Epigenetics in Yeast

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Outline

- **Genetic and epigenetic regulation of gene expression.**
- **Mating-type switching in budding yeast.**
- **Positive and negative regulation of mating-type switching.**
- **Epigenetics and heterochromatin formation in fission yeast.**
- **Non-coding RNAs and gene regulation in fission yeast.**

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Gene expression regulation

- **The study of how genes are turned ‘ON’ or ‘OFF’ in response to a signal.**

- The Operon model (Jacob & Monod, 1961):

  “*the gene was something in the minds of people…which was as inaccessible, by definition, as the material of the galaxies. That experiments we were doing would involve an actual physical interaction between a compound in the cell and actually the gene itself, was something extremely difficult to come to.*’’

Gene regulation is fundamental

Genetic / Epigenetic control of gene expression is important for many fundamental processes:

- **Embryonic development.**
- **Maintenance and differentiation of pluripotent stem cells.**

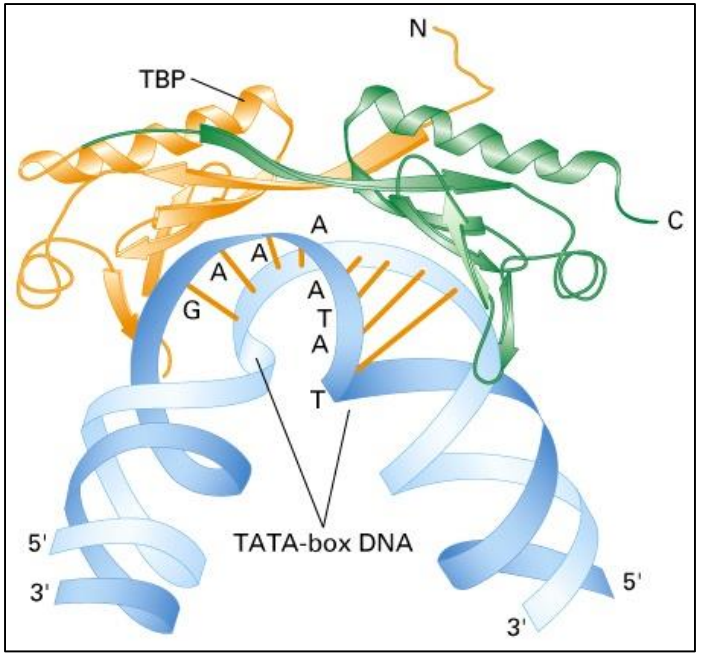
Dysfunctional in:

- **Cancer.**
- **Mental retardation.**
- **…**

Genetic regulation of gene expression

Mechanisms:

- **Promoter / enhancer.**
- **Transcription factor binding sites.**



Definition of Epigenetics

- **Heritable differences in genome function that occur without a change in DNA sequence.**

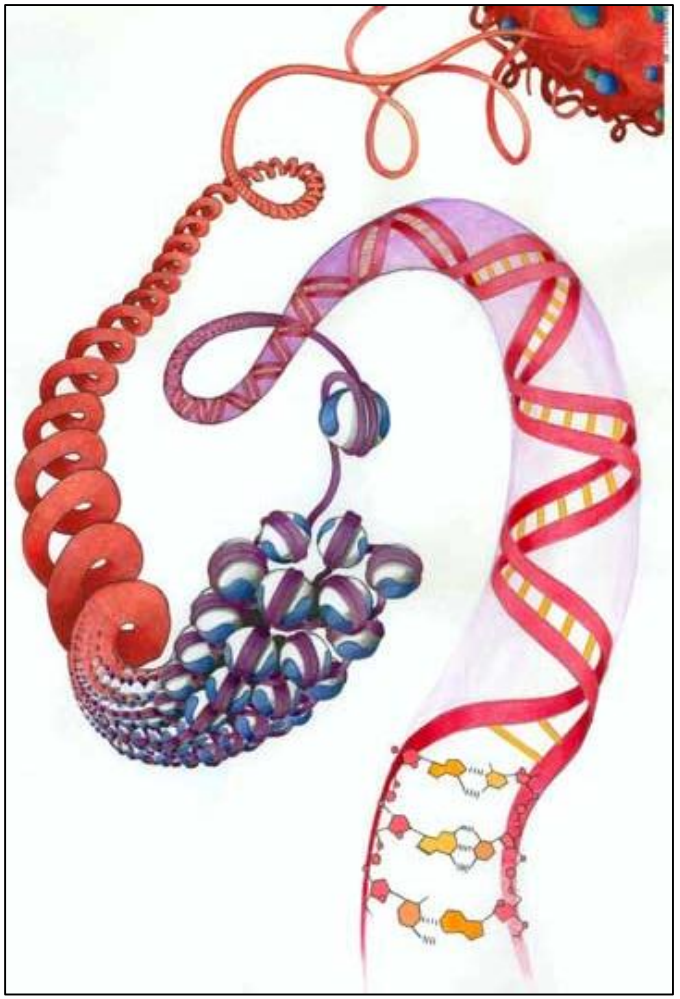
- *"… all the weird and wonderful things that can't be explained by genetics.*"

Denise Barlow

Epigenetic regulation of expression

Mechanisms:

- **DNA methylation.**
- **Histone modifications.**
- **Nucleosome positioning and remodelling.**
- **Non-coding RNAs.**
- **Prions.**

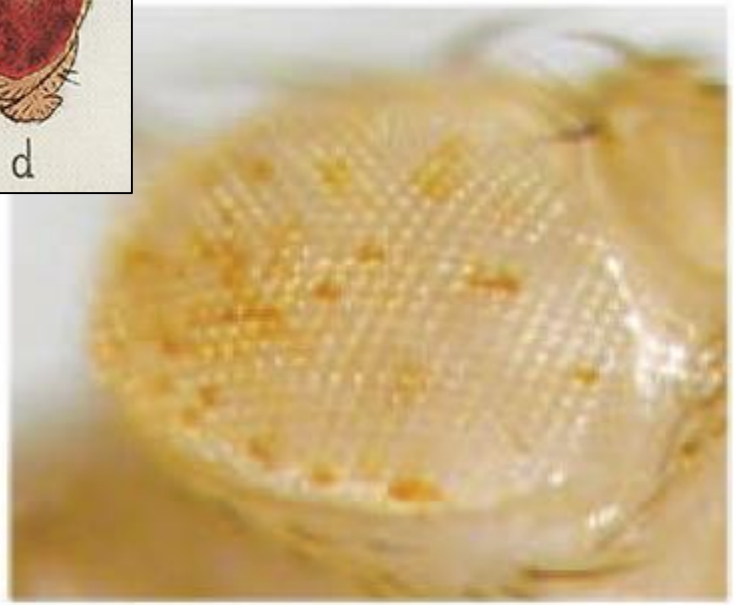


Models to study epigenetic regulation of gene expression

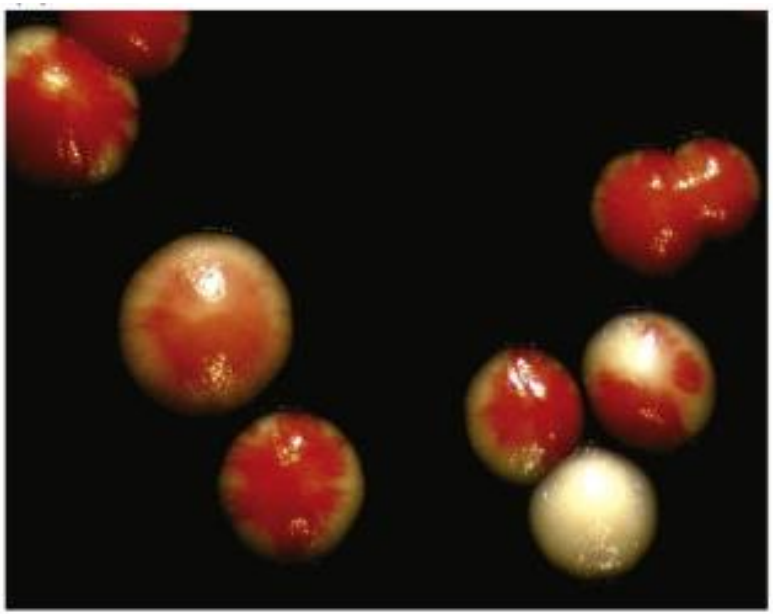
- **Budding yeast (*S. cerevisiae*)**: repression of telomeres, mating-type switching.
- **Fission yeast (*S. pombe*)**: repression of centromeres & telomeres, RNAi, mating-type switching.
- **Fungus (*Neurospora*)**: DNA methylation.
- **Ciliates (*Tetrahymena*)**: Histone modifications + variants.
- **Flies (*Drosophila*)**: Histone modifications, chromatin domains.
- **Plants + worms**: DNA methylation, RNAi.

Example of a phenotype revealing epigenetic regulation of gene expression: variegation

Fly eye



Budding yeast



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Mating-type switching in the budding yeast *S. cerevisiae*

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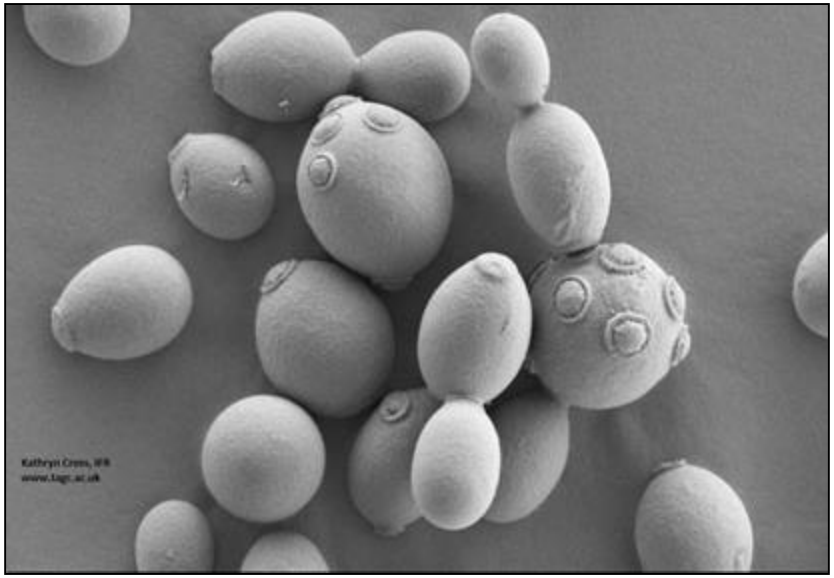
An example of both genetic and epigenetic regulation

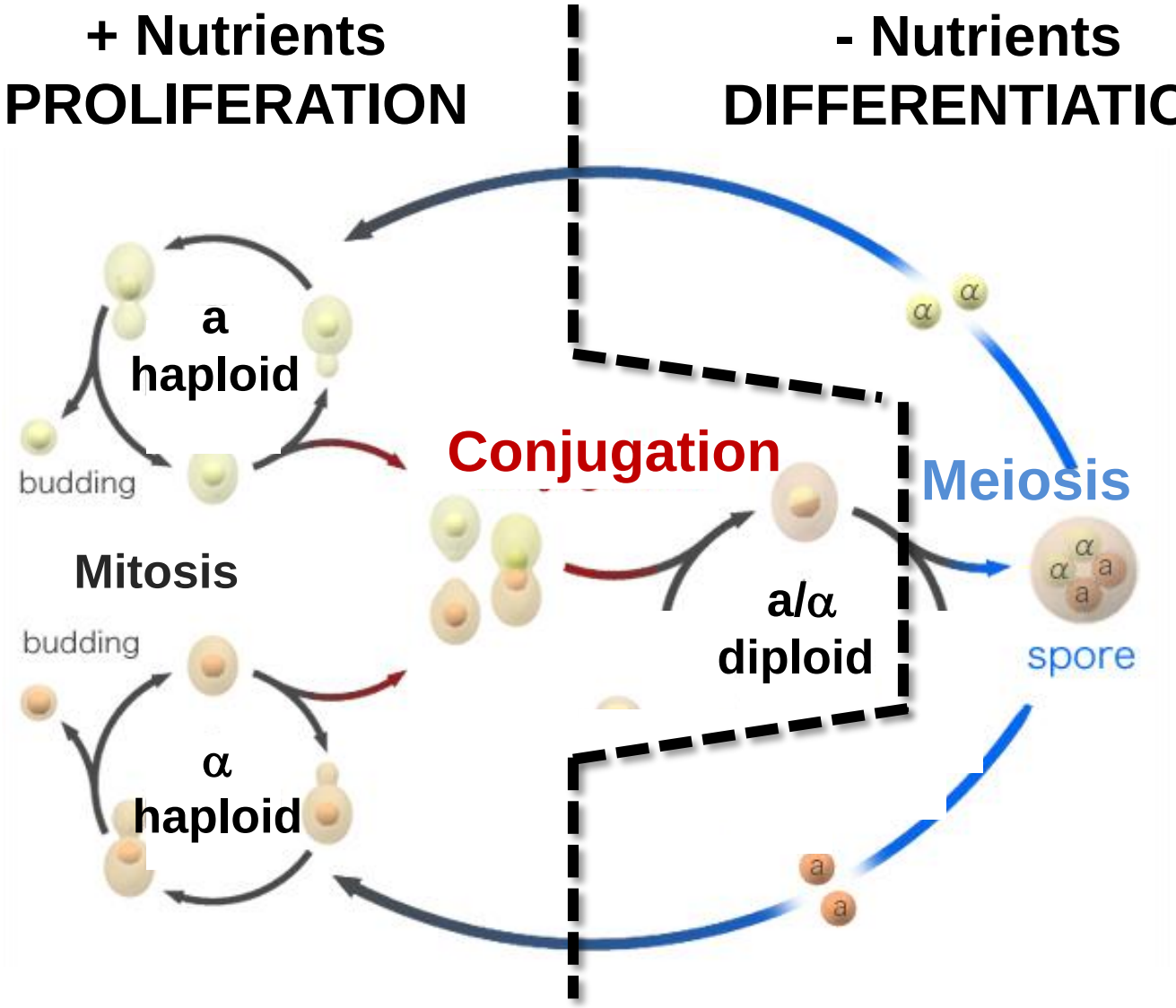


Key concept

- **Allele translocation (= gene conversion) between a transcriptionally silent and an active locus.**
- **States are determined by chromatin structure and epigenetic modifications.**

The study of this phenomenon, particularly using classical genetics starting in the early '80s, has led to the discovery of several fundamental, conserved mechanisms and tools, and is continuing to do so.

S. cerevisiae life cycle

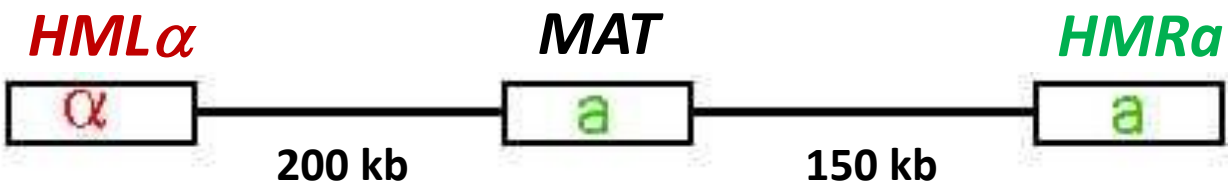


Requirements to switch mating-type

- **One active copy of mating-type genes (‘playback’)**
- **One silent copy of 2 other mating-type genes (‘storage’).**
- **Regulated, targeted homologous recombination.**
- **Mix progeny after division to allow different mating types to ‘meet’.**
- ***Mechanisms to allow directional switching and to choose the correct ‘storage’ copy.***

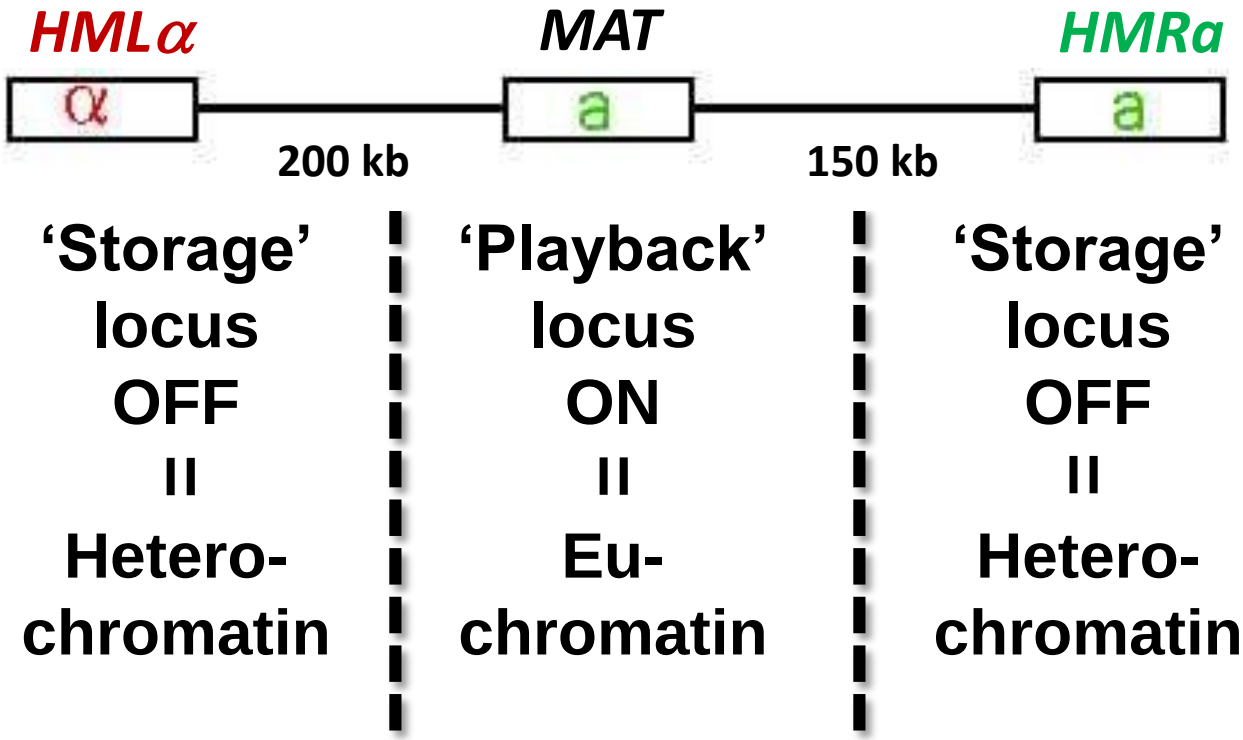
MAT locus alleles

- **Mating-type is determined by the presence of a specific allele present at the *MAT* locus, which is <u>expressed</u>.**



MAT locus alleles

- **Additional, but <u>silent</u>, copies of this locus (= cassettes), 5' and 3' of *MAT*.**



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- Genetic and epigenetic regulation of gene expression.
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- **Positive** and negative **regulation of mating-type switching.**
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- Non-coding RNAs and gene regulation in fission yeast.

