Ch 2 - L2.1

chromatin states establishment and maintenance

Epigenetic inheritance

- Mitotic inheritance
- Trans-generational inheritance

Your Review TextBook contains an important discussion on trans-generational transmission of epigenetic characters.

This is not relevant to what we discuss today, but it will become very important for Lesson 4.

Box 1. Transgenerational inheritance; considering caveats and alternative mechanisms

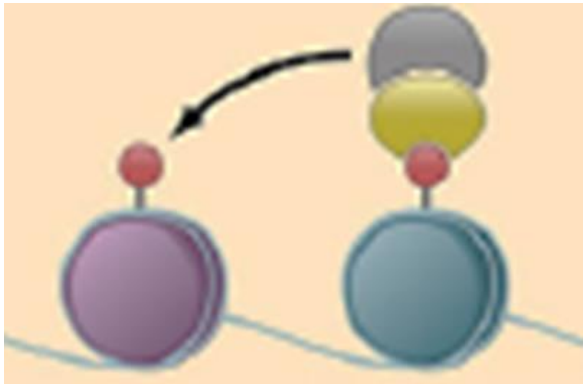
Non-chromatin based mechanisms likely contribute to transgenerational inheritance. For example, some of these phenotypes might arise from cryptic genetic variation given that inbred strains, nearly identical clones or even neighboring cells in the same organism may possess marked genetic differences [108]. Such genetic variation could be passed on to offspring or arise *de novo* (e.g., transposable elements, mutations) and account for differences. Unfortunately, these alternatives are seldom examined in transgenerational studies. Furthermore, establishing transgenerational inheritance in its purest sense is often confounded by maternal care, social transmission, or other variables that may propagate a phenotype without requirement for epigenetic memory *per se*. Indeed recent studies suggest that maternal care may play a significant role even in the transmission of phenotypes originating from the father [109].

Even if a phenotype is transmitted in a transgenerational epigenetic fashion, chromatin events may not always be responsible for their propagation. Transcriptional loops are one example [110]. As in somatic tissue, noncoding RNAs such as siRNA, piRNAs as well as miRNA contribute to inheritance and might function independently of changes at the level of chromatin (recently reviewed by [63]). In fact, a recent study showed that miRNAs are important for transmitting the experience of trauma to progeny through the paternal lineage [65]. Studying the importance of these varied contributions to transgenerational inheritance is important in understanding whether they are truly epigenetic.

Campos et al., 2014

From Lesson 1, we have learnt that the epigenetic marks to histones, as well as CpG methylation, are reproduced over the new DNA (chromatin) after DNA chromatin) replication in a period of time that can extend to the next G1.

Copying pre-existing meCpG and PTMs to new «naif» DNA and nucleosomes implies a general property of chromatin that we can call: «spreading» of chromatin states (by R/W/E complexes).



maintenance

The R/W/E complex model

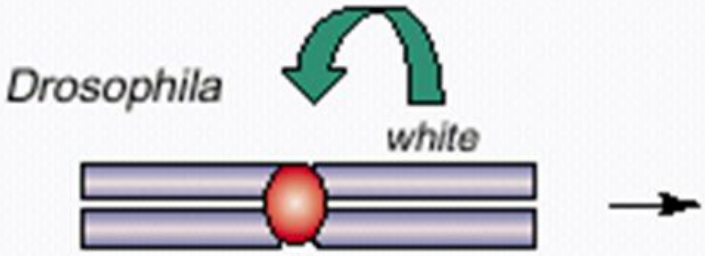
Let’s examine some *classical* examples and knowledge from genetical research, quoted in many reviews and books.

These are examples taken from older literature and model systems, are often quoted even simply by the acronym. Therefore, it is possibly better to see them and understand the underlaying principles, even when you won’t be required to tell about at the exam.

The first data concerns the historical concept in Genetics of the so-called «positional effect variegation» (**PEV)** in *D. melanogaster (…and other model organisms).*

Drosophila ?
Please note that Insects present very low levels of cytosine methylation. It has been mainly explained for transposon silencing.

PEV = Positional Effect Variegation Silencing effects of heterochromatin in different organisms.



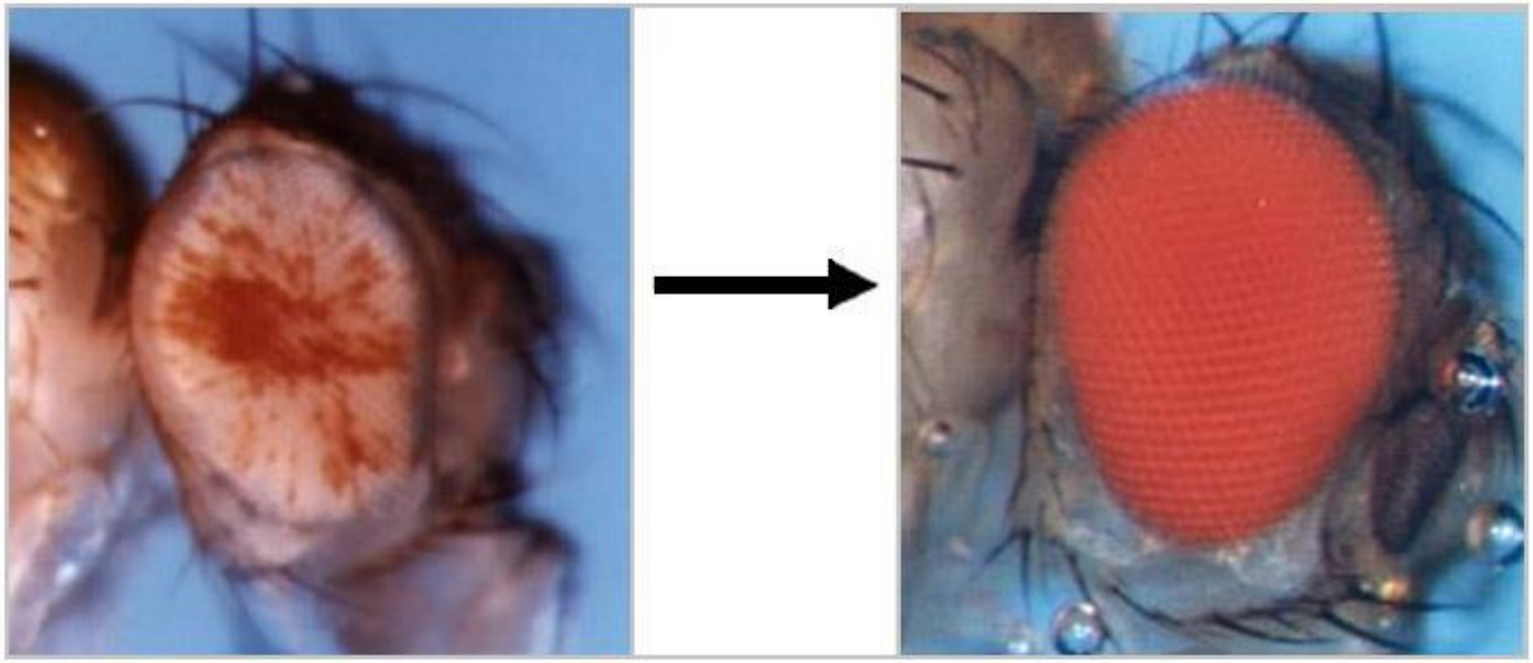
Translocation of the Drosophila *white* gene close to the centromere results in mosaic (variegated) expression in the eye.

Insertion of transgenes into peri-centromeric regions in mice gives expression in a proportion of cells from expressing tissues. Expression is clonally stable through multiple cell divisions but also undergoes stochastic switching between the active and inactive states.

Insertion of the Ade2 gene into the telomeric region of Saccharomyces cerevisiae causes metastable silencing of the gene resulting in sectored colonies containing white (expressing) and red (nonexpressing) cells.

Variegated

Actually, looking at the eyes of these animals, we observe patches of pigmented cells among a general white color of other cells.



