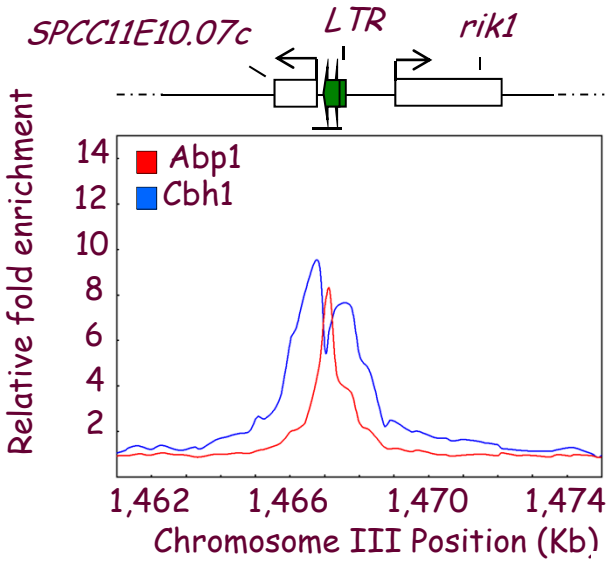


All retroelements are bound by transposase-derived CENP-B proteins (Cam et al Nature 2008)

CENP-Bs localize to retrotransposons and their remnants in the S. pombe genome

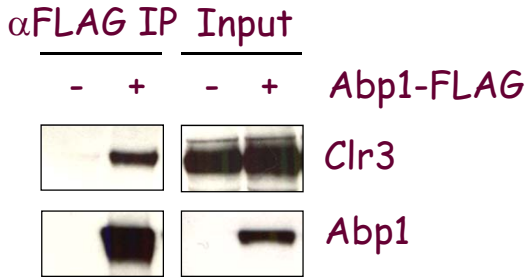


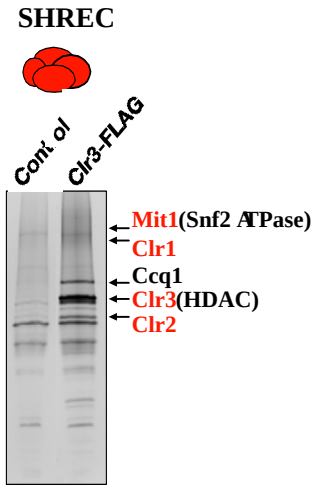
CENP-Bs recruit Clr3 and Clr6 histone deacetylases to repress Tf2 retroelements

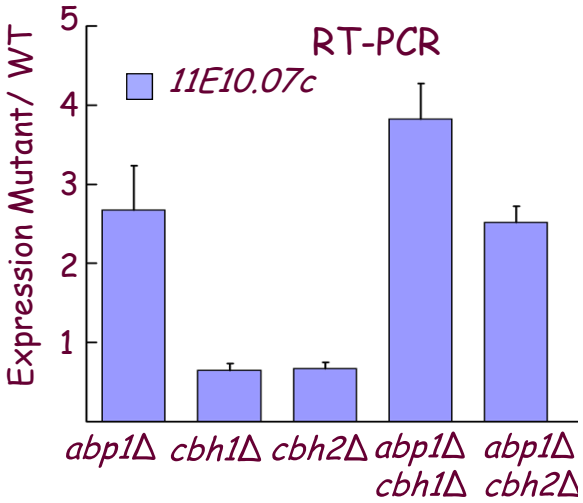


IP Western

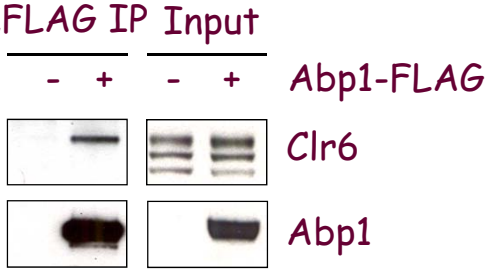
Clr3 = SHREC

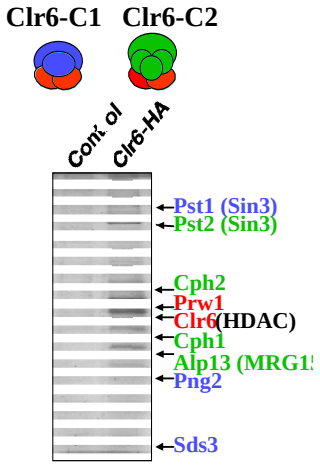




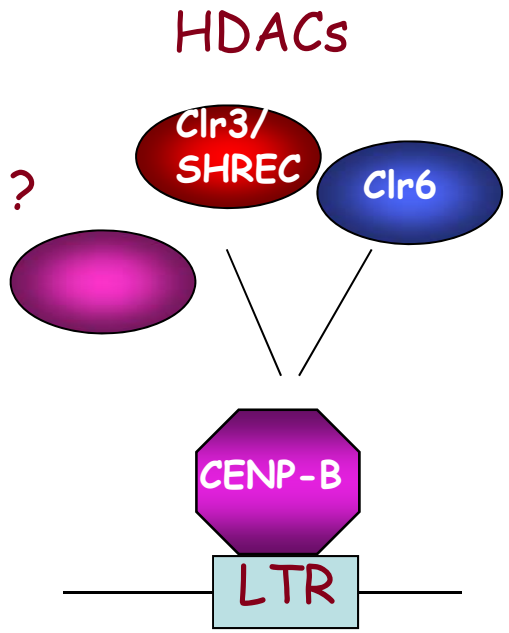


Clr6 HDAC





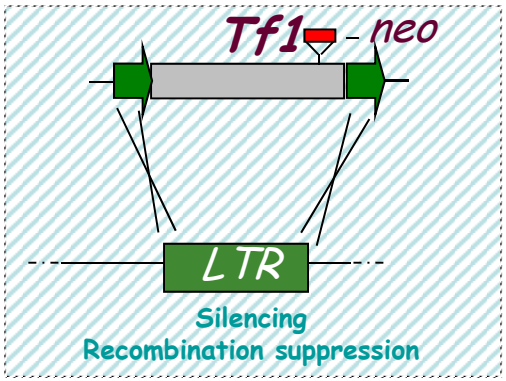
CENP-Bs recruit SHREC and Clr6 histone deacetylases to repress Tf2 retroelements