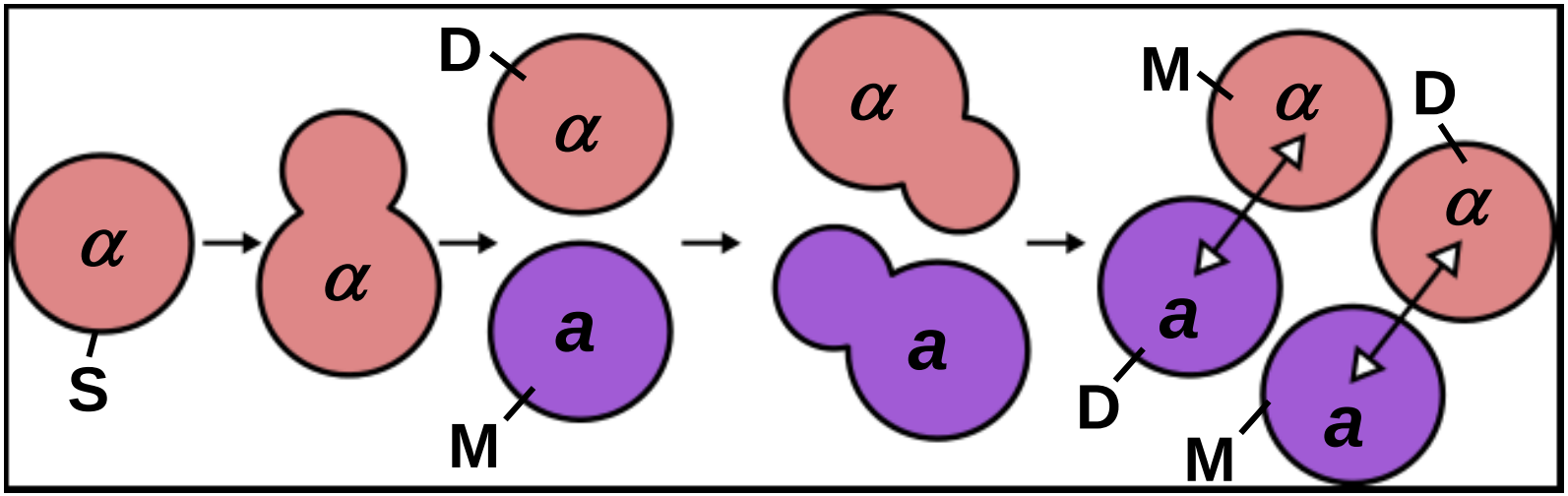
Controlling mating-type switching

- **A yeast is able to switch mating-type, after each division (aka. homothallism),**
- **Through homologous recombination (= DNA damage repair mechanism).**

Regulation of the switch

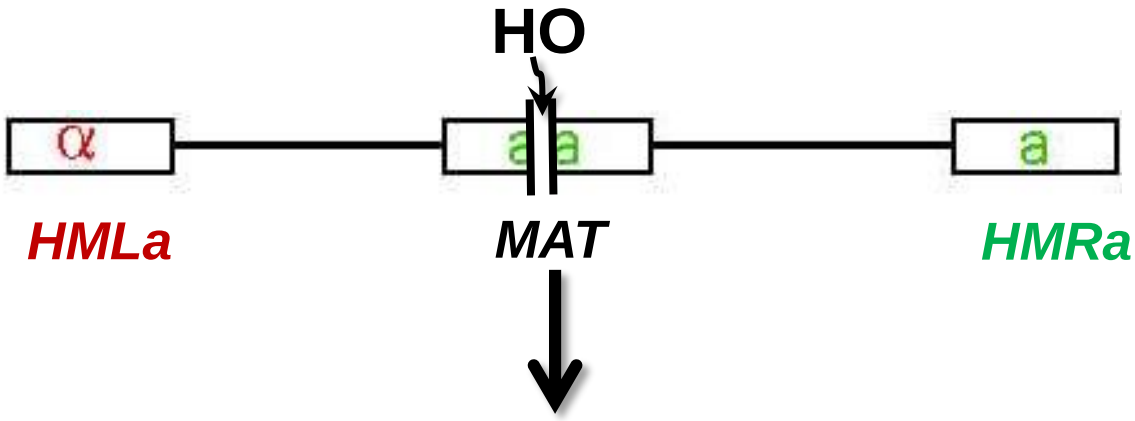
- **Tight regulation: happens after each mitosis but only in one of the 2 cells (= the mother).**

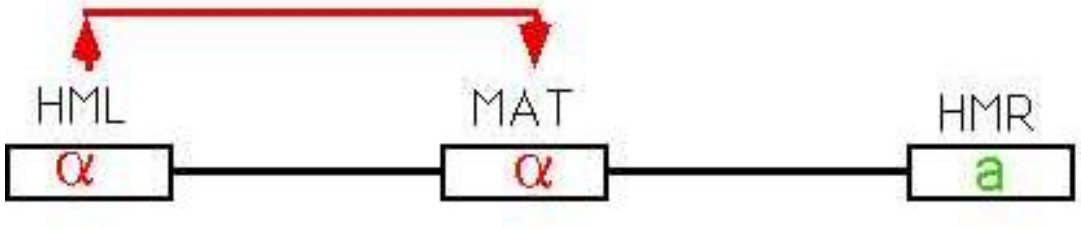
S = spore M = mother cell D = daughter cell



Mechanism of the switch

- **Double-strand break at the *MAT* locus, generated by an endonuclease (HO).**
- **Homologous recombination using *HMLa* or *HMRa* as donor DNA.**



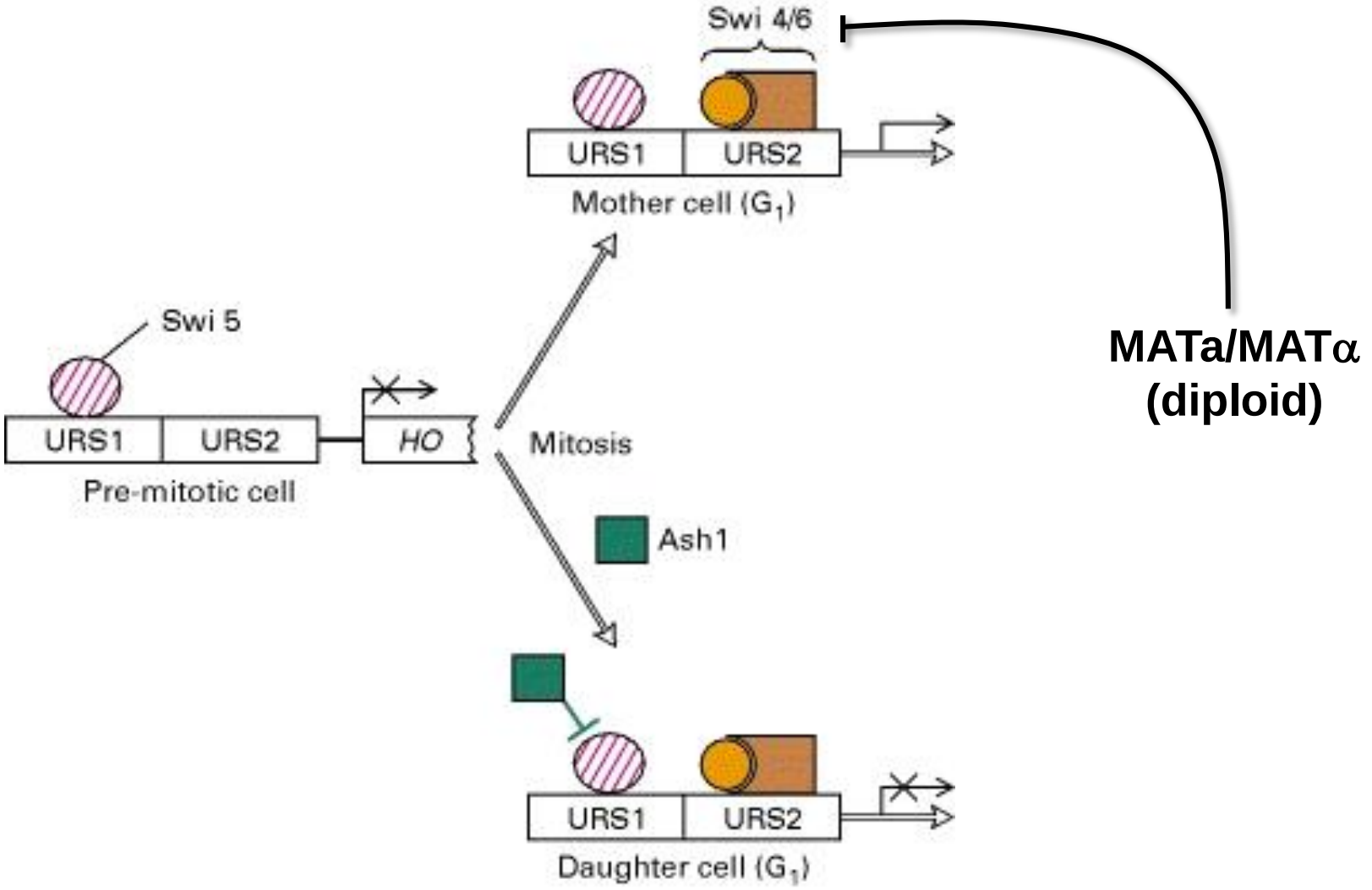


Therefore:

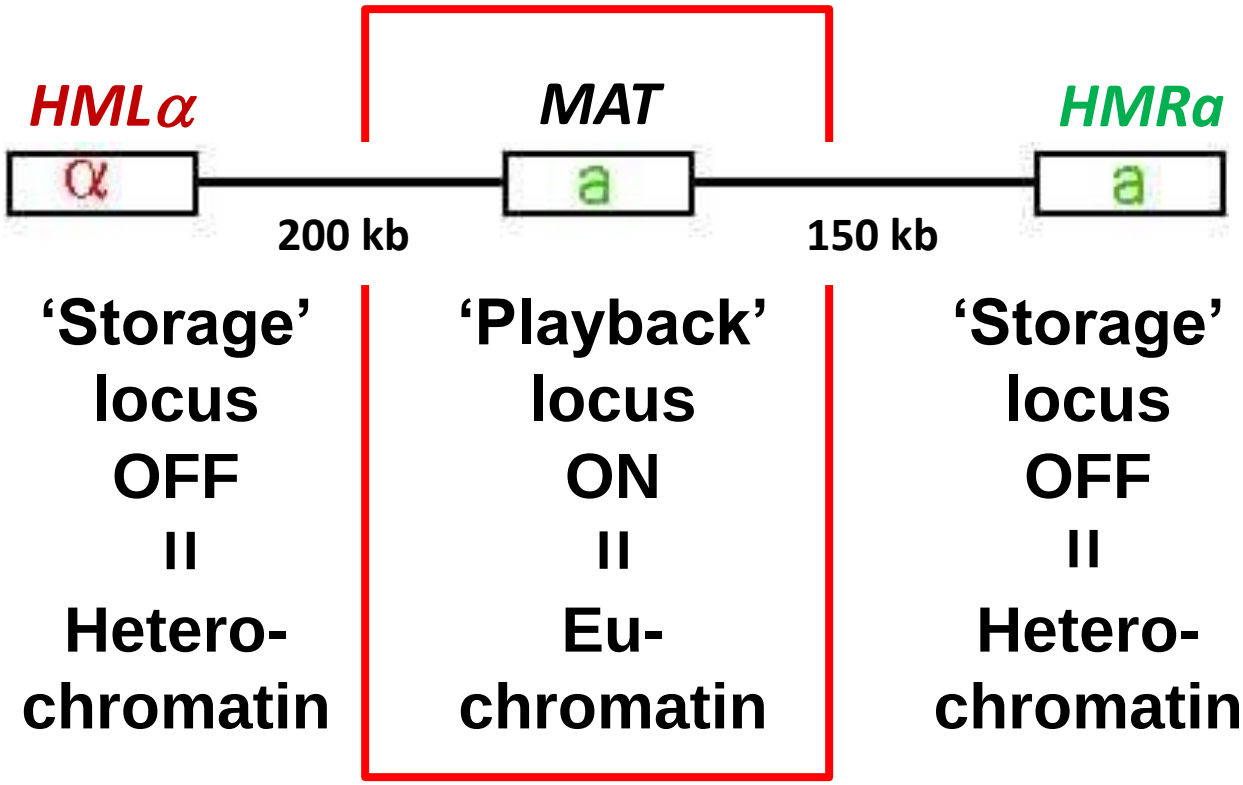
Regulation of mating type identity depends on the regulation of *HO* expression:

- Expressed in mother cell, not in daughter cell;
- Expressed in haploid cell, not in diploid cell;
- Expressed in late G1 phase of the cell cycle (before DNA is replicated).

Molecular mechanism of *HO* induction: genetic regulation

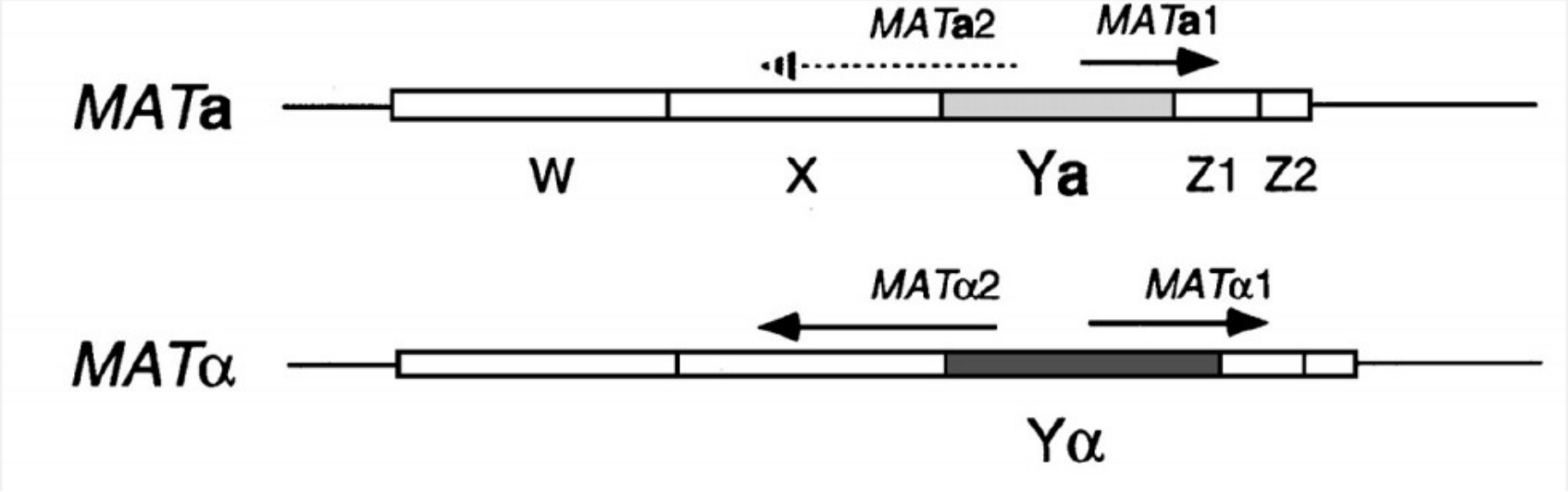


How is expression regulated at the ‘playback’, *MAT* locus?

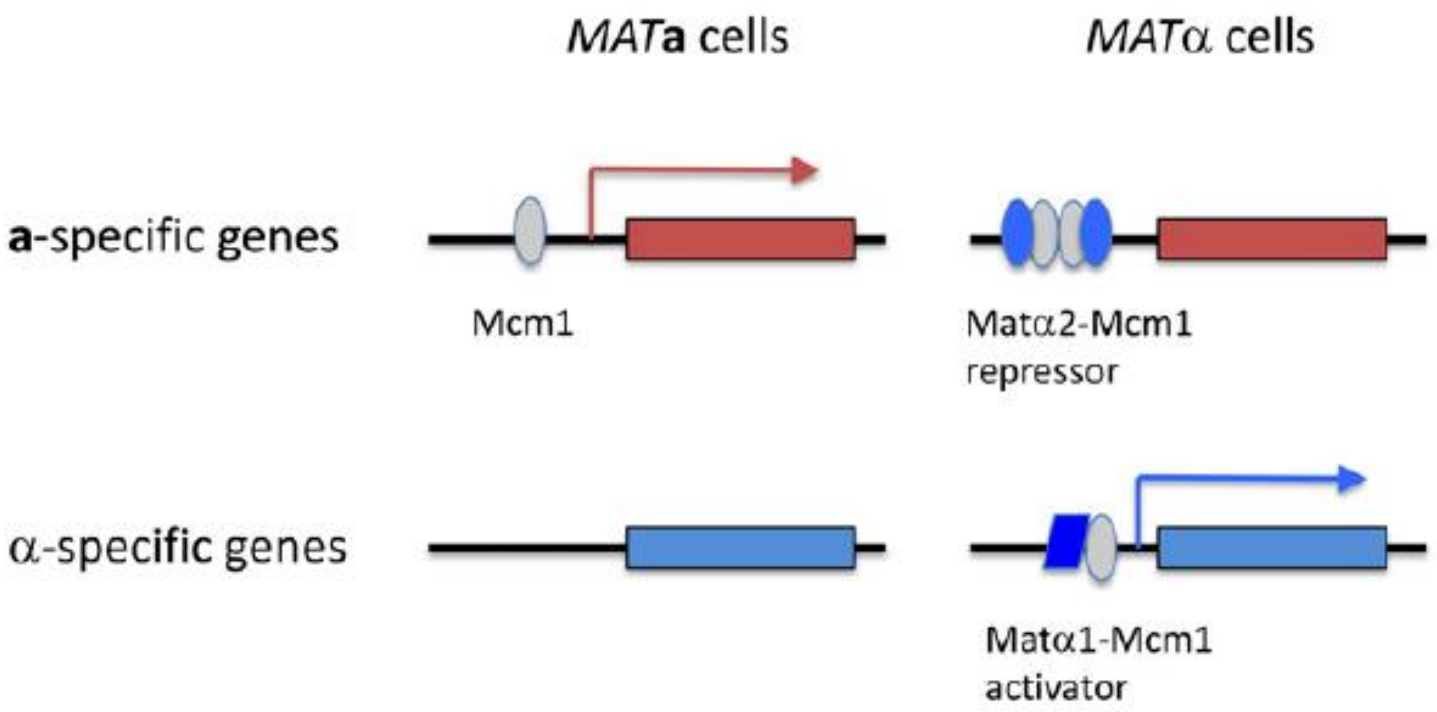


Two genes at the *MAT* locus

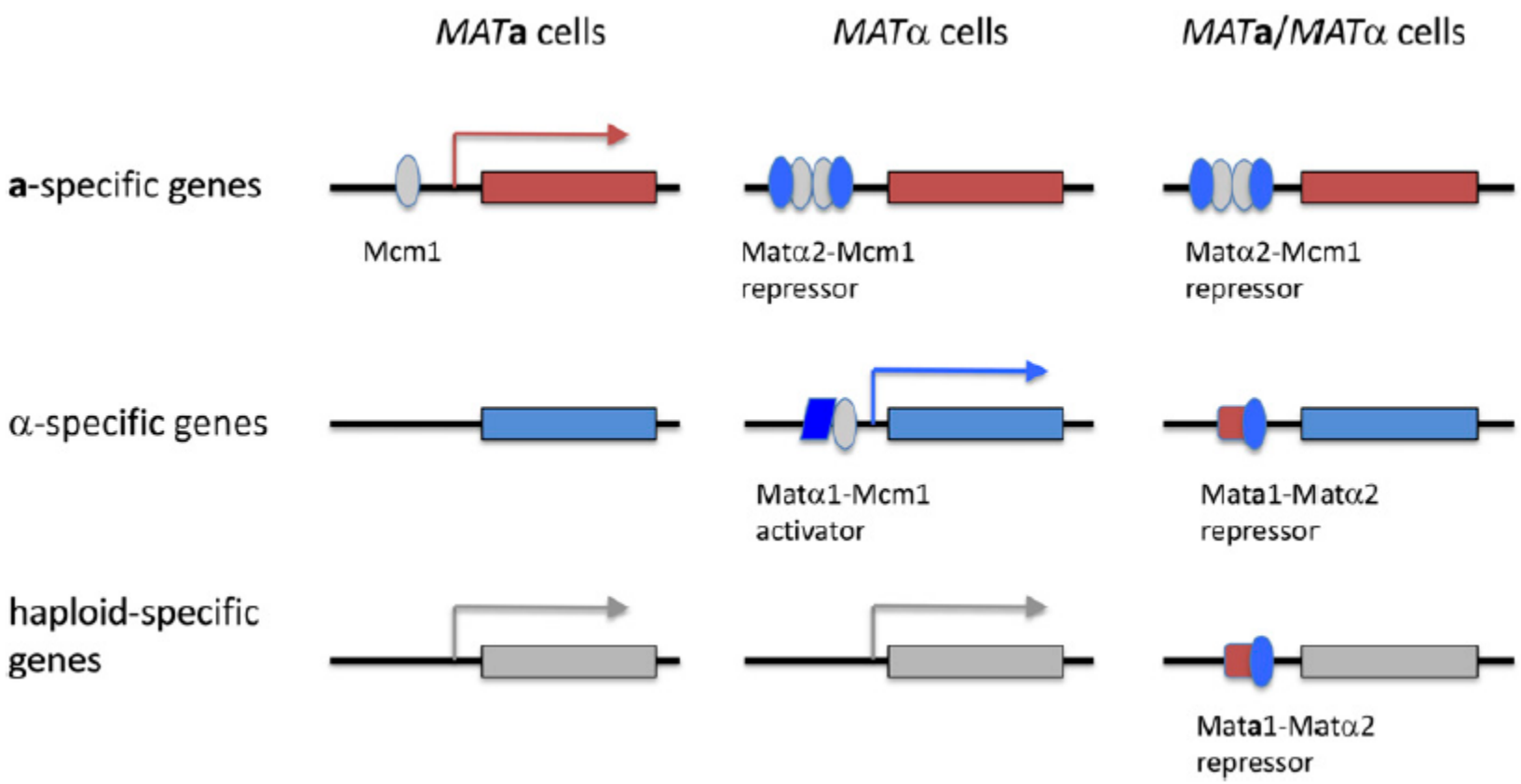
- **Gene structure of the expressed, *MAT* locus:**