Washington University Department of Biology - Mutations in HP1 lead to a loss of silencing, a suppression of PEV.

Mendez et al, 2011

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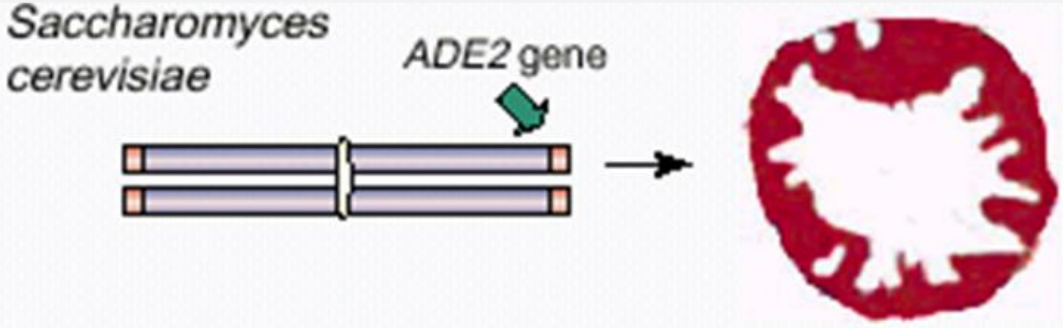
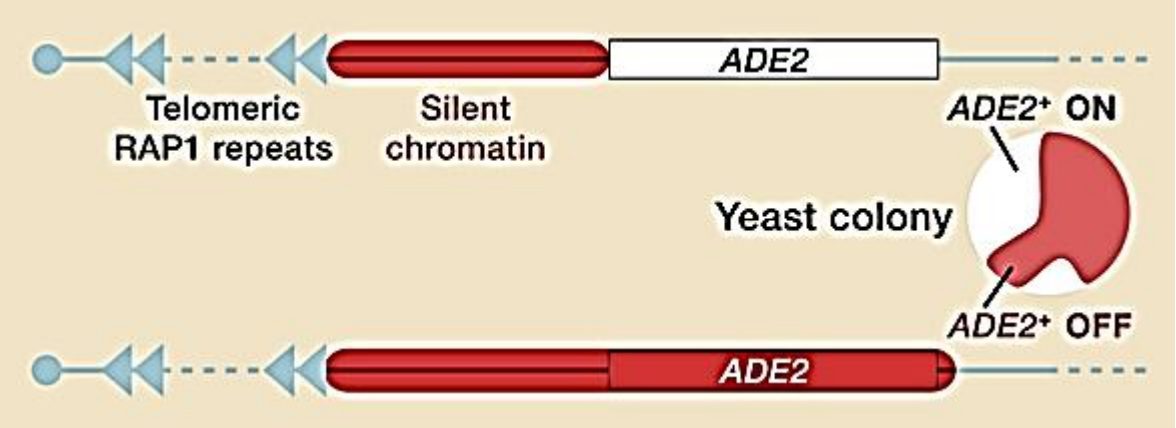
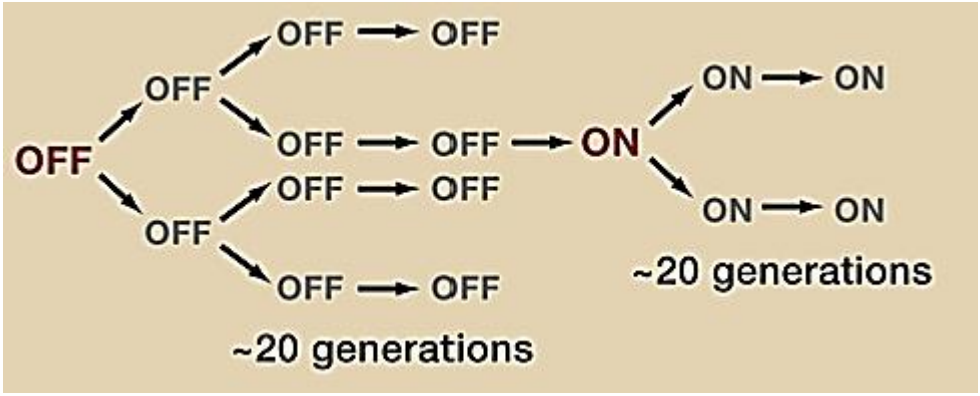


Insertion of the Ade2 gene into the telomeric region of Saccharomyces cerevisiae causes **metastable** silencing of the gene resulting in sectored colonies containing white (expressing) and red (nonexpressing) cells.

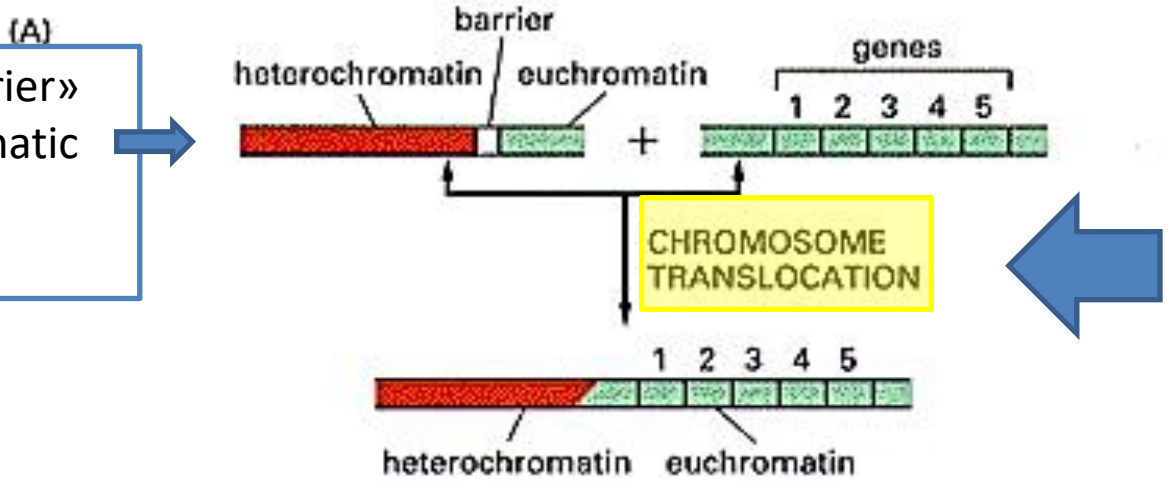


PEV

This variegation results from stochastic loss and re-establishment of silent chromatin and indicates that following a switch in gene expression, the daughters of the switching cell have **a memory of the expression state** of the mother cell.