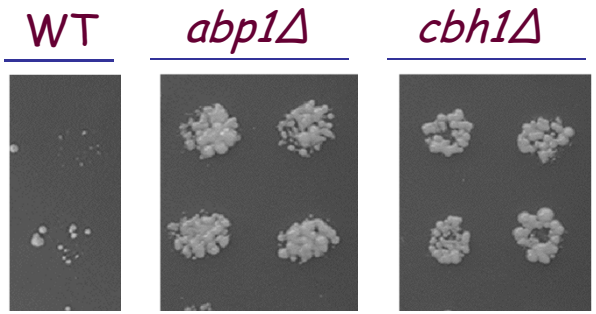


Transcriptional and recombinational suppression

CENP-Bs and their associated HDACs protect integrity of the genome

Tf1-neo expression





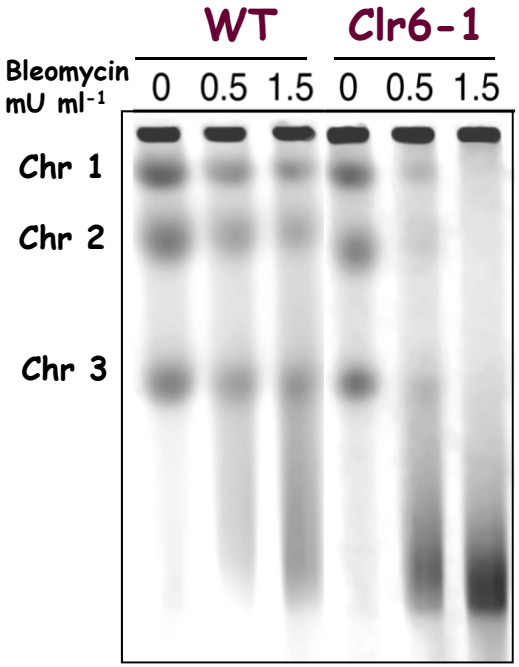
neo^R colonies

CENP-B proteins → Clr3 & Clr6 HDACs, other effectors

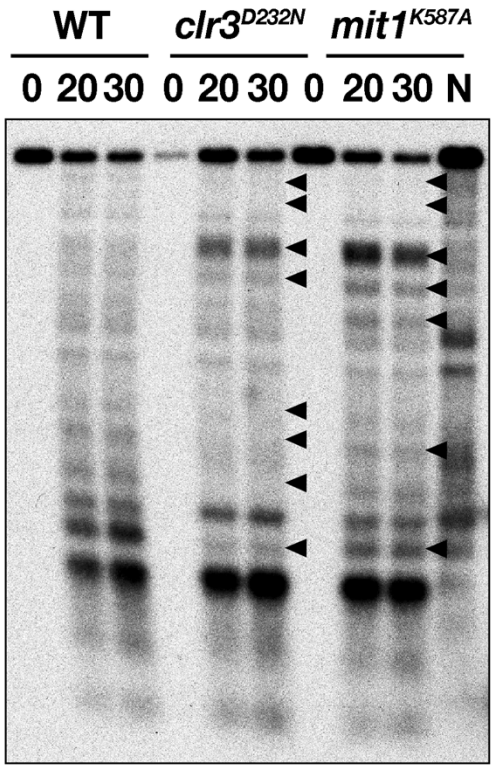
Gene regulatory networks

Maintenance of genome stability

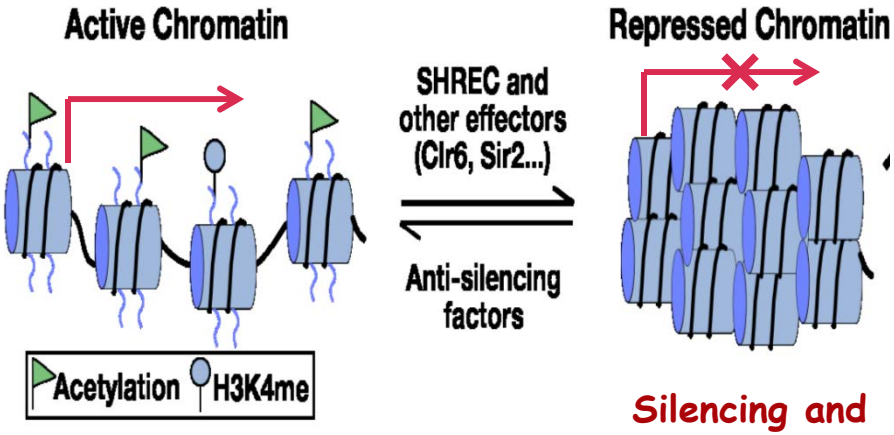
Pulse-field Electrophoresis



SHREC activities facilitate positioning of nucleosomes to suppress transcriptional "noise"