Mating-type identity in haploids



Mating-type identity in diploids



Examples of such downstream targets

- **a/α-specific genes: pheromones and their receptors (discovery of GPCR signaling).**

- **Haploid-specific: HO endonuclease (= tool to study DNA repair after DBSs).**

Impact of these studies

- **Role of transcription factors in cell fate decisions.**
- **Transcription factors act by binding specific DNA sequences (promoters).**
- **Their functions can be modulated through hetero-dimerization (repressors – activators).**
- **Recently: role of non-coding RNAs in repression of meiosis-specific genes in haploid cells.**
- **GPCRs + kinases (MAPK) transmit the pheromone signal to induce mating (cell fusion).**

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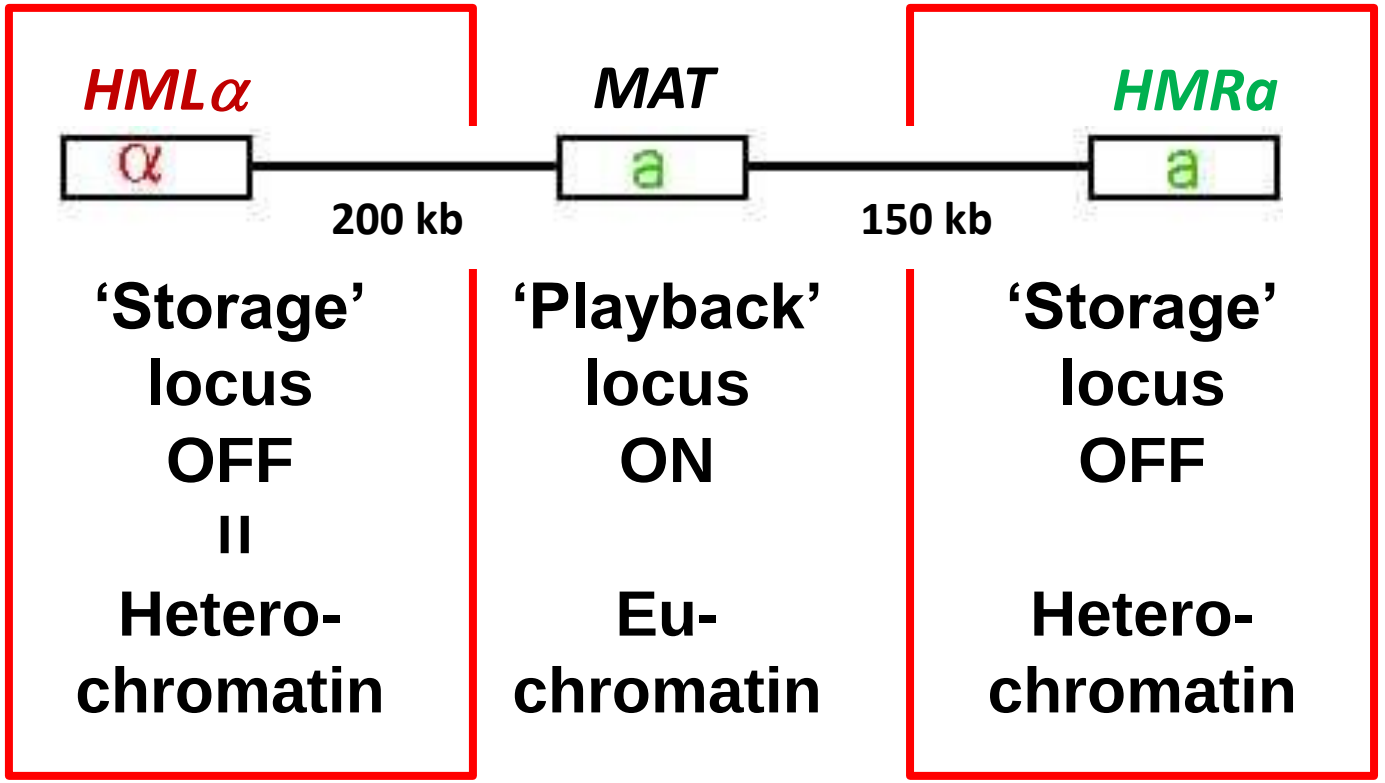
Repression of the storage ‘cassettes’: epigenetic regulation



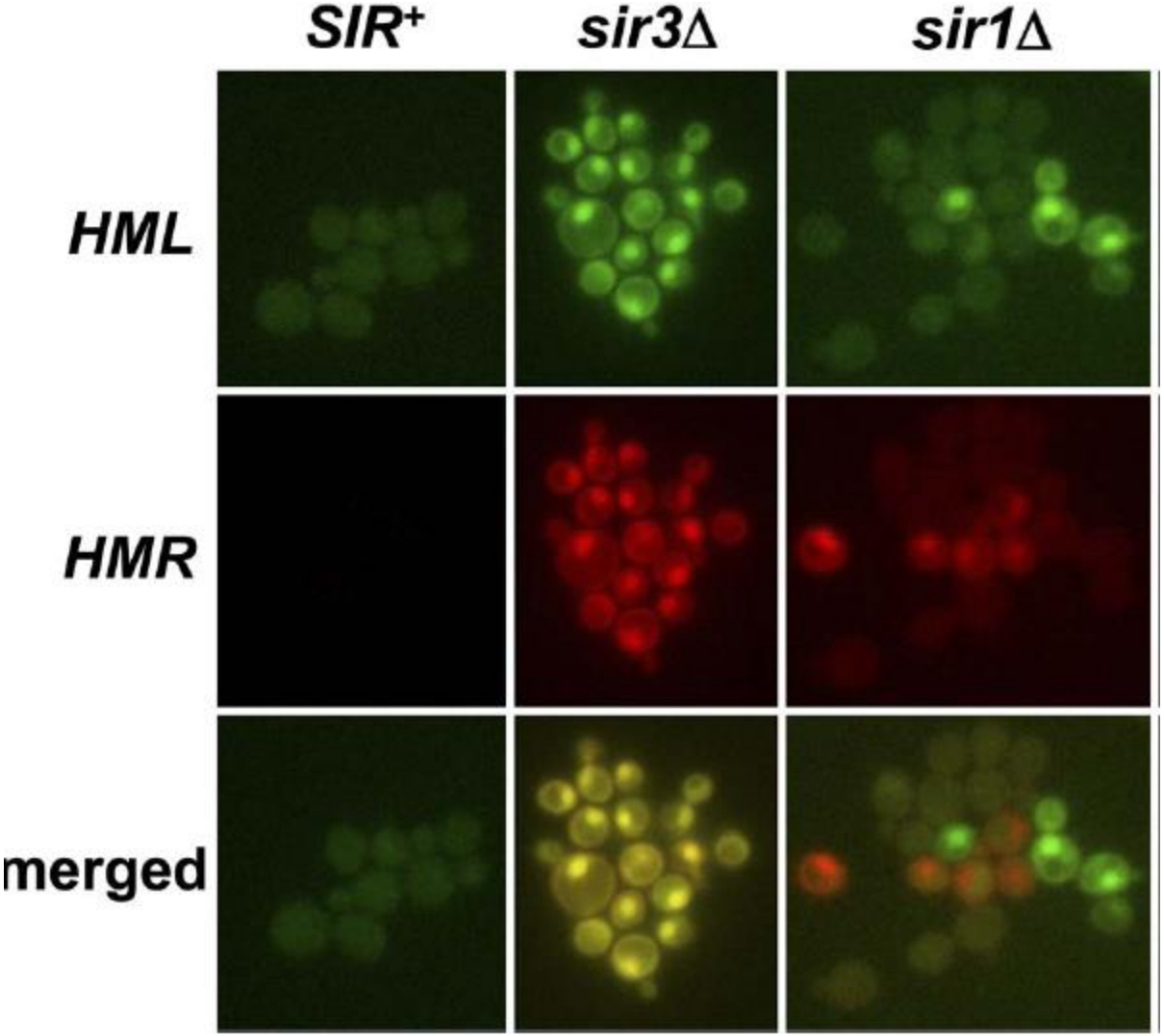
Identification of other factors controlling silent cassettes = identification of an important, conserved epigenetic regulator

Genetic screen: identify mutations that allow mating even in the absence of a functional (expressed) *MAT* locus.

↓

***SIR* genes (Silent Information Regulator): Sir1-4**

SIR mutant phenotypes



Sir2

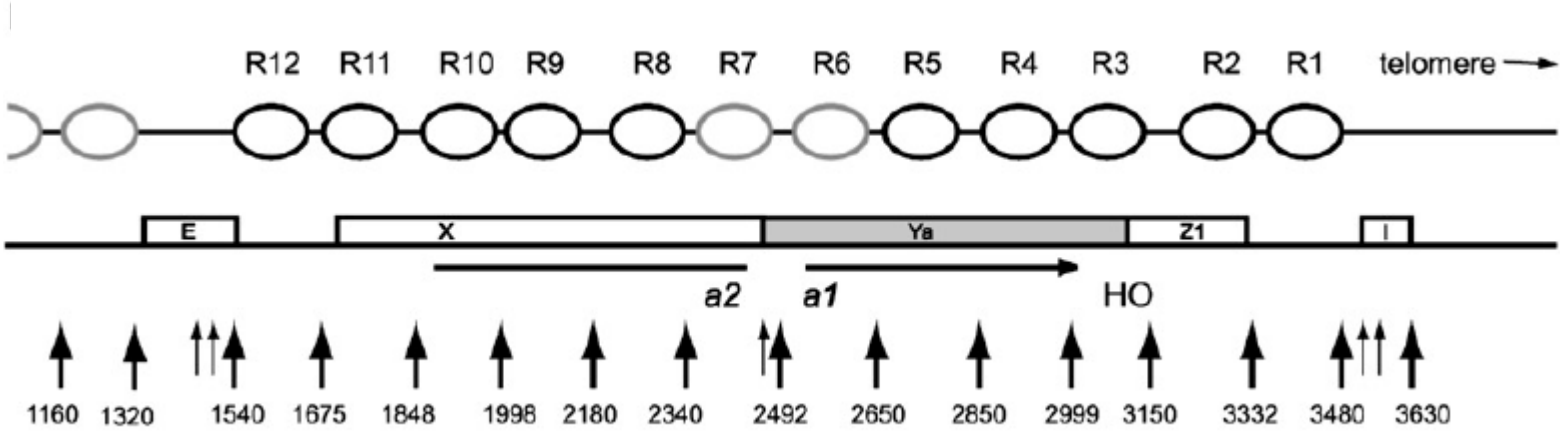
- **A very important and well-studied epigenetic regulator.**
- **The first HDAC (Histone Deacetylase) identified with a role in silencing:**

  De-acetylates histones H3 and H4 = repression of transcription.

- **= human SIRT1 (sirtuin), involved in aging, metabolic diseases, inflammation, Alzheimer's, …**

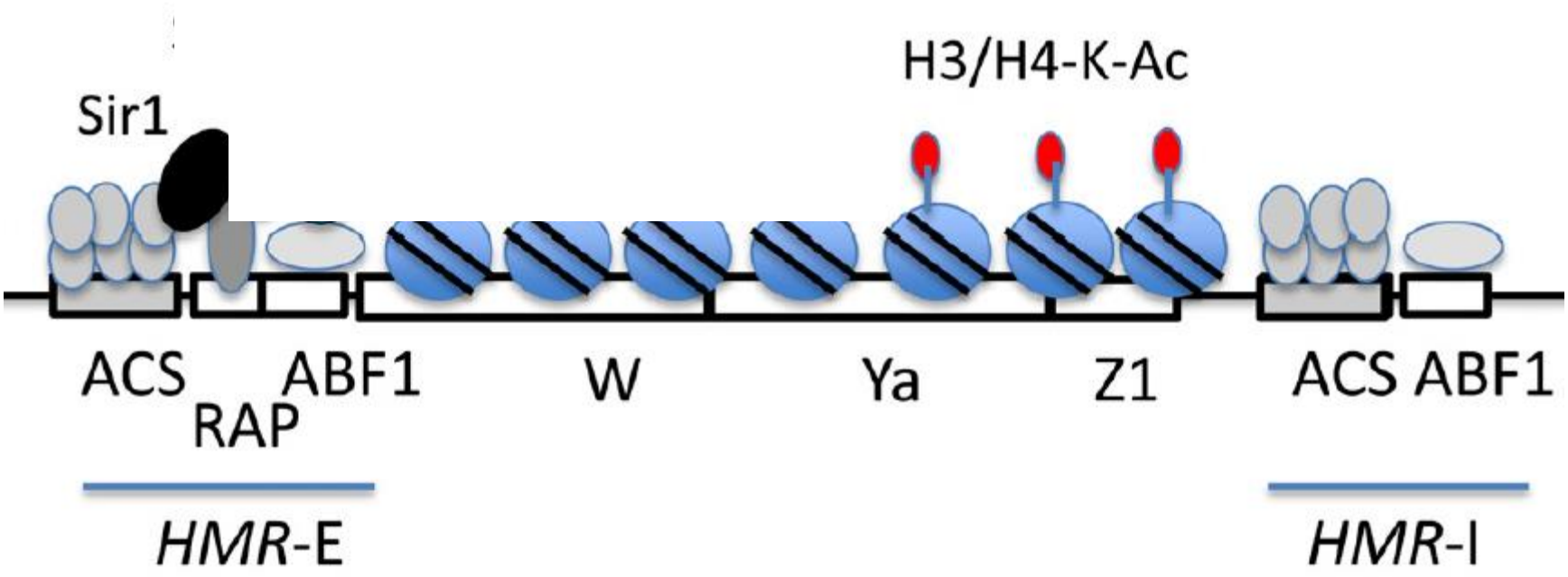
Making a silent domain = making heterochromatin

- **Precise positioning of nucleosomes within the silent locus (example of *HMRa*):**



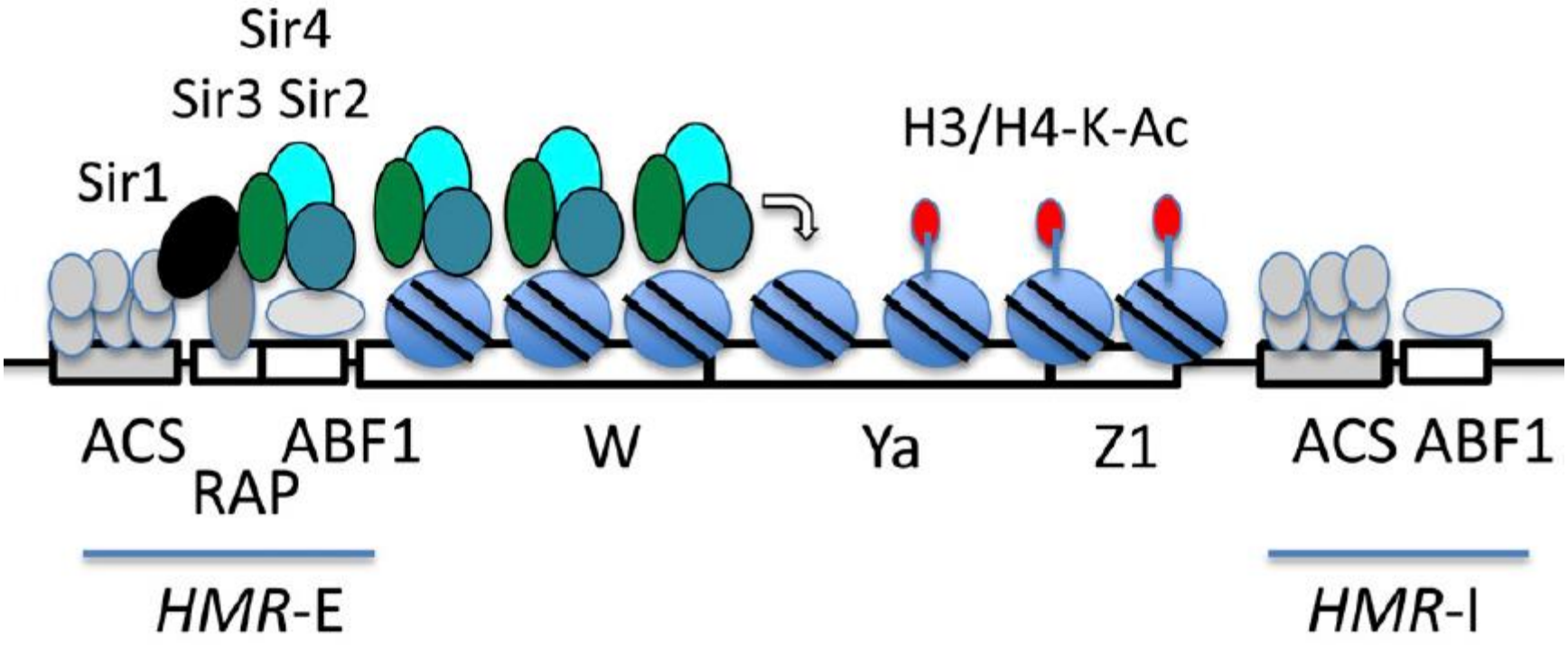
Establishment of heterochromatin

- **Sir1 is recruited to an 'initiation site' (*HMR-E* and *HMR-I*):**



Spreading, maintenance, and inheritance of heterochromatin

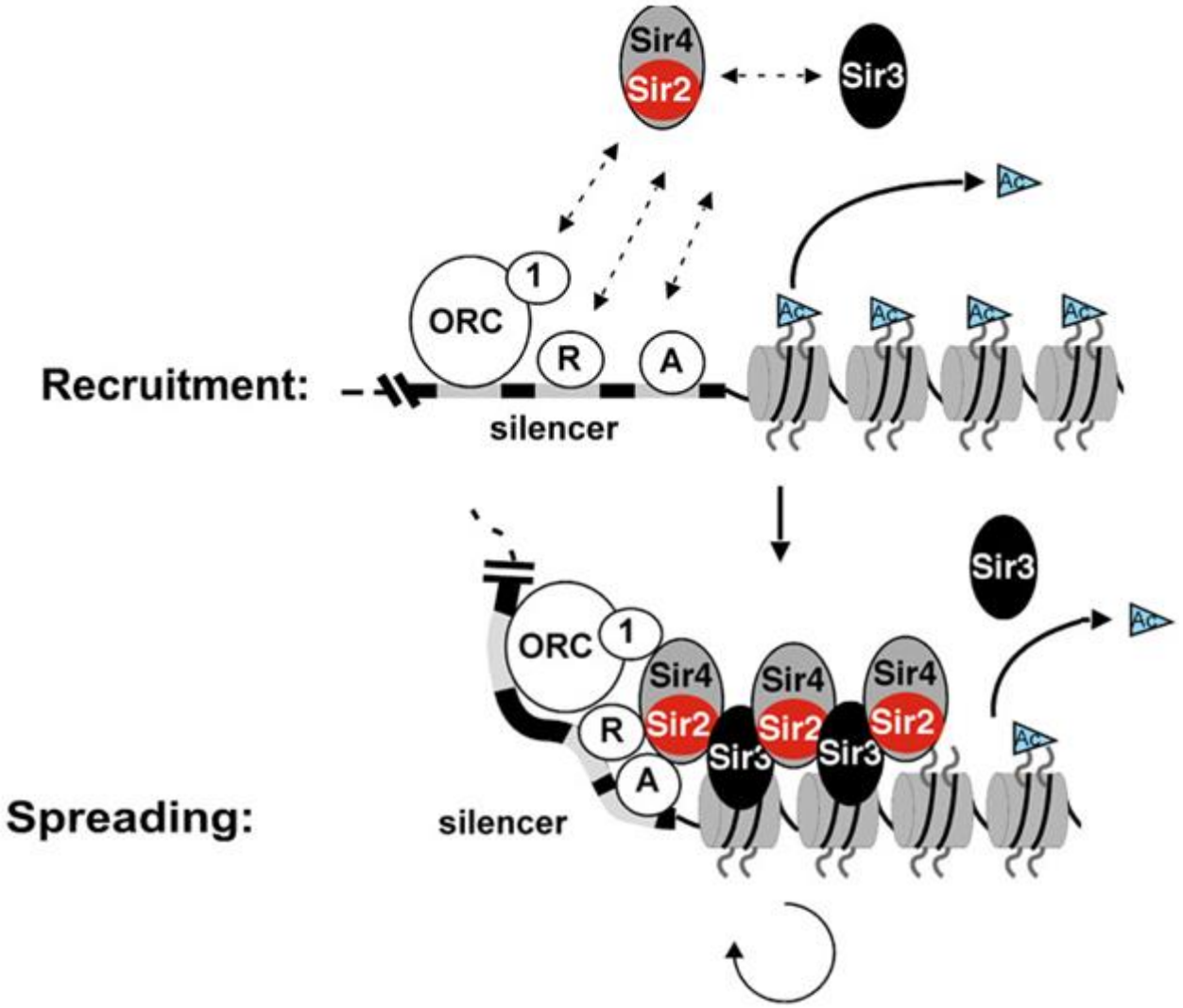
- **Sir1 recruits Sir2 (deacetylate H3/H4), then Sir3/Sir4 recognize deacetylated H3/H4, bringing new Sir2, …**