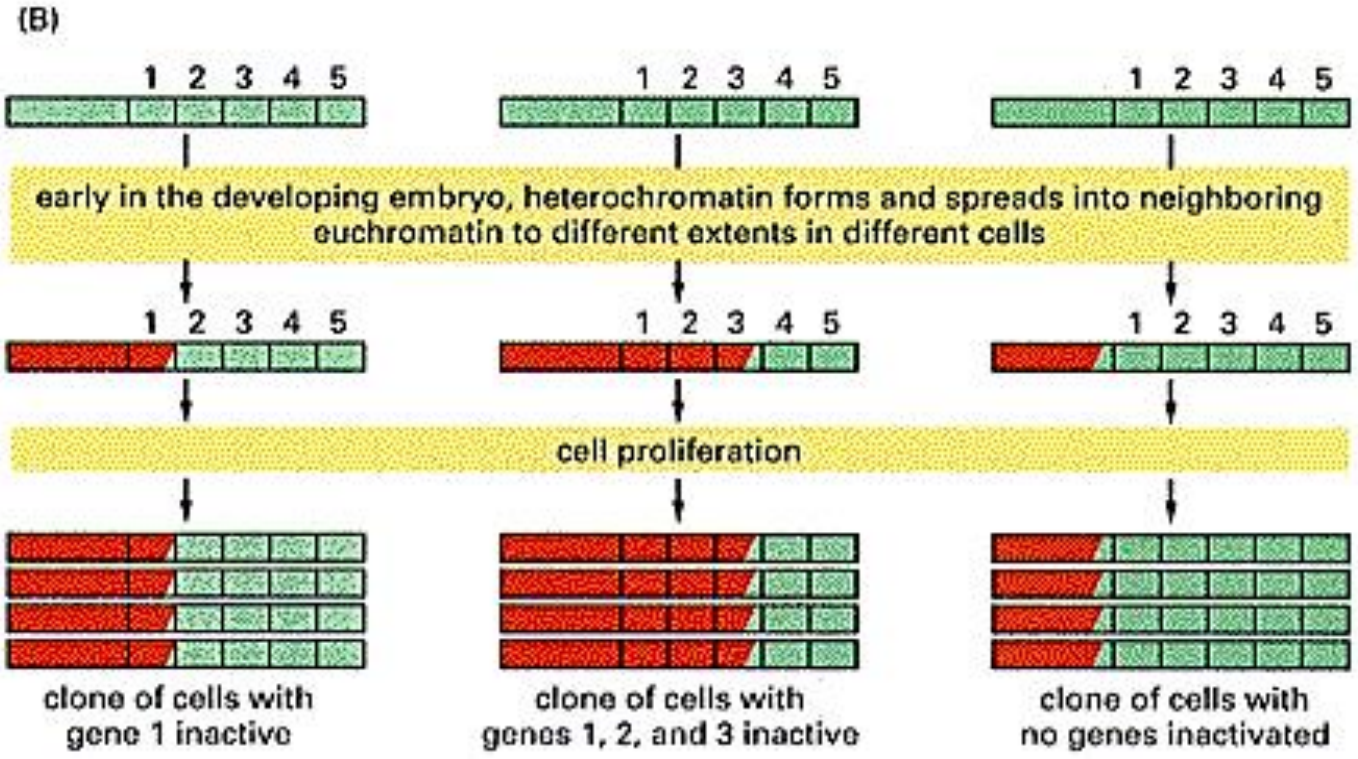


In normal situation, a «barrier» exist between heterochromatic and euchromatic domains
We call it «insulator».



Establishement →

Maintenance →



These results tell us that there are distinct phenomena:

- Establishment (or programming)
- Maintenance (mitotic epigenetic inheritance)
- Re-programming

Establishment

Where do chromatin states start from ?

In other words, what is the original signal or event that will start formation of heterochromatic / euchromatic domains ?

What are the **determinants** ?

cis & *trans*

Again, classical models have illustrated the mechanism

Essentially in the Yeasts:

- simpler, smaller genomes, less regulators on the plot
- *high rates of homologous recombination simplify experiments*

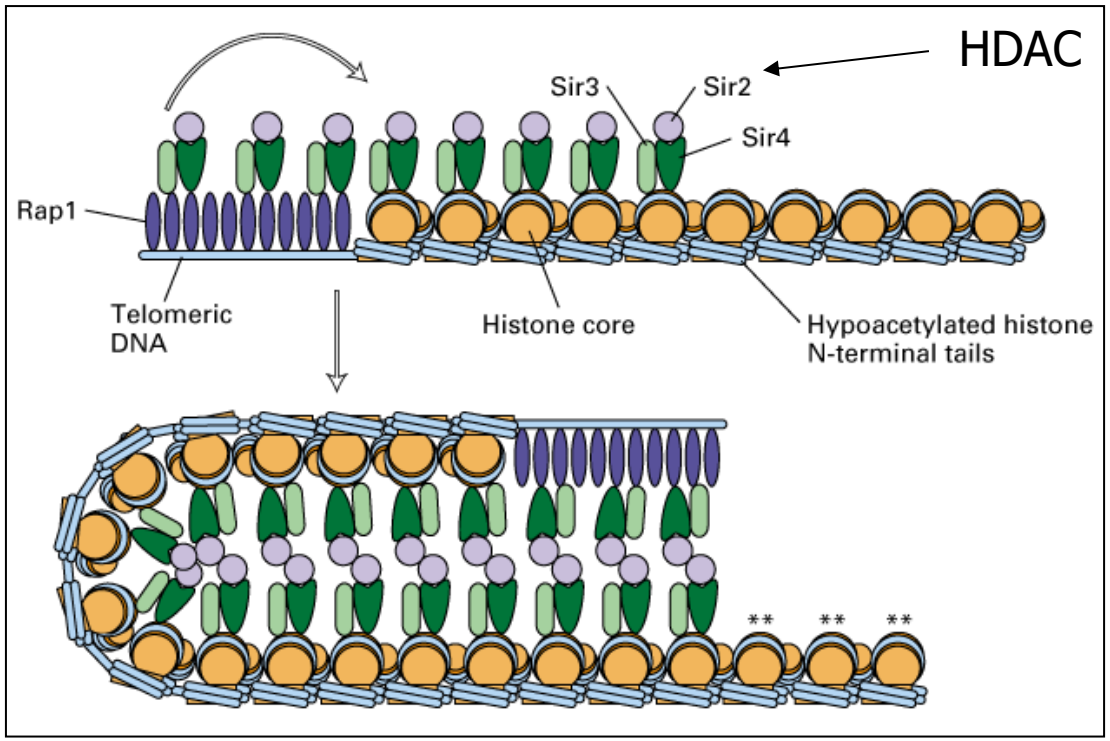
Most studied models:

- Telomeric heterochromatin (S. cerevisiae)
- MAT locus (S. cervisiae)
- Centromeric heterochromatin (S. pombe)

The model emerging includes:

1. ***cis*-determinants** (DNA sequence)
2. **specificity factors** (Sequence reader)
3. **bridging proteins** (connector)
4. **Effector enzymes** (histone modifiers, chaperons, DNTM)