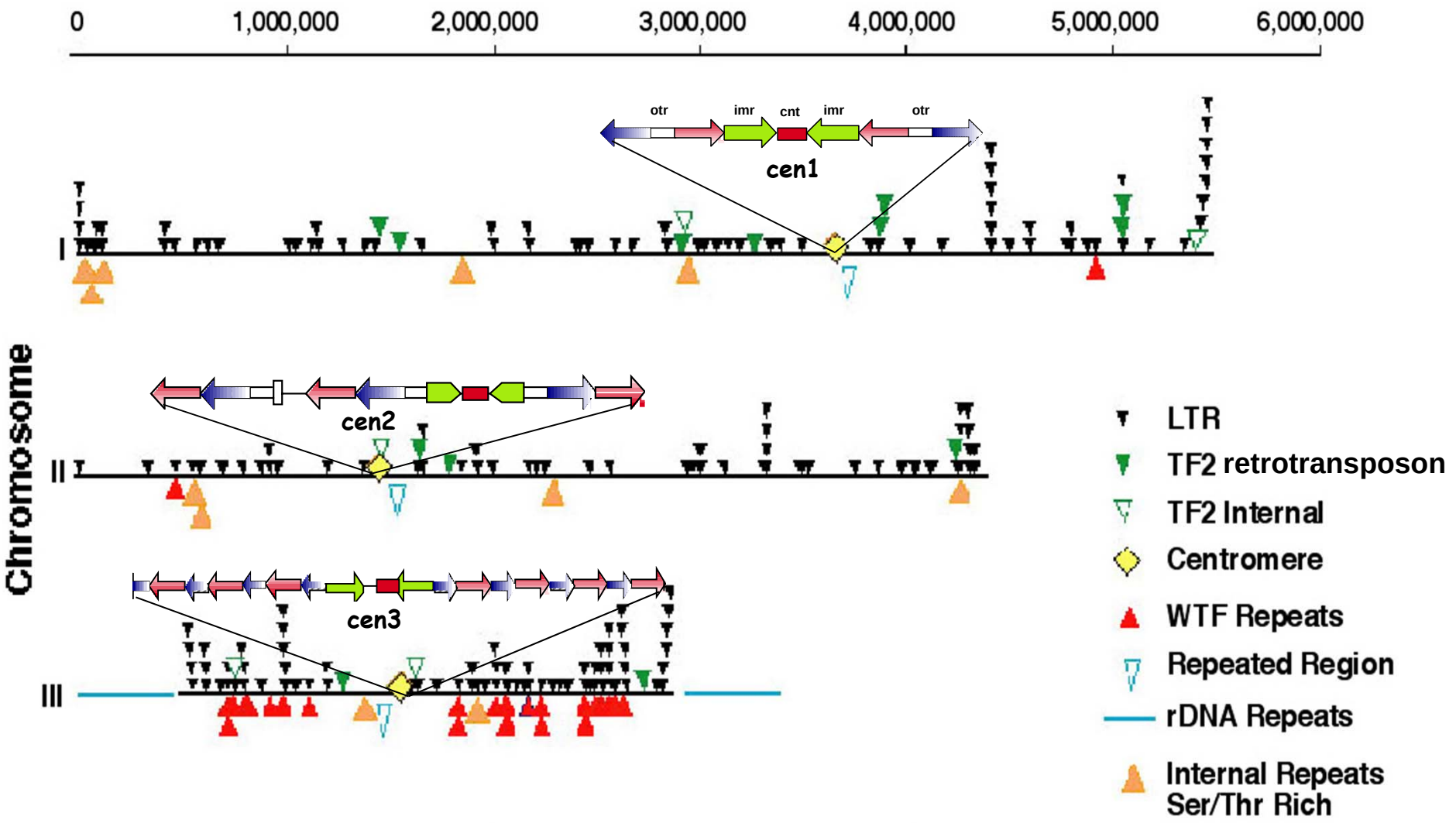


Micrococcal nuclease digestion patterns

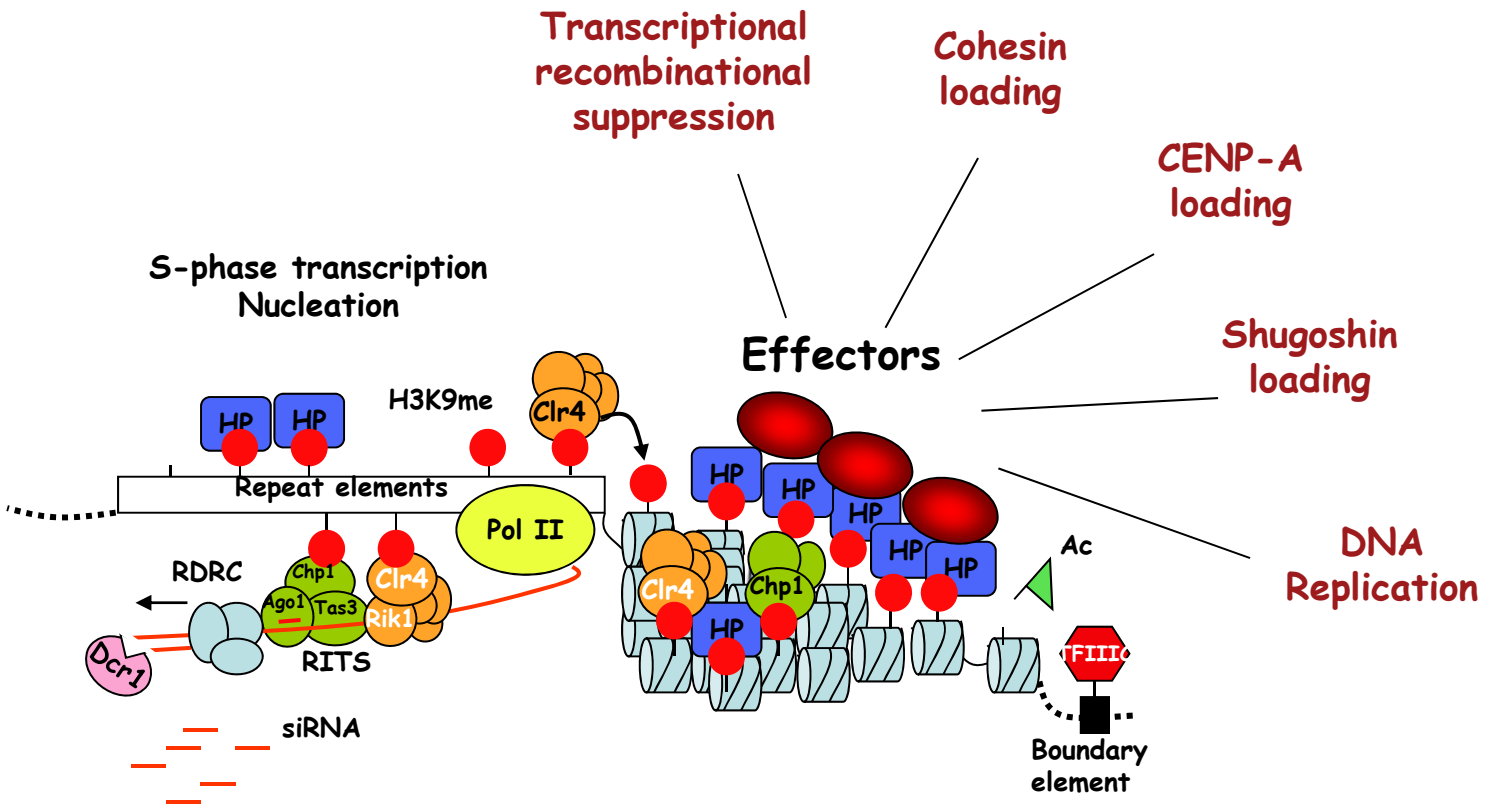


Silencing and recombination suppression

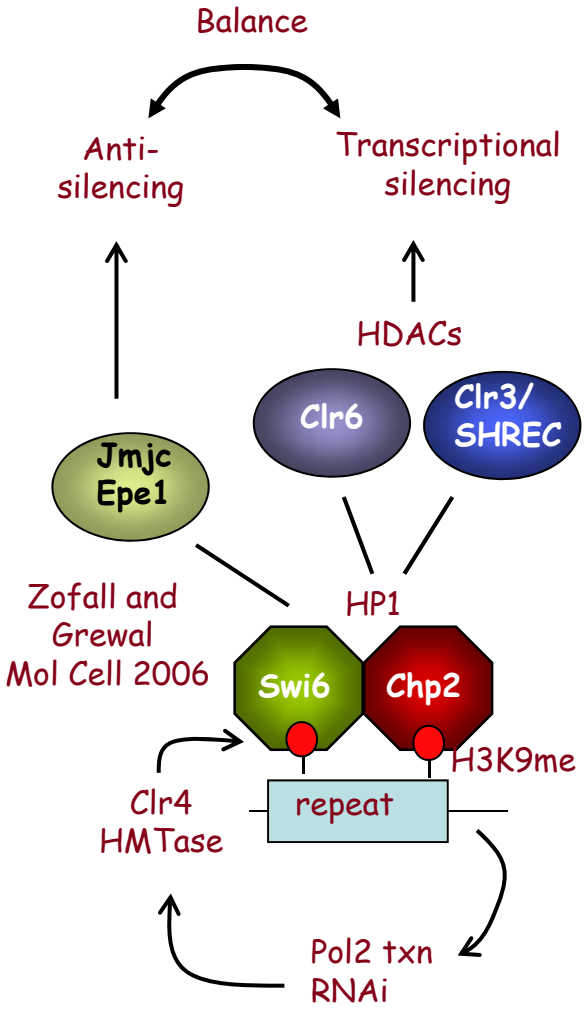
S. pombe genome contains several classes of repeat elements that are assembled in repressive chromatin