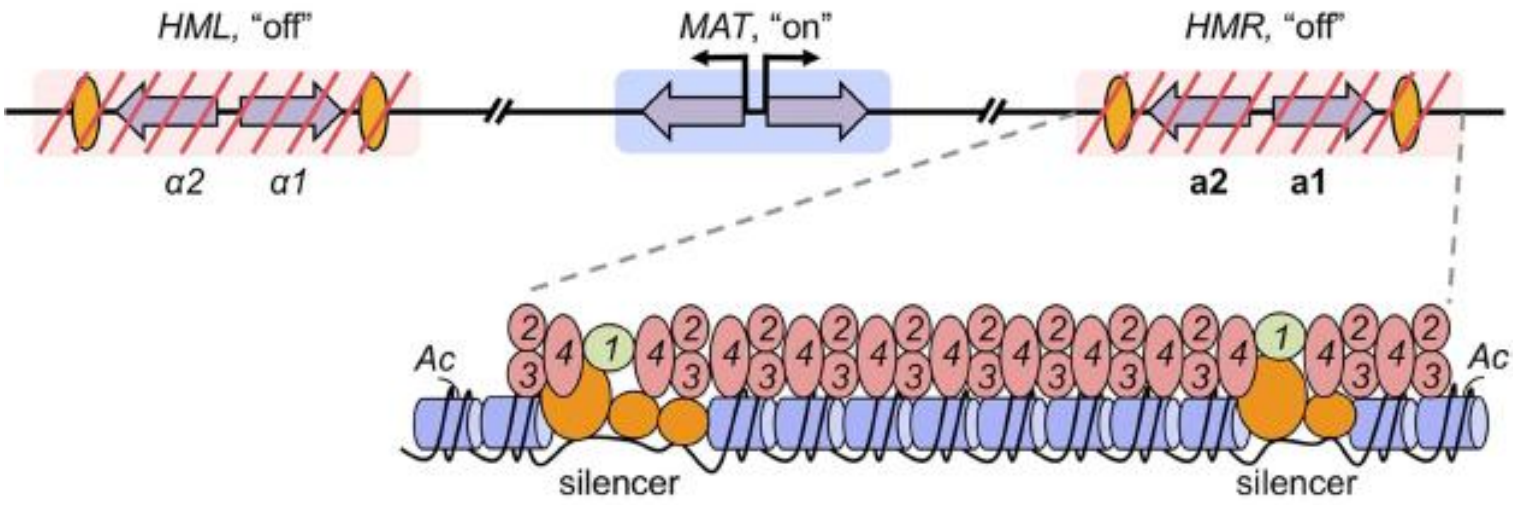
Heterochromatin properties *HML*/*HMR*

- **Limited to 3 kb (2 flanking silencer regions).**
- **No transcription.**
- **No access to *HO*, but to homologous recombination factors.**
- **Same properties mark / same factors form heterochromatin at telomeres in *S. cerevisiae*.**

Conclusion: working model



But: is silent chromatin really inert and unchanging?



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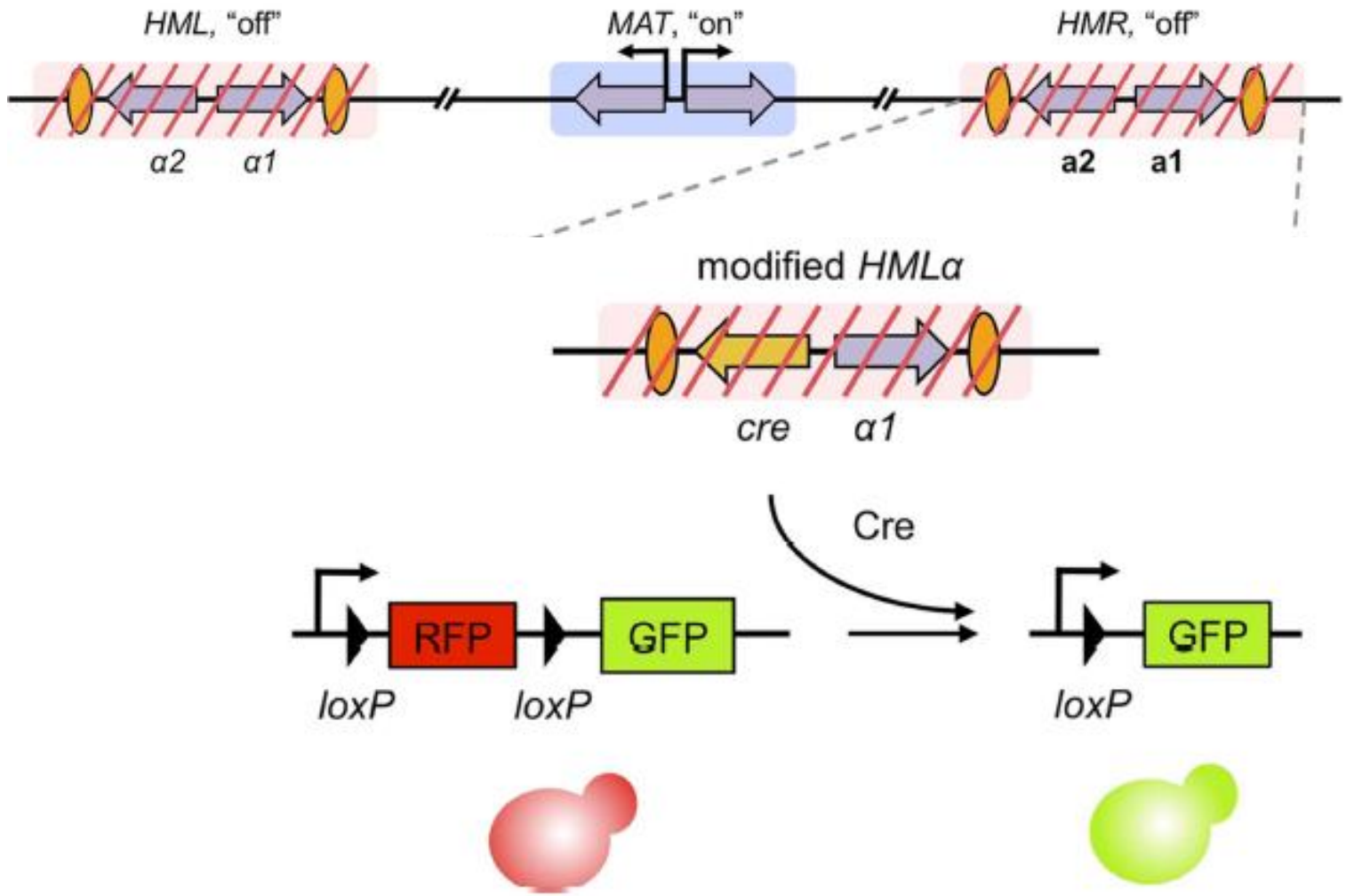
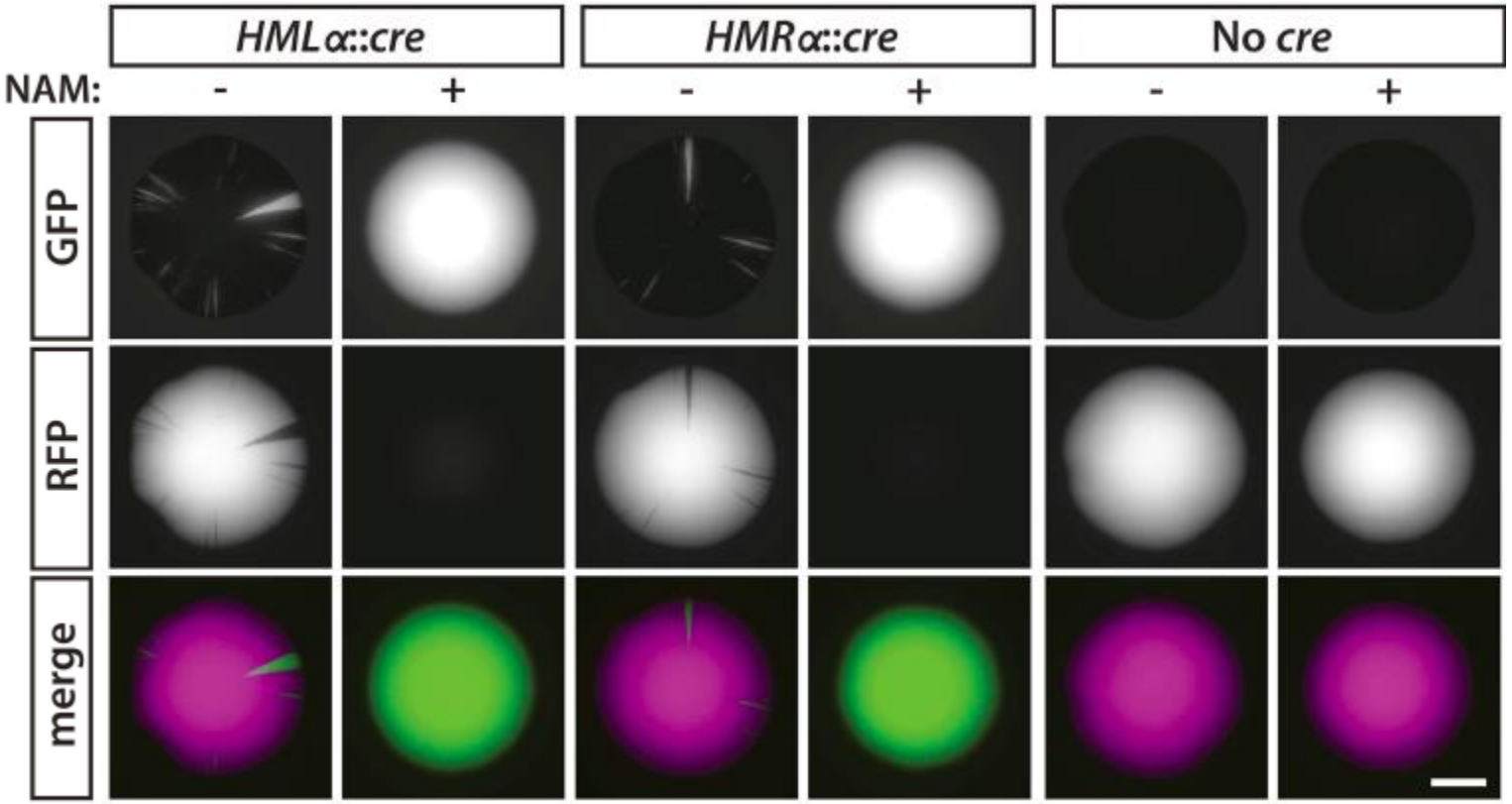
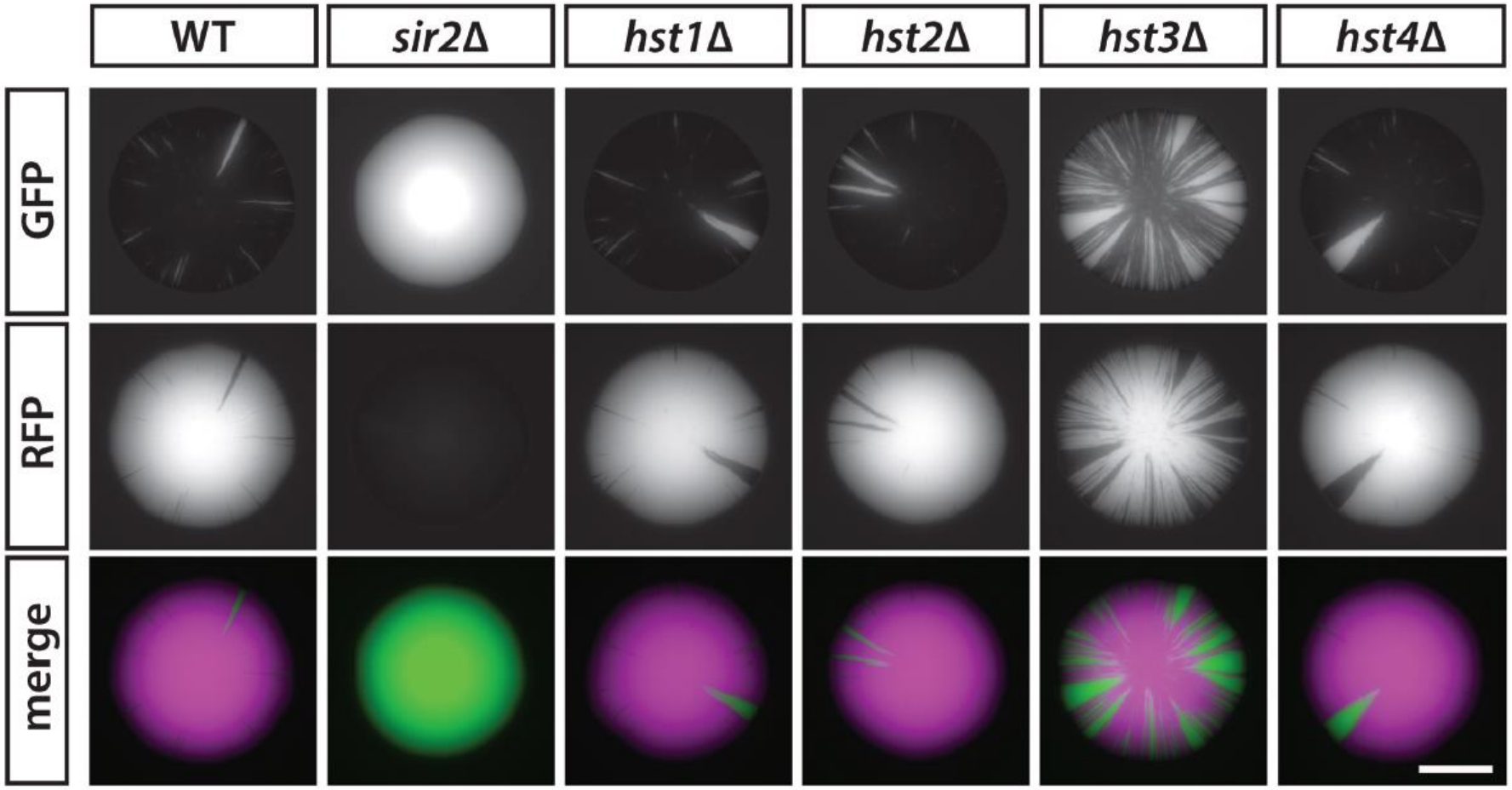


Sectoring assays in WT cells: rare, but detectable loss of silencing



Uncovering novel regulators of silencing maintenance/inheritance



Role of these factors?

Required for proper maintenance / inheritance, re-establishing heterochromatin after disruptions:

- **Cell cycle stage (replication).**
- **Dynamic recycling of nucleosomes /silencing factors.**

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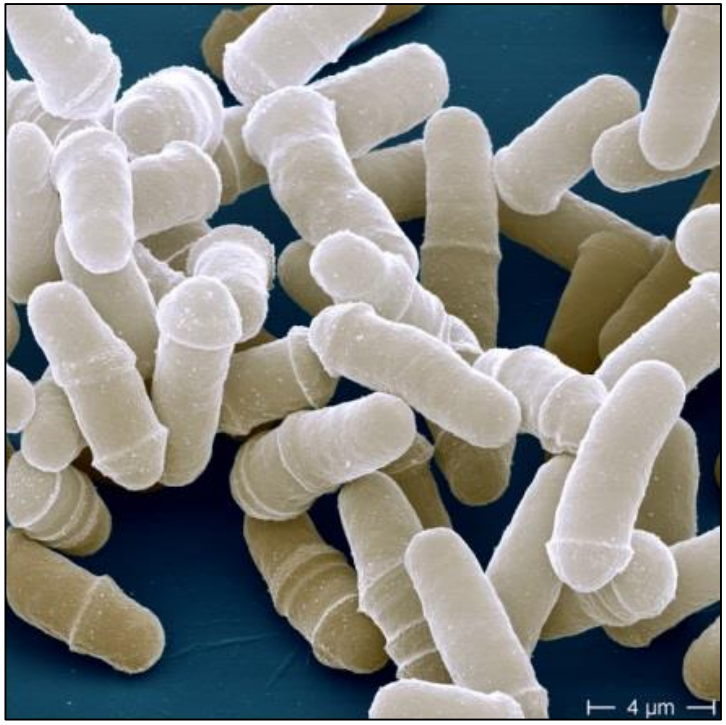
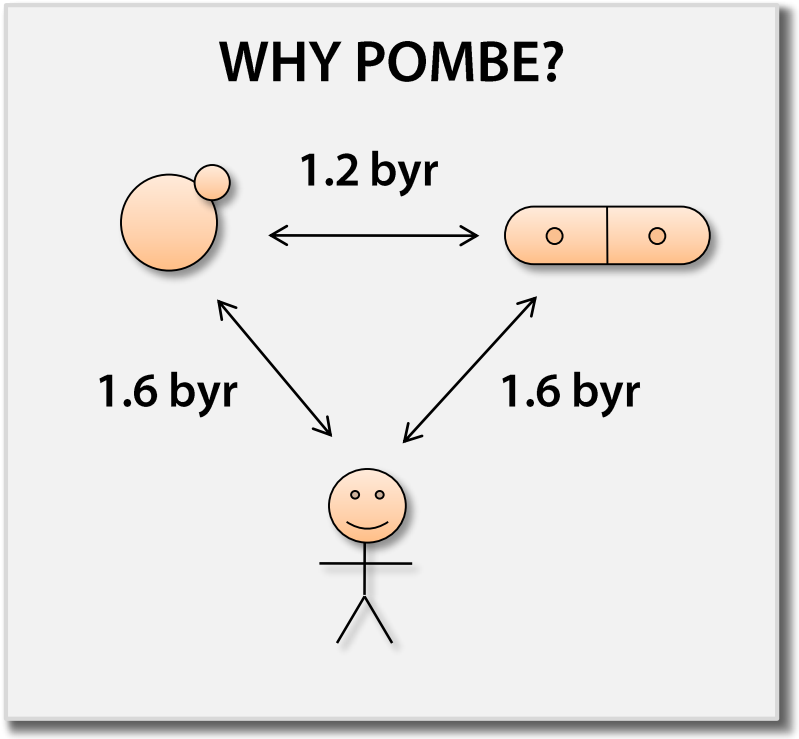
What is fission yeast?