First model system was budding yeast telomeric heterochromatin



Linear chromosome replication

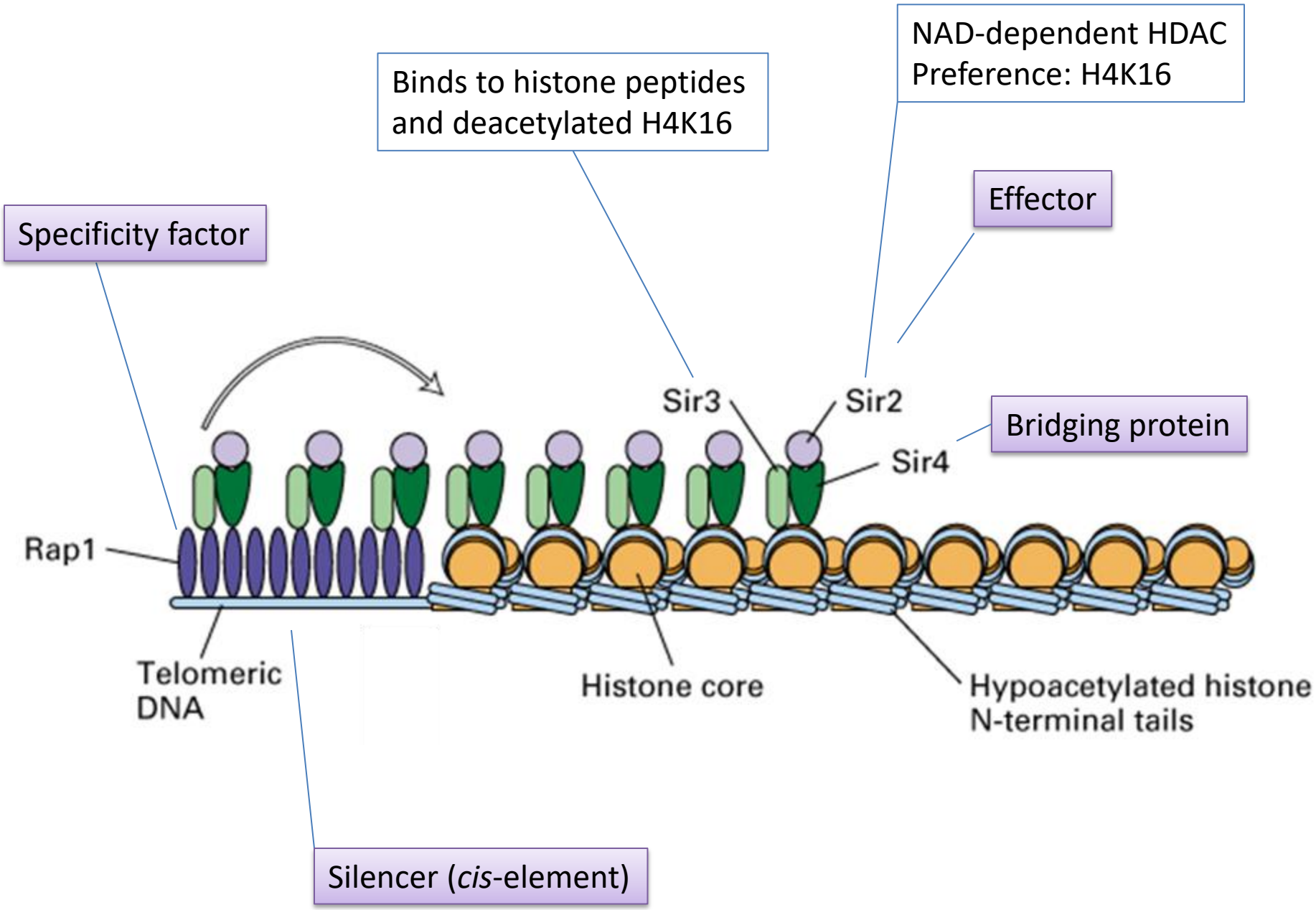
Telomere structure

Telomerase

Telomeric DNA is repetitive (microsatellite)
Rap1 protein binds to repetitive telomeric sequence
Rap1 interacts with Sir3 and Sir4 (silence inducing repressor) that, in turn, interact with Sir2 (HDAC)
When repetitive DNA finishes Sir3/4 interact with hypoacetylated histones and associate Sir2 to deacetylate adjacent nucleosomes

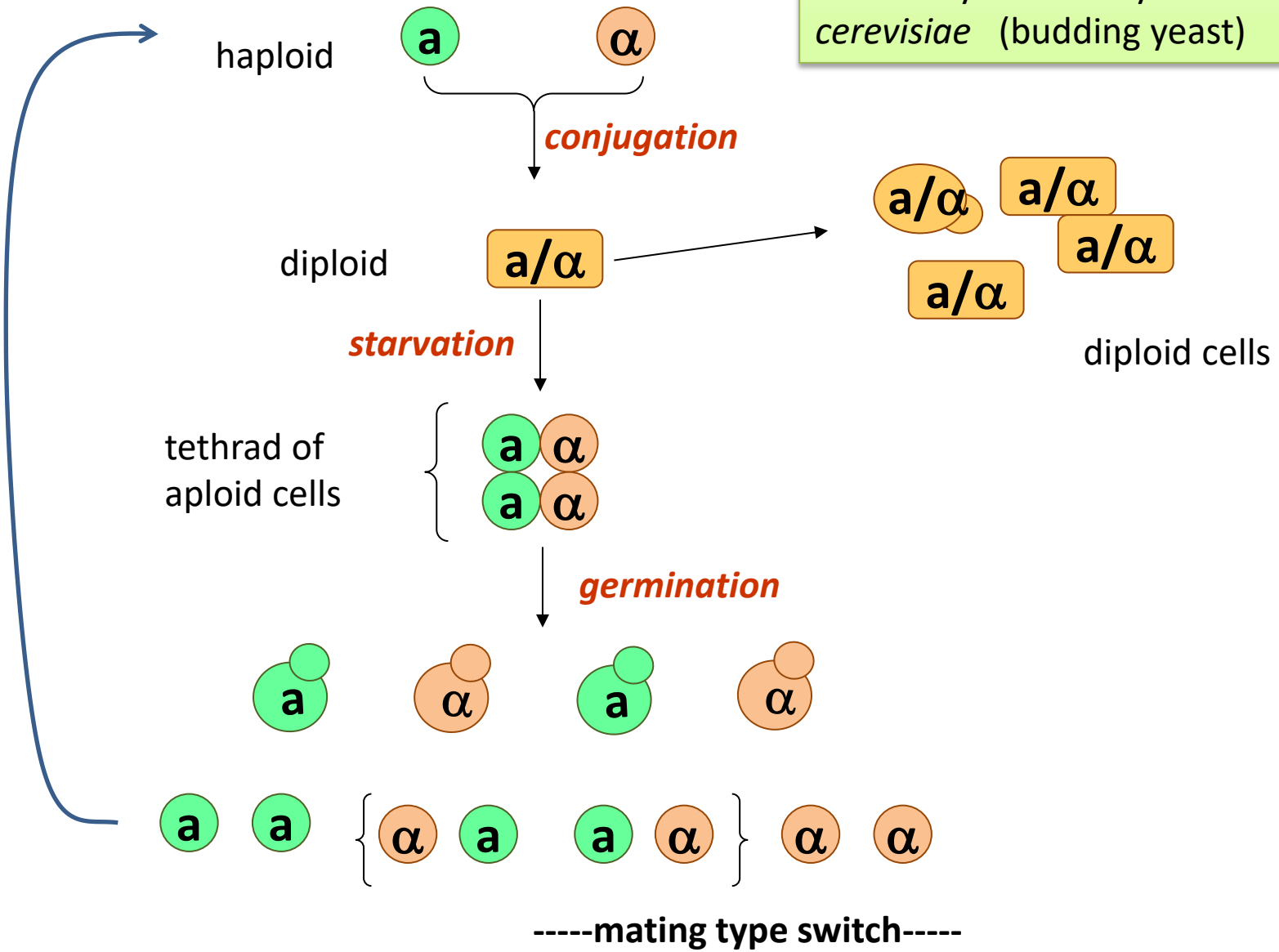
Experimental:
Delete SIR3 → no longer telomeric chromatin
SIR3 overexpression → heterochromatin extended up to some 16–20 kb from the telomere.