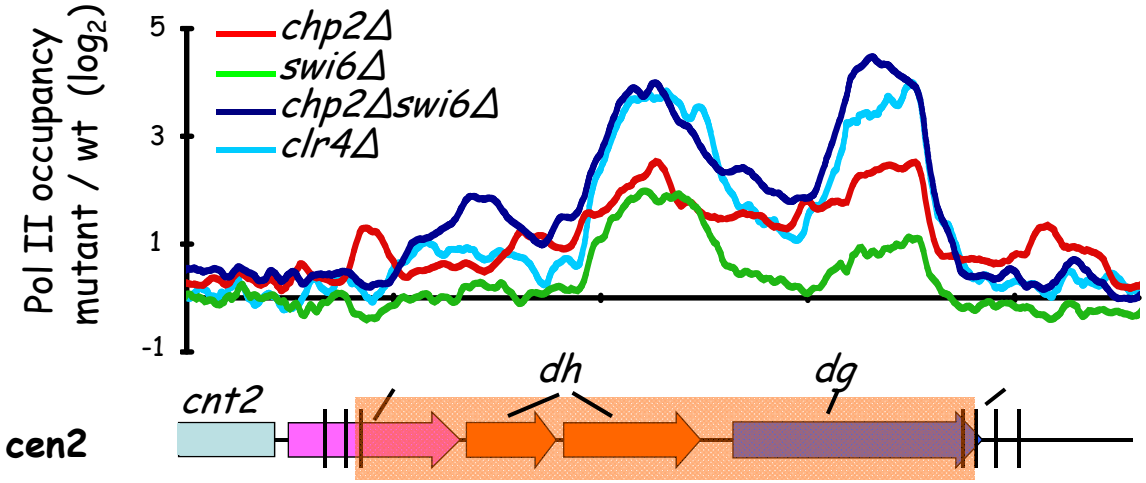


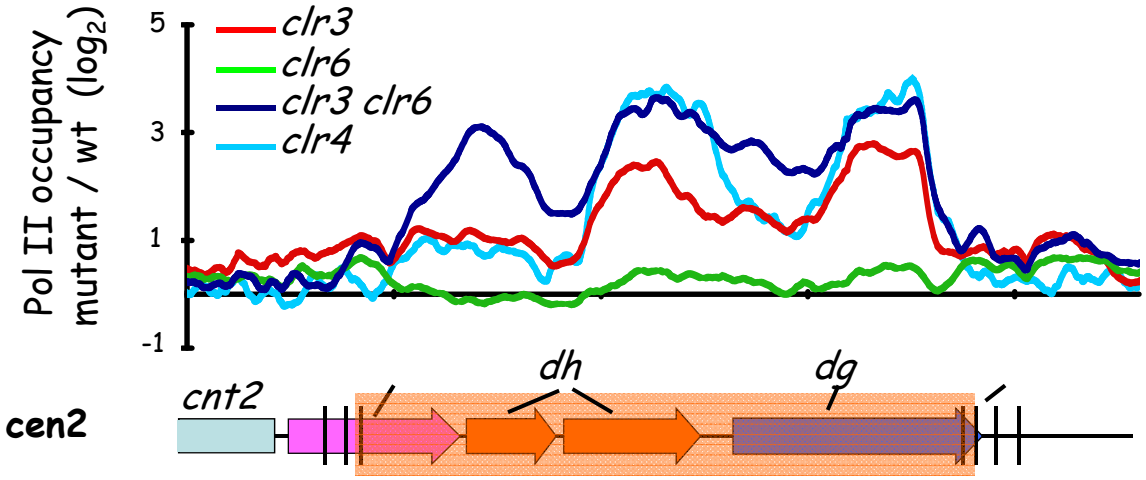
Heterochromatin: a versatile recruiting platform



HP1 proteins and their associated HDACs collaborate to enforce heterochromatic transcriptional silencing





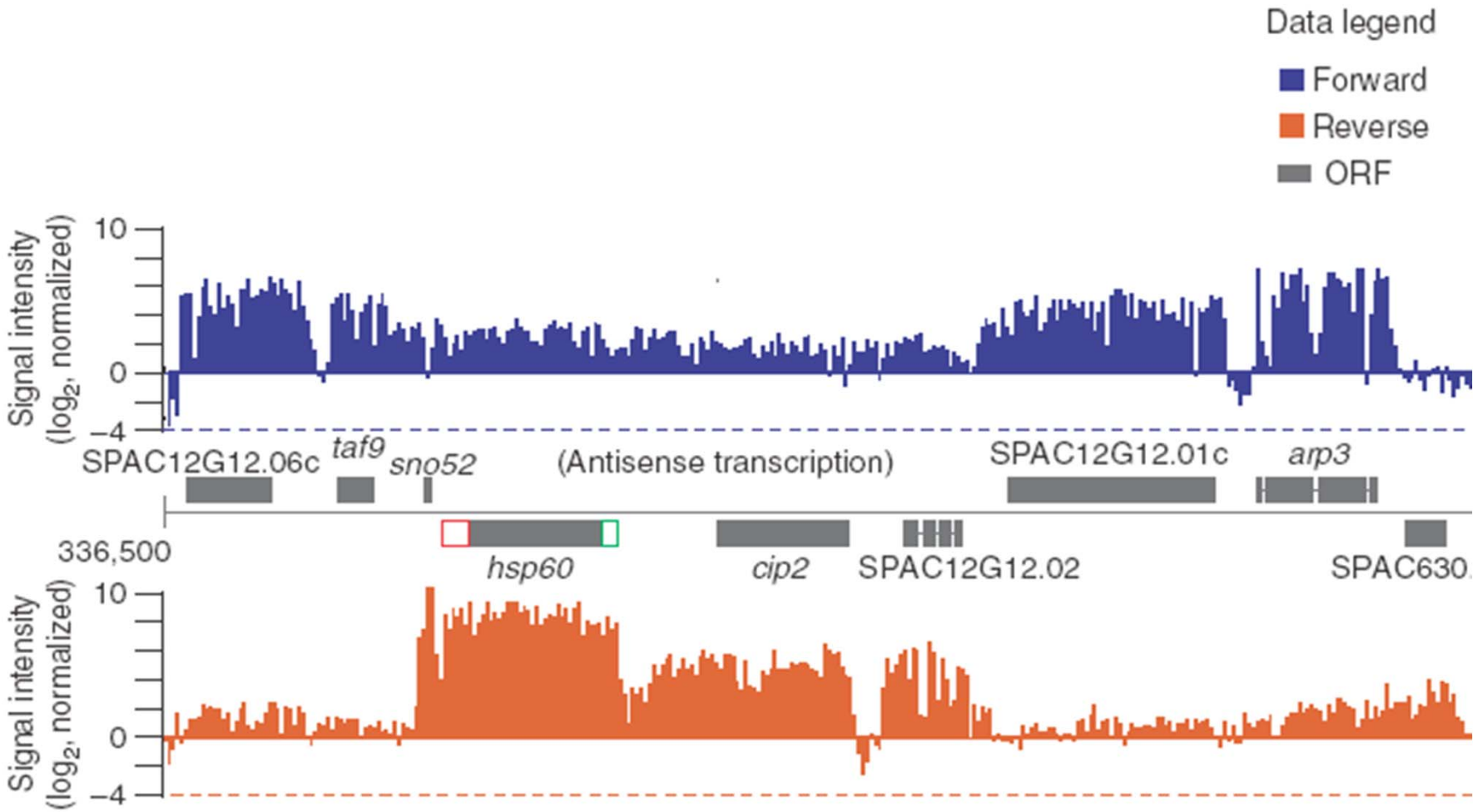


Topics:

- Silencing of retrotransposons and repeat elements by RNAi and chromatin-modifying factors

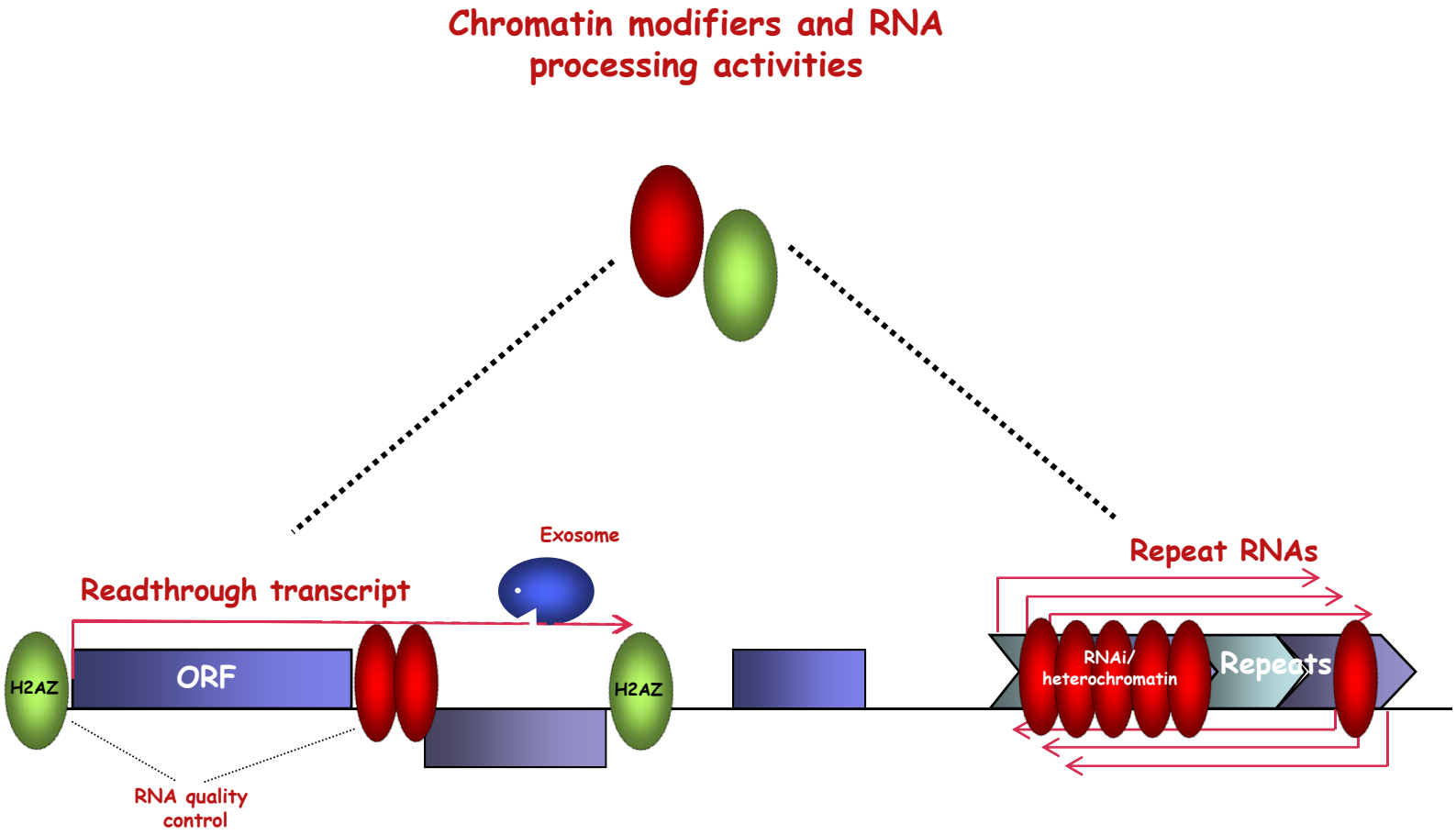
- Genome-wide suppression of aberrant RNAs by a variant histone and the RNAi machinery

Large proportion (>90%) of the S. pombe genome including the intergenic regions are transcribed in both directions



Dutrow et al 2008 (Cairns lab)
Wilhelm et al 2008 (Bahler lab)
Nicolas et al 2007 (Our lab)

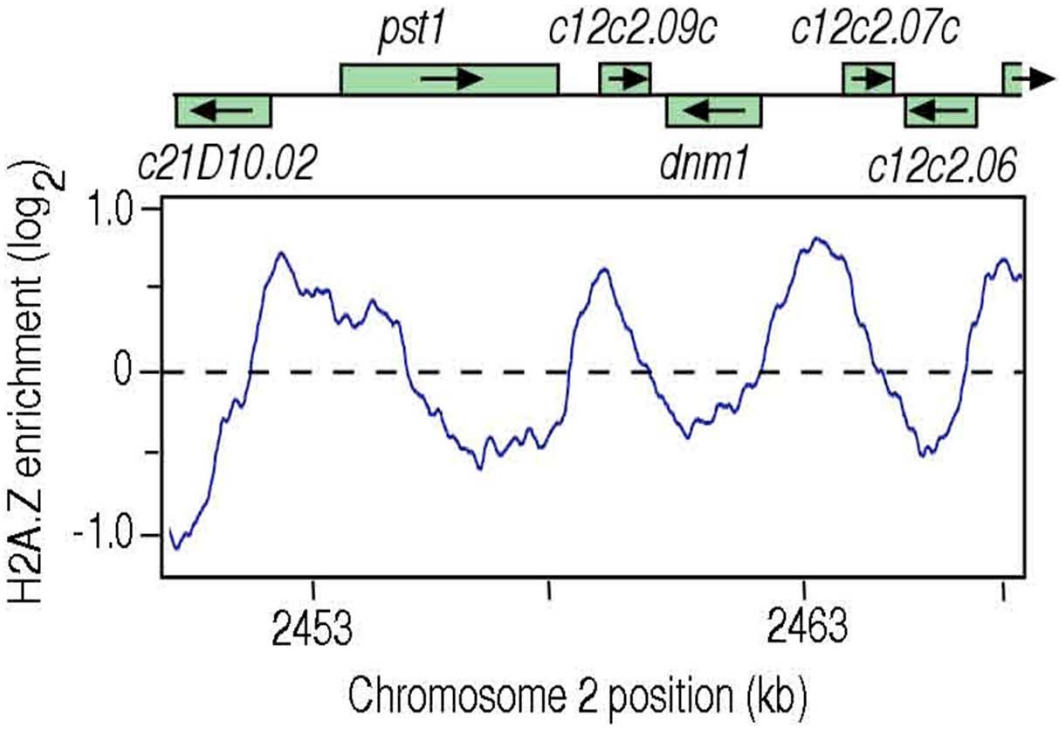
RNAi and heterochromatin factors cooperate with a variant histone H2A.Z to suppress antisense RNAs



Accumulation of aberrant RNAs can lead to genomic instability

H2A.Z is enriched at 5' ends of genes

ChIP profiling of H2A.Z



- **H2AZ is a histone H2.A variant that is deposited onto chromatin by the SWR-C**
- **Loss of H2A.Z affects various chromosomal processes but its exact function is not known**