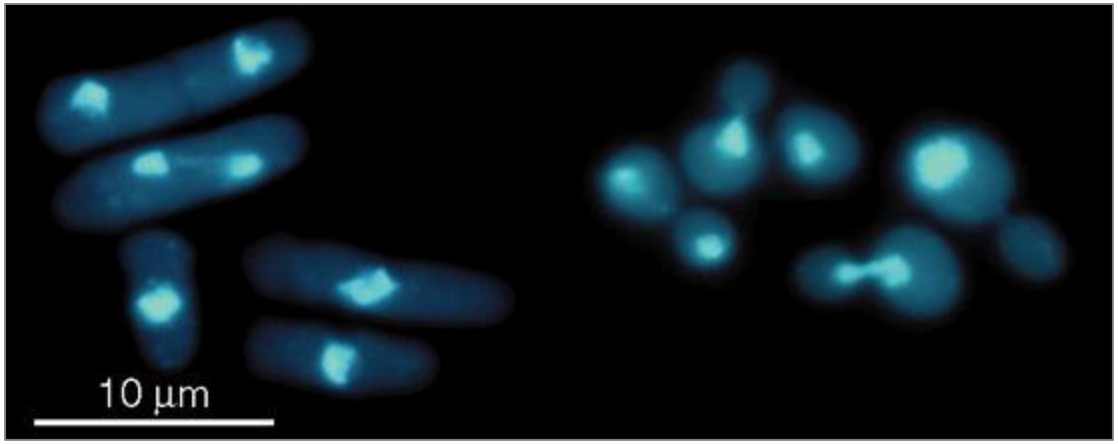




Similarities budding *vs* fission yeast

- **Unicellular yeast.**
- **Very easy to manipulate for genetics, biochemistry, and (epi)genomics.**

➔ **Conservation across eukaryotes.**



Differences budding *vs* fission yeast

	S. pombe	*S. cerevisiae*	Mammals
Chromatin - histone marks	H3K9 methylation	absent	H3K9 methylation
	Clr4 (H3K9 HMT)	absent	Suv3-9, G9a
	absent	Histone H1 (Hho1)	Histone H1 linker
Heterochromatin components	Swi6 (3 isoforms)	absent	HP1 (3 isoforms)
RNAi machinery	present (1 copy of Dcr, Ago)	absent	present
Transcription start site selection	TATA is -30 to -70 bp from +1	TATA is -40 to -200bp from +1	TATA is -30bp from +1
Centromere structure	35-110kb, repetitive elements	125bp, no repeats	6-7Mb, rep. elements
Coding genes w/ introns	43%	5%	99.99%

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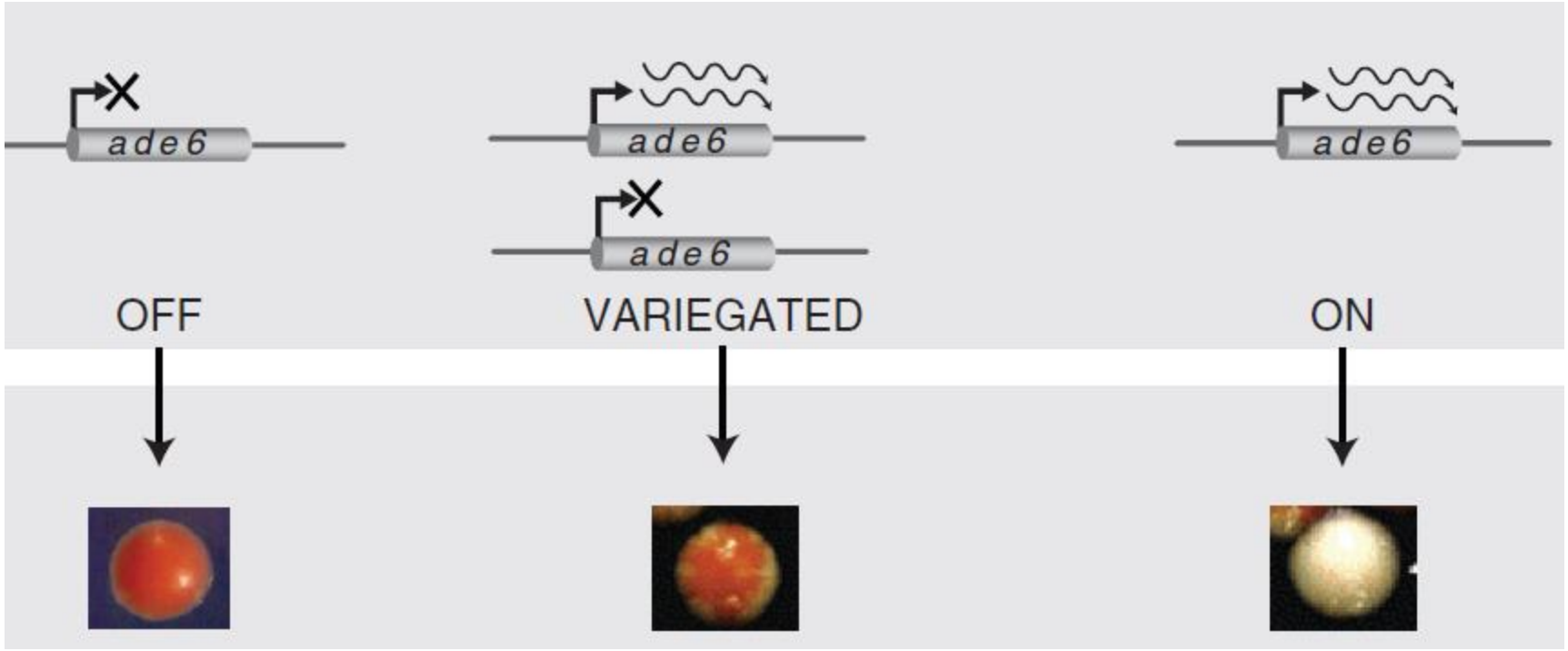
Epigenetic regulation of chromatin states in *S. pombe*

- **Discovery of a <u>Position Effect Variegation</u> phenotype in *S. pombe*, similar to *Drosophila*.**



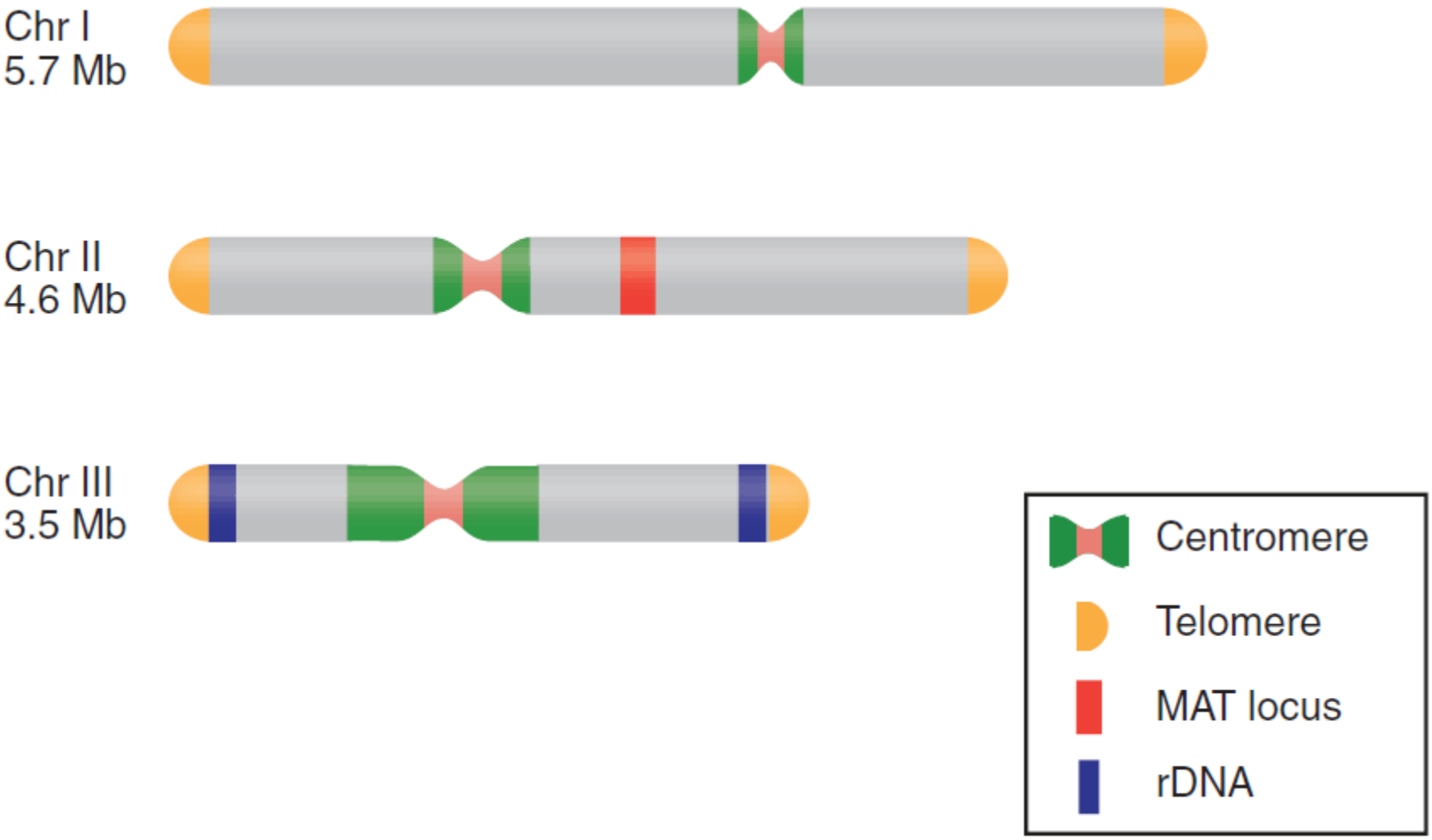
Epigenetic regulation of chromatin states in *S. pombe*

- **Discovery of a Position Effect Variegation phenotype in *S. pombe*, similar to *Drosophila*.**
- **Transgene expression state is variable.**
- **Stably transmitted in subsequent generations.**
- **Happens if transgene inserted near/in heterochromatic regions.**

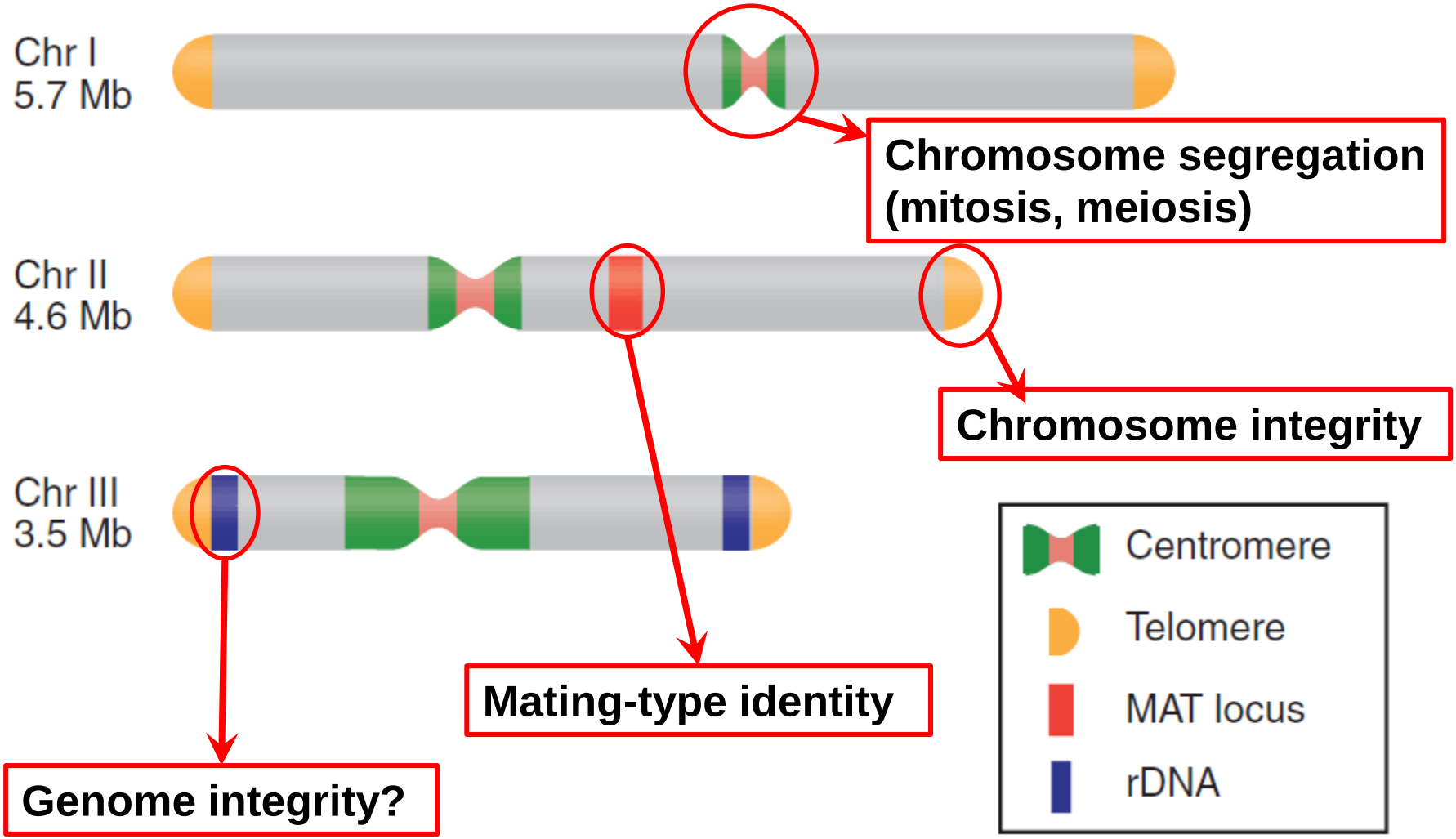
➔ **Exploited for understanding establishment, maintenance and repression mechanisms.**

➔ **Found conserved properties and mechanisms.**

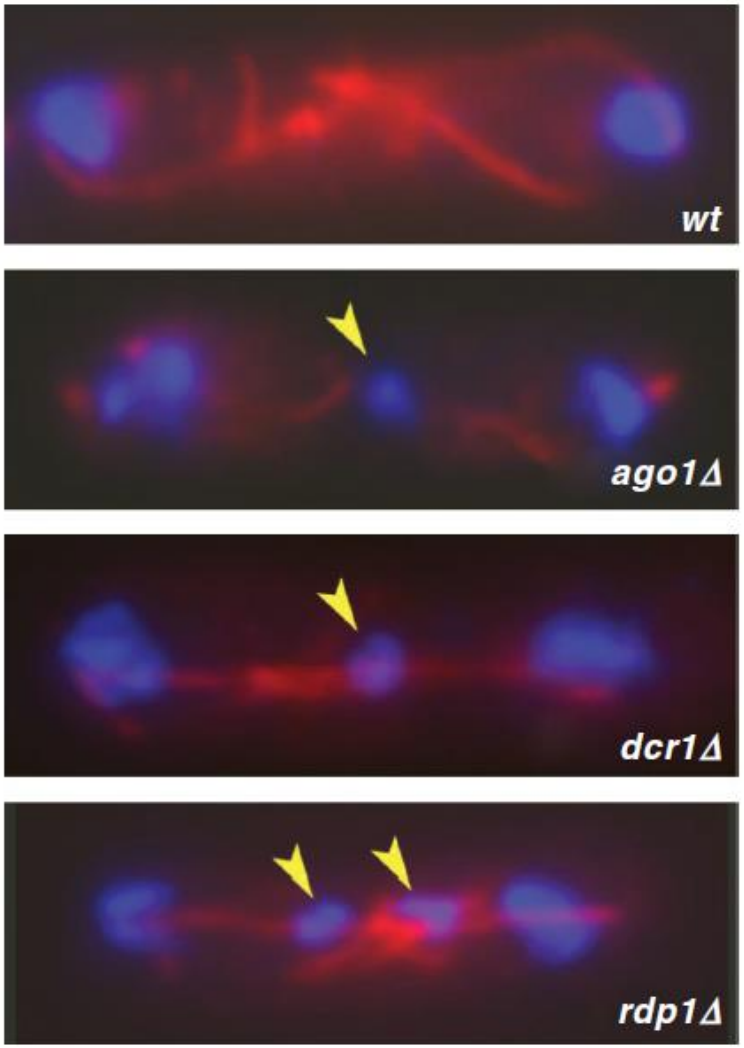
Heterochromatin / silenced regions in *S. pombe*

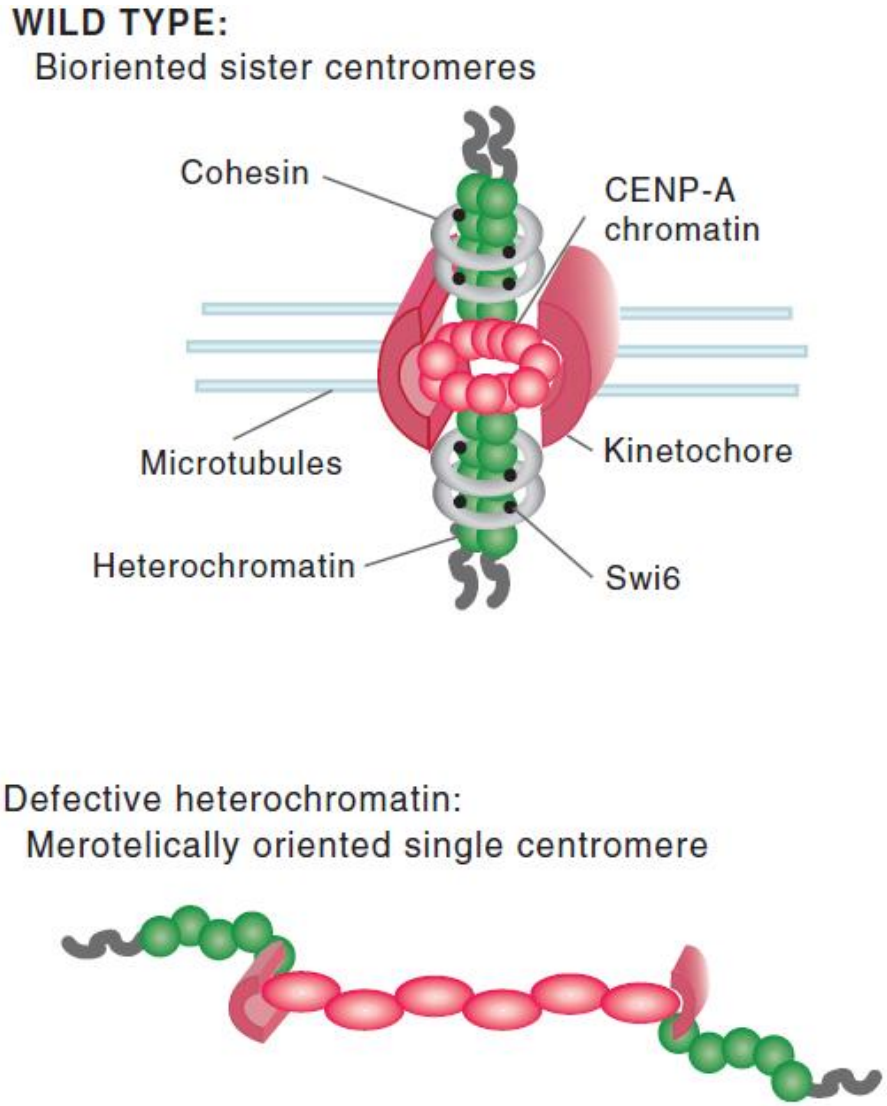


Functional roles of heterochromatin in *S. pombe*



Defective heterochromatin leads to abnormal centromere structures





Epigenetic regulators identified

Gene product	Molecular function	Reference(s)
Clr1 (Cryptic loci regulator 1)	Zinc ion binding protein, part of the SHREC complex	Thon and Klar 1992
Clr2 (Cryptic loci regulator 2)	Transcription-silencing protein Clr2; part of the SHREC complex	Ekwall and Ruusala 1994; Thon et al. 1994
Clr3 (Cryptic loci regulator 3)	Histone deacetylase class II (H3-K14-specific); part of the SHREC complex	Ekwall and Ruusala 1994; Thon et al. 1994; Grewal et al. 1998
Clr4 (Cryptic loci regulator 4)	Histone methyltransferase KMT activity (H3-K9-specific)	Ekwall and Ruusala 1994; Thon et al. 1994
Rik1	DNA-binding protein; part of the CLRC ubiquitin ligase complex	Egel et al. 1989; Ekwall and Ruusala 1994
Swi6	Chromo domain/shadow protein, heterochromatin protein 1 homolog	Ekwall and Ruusala 1994; Lorentz et al. 1994
Clr6 (Cryptic loci regulator 6)	Histone deacetylase class I	Grewal et al. 1998