S. Cerevisiae telomere HC

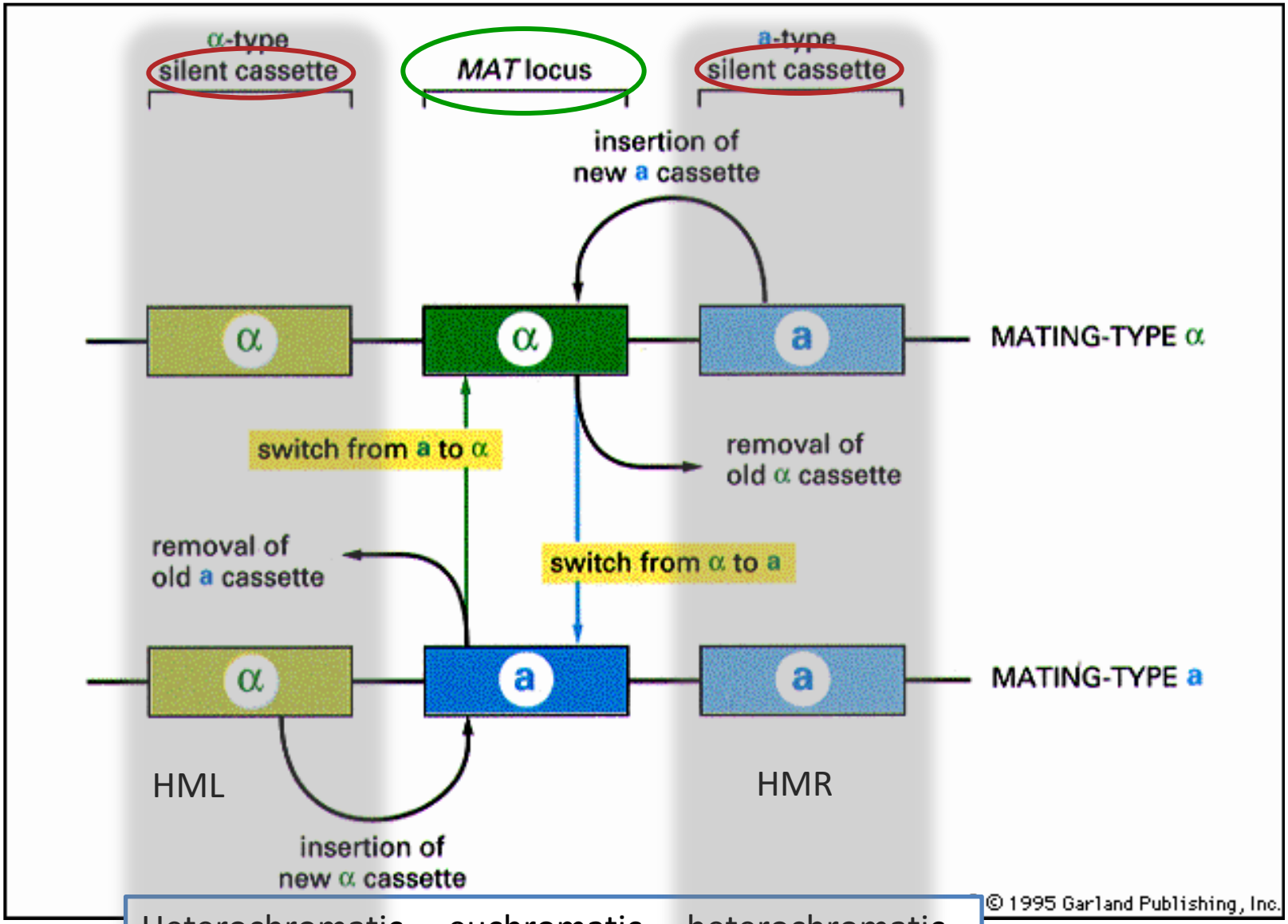


2nd model system was the Mating type locus in budding yeast

The life cycle of the yeast *Saccaromyces cerevisiae* (budding yeast)

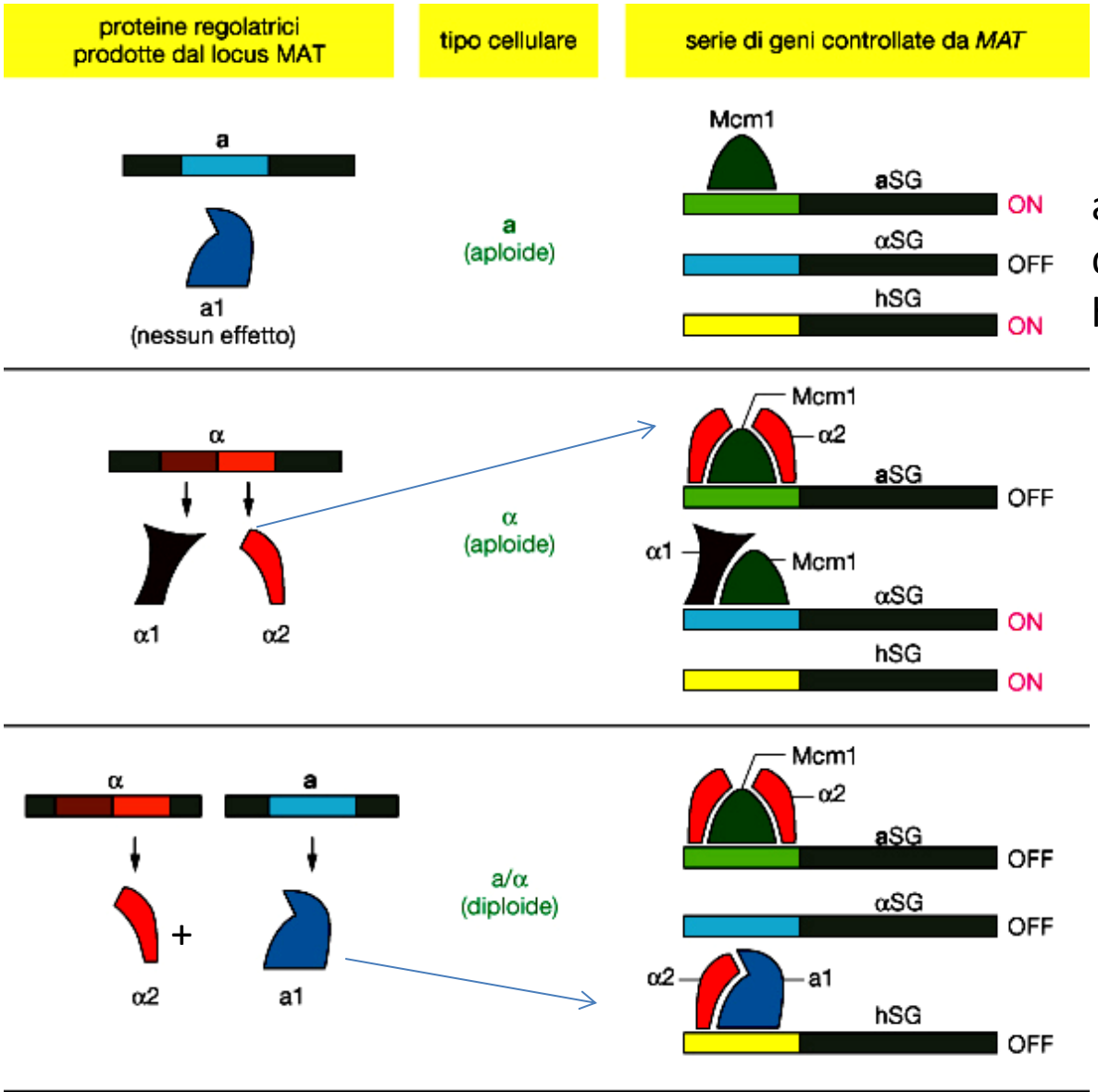


HMR/HML loci in yeast have heterochromatic features, e.g. are resistant to endonuclease digestion and **silence constructs placed within**



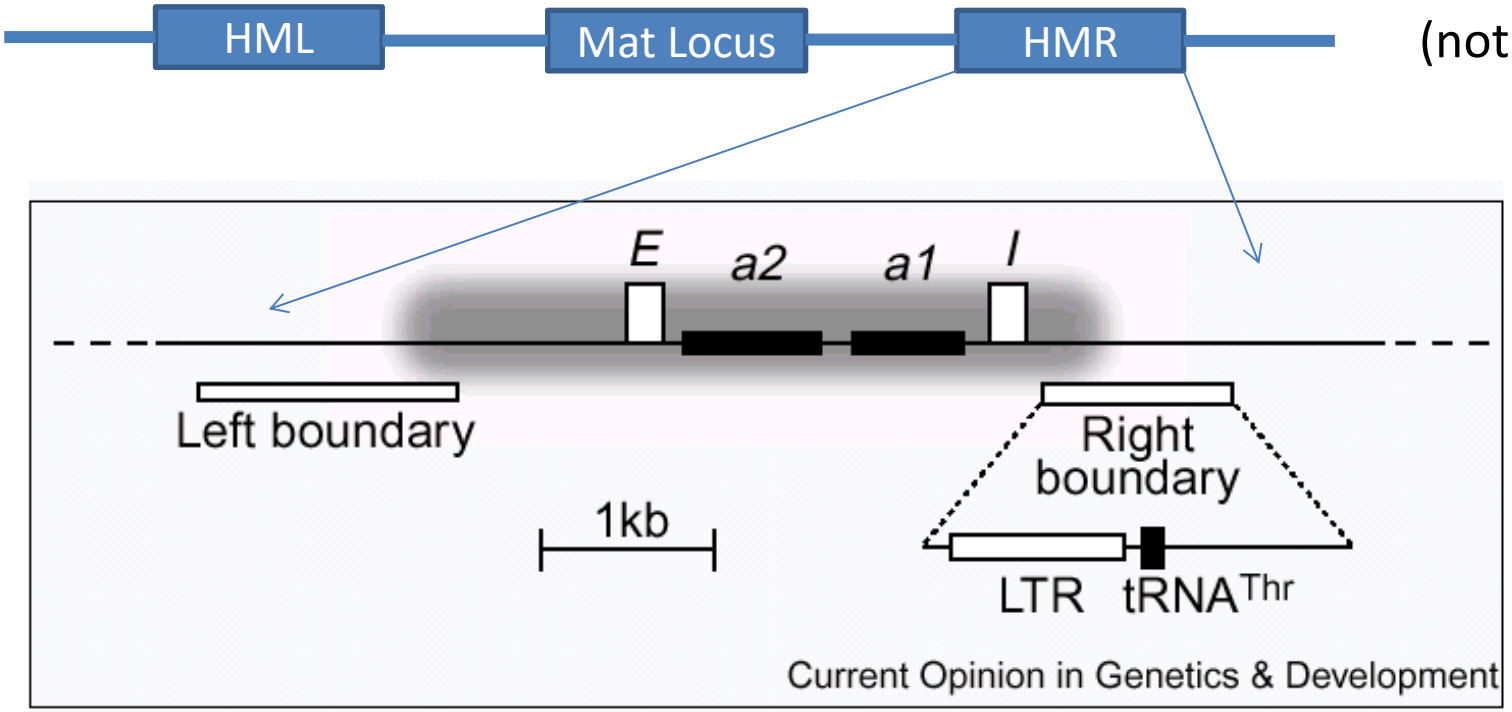
Heterochromatic euchromatic heterochromatic