Loss of H2A.Z (pht1) causes disproportionate increase in antisense transcripts at convergent gene loci

Strand-specific RT-PCR

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

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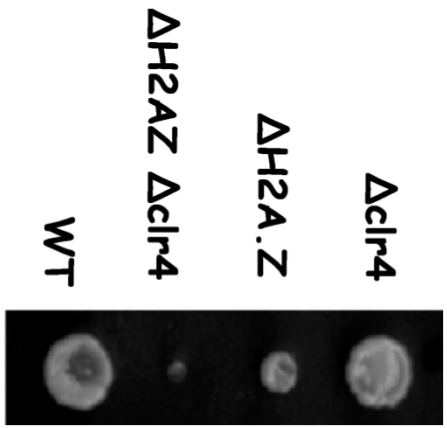
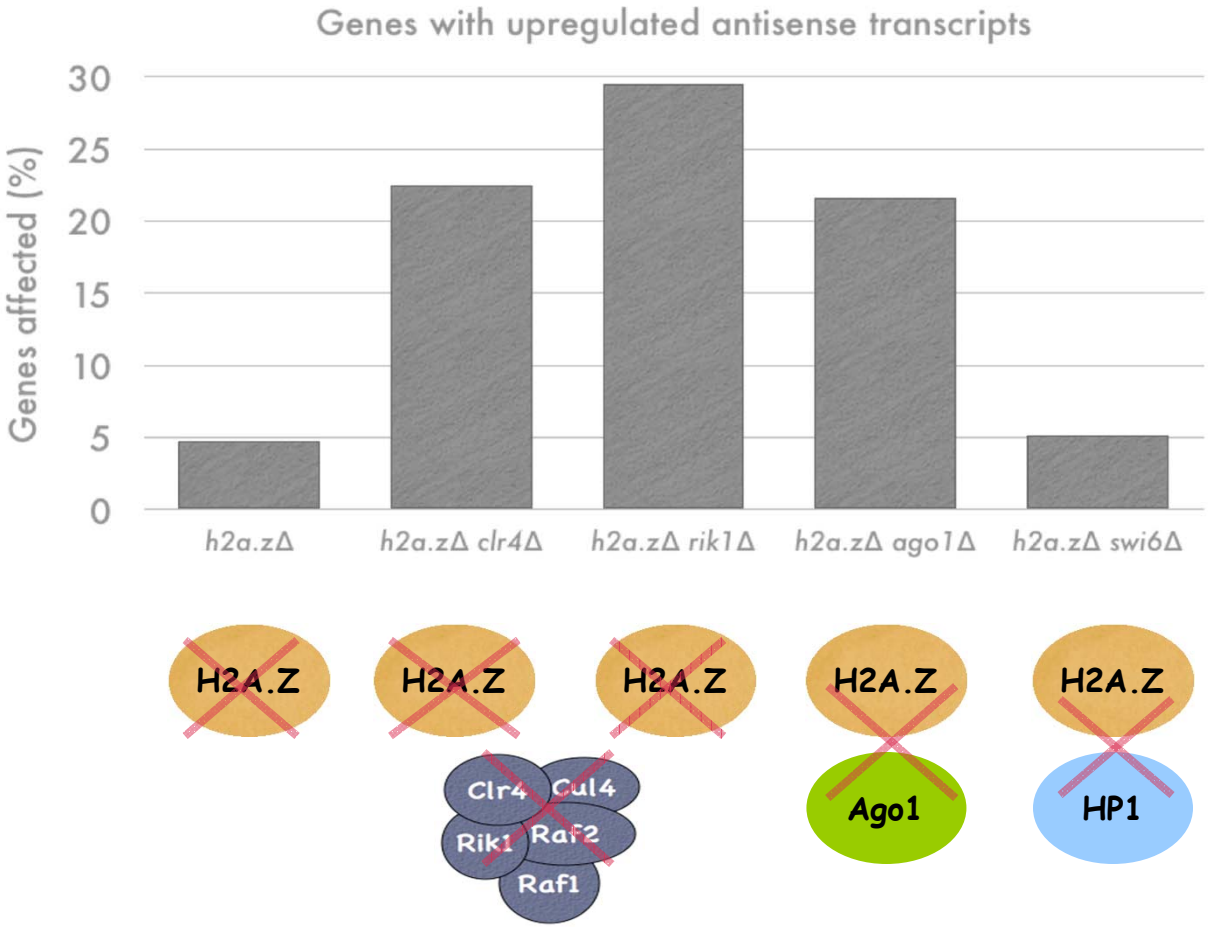
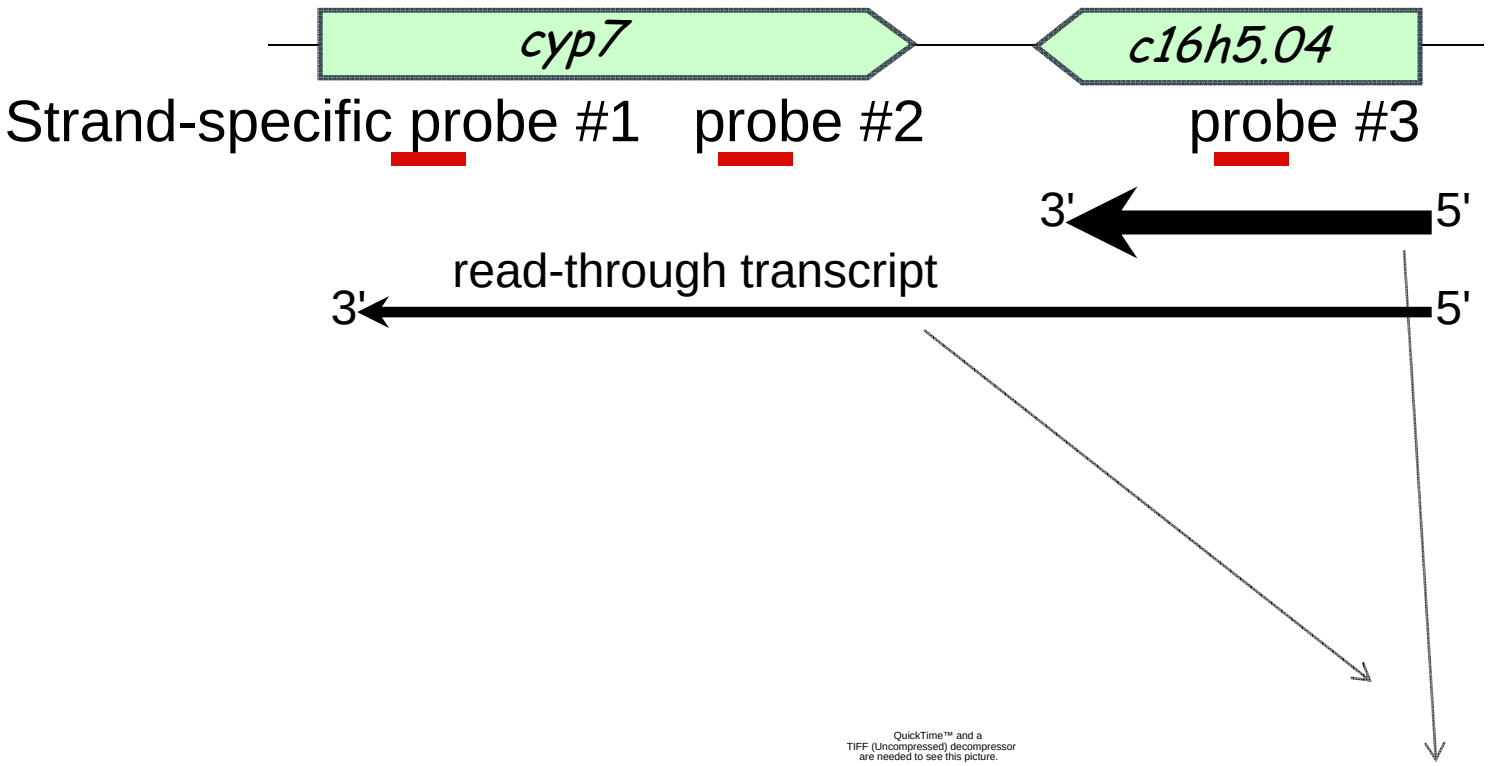
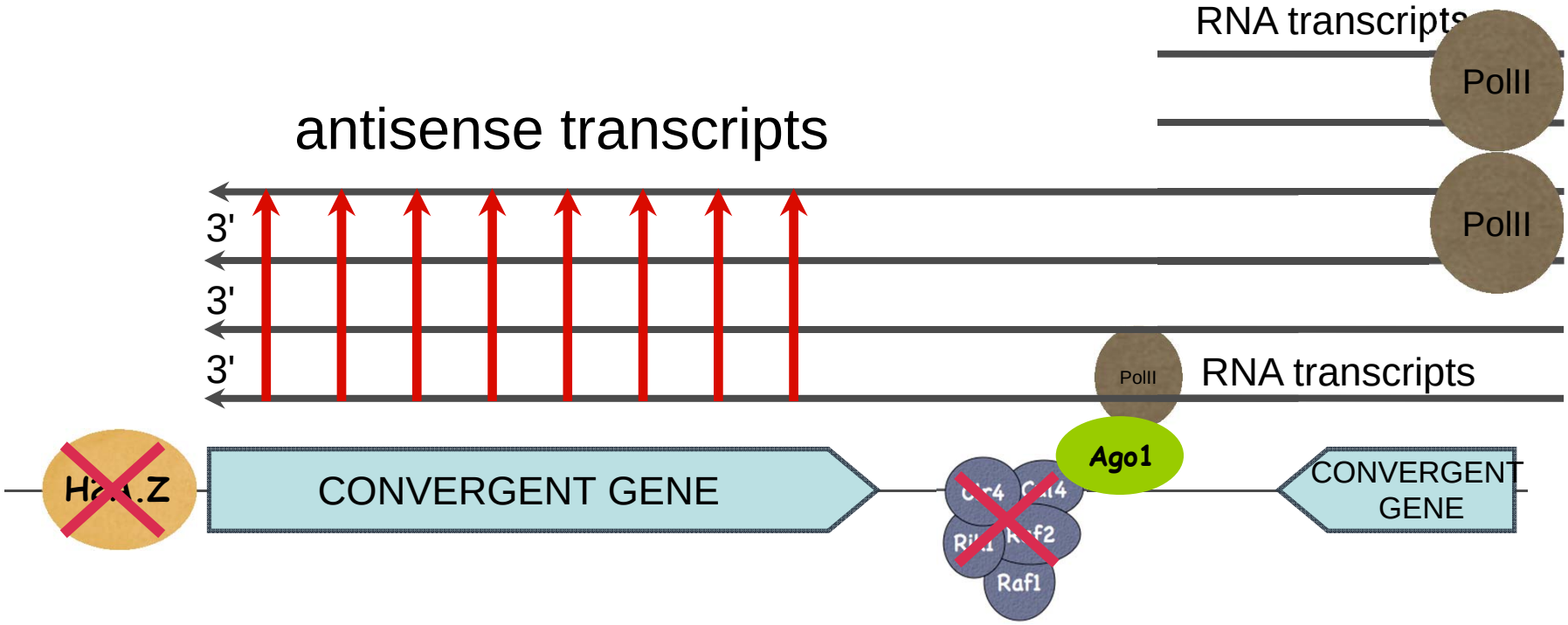
H2A.Z acts synergistically with Clr4 and Ago1 to suppress antisense transcripts