➔ **Identified as mating-type region silencer mutants.**

➔ **Mutants also defective for centromeric silencing (distinction from *S. cerevisiae*).**

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Clr4 (Cryptic loci regulator 4)	Histone methyltransferase KMT activity (H3-K9-specific)	Ekwall and Ruusala 1994; Thon et al. 1994
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Ago1 was identified as Csp9 (centromere: suppressor of position effect 9)	Argonaute protein, part of the RITS complex	Ekwall et al. 1999; Volpe et al. 2003
Rpb7 was identified as Csp3 (centromere: suppressor of position effect 3)	DNA-directed RNA polymerase II subunit	Ekwall et al. 1999; Djupedal et al. 2005
Rpb2	DNA-directed RNA polymerase II subunit	Kato et al. 2005
Epe1 (Enhanced position effect 1)	jmjC domain protein	Ayoub et al. 2003
Cwf10 was identified as Csp4	Splicing factor	Bayne et al. 2008; Ekwall et al. 1999
Prp39 was identified as Csp5	Splicing factor	Bayne et al. 2008; Ekwall et al. 1999

Key factors

Gene product	Molecular function	Reference(s)
Clr1 (Cryptic loci regulator 1)	Zinc ion binding protein, part of the SHREC complex	Thon and Klar 1992
Clr2 (Cryptic loci regulator 2)	Transcription-silencing protein Clr2; part of the SHREC complex	Ekwall and Ruusala 1994; Thon et al. 1994
Clr3 (Cryptic loci regulator 3)	Histone deacetylase class II (H3-K14-specific); part of the SHREC complex	Ekwall and Ruusala 1994; Thon et al. 1994; Grewal et al. 1998
Clr4 (Cryptic loci regulator 4)	Histone methyltransferase KMT activity (H3-K9-specific)	Ekwall and Ruusala 1994; Thon et al. 1994
Rik1	DNA-binding protein; part of the CLRC ubiquitin ligase complex	Egel et al. 1989; Ekwall and Ruusala 1994
Swi6	Chromo domain/shadow protein, heterochromatin protein 1 homolog	Ekwall and Ruusala 1994; Lorentz et al. 1994
Clr6 (Cryptic loci regulator 6)	Histone deacetylase class I	Grewal et al. 1998
Ago1 was identified as Csp9 (centromere: suppressor of position effect 9)	Argonaute protein, part of the RITS complex	Ekwall et al. 1999; Volpe et al. 2003
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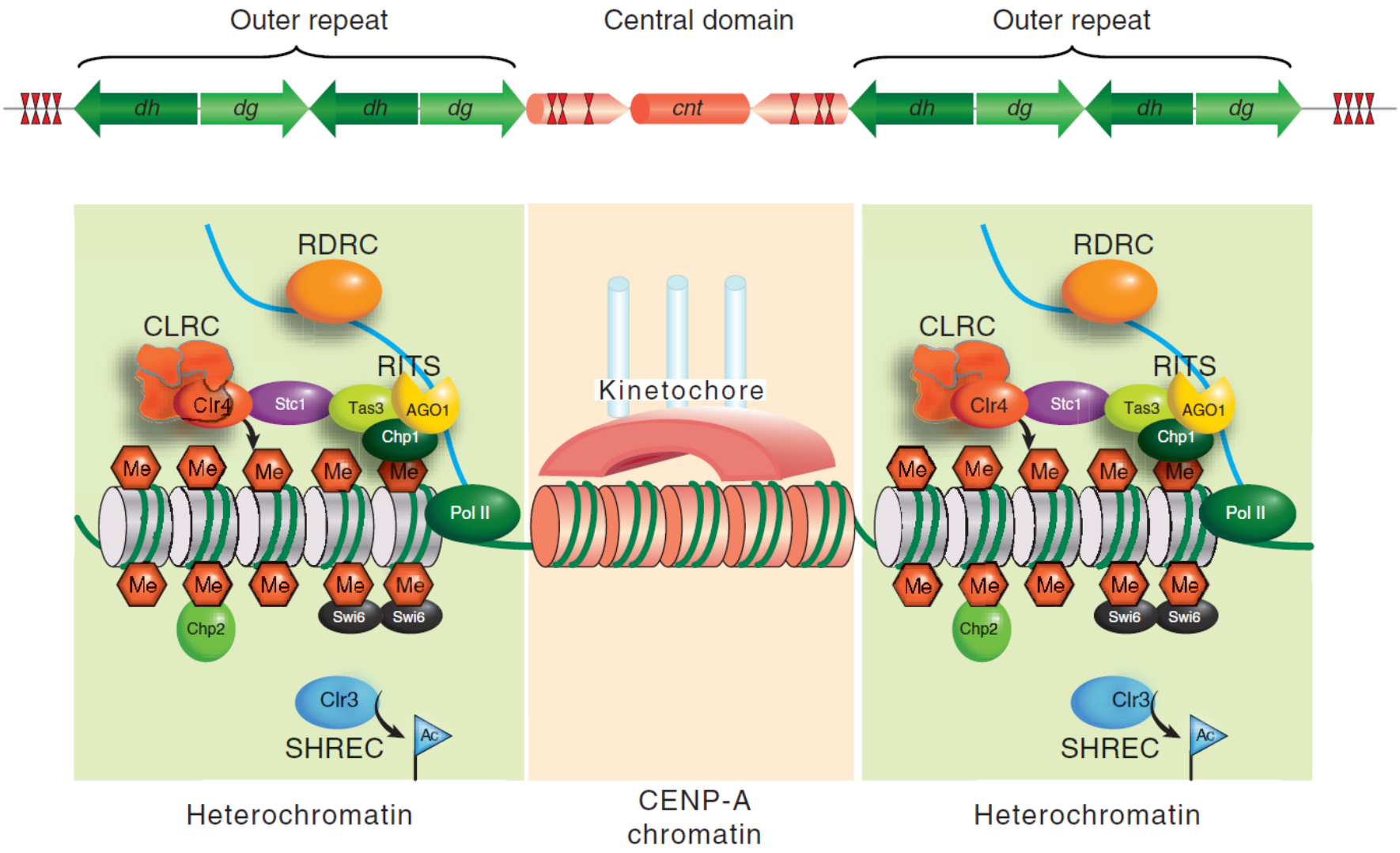
Key findings

Heterochromatin silencing in *S. pombe* is different from *S. cerevisiae*, but similar to plants and metazoan:

- **Histone modifications (deposition & recognition of H3-K9me3).**
- **RNA interference (RNAi) machinery.**
- **RNA Pol II transcription.**
- ***But without DNA methylation.***

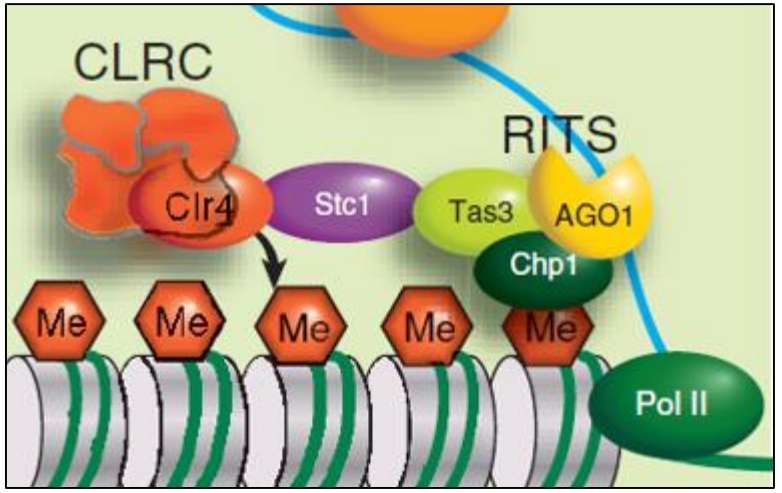
Asembly of heterochromatic domains at centromeres



Establishment *vs* maintenance

Two ‘establishment’ models ongoing:

- **siRNAs recognize nascent transcripts, loading RITS.**
- **siRNAs originate from degradation or Dicer activity.**
- **Supported by genetics: Clr4 tethering bypass requirement for RNAi.**

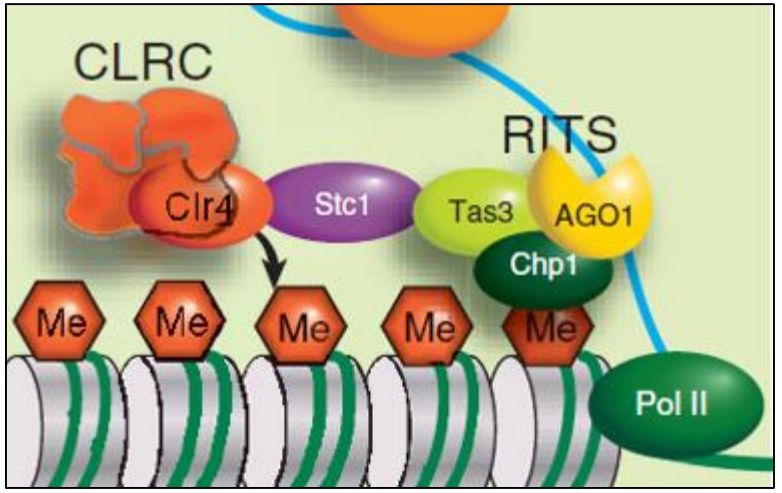


Establishment *vs* maintenance

Two 'establishment' models ongoing:

- **siRNAs recognize nascent transcripts, loading RITS.**
- **siRNAs originate from degradation or Dicer activity.**
- **Supported by genetics: Clr4 tethering bypass requirement for RNAi.**

- **BUT: no siRNAs in *clr4*Δ!**

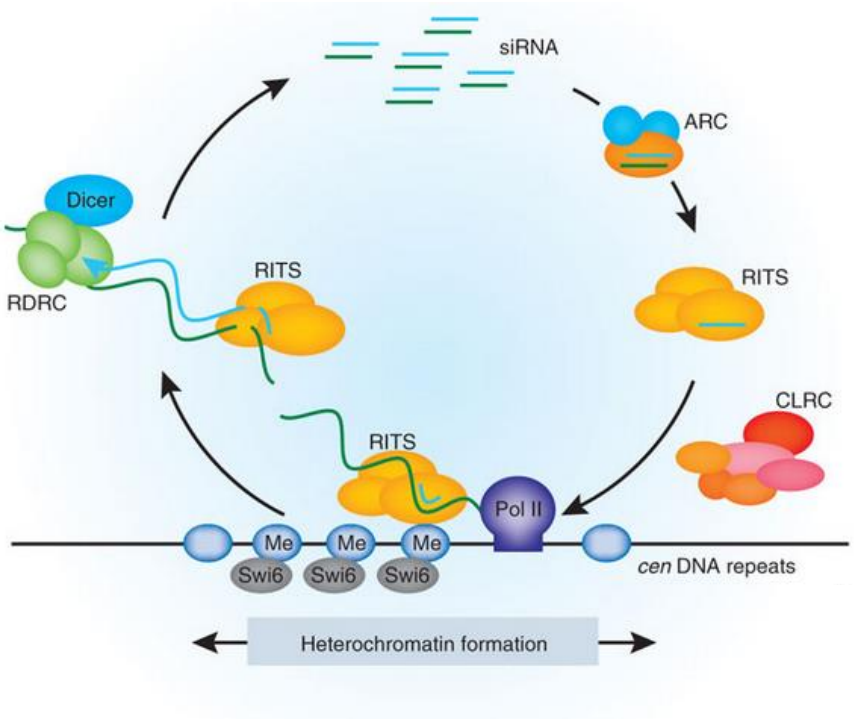


Establishment *vs* maintenance

Second 'establishment' model:

- **H3K9me required for siRNA generation.**
- **Feedback amplification.**

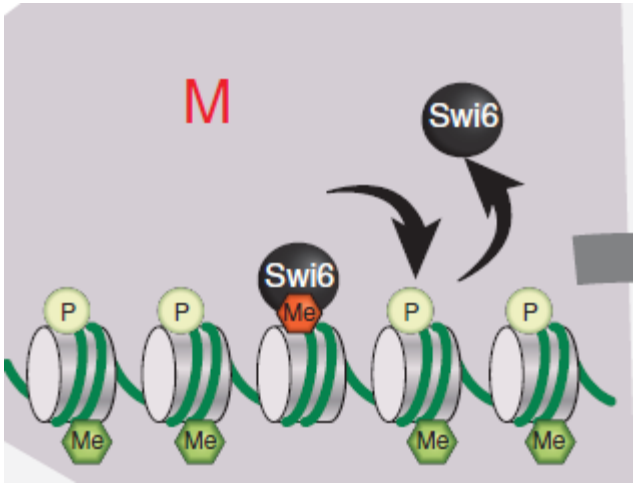
➔ ***Chicken vs egg?***



Establishment *vs* maintenance

Maintenance through cell divisions (epigenetic):

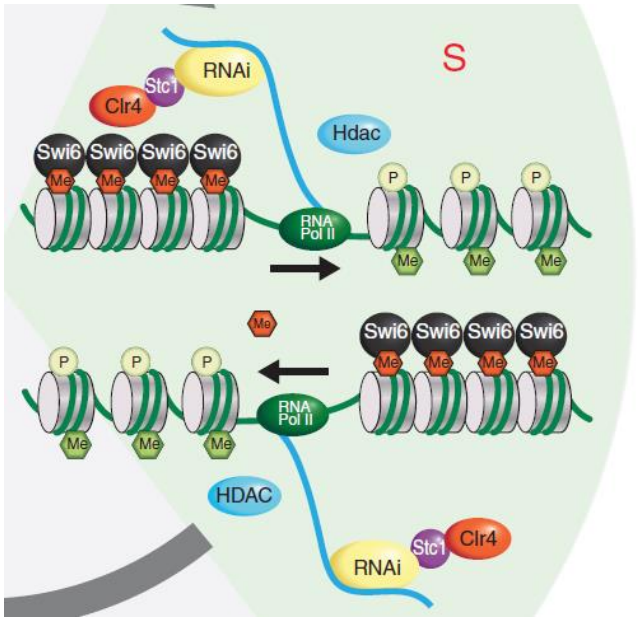
- **Swi6 detached during mitosis.**



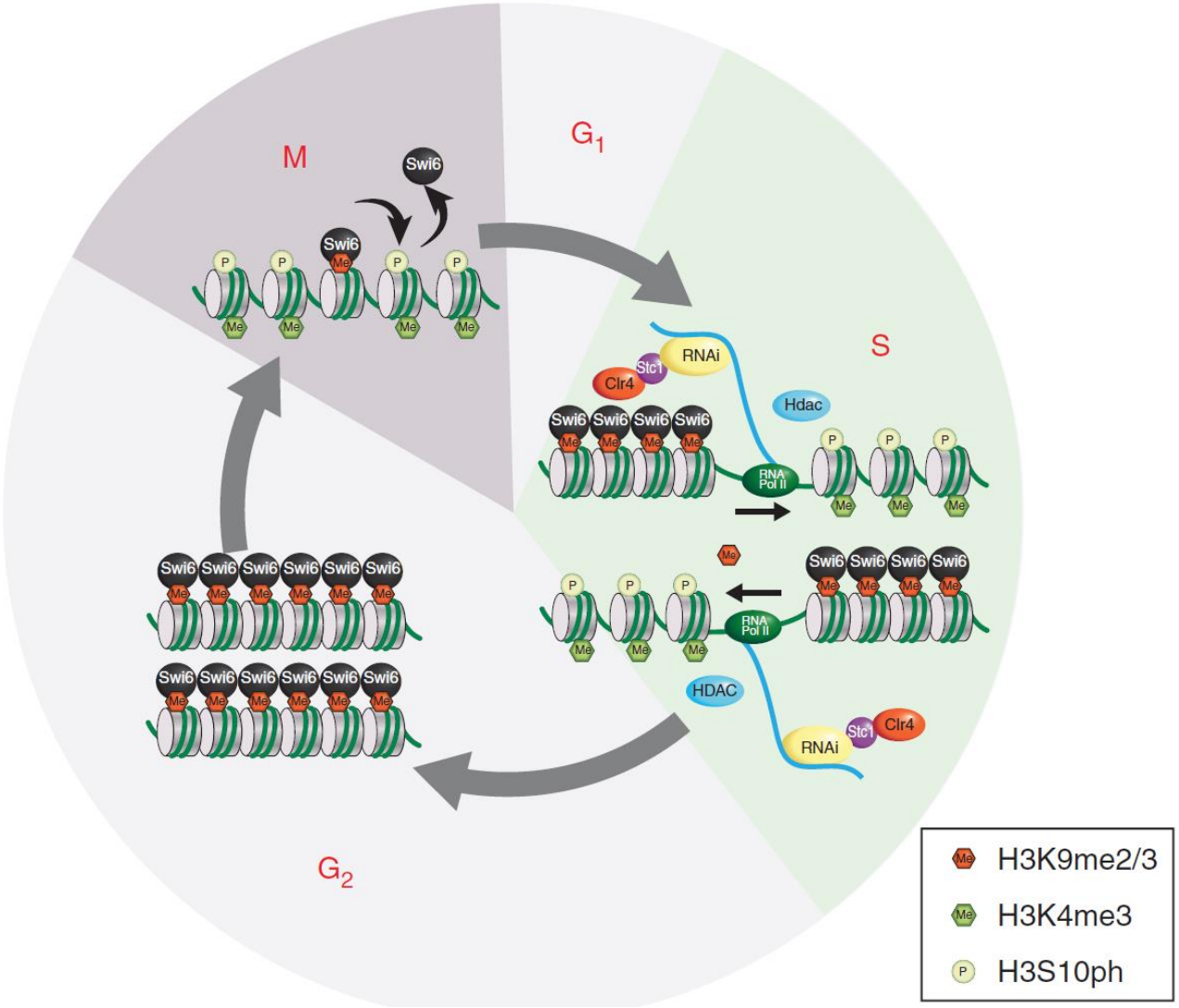
Establishment *vs* maintenance

Maintenance through cell divisions (epigenetic):