A and α cassettes encode transcription factors that regulate aploid and mating type-specific genes



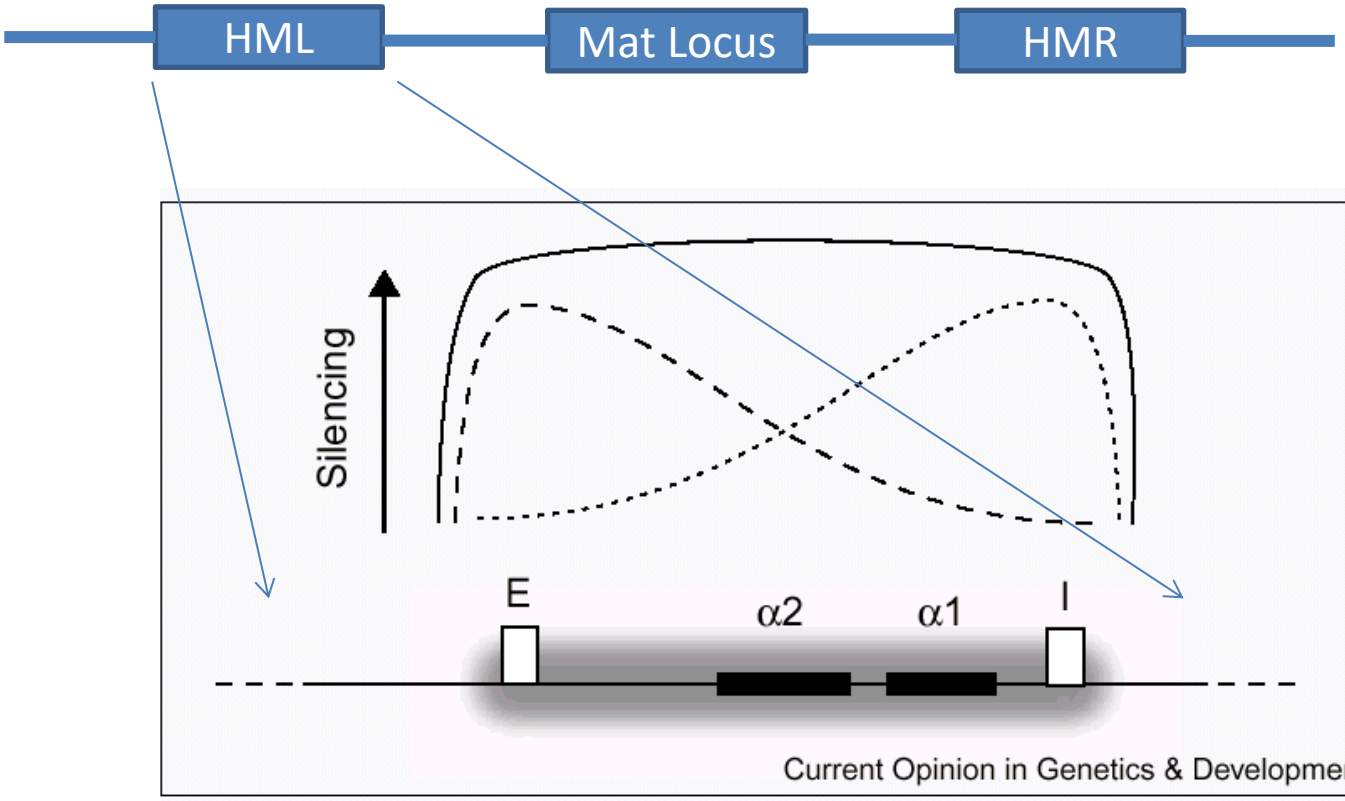
a-specific genes (aSG)
α-specific genes (αSG)
haploid-specific genes (hSG)

Mcm1 is constitutive



HMR

Organization of heterochromatin barriers surrounding *HMR*. The *HMR* locus is diagrammed, showing the location of the mating type genes *a1* and *a2*, the *E* and *I* heterochromatin organizing centers (silencers) and the left and right heterochromatin barriers as defined in [1••]. Background shading indicates the extent of the repressed domain. An expansion of the right barrier shows the location of a Ty1 LTR and the gene for $tRNA^{Thr}$. Earlier evidence suggested that both these elements contribute to barrier activity [1••] but more recent data indicate that the $tRNA^{Thr}$ gene is necessary and sufficient for full barrier activity

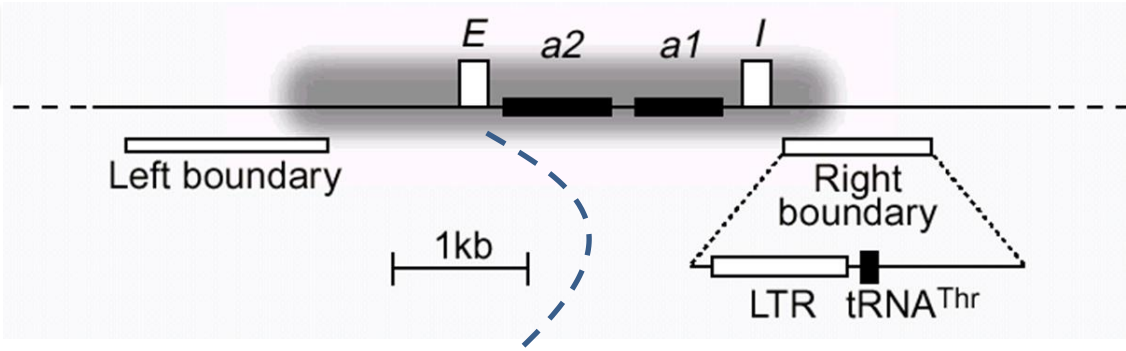


E and I are mixed Silencers/barriers

HML

Domain organization by directional initiation of heterochromatin. The *HML* locus is diagrammed, showing the location of the mating type genes, α*1* and α*2*, and the *E* and *I* heterochromatin organizing centers (silencers). Background shading indicates the extent of the repressed domain. Repression emanating from *E* is represented as a dashed line, whereas that emanating from *I* is represented as a dotted line. The sum of the effects of the two organizing centers, shown as a solid line, results in uniformly high repression between the two centers with repression dropping off sharply outside the domain. This model emerges from studies on the domain organization of HML presented in [35•].