Antisense RNAs correspond to read-through transcripts rather than new initiation events

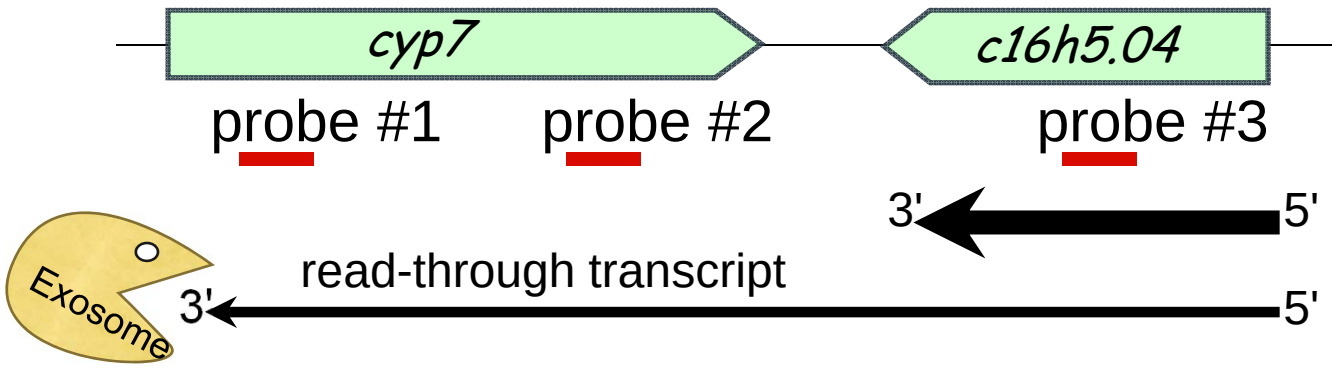




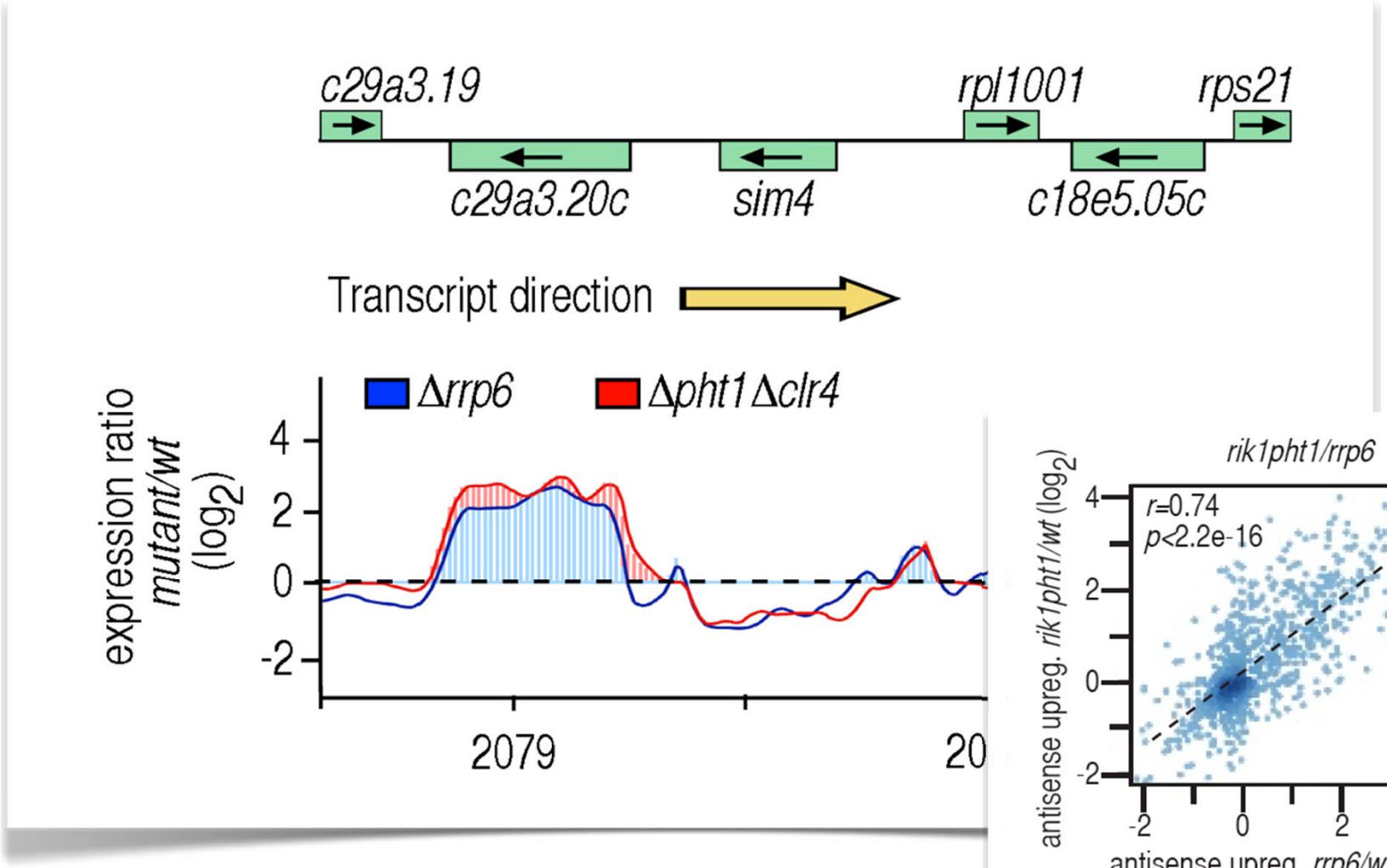
elevated RNA level → increased transcription

elevated RNA level → increased RNA stability

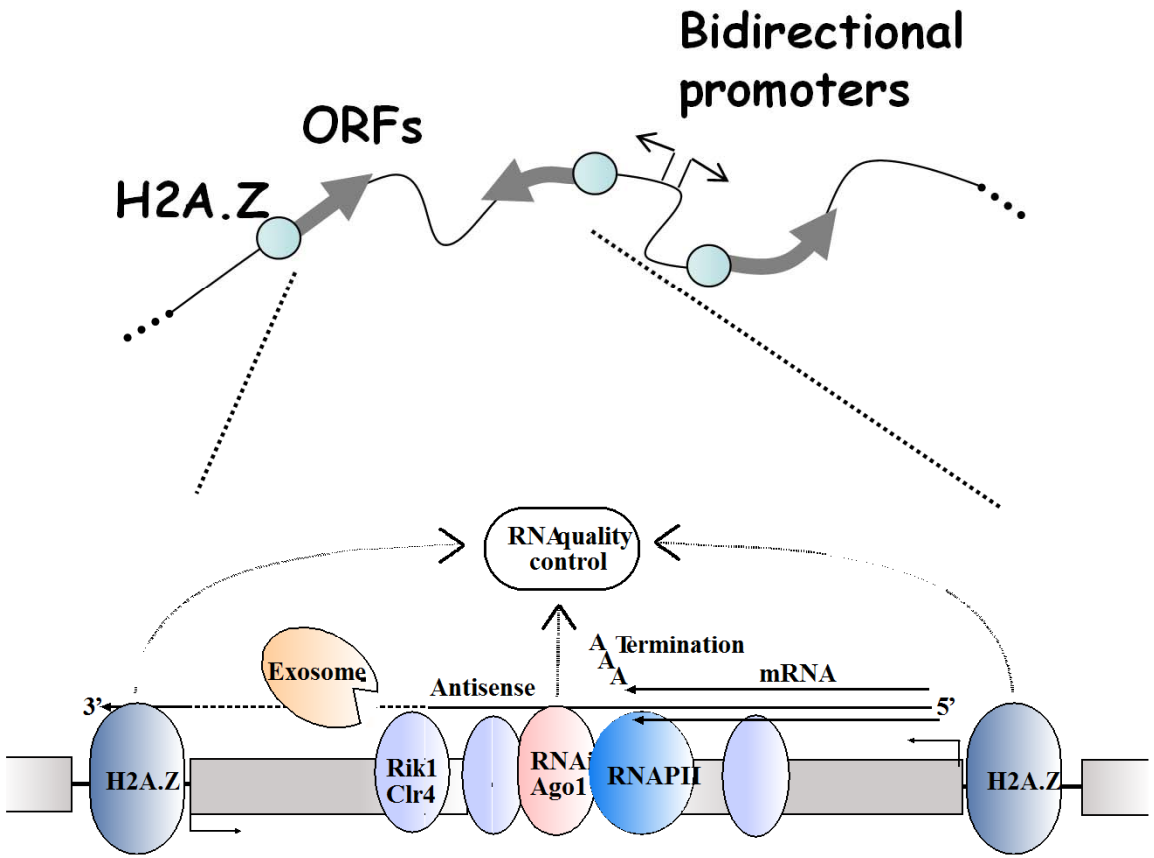
Readthrough antisense RNAs accumulate in exosome (rrp6) mutant



Loss of exosome causes upregulation of antisense transcripts in a pattern identical to H2A.Z clr4 mutant



H2A.Z and Pol II-associated Ago1 are components of RNA quality control mechanism involved in antisense suppression



Summary

- Transposon-derived CENP-B proteins and RNAi target chromatin modifying activities which in turn facilitate nucleosome positioning to suppress transcriptional noise at repeat elements

- H2A.Z is a component of genome indexing mechanism that cooperates with RNAi and heterochromatin factors to suppress antisense RNAs

Acknowledgments

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Estelle Nicolas (CNRS, Toulouse)
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Collaborators

Peter FitzGerald (NCI)
Tim Veenstra and Ming Zhou (NCI)

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