- **Swi6 detached during mitosis.**
- **Heterochromatin decondensation during S phase allows RNA Pol II transcription.**
- **siRNA production.**
- **RITS-RDRC-CLRC recruitment.**



Cell-cycle regulation of centromere heterochromatin assembly



Summary

What we have learned from *S. pombe*:

- **Heterochromatin is found *mainly* at centromeres, telomeres, ribosomal DNA, and mating-type regions.**
- **Heterochromatin assembly requires RNAi machinery, H3K9me3 mark, and transcription (!).**

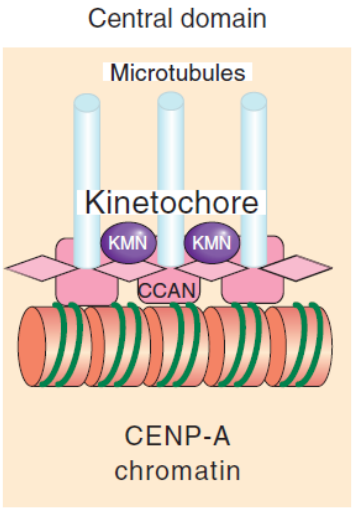
Summary

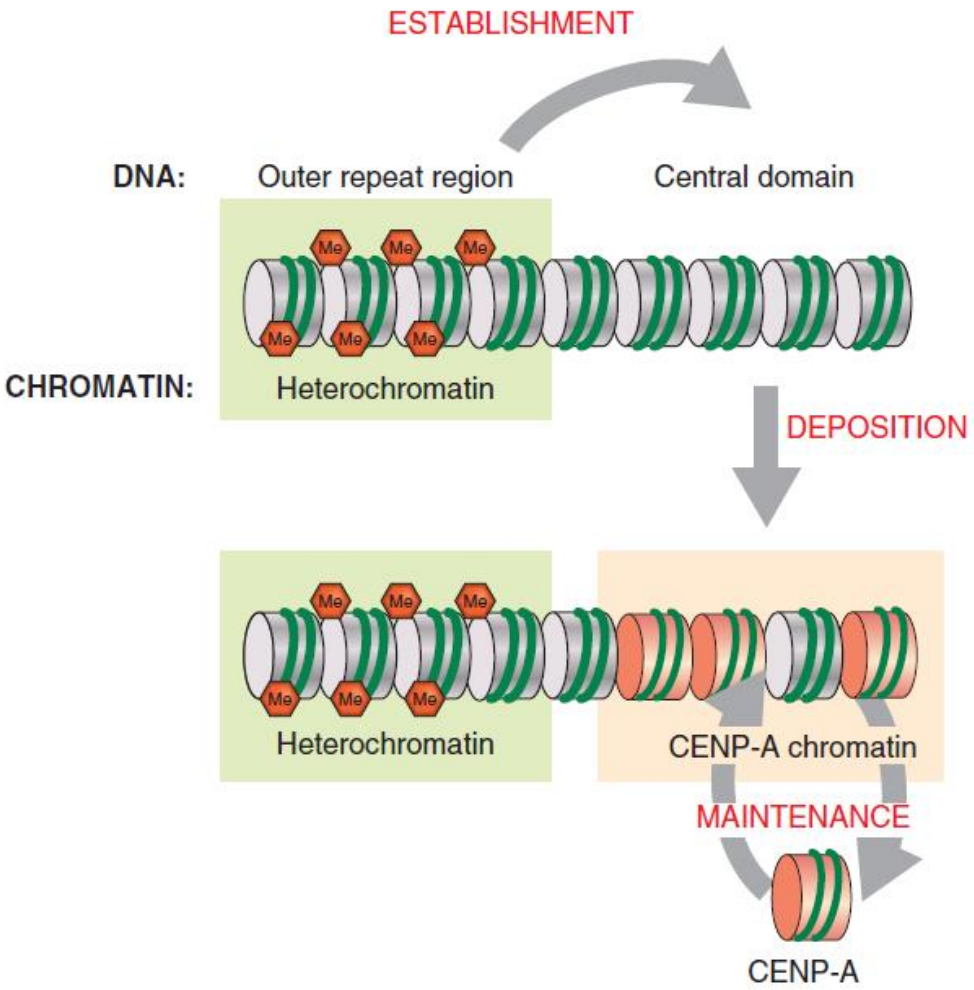
What we have learned from *S. pombe*:

- **Heterochromatin is found *mainly* at centromeres, telomeres, ribosomal DNA, and mating-type regions.**
- **Heterochromatin assembly requires RNAi machinery, H3K9me3 mark, and transcription (!).**

- **Boundaries (role Epe1 and Cullin ligases).**
- **Epigenetic inheritance of centromere identity through deposition of CENP-A.**

Epigenetic regulation of chromatin states in *S. pombe*





Outline

- Genetic and epigenetic regulation of gene expression.
- Mating-type switching in budding yeast.
- Positive and negative regulation of mating-type switching.
- Epigenetics and heterochromatin formation in fission yeast.
- **Non-coding RNAs and gene regulation in fission yeast.**

Article presentation

lncRNA recruits RNAi and the exosome to dynamically regulate *pho1* expression in response to phosphate levels in fission yeast

Sneha Shah, Sina Wittmann,[1] Cornelia Kilchert,[1] and Lidia Vasiljeva[2]

Department of Biochemistry, University of Oxford, Oxford OX1 3QU, United Kingdom

noncoding RNAs:

- **Genomes are highly transcribed, including many noncoding RNAs.**
- **From short to very long ncRNAs (21nt. to ~300 bp, up to >10 kb).**
- **Typically rapidly processed (eg. exosome degradation).**
- **Noise or functional roles: still poorly understood.**

Examples of regulatory roles:

Several examples, different mechanisms:

- **Cis-acting, transcriptional interference (*SER3*, *IME4*).**
- **dsRNA-mediated silencing (sense/anti-sense).**
- **Recruitment and establishment of epigenetics marks (HOTAIR-PRC2 at HOX loci).**
- **Silencing: piRNAs in germ line cells in animals.**
- **Constitutive repression: RNAi-dependent heterochromatin formation in fission yeast.**

Examples of regulatory roles:

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- **Silencing: piRNAs in germ line cells in animals.**
- **Constitutive repression: RNAi-dependent heterochromatin formation in fission yeast.**

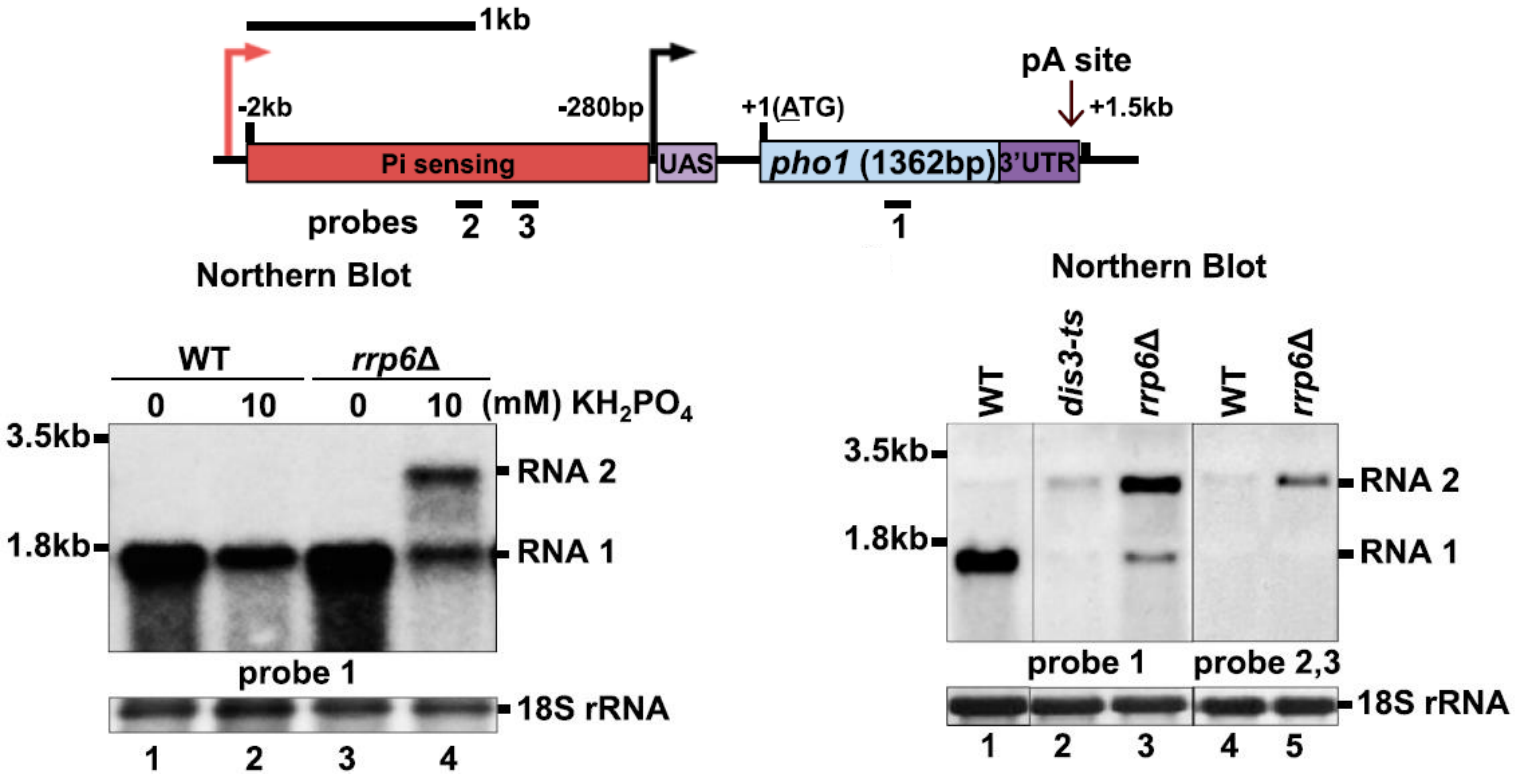
At facultative heterochromatin?

This is the question that this paper asks.

Key findings:

- **ncRNA: promoter-produced, cis-acting, nutrient (phosphate)-regulated.**
- **Repressing by heterochromatin formation.**
- **Repression is RNAi-dependent.**
- **Recognized by RNA binding protein (Mmi1).**
- **Degraded (and terminated?) by exosome.**

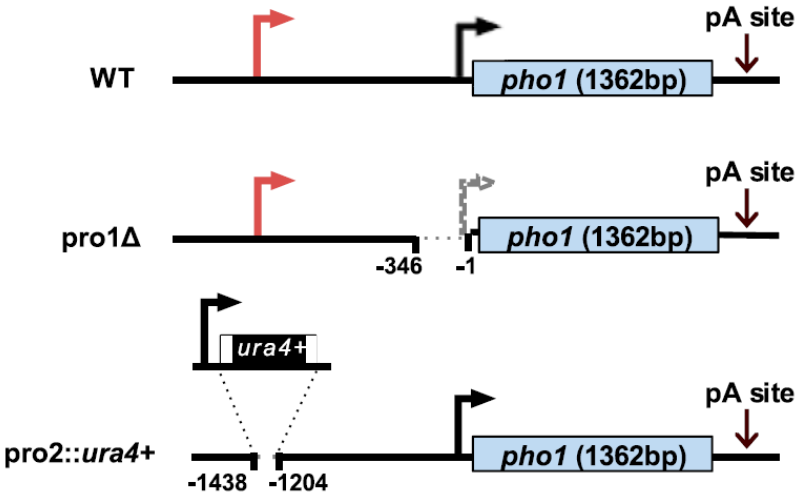
Figure 1: Two overlapping transcripts detected at *pho1*.

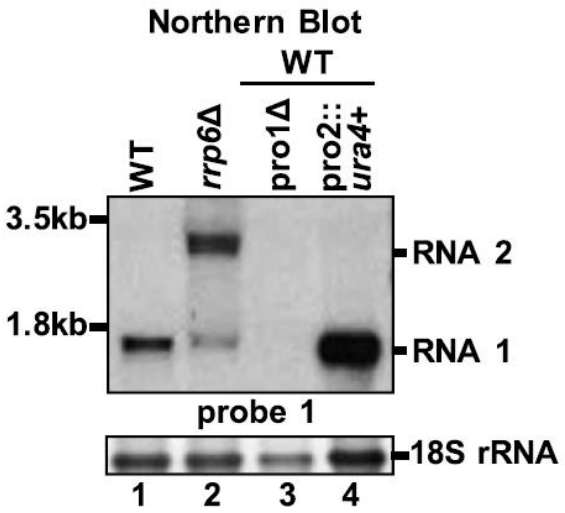


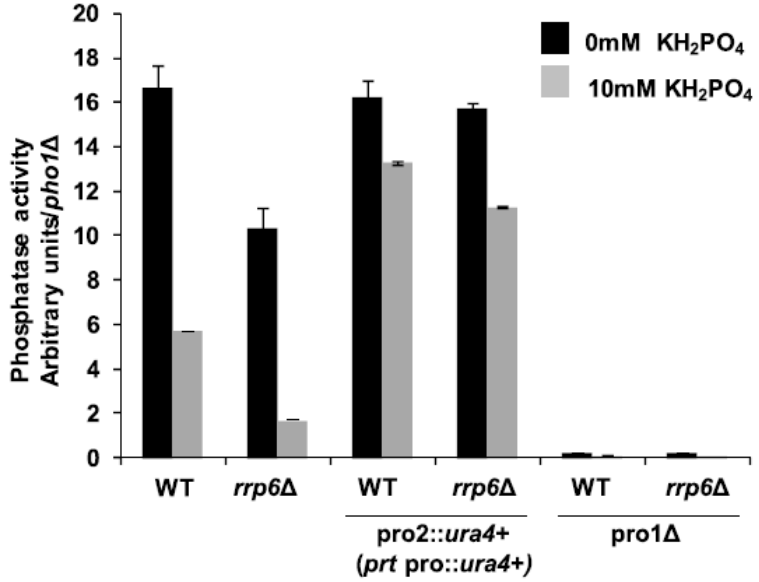
Caution: examples of bad Photoshop cropping!

Upstream ncRNA confirmed by 5' + 3' RACE.

Figure 2: upstream ncRNA represses downstream mRNA.







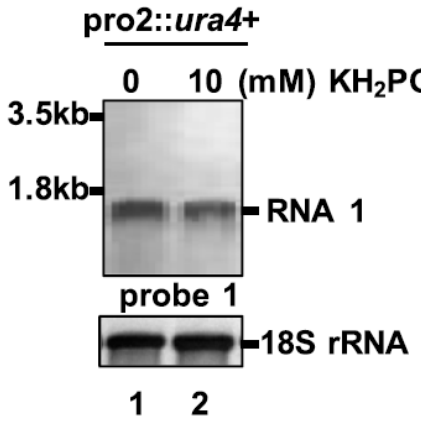
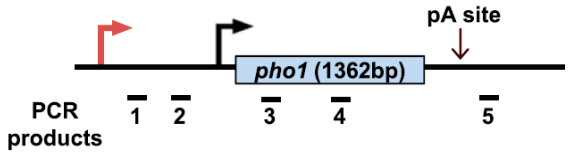
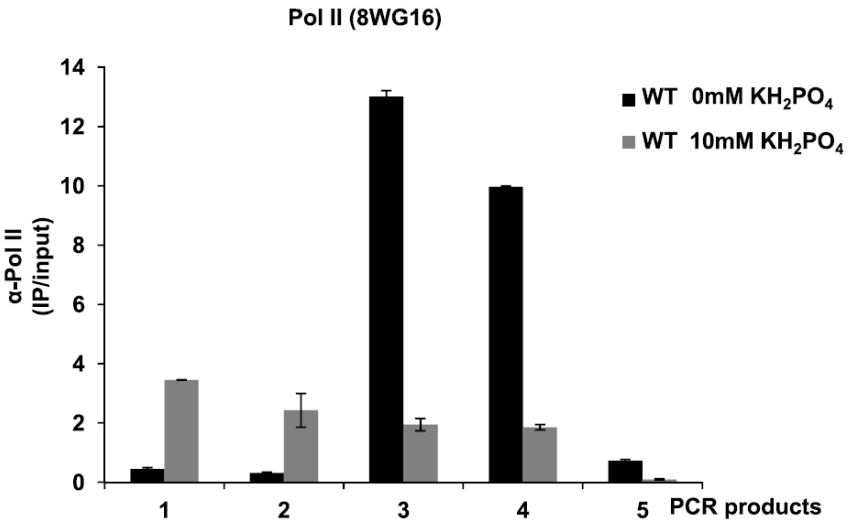
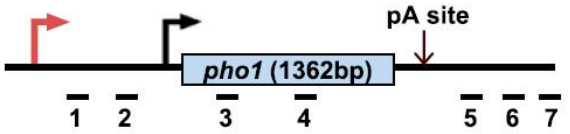


Figure 1 + 5: transcription of *pho1* locus.

ChIP + Run-on (TRO) experiments:







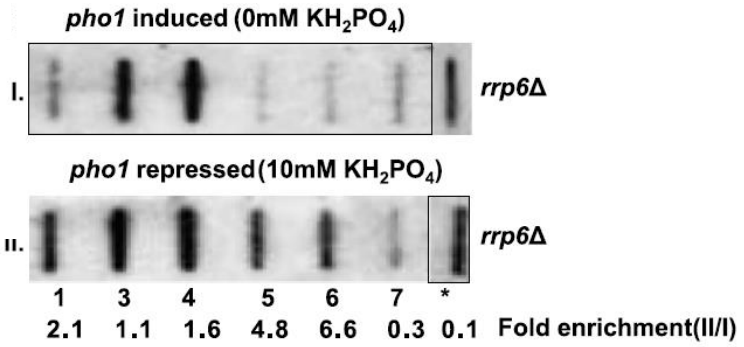


Figure 4: Deposition of H3K9me2 marks across *pho1*,

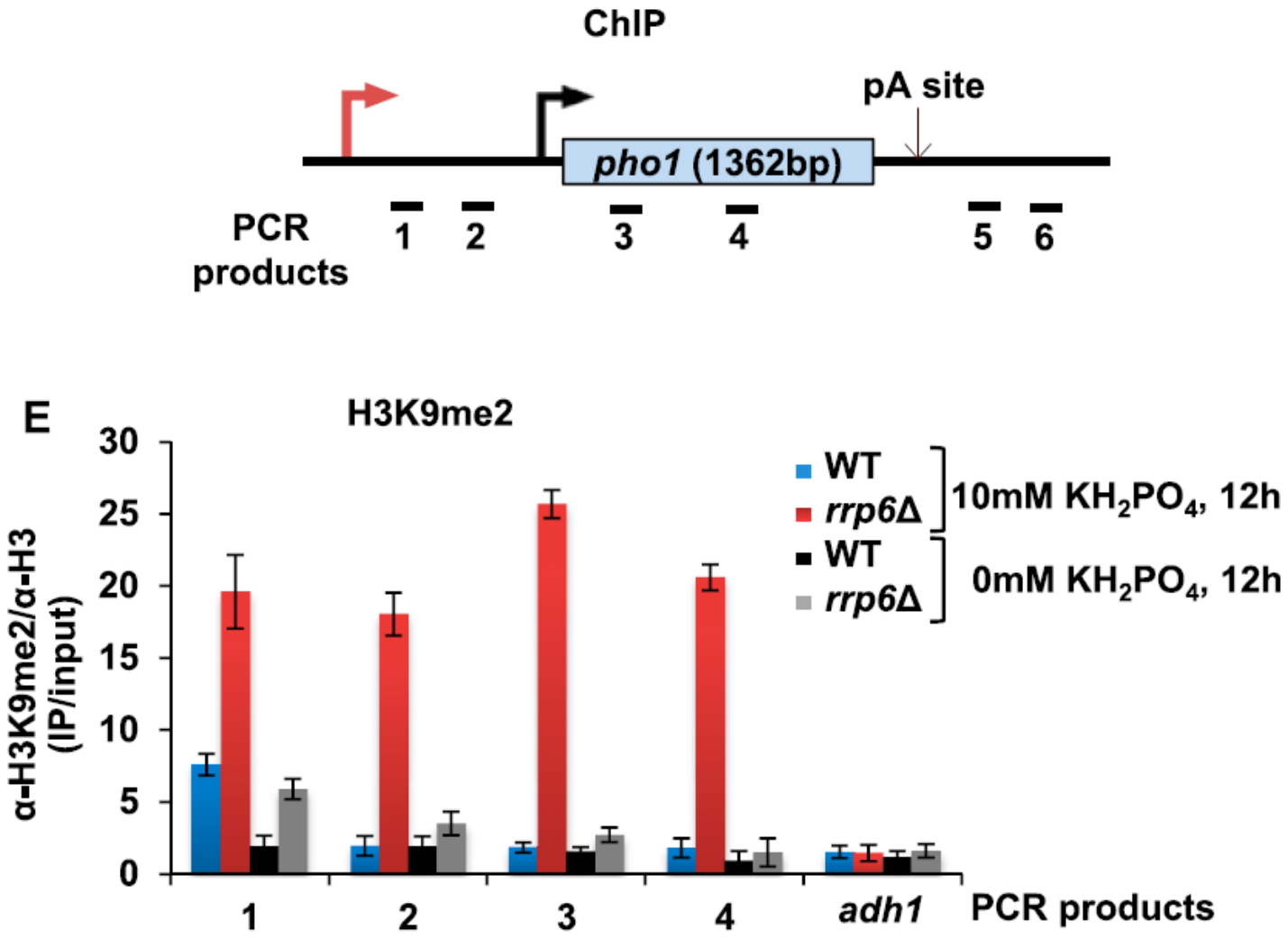
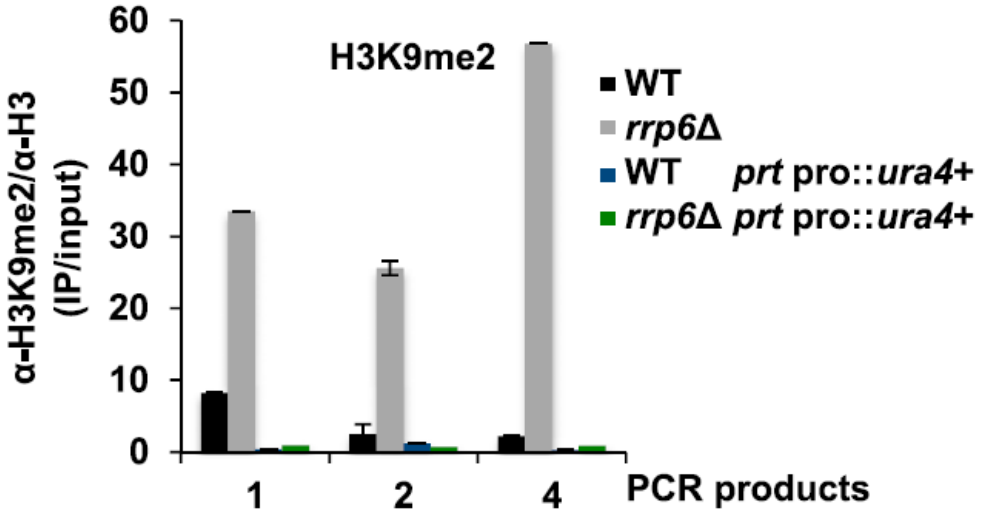


Figure 4: Deposition of H3K9me2 marks across *pho1*, upon ncRNA expression.



Depends also on RNAi (Ago1, Dcr1) and on Red1 (degradation machinery for meiotic transcripts).

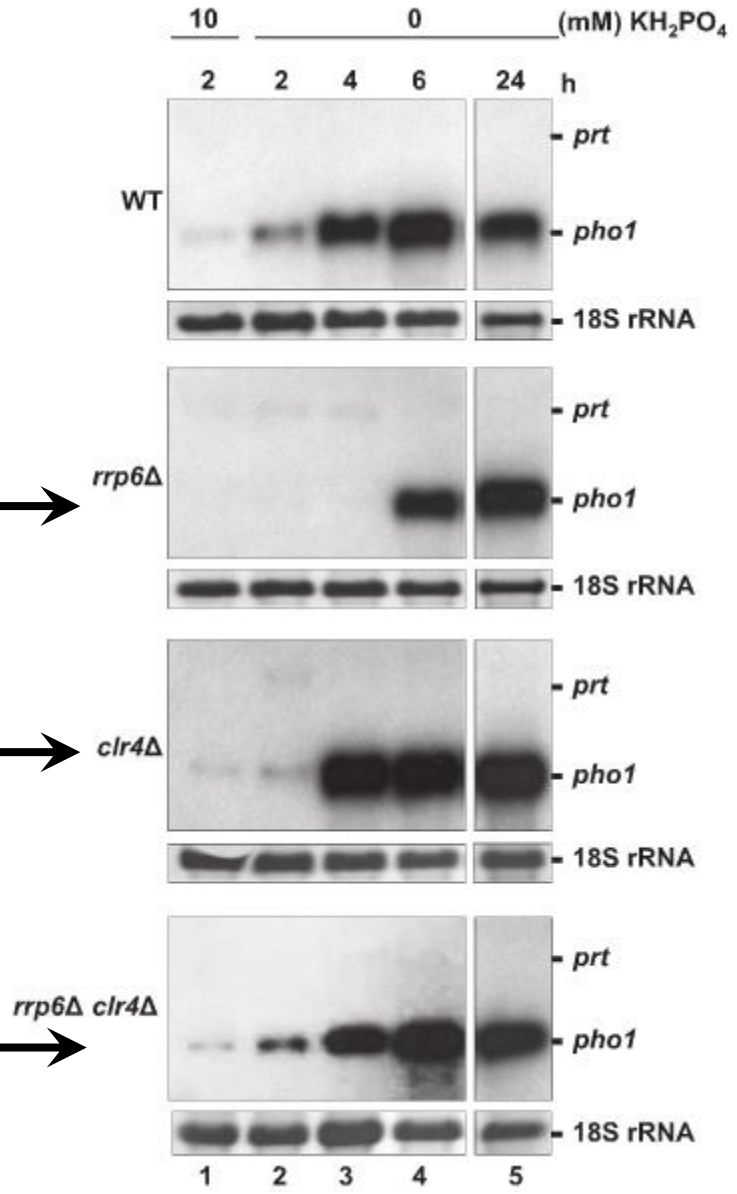
Figure 3: kinetics of *pho1* expression.

3 important observations:

1. Rrp6 promotes induction:

2. Clr4 (H3K9me) inhibits induction:

3. Rrp6 and Clr4 act independently (problem!)

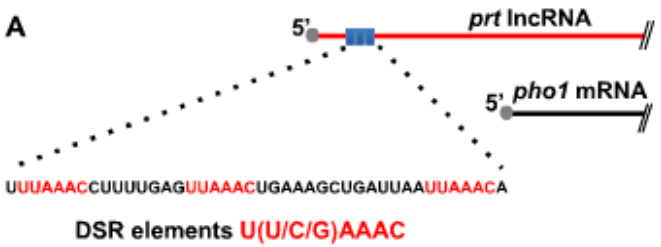


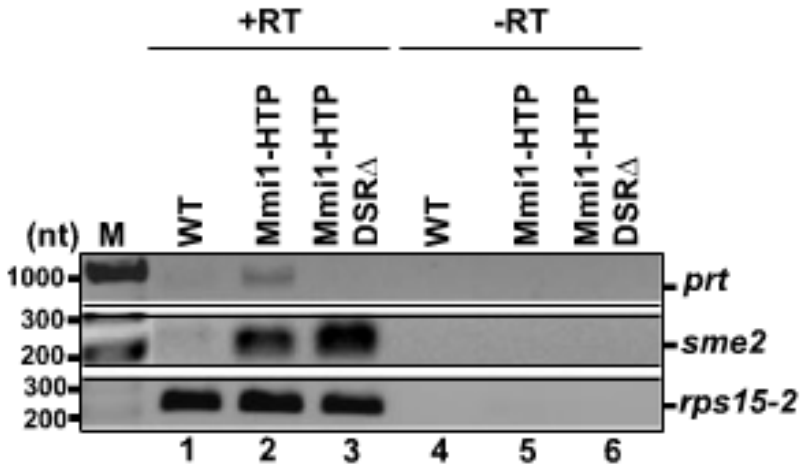
Another problem:

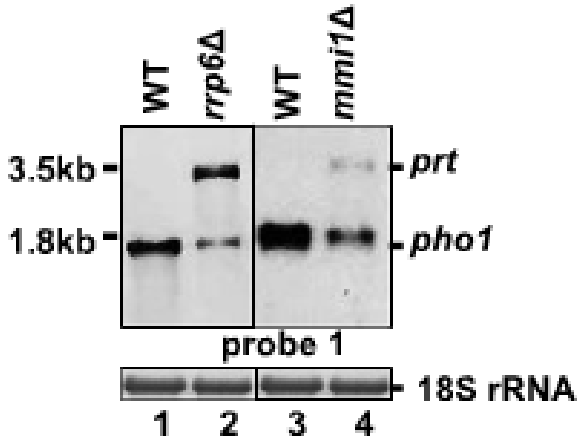
- **Two overlapping transcripts: one ncRNA regulating one mRNA.**
- **Share the same 3’ end, differ 5’.**
- **Degradation (and termination) only of the ncRNA depends on exosome.**

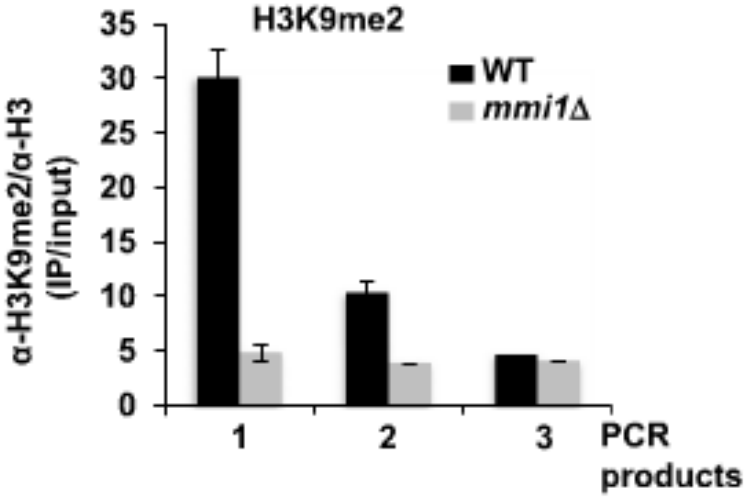
How does the exosome knows which is the ncRNA?

Figure 6: 5’ sequence motif suggest role of Mmi1. (Found in Yamamoto lab: Harigaya Y, *Nature*, 2006)



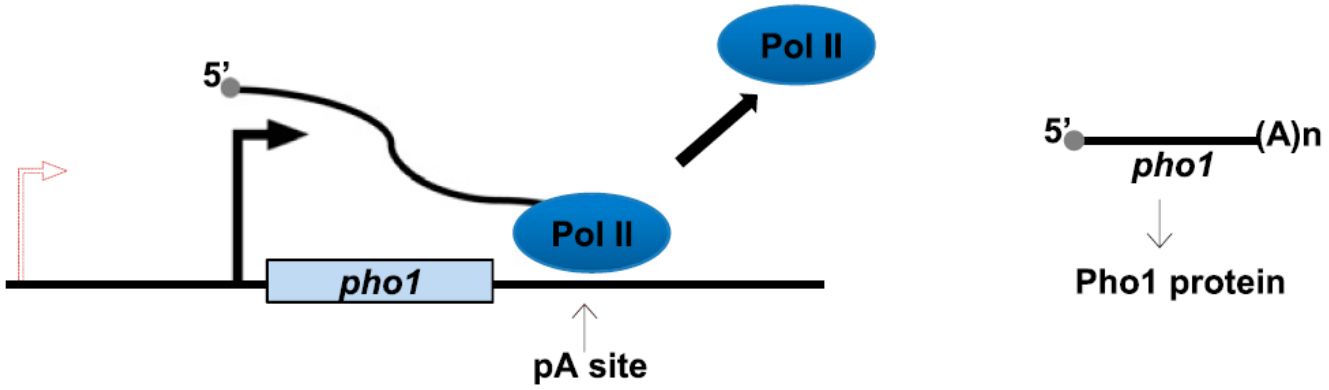




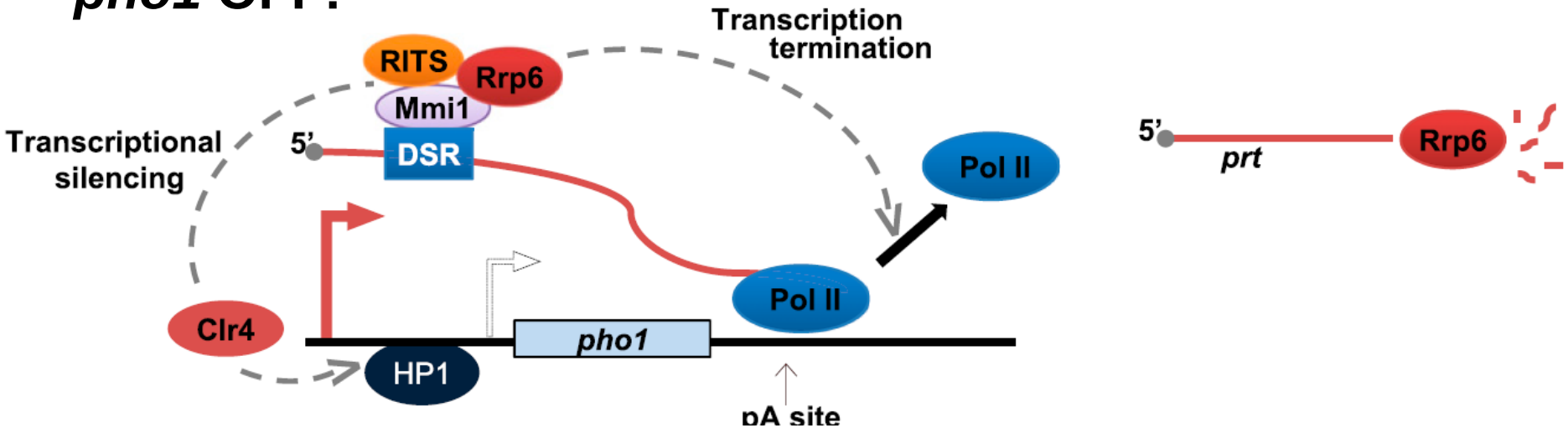


Working model

pho1 ON:



pho1 OFF:



Conclusions

- **Describes new role for a ncRNA.**
- **Mechanism of transcriptional repression at constitutive heterochromatin may be more general.**
- **Confirmed by several other studies at meiotic genes.**

- **Poor choice of inducible gene: strongly Rrp6-dependent, but not so much phosphate-regulated.**
- **Some contradictory results ignored.**
- **Writing…**

Thank you!

Now –which one is the ‘higher’ eukaryote?!

Questions?

If they ask you anything you don't know, just say it's due to epigenetics.

Learn more

- **Ask me:**

dom@helmlinger.com

- **Reference articles:**

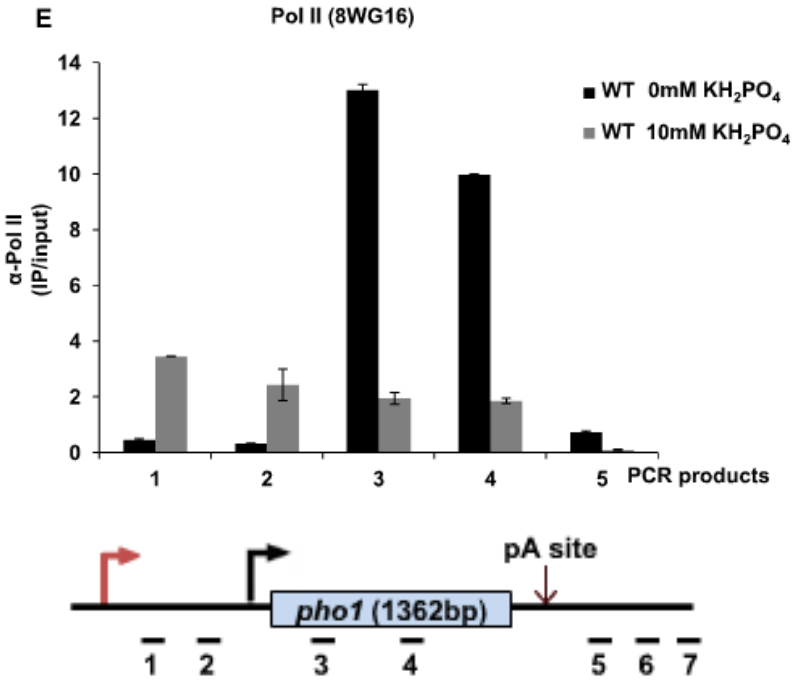
http://www.ncbi.nlm.nih.gov/pubmed/22555442

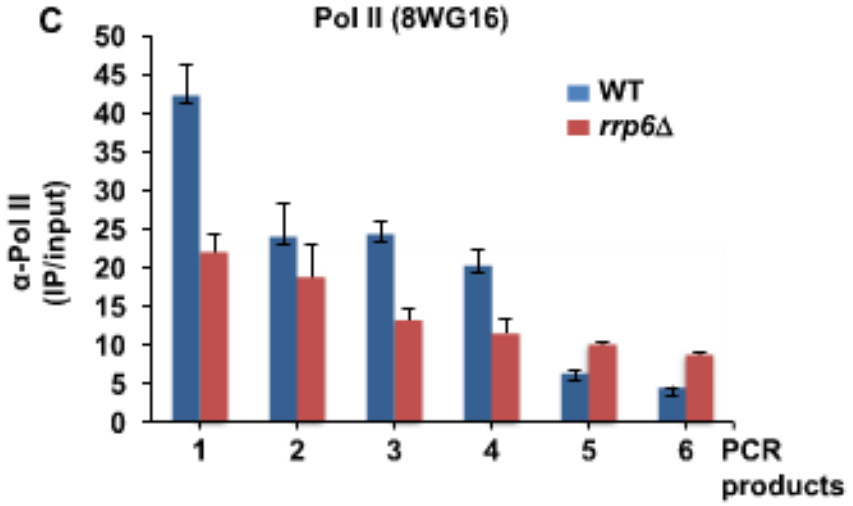
http://elifesciences.org/content/early/2015/01/12/eLife.05007

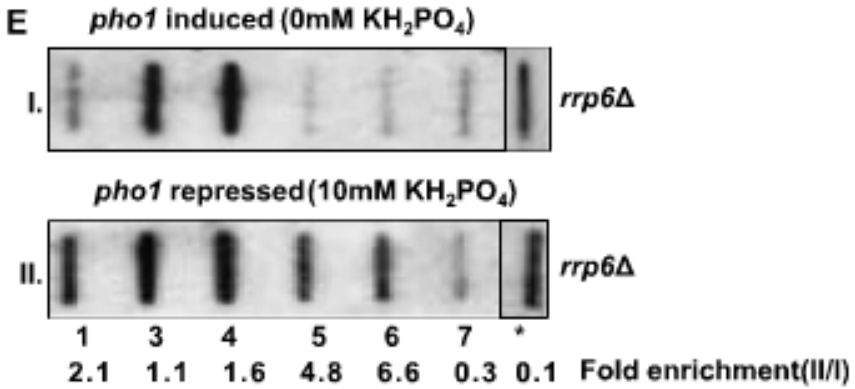
https://www.ncbi.nlm.nih.gov/pubmed/26134317

http://www.ncbi.nlm.nih.gov/pubmed/24493644

Figure 1 + 5: transcription of *pho1* locus.







- Discrepancy ChIP / TRO.
- Termination defect?

Unclear…

Transcription run-on assay