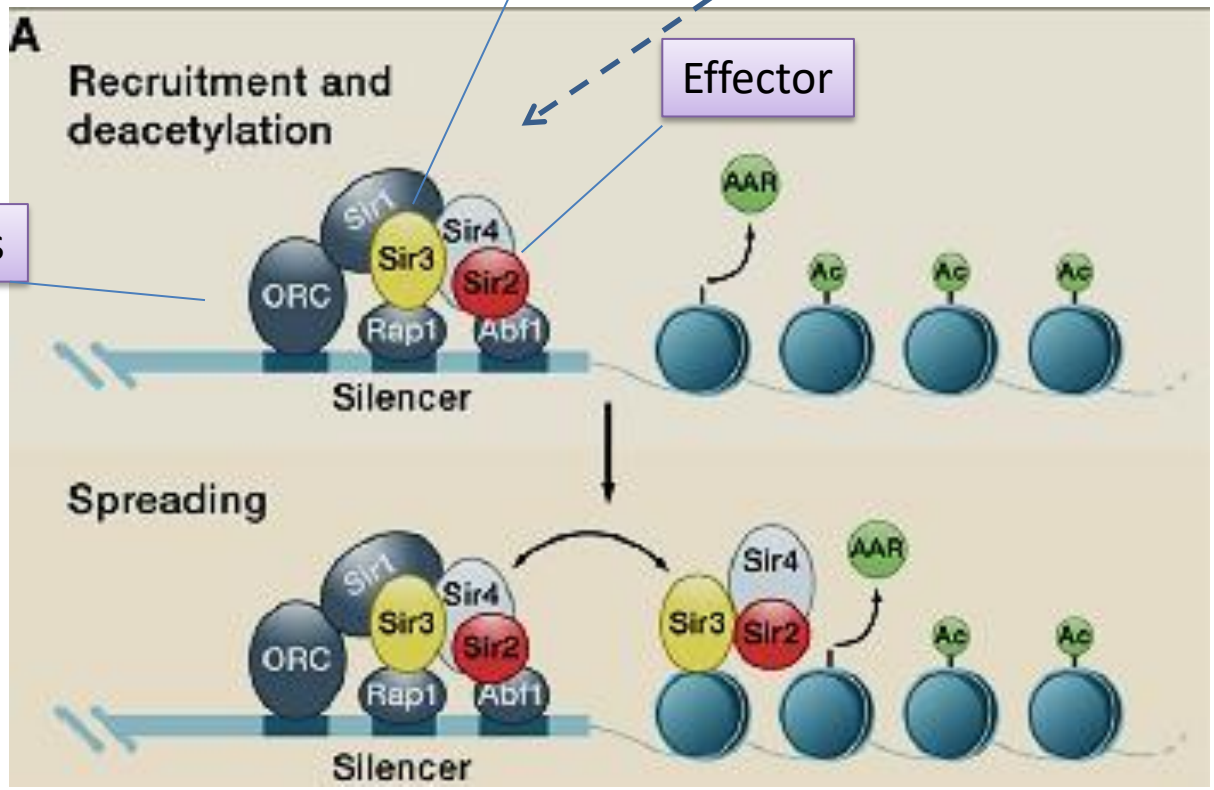
S. Cerevisiae MAT locus HC



Bridging protein

Effector

Specificity factors

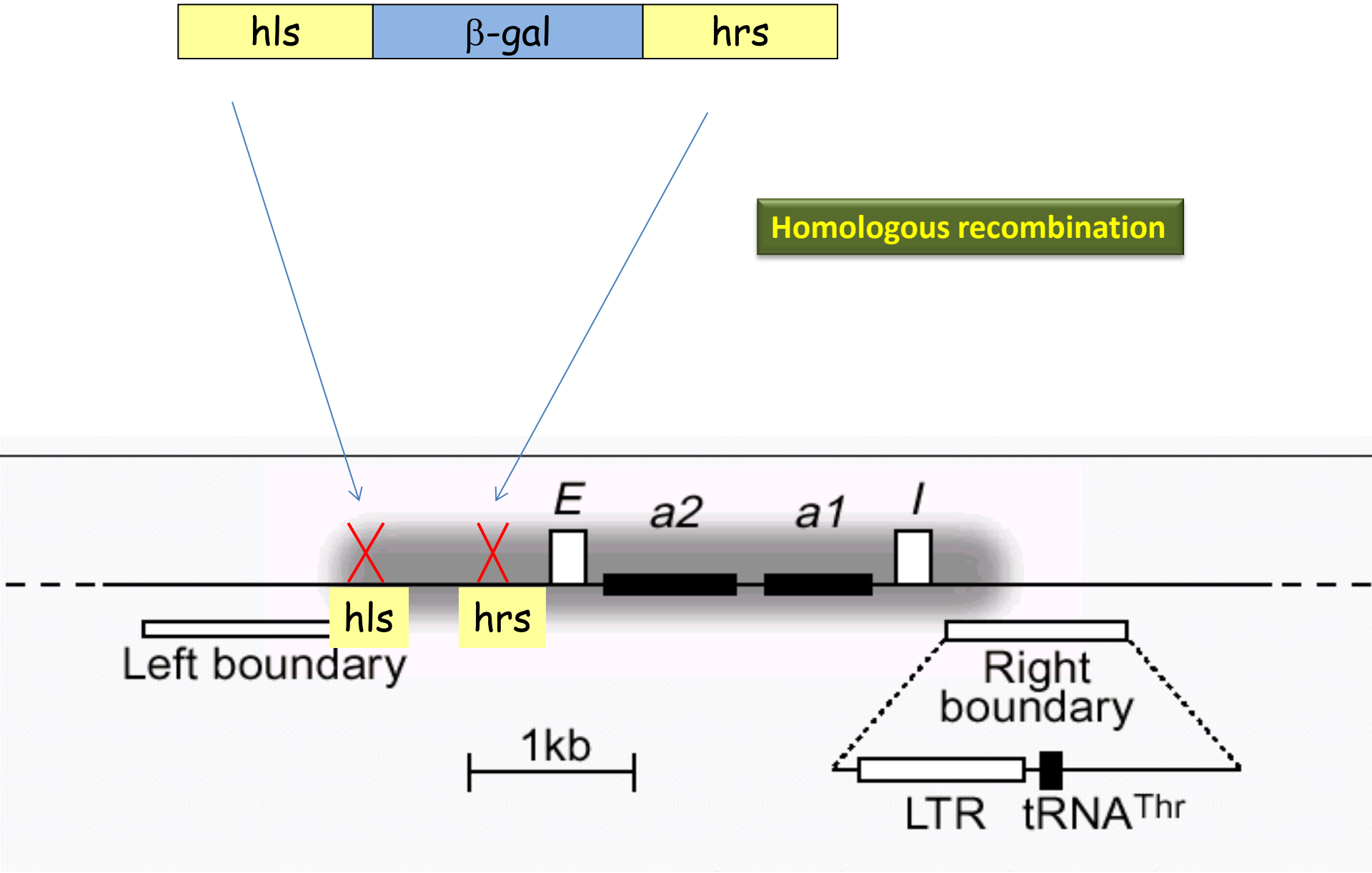


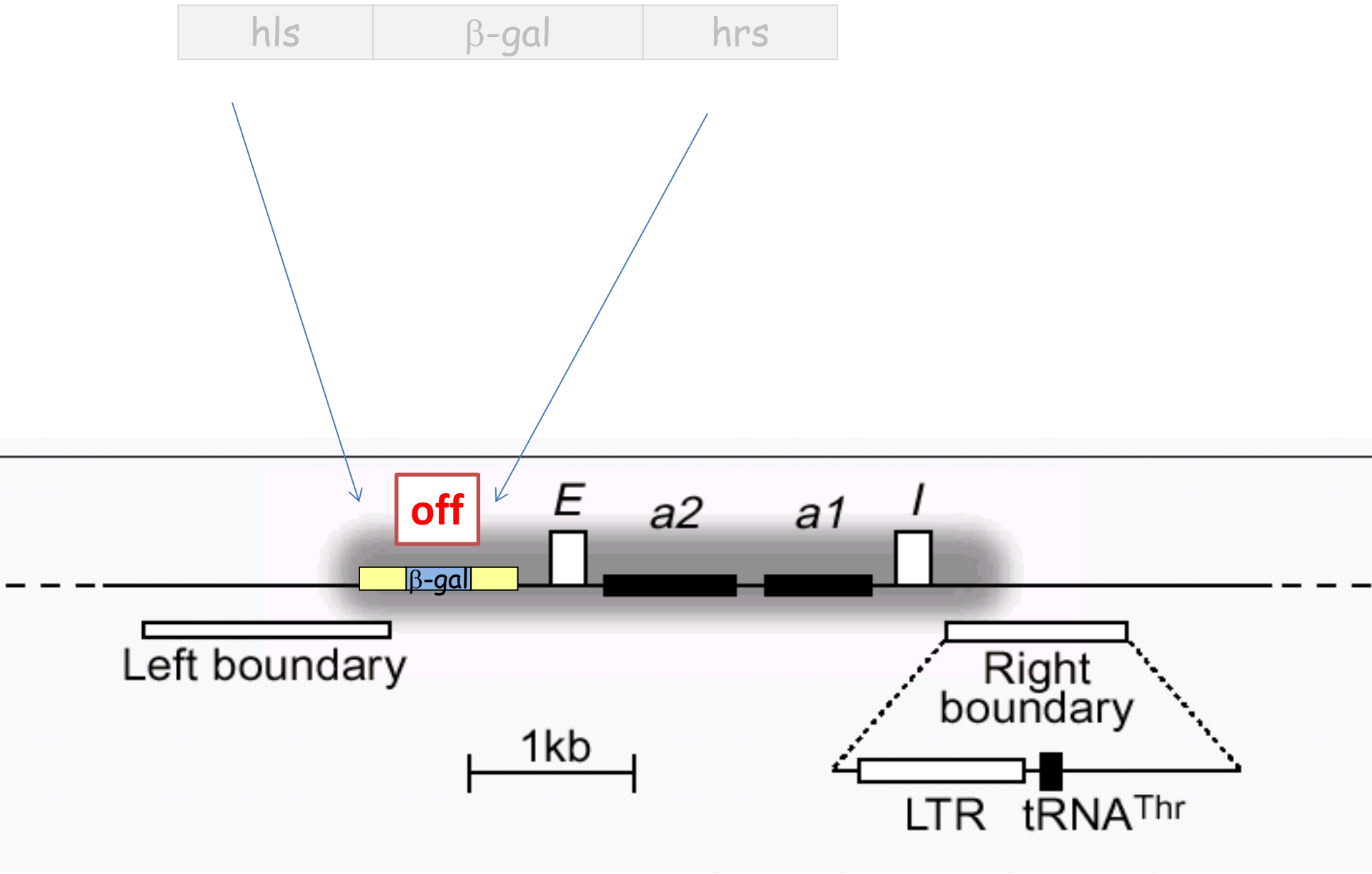
Silencer (*cis*-element)

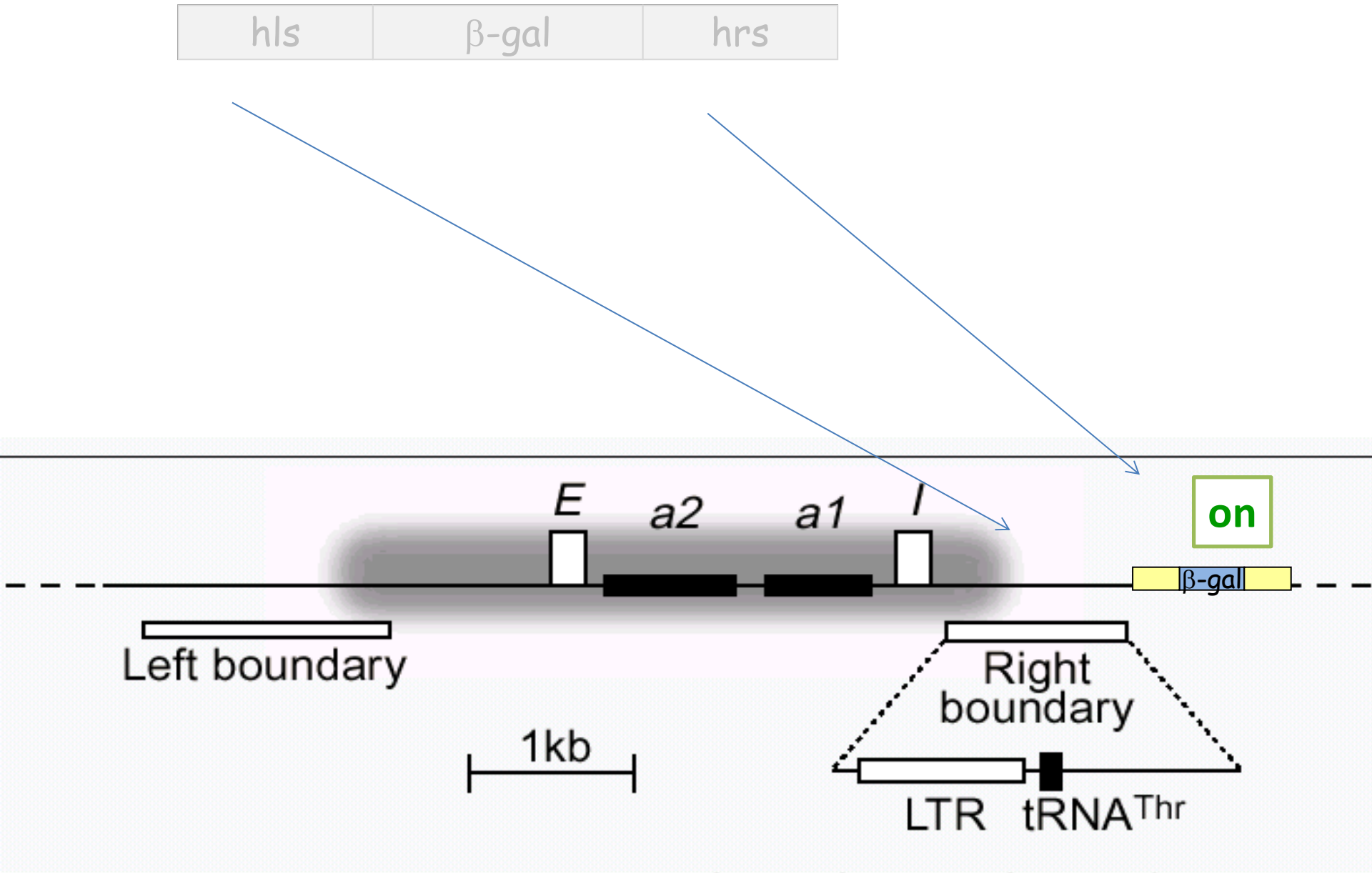
Methodology

High efficiency of Homologous Recombination in Yeast makes easy to study the effects of Heterochromatizatin / euchromatization, using reporter genes such as:

- Beta-galactosidase (blue color)
- Ura4 (S. pombe) or Ura3 in S.cerevisiae, is required to grow in absence of uracil and renders cells sensitive to toxicity of 5-FOA
- …other


hls
β-gal
hrs
Homologous recombination
E
a2
a1
I
hls
hrs
Left boundary
1kb
Right boundary
LTR
tRNA Thr


hls
β-gal
hrs
off
E
a2
a1
I
β-gal
Left boundary
Right boundary
1kb
LTR
tRNA Thr


hls
β-gal
hrs
E
a2
a1
I
on
β-gal
Left boundary
Right boundary
1kb
LTR
tRNA Thr

Looking to these model systems, we can conclude:

- A DNA element that we can call «silencer» (talking heterochromatin)
- A molecule recognizing this DNA element, called «specificity factor»
- A bridging protein
- A chromatin competent enzyme

Chromatin states establishment in animals ?

- A DNA element that we can call «silencer» (talking heterochromatin)
- A molecule recognizing this DNA element, called «specificity factor»
- A bridging protein
- A chromatin competent enzyme

TF binding sites

Transcription Factors

co-regulator

e.g. PRC2 ?